



# **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.8 (AS)**

#### **Environmental Fate & Behaviour**

Great Britain

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**Version History**

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## B.8. Environmental Fate and Behaviour

Inpyrfluxam, also known as S-2399, is a fungicide proposed for use on cereal crops. Single spray applications to winter and spring wheat, winter and spring barley and durum wheat at a rate of up to 1.5 L product/ha (90 g a.s./ha) are proposed for a spring application window of BBCH 30-71.

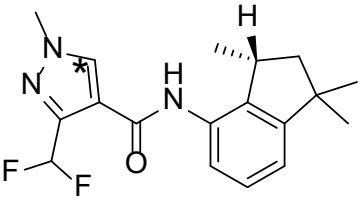
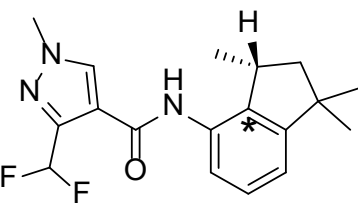
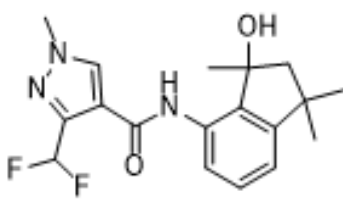
All studies supplied by the applicant were conducted in accordance with the requirements of assimilated Regulation (EU) 1107/2009. The environmentally relevant properties were investigated using inpyrfluxam radiolabelled in both the phenyl and pyrazolyl ring structures. The studies were used to elucidate a full metabolic profile for inpyrfluxam in the environment and to propose environmentally relevant exposure concentrations for the proposed use pattern.

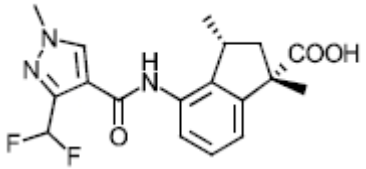
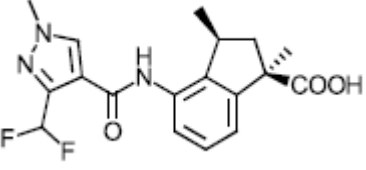
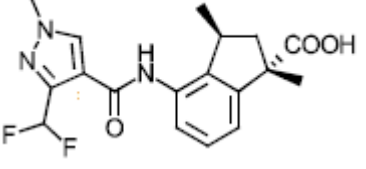
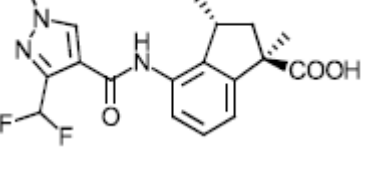
Inpyrfluxam contains a chiral centre and exists as both R- and S-isomers. The enantiomeric ratio of parent and 1'COOH-S-2840 (and, where appropriate, chiral minor metabolites) were monitored throughout the studies via chiral HPLC analysis. The active substance is present predominantly as the R-isomer and the ratio of the two isomers did not change during the studies. Inpyrfluxam degrades via two main mechanisms, forming 3'-OH-inpyrfluxam via one route of degradation and forming 1'COOH-S-2840 (and associated minor metabolites) via the other route. Conclusions regarding chirality for 3'-OH-S-2840, are the same as for the parent. Metabolites formed by the second route however also contain an additional chiral carbon that results in the formation of two sets of isomer pairs and further complicates stereoisomer considerations.

An overview of the active substance, metabolites and their radiolabel positions is discussed below in Table B.8.01.

Inpyrfluxam contains a fluorinated methyl group but it does not meet the definition of a PFAS according to the OECD criteria.

Table B.8-1 Summary of active substance and metabolites

Substance name (plus synonyms)	Compartments assessed	Structure
<b>Parent</b>		
Inpyrfluxam (S-2399)	Soil  Surface water  Groundwater  Air	 <p>[pyrazolyl-4-<sup>14</sup>C] inpyrfluxam</p>  <p>[phenyl-U-<sup>14</sup>C] inpyrfluxam</p> <p>Radiolabel position denoted by *</p>
<b>Metabolites</b>		
3'-OH-S-2840	Soil  Surface water  Groundwater  Air	

Substance name (plus synonyms)	Compartments assessed	Structure
1'-COOH-S-2840A isomer	Soil  Surface water  Groundwater  Air	 
1'-COOH-S-2840B isomer		 

For the soil and water environmental compartments, the assessment of the rate of degradation included the derivation of several endpoints through kinetic evaluation in accordance with the FOCUS Kinetics Guidance (2006; 2014). Regulatory endpoints, also known as trigger endpoints, were derived from best fit kinetics as regulatory endpoints used as triggers for higher-tier experiments. The endpoints are also used for predicting the environmental concentration of the active substance in soil ( $PEC_{soil}$ ). The triggers for additional studies on the environmental fate and behaviour of substances are as follows:

- Field dissipation studies are required for the active substance or metabolites when the  $DT_{50}$  exceeds 60 days (20°C, pF2) or 90 days (10°C, pF2) in a laboratory study;
- Field dissipation studies are required for the active substance or metabolites when the  $DT_{90}$  exceeds 200 days (20°C, pF2) or 300 days (10°C, pF2) in a laboratory study;
- Soil accumulation studies are required when the field  $DT_{90}$  exceeds one year.

Degradation rates based on best fit kinetics are also derived for the consideration of persistence criteria, known as persistence endpoints. These are used to conclude whether an active substance is a persistent organic pollutant (POP), or whether it fulfils persistence, bioaccumulation and toxicity (PBT) and very persistent, very bioaccumulative (vPvB) criteria.

Additionally, degradation rates are derived for use as input for pesticide fate models, also known as modelling endpoints. These are used to determine predicted environmental concentrations (PECs) in surface water, sediment and groundwater and are normally based on single first order kinetics.

### **B.8.1. Fate and behaviour in soil**

The applicant submitted laboratory and field studies to determine the fate and behaviour of inpyrfluxam in the environment. The route and rate of degradation and dissipation was studied for parent and metabolites.

The route and rate of degradation are summarised below including a summary of FOCUS Kinetics and the endpoints selected for use in the exposure assessment.

#### **Route of degradation**

The applicant submitted studies to investigate the route of degradation in soil for the parent and for metabolites. These are summarised in Table B.8.1-1. Two major metabolites, 3'-OH-S-2840 and 1'-COOH-S-2840 were observed in the parent applied study. Metabolite applied studies for these metabolites were also supplied. The applicant also submitted anaerobic degradation studies and a photolysis study for parent inpyrfluxam.

**Table B.8.1-1 Laboratory studies investigating the route of inpyrfluxam degradation in soil.**

<b>Laboratory soil study</b>	<b>Study type</b>	<b>Comments</b>
██████████ 2017 KCA 7.1.1.1/01	Aerobic degradation	Parent applied route and rate of degradation study
██████████ 2017a KCA 7.1.1.1/02	Aerobic degradation	Parent applied route and rate of degradation study
██████████ 2017a KCA 7.1.2.1.2/01	Aerobic degradation	3'-OH-S-2840 applied route and rate study
██████████ 2017b KCA 7.1.2.1.2/02	Aerobic degradation	1'-COOH-S-2840 applied route and rate study.
██████████, 2017a KCA 7.1.1.2/01	Anaerobic degradation	Parent applied route and rate of degradation study
██████████ 2017b KCA 7.1.1.2/02	Anaerobic degradation	Parent applied route and rate of degradation study
██████████ 2014 KCA 7.1.1.3/1	Soil photolysis	Parent applied study

Aerobic degradation is considered to be a major route of degradation for inpyrfluxam. The aerobic degradation of inpyrfluxam was investigated under laboratory conditions in 2 European and 2 US soils. Inpyrfluxam was labelled in both the phenyl and pyrazolyl rings to allow the metabolism of the entire molecule to be monitored throughout the studies. By study end (120 to 182 days), inpyrfluxam accounted for 41.8 to 53.5 % AR, while unextracted residues and CO<sub>2</sub> increased during the study to 8.9 to 12.2 % AR and 0.3 to 0.8 % AR respectively. Two major metabolites were detected, both generally increasing during the study: 3'-OH-S-2840 reached a maximum of 20.7 % AR and 1'-COOH-S-2840 (A and B combined) reached a maximum of 30.1 % AR by study end. Other metabolites DFPA, N-des-Me-DFPA

and N-des-Me-S-2840 were also detected in minor amounts ( $\leq 3.3$  % AR). HSE notes that the route diagram presented in the laboratory degradation studies of inpyrfluxam, where metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 degrade to form DFPA and then N-des-Me-DFPA, is not corroborated by the 3'-OH-S-2840 and 1'-COOH-S-2840 dosed laboratory studies. In the 3'-OH-S-2840 dosed study, only minor unknown compounds were detected, but in the 1'-COOH-S-2840 dosed study, a further metabolite, 1'-keto-S-2840, was detected at up to 21.7 % AR. The applicant has also provided a new degradation scheme including 1'-keto-S-2840 (see Figure B.8.1.1.2-08).

Metabolite 1'-keto-S-2840 also has the potential to be a major metabolite in soil at up to 6.5 % AR based on the amount of 1'-COOH-S-2840 remaining in the parent applied study, but a case has been made to show that it is not necessary to include this metabolite in the exposure assessment. This is because this metabolite was not observed in field studies and is also less mobile than its precursor and the risk to groundwater is therefore considered covered by the assessment for 1'-COOH-S-2840. DFPA, N-des-Me-DFPA and N-des-Me-S-2840 were not detected as major metabolites in any of the soil studies submitted and the available studies do not therefore give a full picture of the degradation processes occurring in soil. Around 80 % AR is still present at study end as parent and the two major metabolites, in which the amide bridge is still intact. Limited cleaving of the amide bridge to form the minor metabolites has therefore occurred during the study. Furthermore, these minor metabolites are not detected in the metabolite applied studies. The degradation studies do not therefore give a complete picture of the degradation in soil and is unclear to what extent cleavage of the amide bridge and formation of the minor metabolites would occur over longer durations. Further formation of the minor metabolites may have occurred had the study had been continued.

The anaerobic metabolism of inpyrfluxam was studied in 2 European and 2 North American soils under laboratory conditions. Inpyrfluxam was radiolabelled in the phenyl and pyrazolyl rings for one soil and in the pyrazolyl position only in the 3 further soils. An aerobic phase of 28 days was implemented prior to soil flooding to induce anaerobic conditions for the remaining 125 to 127 days. At study end inpyrfluxam accounted for 60.6 to 86.8 % AR with the majority of the degradation occurring in the aerobic phase. Both unextracted residues and CO<sub>2</sub> remained low in all soils: unextracted residues reached a maximum of 1.2 to 7.7 % AR while CO<sub>2</sub> reached 0.1 to 0.5 % AR by study end. It was concluded that anaerobic metabolism is not a major route of degradation for inpyrfluxam.

The soil photolysis of inpyrfluxam radiolabelled in the pyrazolyl- and phenyl-positions was investigated in one soil over 12 or 13 days under continuous artificial light. In light exposed samples, inpyrfluxam was present at 84.0 to 87.8 % AR at study end, with 3'-OH-S-2840 the only metabolite detected at 7.7 to 8.3 % AR at study end; TLC analysis showed that this HPLC peak consisted of two components present in

roughly equal amounts although the second component was not identified. Unextracted residues reached 1.9 to 2.0 % AR at study end, while total volatiles reached 0.2 to 0.5 % AR. It was therefore concluded that photodegradation is not expected to be a major degradation process in soil for inpyrfluxam.

### Rate of degradation

Trigger and modelling endpoints were calculated for the laboratory degradation studies described above. These are summarised below for inpyrfluxam and its metabolites 3'-OH-S-2840 and 1'-COOH-S-2840. Modelling and best fit endpoints are the same and therefore only one set of endpoints is provided.

As the longest DT<sub>50</sub> and DT<sub>90</sub> values for inpyrfluxam, 3'-OH-S-2840 and 1'-COOH-S-2840 exceeded the field dissipation study triggers, studies were provided covering 4 European and 5 North American sites (see Table B.8.1-7 and Table B.8.1-8 for summary results).

**Table B.8.1-2 Summary of trigger/persistence and modelling endpoints for inpyrfluxam and its two metabolites derived from the parent applied aerobic degradation study (conducted at 20°C and pF 2); modelling and best fit endpoints are the same.**

Inpyrfluxam	Dark aerobic conditions							
Soil	pH (H <sub>2</sub> O)	pH (CaCl <sub>2</sub> *)	Temp °C	% MWHC	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	St. (χ <sup>2</sup> )	Method of calculation
Atwater (sandy loam)	7.5	7.0	20	pF2	121	402	1.14	SFO
Newhaven (silt loam)	5.7	5.1	20	pF2	69.7 (Pseudo DT <sub>50</sub> = 1000 d)	>1000	0.662	DFOP (k <sub>2</sub> fixed 1000d)
Penn (loam)	6.8	6.3	20	pF2	254 (Pseudo DT <sub>50</sub> = 1000 d)	>1000	1.02	DFOP (k <sub>2</sub> fixed 1000d)
Woodside (loam)	7.5	7.0	20	pF2	86.1	>1000	0.680	DFOP (k <sub>2</sub> fixed 1000d, 3' OH S 2840 M <sub>0</sub> fixed to 1.9% AR)

Maximum (non-normalised)					254	>1000		
3'-OH-S-2840	Dark aerobic conditions							
Soil	pH (H <sub>2</sub> O)	pH (CaCl <sub>2</sub> ) *	Temp °C	% MWHC	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	St. (χ <sup>2</sup> )	Method of calculation
Atwater (sandy loam)	7.5	7.0	20	pF2	No endpoints derived (t-test failed)			
Newhaven (silt loam)	5.7	5.1	20	pF2	No endpoints derived (t-test failed)			
Penn (loam)	6.8	6.3	20	pF2	No endpoints derived (Poorly defined decline phase)			
Woodside (loam)	7.5	7.0	20	pF2	No endpoints derived (Visually unacceptable fit)			
Maximum (non-normalised)					N/A	N/A		
1'-COOH-S-2840	Dark aerobic conditions							
Soil	pH (H <sub>2</sub> O)	pH (CaCl <sub>2</sub> )	Temp °C	% MWHC	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	St. (χ <sup>2</sup> )	Method of calculation
Atwater (sandy loam)	7.5	7.0	20	pF2	No endpoints derived (Poorly defined decline phase)			
Newhaven (silt loam)	5.7	5.1	20	pF2	207	689	5.18	DFOP(k <sub>2</sub> fixed)-SFO
Penn (loam)	6.8	6.3	20	pF2	No endpoints derived (Poorly defined decline phase)			
Woodside (loam)	7.5	7.0	20	pF2	840	>1000	2.65	DFOP(k <sub>2</sub> fixed)-SFO
Maximum (non-normalised)					840	>1000		

\*Not given, calculated using the equation in the EFSA PEC<sub>soil</sub> guidance (2017)



**Table B.8.1-3 Summary of trigger/persistence endpoints for 3'-OH-S-2840 and 1'-COOH-S-2840 derived from the metabolite applied aerobic degradation studies (conducted at 20°C and pF 2).**

1'-COOH-S-2840	Dark aerobic conditions							
Soil	pH (H <sub>2</sub> O)	pH (CaCl <sub>2</sub> )	Temp °C	% MWHC	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	St. (χ <sup>2</sup> )	Method of calculation
Speyer 5M	8.3	7.3	20	pF2	91.3	303	3.56	SFO
Newhaven	6.2	5.5	20	pF2	24.5	623	3.39	DFOP*
Atwater	7.1	6.3	20	pF2	148	491	2.59	SFO
Maximum (non-normalised)					148	623		
3'-OH-S-2840	Dark aerobic conditions							
Soil	pH (H <sub>2</sub> O)	pH (CaCl <sub>2</sub> )	Temp (°C)	% MWHC	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	St. (χ <sup>2</sup> )	Method of calculation
Speyer 5M	8.3	7.3	20	pF2	369	>1000	2.5	SFO
Newhaven	6.2	5.5	20	pF2	303	>1000	3.05	SFO
Atwater	7.1	6.3	20	pF2	276	917	0.863	SFO
Maximum (non-normalised)					369	>1000		

\*For the PECsoil endpoint, a pseudo SFO should be calculated by back calculating PECsoil using the tier 1 spreadsheet, or the kinetic parameters should be used if using ESCAPE

Anaerobic degradation occurred slowly for inpyrfluxam with a DT<sub>50</sub> of >1000 days for all soils (Table B.8.1-4). The photolysis DT<sub>50</sub> values were extrapolated beyond the study duration which adds uncertainty. When however these are used to estimate photolytic degradation under natural summer sunlight conditions from 30°N to 50°N the DT<sub>50</sub> values ranged from 641 d to 6863 d (Table B.8.1-5). These results suggest that the main route of degradation in soils will be via aerobic processes.

**Table B.8.1-4 Summary of trigger/persistence endpoints for inpyrfluxam in anaerobic conditions**

<b>Inpyrfluxam</b>	<b>Dark anaerobic conditions</b>						
<b>Soil type</b>	<b>pH (H<sub>2</sub>O)</b>	<b>Temp °C</b>	<b>% MWHC</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>	<b>St. (X<sup>2</sup>)</b>	<b>Method of calculation</b>
Penn	6.1	20	Flooded Soil	>10,000	>10,000	1.51	SFO
<b>Maximum (non-normalised)</b>				<b>4278</b>	<b>&gt;10,000</b>		

**Table B.8.1-5 Summary of trigger/persistence endpoints for the photolytic degradation of inpyrfluxam**

<b>Inpyrfluxam</b>	<b>Photolysis study</b>						
<b>Experiment (Penn soil)</b>	<b>Visual fit</b>	<b>Model</b>	<b><math>\chi^2</math> (%)</b>	<b>DT<sub>50</sub> (days)</b>	<b>Summer Days at 30°N (290-800 nm)</b>	<b>Summer Days at 40°N (290-800 nm)</b>	<b>Summer Days at 50°N (290- 800 nm)</b>
<b>Phenyl-label</b>							
Light	Good	SFO	3.37	73			
Dark control	Good	SFO	1.56	233			
<b>Photolysis only degradation rate</b>				106	763	688	641
<b>Pyrazolyl-label</b>							
Light	Good	SFO	0.948	97.7			
Dark control	Good	SFO	1.98	88.8			
				98	6863	6192	5772

## Enantiomeric ratio changes

### *Inpyrfluxam*

The enantiomeric ratio was analysed via chiral HPLC in laboratory soil studies. In the laboratory aerobic degradation studies (KCA 7.1.1.1/01 and 02), the stereochemical purity of the test substances in stock solutions was tested prior to application and showed that for the phenyl-labelled inpyrfluxam 100 % of the radiolabelled molecules were present as the R-isomer, while for the pyrazolyl-labelled inpyrfluxam 99.3 % was R-isomer and 0.7 % was S-isomer. Chiral HPLC analysis confirmed that the ratio of S- and R-isomers did not change during the course of the study. Similarly in the anaerobic degradation studies, chiral analysis of the stock solution prior to dosing and on soil extracts for the final time point showed that the inpyrfluxam consisted only of the R-isomer (100 % and 99.46 % respectively).

In the soil photolysis study, extracts from both the day 0 and day 12 (study end) were analysed by chiral HPLC. Extracts showed no change in the enantiomeric ratio during the study, with 95.8 % of inpyrfluxam present as the R-isomer at day 0 and 95.5 % at study end.

It is therefore concluded that changes in the enantiomeric ratio are not to be expected during microbially mediated metabolism or soil photolysis processes.

### *1'-COOH-S-2840A and B*

The applicant has proposed that one of the pathways via which inpyrfluxam degrades is through the oxidation of one of the 1'-CH<sub>3</sub> groups of the indenyl ring into a 1'-COOH group. As a result of this oxidative process two isomeric metabolites, 1'-COOH-S-2840A and 1'-COOH-S-2840B, were formed. Both of these metabolites contain two chiral centres; the metabolite 1'-COOH-S-2840A had either 1'S, 3'R or 1'R, 3'S configuration, or a mixture of both enantiomers. Similarly, the metabolite 1'-COOH-S-2840B had either 1'R, 3'R or 1'S, 3'S configuration, or a mixture of both enantiomers. The applicant reported that the analytical methods used could not determine which of the enantiomers of 1'-COOH-S-2840A (and 1'-COOH-S-2840B) were formed from inpyrfluxam in the parent applied study. In the 1'-COOH-S-2840 applied study, the amounts of isomers A and B were recorded at each timepoint in all 3 soils. In 2 of the soils, the ratio between the isomers remained approximately constant throughout the study at 50:50. In the final soil there was a shift of 7.1 %, with the B isomer increasing relative to the A isomer. As this is less than the 10 % change specified in the EFSA Guidance document (Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers' EFSA Journal 2019:17(8):5804), HSE does not consider that this is a significant change.

Changes in enantiomeric excess for 1'-COOH-S-2840 were considered in accordance with the principles outlined in the EFSA Guidance document, 'and the 'GB Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers'. Changes in enantiomeric excess occurred only in the Newhaven soil. A transient change in enantiomeric excess of 11.8 % was observed but enantiomeric excess was <10 % at study end.

Degradation of 1'-COOH-S-2840 was best described by DFOP kinetics, in contrast to the SFO kinetics used for the other two soils. The behaviour of both isomers individually was also biphasic, showing that the biphasic behaviour is due to factors other than behaviour of the individual isomers. The k values for the fast and slow phases were similar for both isomers. The overall degradation behaviour of 1'-COOH-S-2840 (A and B combined) follows SFO kinetics which would not be the case if the stereoisomers showed different degradation behaviour in soil. It is not therefore considered that the changes in the enantiomeric excess for 1'-COOH-S-2840A and 1'-COOH-S-2840B are indicative of isomeric conversion.

The changes in enantiomeric excess observed in the field studies have also been considered in line with the Guidance of EFSA. The enantiomeric excess by study end is <10 % at all sites, with any values >10 % being transient. The field study data therefore corroborates the data from the laboratory degradation study.

A single exposure assessment for the sum of the isomers was therefore considered appropriate.

### 3'-OH-S-2840

The metabolite 3'-OH-S-2840 forms via the oxidation of the 3'-position in the indenyl ring of inpyrfluxam. Conclusions are the same as for the parent.

HSE therefore accepts that the degradation behaviour of all substances could be adequately described based on behaviour of the total substance and that isomer specific behaviour did not need to be taken into account for the purposes of either the persistence or modelling assessments.

### Field dissipation studies

The applicant has submitted field studies at 4 European and 5 North American sites to investigate the behaviour of inpyrfluxam under field conditions. Additional studies were submitted in support of the field studies. These are summarised below including the associated FOCUS Kinetics evaluations.

**Table B.8.1-6 Summary of field studies submitted in support of inpyrfluxam**

Field dissipation study	Field sites	Kinetic report(s)	Endpoints calculated?
[REDACTED] 2018a (KCA 7.1.2.2.1/7)	Germany	Initial kinetics reported in [REDACTED] and [REDACTED] 2018b (KCA 7.1.2.2.1/8) and recalculated in [REDACTED] 2023 (KCA 7.1.2.2.1)	Modelling and trigger
	Czech Republic		
	Italy		
	Spain		
[REDACTED] 2017a, b and c (KCA 7.1.2.2.1/1, 2 and 4)	Mississippi	Initial kinetics reported in [REDACTED] 2018b (KCA 7.1.2.2.1/6) and recalculated in [REDACTED]	Modelling and trigger
	California		
	Washington		
	Ontario	[REDACTED]	

Field dissipation study	Field sites	Kinetic report(s)	Endpoints calculated?
[REDACTED] 2018a (KCA 7.1.2.2.1/7)	Germany	Initial kinetics reported in [REDACTED] 2018b (KCA 7.1.2.2.1/8) and recalculated in [REDACTED] [REDACTED] 2023 (KCA 7.1.2.2.1) V., 2023 (KCA 7.1.2.2.1)	Modelling and trigger
	Czech Republic		
	Italy		
	Spain		
[REDACTED] 2017 a and b (KCA 7.1.2.2.1/3 and 5)	North Dakota		Modelling and trigger
[REDACTED] 2017e (KCA 7.1.2.2.1/9)	A study to determine the storage stability in soil.		
[REDACTED] 2018 (KCA 7.1.2.2.1/10)	A study to determine the storage stability in soil.		
[REDACTED] 2018a (KCA 7.1.2.2.1/6)	Normalisation of data from 5 field sites in North America, Ecoregion crosswalk study and determination of normalised DT <sub>50</sub> values for inpyrfluxam and metabolites and FOCUS Kinetic analysis (kinetics later recalculated)		
[REDACTED] 2018b (KCA 7.1.2.2.1/8)	Normalisation of data from 4 field sites in Europe and determination of normalised DT <sub>50</sub> values for inpyrfluxam and metabolites and FOCUS Kinetic analysis (kinetics later recalculated)		
[REDACTED] 2023 (KCA 7.1.2.2.1)	Recalculation of FOCUS Kinetics for field data presented in KCA 7.1.2.2.1/6 and 8		
[REDACTED] 2018b (KCA 7.1.2.2.2)	Soil accumulation study		

The degradation of inpyrfluxam was investigated under field conditions in 4 representative growing regions of Europe (see KCA 7.1.2.2.1/7). At these sites, the test item was incorporated immediately after application to exclude surface processes and to enable a straightforward generation of modelling endpoints to be used for calculation of predicted environmental concentrations as recommended by EFSA [EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT<sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil. (EFSA Journal 2014:12(5):3662). Metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B were all observed at values >LOQ. Minor metabolites identified in the laboratory studies (DFPA, N-des-Me-DFPA and N-des-Me-S-2840) were not detected for in field dissipation studies.

Additional terrestrial field dissipation studies were run in North America according to US EPA (2008) guidelines at 5 different sites without incorporating the substance after application (see KCA 7.1.2.2.1/1, 2, 3, 4 and 5). Plot locations were chosen as representative of the soil used for growing a variety of crops within the proposed product market. The test sites were not cropped and bare soil conditions were maintained. Metabolite 3'-OH-S-2840 only was observed at these sites.

An ecoregion crosswalk exercise was performed with the OECD Europe – North America Soil Geographic Information for Pesticide Studies application (ENASGIPS v3.0) to determine if there are European ecoregions similar to the North American ecoregions containing the inpyrfluxam terrestrial field dissipation trial sites (see KCA 7.1.2.2.1/6). The objective was to demonstrate that the data generated from the terrestrial dissipation study conducted in North America is representative of dissipation in similar European ecoregions. The HSE evaluator also assessed the actual climatic conditions experienced during the field study. It was concluded that of the 5 sites presented, only the Ontario site was representative of European ecoregions, with North Dakota, Washington, California and Mississippi being excluded on the basis that the conditions are not relevant to Europe. The HSE evaluator therefore included only the degradation rates from the Ontario site with data from the European study sites when calculating modelling endpoint geometric means or when considering trigger endpoints.

Kinetic evaluation was performed for inpyrfluxam according to the FOCUS kinetics guidance [FOCUS (2014)] and EFSA guidance [EFSA (2014)]. Best-fit field degradation endpoints as triggers for additional work (trigger endpoints) and normalised degradation endpoints that could be used as input for modelling (modelling endpoints) were derived (see KCA 7.1.2.2.1).

Storage stability studies were submitted in support of the field dissipation studies (see KCA 7.1.2.2.1/9 and KCA 7.1.2.2.1/10), section B.8.1.4.

Consideration of soil accumulation is required if the  $DT_{90}$  value of at least one field soil exceeds one year. For inpyrfluxam, the  $DT_{90}$  value in one European field soil was 1272 days (SFO, non-normalised trigger endpoint) and 1400 days (SFO, normalised modelling endpoint). A field accumulation study was therefore submitted (see KCA 7.1.2.2.2) and in some field soils, inpyrfluxam is considered to have breached the persistence triggers (p and vP).

Table B.8.1-7 presents the final trigger endpoints. Tables B.8.1-8 – 11 present the final modelling endpoints for inpyrfluxam and its metabolites from field dissipation studies. Consideration of laboratory and field derived  $DT_{50}$  values for modelling according to the EFSA guidance (EFSA Journal 2014;12(5):3662) demonstrated that field endpoints should be treated as a separate population and the geometric mean of endpoints from field studies should be used. There was therefore no need to average laboratory and field study derived modelling endpoints. An SFO curve was found to approximate the averaged field study degradation for inpyrfluxam, and was therefore used for modelling. This is discussed further in Table B.8.1-9. Discussion of pH dependence (referenced in the following tables) follows the field dissipation section.

**Table B.8.1-7 Summary of trigger/persistence endpoints for inpyrfluxam (non-normalised)**

Parent	Aerobic conditions								
Field dissipation study	Soil type (indicate if bare or cropped soil was used)	Location (country or North American province)	pH CaCl <sub>2</sub> a	pH H <sub>2</sub> O	Depth (cm) b	St. ( $\chi^2$ )	$DT_{50}$ (d) c	$DT_{90}$ (d) c	Method of calculation
2018a (KCA 7.1.2.2.1/7)	Sandy clay loam, bare soil	Germany	6.0	6.5	0-30	19	117	388	SFO
2017b (KCA 7.1.2.2.1/5)	Clay/silty clay, bare soil	Czech Republic	7.4	7.9	0-40	16.1	322	1069	SFO

Parent	Aerobic conditions								
Field dissipation study	Soil type (indicate if bare or cropped soil was used)	Location (country or North American province)	pH CaCl <sub>2</sub> <sup>a</sup>	pH H <sub>2</sub> O	Depth (cm) <sup>b</sup>	St. (X <sup>2</sup> )	DT <sub>50</sub> (d) <sup>c</sup>	DT <sub>90</sub> (d) <sup>c</sup>	Method of calculation
	Sandy loam, bare soil	Italy	7.5	8.1	0-30	15.2	383	1272	SFO
	Loam, bare soil	Spain	4.4	5.0	0-30	5.78	47.3	753	DFOP
	Sandy loam, bare soil	Ontario	4.8 <sup>d</sup>	5.4	0-15	7.85	10.9	543	DFOP
Longest DT <sub>50</sub>							383		
pH dependence							No		

<sup>a</sup> pH values are mean values for the soil across the depths at which residues were detected.

<sup>b</sup> Residue depth refers to the depths at which residues were detected, plus the following depth where the applicant added values corresponding to 0.5 × LOD.

<sup>c</sup> Soils North Dakota, Washington, California and Mississippi were excluded from consideration of trigger endpoints as it was deemed not ecologically or climatically relevant to Europe following an ENASGIPS crosswalk exercise and consideration of the actual climatic conditions measured during the conduct of the field study.

<sup>d</sup> pH (H<sub>2</sub>O) converted to pH (CaCl<sub>2</sub>) using conversion given in the EFSA PECsoil guidance (2017).



**Table B.8.1-8 Summary of modelling endpoints for inpyrfluxam (time step normalisation performed)**

Parent	Aerobic conditions								
Field dissipation study	Soil type (indicate if bare or cropped soil was used)	Location (country or North American province)	pH CaCl <sub>2</sub> a	pH H <sub>2</sub> O	Depth (cm) b	St. (X <sup>2</sup> )	DT <sub>50</sub> (d) Norm. c	DT <sub>90</sub> (d) Norm.	Method of calculation
<div> <div></div> 2018a (KCA 7.1.2.2.1/7) <div></div> 2017b (KCA 7.1.2.2.1/5) </div>	Sandy clay loam, bare soil	Germany	6.0	6.5	0-30	16.7	78.8	262	SFO
	Clay/silty clay, bare soil	Czech Republic	7.4	7.9	0-40	14.8	169	561	SFO
	Sandy loam, bare soil	Italy	7.5	8.1	0-30	14.9	421	1400	SFO
	Loam, bare soil	Spain	4.4	5.0	0-30	7.54	38 (overall) 3.51 (fast phase) 111 (slow phase) g value 0.37	295	DFOP

Parent	Aerobic conditions								
Field dissipation study	Soil type (indicate if bare or cropped soil was used)	Location (country or North American province)	pH CaCl <sub>2</sub> a	pH H <sub>2</sub> O	Depth (cm) b	St. (X <sup>2</sup> )	DT <sub>50</sub> (d) Norm. c	DT <sub>90</sub> (d) Norm.	Method of calculation
	Sandy loam, bare soil	Canada (Ontario)	4.8 d	5.4	0-15	19.5	104	344	SFO
Geometric mean (if not pH dependent)							139e		
pH dependence							No		

a pH values are mean values for the soil across the depths at which residues were detected.

b Residue depth refers to the depths at which residues were detected, plus the following depth where the applicant added values corresponding to  $0.5 \times \text{LOD}$ .

c Soils North Dakota, Washington, California and Mississippi were excluded from consideration of trigger endpoints as it was deemed not ecologically or climatically relevant to Europe following an ENASGIPS crosswalk exercise and consideration of the actual climatic conditions measured during the conduct of the field study.

d pH (H<sub>2</sub>O) converted to pH (CaCl<sub>2</sub>) using conversion given in the EFSA PECsoil guidance (2017)

e Geometric mean calculated using DFOP DT<sub>90</sub>/3.32 for the Spain site.

### Selection of a model for inpyrfluxam

As the modelling endpoint determination of inpyrfluxam involved the combination of SFO and DFOP models, an SFO approximation of the geomean of the degradation was determined according to the EFSA DegT<sub>50</sub> guidance (2014). The geomean fast phase DT<sub>50</sub> of the field studies is 72.8 days, the slow phase DT<sub>50</sub> is 145.3 days, and the g value is 0.37. This was found to be well approximated by an SFO model with a DT<sub>50</sub> of 121.4 days (See section B.8.1.3, 3CA\_B8).

**Table B.8.1-9 Selection of an SFO approximation of modelling endpoints for inpyrfluxam**

<b>Soil location</b>	<b>Fast phase <math>k_1</math> DT<sub>50</sub> (days)</b>	<b>Slow phase <math>k_2</math> DT<sub>50</sub> (days)</b>	<b>g value</b>	<b>Kine tic</b>
Germany	78.8	78.8	-	SFO
Czech Republic	169.0	169.0	-	SFO
Italy	421.0	421.0	-	SFO
Spain	3.51	111.0	0.37	DFO P
Ontario	104.0	104.0	-	SFO
<b>geomean</b>	72.8	145.3	-	-
<b>arithmetic mean</b>	-	-	0.37	-
<b>SFO approximation used for modelling</b>	<b>121.4</b>	<b>121.4</b>	<b>-</b>	<b>SFO</b>

**Table B.8.1-10 Summary of modelling endpoints for 3'-OH-S-2840 (time step normalisation performed).**

<b>3'-OH-S-2840</b>	<b>Aerobic conditions</b>									
<b>Field dissipation study</b>	<b>Soil type (indicate if bare or cropped soil was used)</b>	<b>Location (country or North American province)</b>	<b>pH CaCl<sub>2</sub> a</b>	<b>pH H<sub>2</sub>O</b>	<b>Depth (cm) b</b>	<b>St. (X<sup>2</sup>)</b>	<b>DT<sub>50</sub> (d) Norm. c</b>	<b>DT<sub>90</sub> (d) Norm.</b>	<b>ff</b>	<b>Method of calculation</b>
<div>2018a (KCA 7.1.2.2.1/7)</div> <div>2017b (KCA 7.1.2.2.1/5)</div>	Sandy clay loam, bare soil	Germany	6.0	6.5	0-30	18.2	96.6	321	0.18	SFO
	Clay/silty clay, bare soil	Czech Republic	7.4	7.9	0-10	19.8	101	335	0.27	SFO
	Sandy loam, bare soil	Italy	7.5	8.1	0-10	24.0	204	678	0.34	SFO
	Loam, bare soil	Spain	4.4	5.0	0-10	15.5	149	495	0.13	SFO
	Sandy loam, bare soil	Ontario	4.8 d	5.4	Not detected					
Geometric mean (if not pH dependent)							131			
pH dependence							No			

a pH values are mean values for the soil across the depths at which residues were detected.

b Residue depth refers to the depths at which residues were detected, plus the following depth where the applicant added values corresponding to  $0.5 \times \text{LOD}$ .

c Soils North Dakota, Washington, California and Mississippi were excluded from consideration of trigger endpoints as it was deemed not ecologically or climatically relevant to Europe following an ENASGIPS crosswalk exercise and consideration of the actual climatic conditions measured during the conduct of the field study.

d pH (H<sub>2</sub>O) converted to pH (CaCl<sub>2</sub>) using conversion given in the EFSA PECsoil guidance (2017).

**Table B.8.1-11 Summary of modelling endpoints for 1'-COOH-S-2840A and B (time step normalisation performed).**

<b>1'-COOH-S-2840A and B</b>		<b>Aerobic conditions</b>								
<b>Field dissipation study</b>	<b>Soil type (indicate if bare or cropped soil was used)</b>	<b>Location (country or North American province)</b>	<b>pH CaCl<sub>2</sub> a</b>	<b>pH H<sub>2</sub>O</b>	<b>Depth (cm) b</b>	<b>St. (X<sup>2</sup>)</b>	<b>DT<sub>50</sub> (d) Norm. c</b>	<b>DT<sub>90</sub> (d) Norm.</b>	<b>ff</b>	<b>Method of calculation</b>
2018a (KCA 7.1.2.2.1/7)	Sandy clay loam, bare soil	Germany	6.0	6.5	0-30	14.4	75.4	250	0.77	SFO
	Clay/silty clay, bare soil	Czech Republic	7.4	7.9	0-30	18.6	24.7	82.1	0.64	SFO
	Sandy loam, bare soil	Italy	7.5	8.1	Not detected					
	Loam, bare soil	Spain	4.4	5.0	0-30	6.44	224	744	0.17	SFO
	Sandy loam, bare soil	Ontario	4.8 d	5.4	Not detected					
Geometric mean (if not pH dependent)							74.7			
pH dependence							No			

a pH values are mean values for the soil across the depths at which residues were detected.

b Residue depth refers to the depths at which residues were detected, plus the following depth where the applicant added values corresponding to 0.5 × LOD.

c Soils North Dakota, Washington, California and Mississippi were excluded from consideration of trigger endpoints as it was deemed not ecologically or climatically relevant to Europe following an ENASGIPS crosswalk exercise and consideration of the actual climatic conditions measured during the conduct of the field study.

d pH (H<sub>2</sub>O) converted to pH (CaCl<sub>2</sub>) using conversion given in the EFSA PECsoil guidance (2017).

## pH Dependence

In physical/chemical property studies it was considered that inpyrfluxam contained no chemical functional groups which would allow ionisation of the test item within the environmentally relevant pH range. As a consequence, the influence of pH on the octanol-water partition coefficient and water solubility of the test item was not determined. The partition co-efficient ( $\log P_{ow}$ ) for inpyrfluxam was 3.65 at pH 7.1 to 7.3 and  $25 \pm 1$  °C (see KCA 2.7/001). The solubility in water of inpyrfluxam is  $1.64 \times 10^{-2}$  g/L at  $20.0 \pm 0.5$  °C with solution pH values between 5.5 and 5.8. Additionally, in the sterile aqueous hydrolysis study no degradation was observed between pH 4, 7 or 9 at 50°C (see B.8.2.1.1). Based on the available studies no influence of pH on degradation rates is therefore anticipated. The HSE evaluator sought to confirm this hypothesis by investigating whether a relationship existed between inpyrfluxam degradation rates and soil pH in the laboratory aerobic degradation and field dissipation studies. Field studies were included in the analysis since these were conducted under a wider pH range compared to the laboratory studies. The Input-Decision 3.3 tool (Federal Environment Agency, Germany) was utilised to conduct the Kendall Test on laboratory-derived and field-derived degradation rates as two separate populations (treating the populations separately was in accordance with the EFSA DegT<sub>50</sub> (2014) guidance).

Aerobic degradation in the laboratory was studied in four soils with a pH (CaCl<sub>2</sub>) range of 5.1 – 7.0. The relationship between laboratory DT<sub>50</sub> values for inpyrfluxam and pH is shown below. As some of the best fit kinetics were biphasic, the relationship between pH and DT<sub>90</sub> is also plotted. In field dissipation studies, five sites were investigated with a pH (CaCl<sub>2</sub>) range of 4.4 – 7.5. The pH dependence for field DT<sub>50</sub> values (normalised/modelling endpoints) is shown in Figure B.8.1-2. The results are reported in Table B.8.1-12 and show no pH dependence for inpyrfluxam degradation. Kendall's Test statistics were calculated for both the laboratory and field populations of inpyrfluxam in Table B.8.1-13. This is to be expected as three of the four soils have the same value, but this step is included for completeness.

HSE notes that the US field dissipation study soil pH value was measured in water only. To harmonise the measurement methods used in the two field dissipation studies a pH values in water were converted into pH values in CaCl<sub>2</sub> by rearranging a

pH conversion equation used to convert a pH-CaCl<sub>2</sub> measurement to pH-H<sub>2</sub>O. This equation was derived from EFSA PEC<sub>soil</sub> guidance (2017) and is shown below:

$$pH(CaCl_2) = \frac{pH(H_2O) - 0.648}{0.982}$$

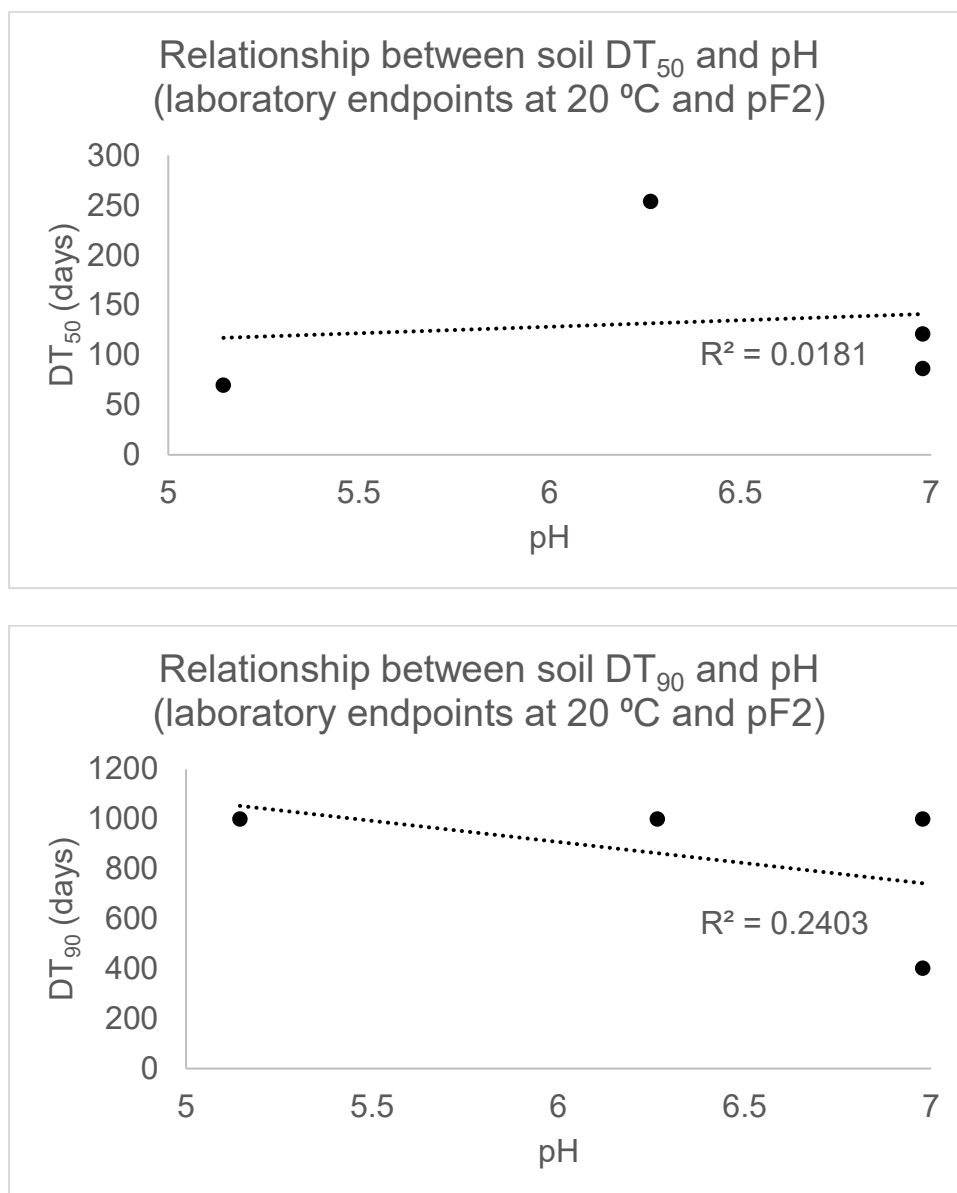
### Inpyrfluxam

**Table B.8.1-12 Inpyrfluxam DT<sub>50</sub> and DT<sub>90</sub> values for comparison against pH**

Inpyrfluxam laboratory endpoints		Dark aerobic conditions		
Soil type (origin)	pH (CaCl <sub>2</sub> )*	Normalised DT <sub>50</sub> (d) – best fit kinetics	Normalised DT <sub>90</sub> (d) best fit kinetics	Normalised pseudo-SFO DT <sub>50</sub> – modelling endpoints
Atwater (sandy loam)	7.0	121	402	Not used – since modelling and best fit endpoints were the same
Newhaven (silt loam)	5.1	69.7	1000	
Penn (loam)	6.3	254	1000	
Woodside (loam)	7.0	86.1	1000	
Inpyrfluxam field endpoints		Field conditions		
Site	pH (CaCl <sub>2</sub> )*	Non-normalised DT <sub>50</sub> (d) – best fit kinetics	Non-normalised DT <sub>90</sub> – best fit kinetics	Normalised pseudo-SFO DT <sub>50</sub> – modelling endpoints
Germany	6.0	Not performed (due to the influence of climate and environmentally variability)		78.8
Czech Republic	7.4			169
Italy	7.5			421
Spain	4.4			89**
Ontario	4.8			104

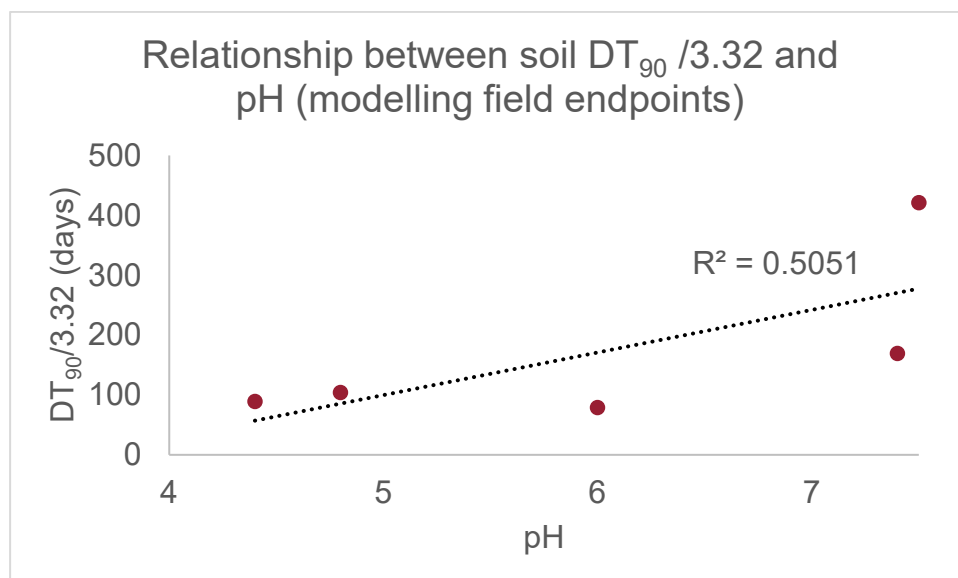
\* pH (H<sub>2</sub>O) converted to pH (CaCl<sub>2</sub>) using conversion given in the EFSA PEC<sub>soil</sub> guidance (2017)

\*\* DT<sub>50</sub> calculated using DFOP DT<sub>90</sub>/3.32



**Figure B.8.1-1 Relationship between laboratory soil degradation and soil pH – best fit  $DT_{50}$  and  $DT_{90}$  endpoints**





**Figure B.8.1-2 Relationship between soil  $DT_{90}/3.32$  and soil pH – normalised modelling endpoints**

**Table B.8.1-13 Kendall's test results investigating pH dependence of the degradation of inpyrfluxam in soil in the laboratory and the field ( $\alpha = 0.05$ ).**

Substance	n	Tau	P	pH dependence?
<b>Laboratory soils</b>				
Inpyrfluxam ( $DT_{50}$ )	4	0.183	1.000	Weak and not statistically significant
Inpyrfluxam ( $DT_{90}$ )	4	-0.516	0.637	Yes (moderate) but not statistically significant
<b>Field soils</b>				
Inpyrfluxam (DFOP $DT_{50}$ derived by $DT_{90}/3.32$ )	5	0.600	0.221	Yes (moderate) but not statistically significant

The relationship between pH and degradation in soil has been considered for both the best-fit endpoints in laboratory studies (DT<sub>50</sub> and DT<sub>90</sub>) and the normalised modelling endpoints from field studies. It is noted that the best fit model and model selected for modelling endpoints for laboratory studies are the same. While a pseudo DT<sub>50</sub> of 1000 days has been derived for laboratory endpoints in some soils, it was concluded that no additional information on pH dependence would be derived from plotting this data.

For laboratory endpoints, the Kendall's test showed a weak positive relationship with pH for DT<sub>50</sub>, but a moderate negative correlation with pH for the DT<sub>90</sub>. Neither relationship was statistically significant and the opposite direction of the correlation is noted. The lack of a correlation with pH was corroborated by the linear regression with a maximum R<sup>2</sup> value of only 0.2403.

For field studies, the Kendall's test indicated a only a moderate positive relationship for the normalised modelling endpoints (DT<sub>50</sub> derived from DFOP kinetics using DT<sub>90</sub>/3.32 at one site). This relationship was not statistically significant. The linear regression analysis showed similar relationships with R<sup>2</sup> of 0.5051 for modelling endpoints. For field studies the influence of climate and other environmental factors may not be completely removed by the time step normalisation process which adds a degree of uncertainty to the analysis of pH effects. Combined with the lack of mechanistic data it is concluded that, based on a weight of evidence approach, inpyrfluxam does not show pH dependent degradation in soil.

#### *3'-OH-S-2840 and 1'-COOH-S-2840*

The degradation behaviour of both metabolites was studied in three laboratory soils under dark conditions. As there are only three data points, it is considered that there is insufficient data to determine if a statistically reliable correlation with pH exists. Based on the available data however, there is no indication of any impact of pH. Consequently no analysis has been conducted and no further information is required.

A summary of the degradation endpoints for use in the exposure assessment is shown below.

**Table B.8.1-14 Summary of final degradation endpoints in soil (modelling and PECsoil calculation)**

Modelling endpoints					
Compound	DegT <sub>50</sub> endpoint (d)*	Formation fraction**	Kinetics	Source	
Inpyrfluxam	121.4	-	Pseudo-SFO (DFOP DT <sub>90</sub> /3.32)	Geometric mean (field)	
3'-OH-S-2840	131.0	0.23	SFO		
1'-COOH-S-2840	74.7	0.53	SFO		
PECsoil					
	Degradation rate***		Max. (% AR)	Kinetics	Source
	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)			
Inpyrfluxam	383	>1000	-	SFO	Field dissipation
3'-OH-S-2840	369	1226	20.7	SFO	Max, lab (metabolite applied)
1'-COOH-S-2840	840	>1000	30.1	SFO	

\*Geometric mean

\*\*Arithmetic mean

\*\*\*Longest values

**Sorption behaviour of inpyrfluxam**

The applicant submitted one laboratory adsorption study for inpyrfluxam and one for each of metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 (1'-COOH-S-2840A and 1'-COOH-S-2840B combined). Table B.8.1-15 provides details, with each study summarised below.

**Table B.8.1-15 Laboratory studies investigating the sorption behaviour of inpyrfluxam and its metabolites**

<b>Laboratory soil study</b>	<b>Study type</b>
<div style="background-color: black; width: 150px; height: 15px; margin-bottom: 5px;"></div> 2017 KCA 7.1.3.1.1/1	Adsorption study for inpyrfluxam
<div style="background-color: black; width: 100px; height: 15px; margin-bottom: 5px;"></div> 2017c KCA 7.1.3.1.2/1	Adsorption study for 1'-COOH-S-2840

2017 KCA 7.1.3.1.2/2	Adsorption study for 3'-OH-S-2840
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The sorption behaviour of inpyrfluxam was investigated in seven soils (4 UK, 2 North American, one Japanese), using the batch equilibrium test [see KCA 7.1.3.1.1/1]. The study was conducted in accordance with the OECD 106 guidelines. The results are reported in Table B.8.1-16. The soils included a range of organic carbon contents and both basic and acidic soils.

**Table B.8.1-16 Overview of adsorption isotherms for inpyrfluxam on 7 soils**

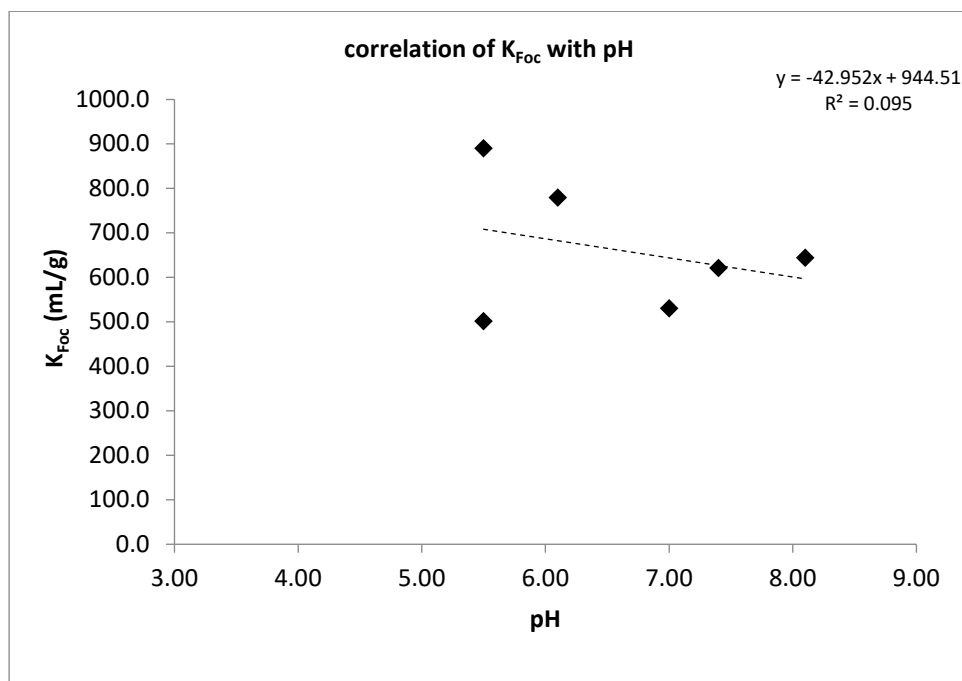
Soil	Soil type (USDA)	C <sub>org</sub> (%)	pH (CaCl <sub>2</sub> )	K <sub>f</sub> (mL/g)	K <sub>foc</sub> (mL/g)	1/n	R <sup>2</sup>
<b>Brierlow</b>	Silt loam	2.4	6.1	18.71	780	0.956	0.9997
<b>Kenslow</b>	Loam	3.8	5.5	19.02	500	0.939	0.9967
<b>Clipstone</b>	Loamy sand	1.2	5.5	10.70	891	0.996	0.9973
<b>Hareby</b>	Clay loam	1.6	7.4	9.91	619	0.932	0.9994
<b>LAD-SCL-PF</b>	Clay or clay loam	0.9	8.1	5.79	643	0.992	0.9993
<b>Atwater</b>	Sandy loam	0.3	7.0	1.59	531	0.942	0.9993
<b>Ibaraki*</b>	Sandy loam	2.6	7.3	17.47	672	0.962	0.9990
<b>Geometric mean</b>					647		
<b>Arithmetic mean</b>						0.960	

\*Volcanic soil; not considered in calculation of mean values. The impact of excluding this soil was considered. With the Ibaraki soil, the geometric mean K<sub>foc</sub> is 650 mL/g and the arithmetic mean 1/n is 0.962, while with the Ibaraki soil excluded the geometric mean K<sub>foc</sub> is 647 mL/g and the arithmetic mean 1/n is 0.960. The soil is well within the range of values derived and the inclusion or exclusion of the soil has minimal impact on the adsorption parameters.

## pH dependence

HSE calculated a Kendall rank correlation coefficient for  $K_{oc}$  of 0.00 using the German support tool (pH Dependence Calculator), this indicates that there is no correlation between sorption and pH. The corresponding p-value was 1.00 which is  $> 0.05$  also confirming that the correlation between pH and  $K_{foc}$  is not statistically significant.

This dependence was further investigated by creating a linear regression plot (see Figure B.8.1.4.1-11) and conducting statistical analysis using Excel. The p-value for this regression is 0.5523 which is above the 0.05 guideline for statistical significance. The linear plot indicates that there is no relationship between  $K_{foc}$  and pH. The  $R^2$  value is small (0.095) indicating there is no relationship between sorption and pH. HSE considered the results from both analyses and agrees that the adsorption of inpyrfluxam is not dependent on pH.



**Figure B.8.1-3 Linear regression investigating the relationship between pH and  $K_{foc}$  in [ $^{14}C$ ] inpyrfluxam on seven soils**

It was therefore concluded that pH dependence was not observed. As there is a relationship between adsorption partition coefficients and organic carbon content, it is considered appropriate to calculate the organic carbon normalised partition coefficients (i.e.  $K_{foc}$ ).

The sorption behaviour of 1'-COOH-S-2840 A and B was investigated separately in 5 soils (4 European, one North American), using the batch equilibrium test [see KCA 7.1.3.1.2/1]. The study was conducted in accordance with the OECD 106 guidelines.

The results are reported in Table B.8.1-17 and Table B.8.1-18. The soils included a range of organic carbon contents and both basic and acidic soils. It was concluded that pH dependence was not observed (see section B.8.1.5.2 for further details of pH dependence for metabolites). A relationship between  $K_f$  and organic carbon content was observed.

**Table B.8.1-17 Overview of adsorption isotherms for 1'-COOH-S-2840A on 5 soils**

Soil	Soil type (USDA)	C <sub>org</sub> (%)	pH (CaCl <sub>2</sub> )	K <sub>f</sub> (mL/g)	K <sub>foc</sub> (mL/g)	1/n	R <sup>2</sup>
Quilen (Pas de Calais, France)	Silt loam or Loam	2.9	7.3	1.057	35	0.939	0.9989
Hareby (Lincolnshire, UK)	Clay loam	1.6	7.4	0.190	11	0.966	0.9987
Clipstone (Nottinghamshire, UK)	Loamy sand	1.2	5.5	0.409	33	0.937	0.9990
Speyer (Rheinland-Pfalz, Germany)	Sandy loam	0.7	5.8	0.080	11	0.962	0.9941
Atwater (California, USA)	Sandy loam	0.3	7.0	0.094	28	0.945	0.9913
pH dependence					No		
Geometric mean					20.8		
Arithmetic mean						0.950	

**Table B.8.1-18 Overview of adsorption isotherms for 1'-COOH-S-2840B on 5 soils**

<b>Soil</b>	<b>Soil type (USDA)</b>	<b>C<sub>org</sub> (%)</b>	<b>pH (CaCl<sub>2</sub>)</b>	<b>K<sub>f</sub> (mL/g)</b>	<b>K<sub>foc</sub> (mL/g)</b>	<b>1/n</b>	<b>R<sup>2</sup></b>
<b>Quilen (Pas de Calais, France)</b>	Silt loam or Loam	2.9	7.3	1.30	45	0.927	0.9949
<b>Hareby (Lincolnshire, UK)</b>	Clay loam	1.6	7.4	0.25	16	0.949	0.9881
<b>Clipstone (Nottinghamshire, UK)</b>	Loamy sand	1.2	5.5	0.52	44	0.972	0.9915
<b>Speyer (Rheinland-Pfalz, Germany)</b>	Sandy loam	0.7	5.8	0.10	15	0.923	0.9905
<b>Atwater (California, USA)</b>	Sandy loam	0.3	7.0	0.09	40	0.940	0.9905
<b>pH dependence</b>					<b>No</b>		
<b>Geometric mean</b>					28.6		
<b>Arithmetic mean</b>						0.942	

The need for including both diastereomers separately or using mean values of the adsorption parameters of 1'-COOH-S-2840A and B in the exposure assessment has been considered in line with both EFSA and GB guidance on stereoisomers. In line with the GB guidance, the susceptibility of the compound to leaching based on the K<sub>foc</sub> values, degradation rate and the overall leaching potential have been

considered. As 1'-COOH-S-2840 is of very high mobility, small differences in the sorption parameters can have a large influence on the behaviour of the substance in leaching simulations. The environmental exposure assessments were therefore conducted for both diastereomers separately and with mean values for the diastereomers combined to determine the impact of combining the diastereomer data on modelling outcomes.

**Table B.8.1-19 Comparison of 1'-COOH-S-284A , 1'-COOH-S-2840B and mean sorption endpoints (HSE data)**

	<b>1'-COOH-S-2840A</b>	<b>1'-COOH-S-2840B</b>	<b>Mean of both diastereomers*</b>
<b>Adsorption</b>			
<b>K<sub>foc</sub> (L/kg)</b>	20.8	28.6	24.4
<b>1/n</b>	0.950	0.942	0.946

\*Means of the two individual mean values for two isomers; K<sub>foc</sub> geometric mean, 1/n arithmetic mean HSE data for Atwater soil, 1'-COOH-S-2840B, all other data applicant data

The sorption behaviour of 3'-OH-S-2840 was investigated in three soils (2 UK, one French), using the batch equilibrium test [see KCA 7.1.3.1.2\_02]. The study was conducted in accordance with the OECD 106 guidelines. The results are reported in Table B.8.1-20. The soils included a range of organic carbon contents and both basic and acidic soils. It was concluded that pH dependence was not observed (see section B.8.1.5.2 for further details of pH dependence for metabolites). The data indicated a possible relationship between adsorption partition coefficients and organic carbon content.



**Table B.8.1-20 Overview of adsorption isotherms for 3'-OH-S-2840 on 3 soils**

<b>Soil</b>	<b>Soil type (USDA)</b>	<b>C<sub>org</sub> (%)</b>	<b>pH (CaCl<sub>2</sub>)</b>	<b>K<sub>f</sub> (mL/g)</b>	<b>K<sub>foc</sub> (mL/g)</b>	<b>1/n</b>	<b>R<sup>2</sup></b>
<b>Quilen</b>	Silt loam	2.9	7.3	14.26	492	0.879 1	0.9942
<b>Hareby</b>	Clay loam	1.6	7.4	5.58	349	0.956 1	0.9993
<b>Clipstone</b>	Loamy sand	1.2	5.5	4.81	401	0.972 9	0.9983
<b>Geometric mean</b>					410		
<b>Arithmetic mean</b>						0.936	

Column leaching, lysimeter and field leaching studies were not required or submitted for inpyrfluxam or its metabolites.

**B.8.1.1. Laboratory route and rate of degradation in soil****B.8.1.1.1. Route of degradation in soil****B.8.1.1.1.1. Aerobic route of degradation of the active substance, 1 US soil**

<b>Data point</b>	KCA 7.1.1.1/01
<b>Study Title:</b>	Aerobic Soil Metabolism of [Phenyl- <sup>14</sup> C] S-2399 and [Pyrazolyl-4- <sup>14</sup> C] inpyrfluxam Amended Report
<b>Author &amp; year:</b>	██████████ (2017)
<b>Address:</b>	Valent Technical Center (VTC), 6560 Trinity Court, Dublin, CA 94568
<b>Study No:</b>	VP-38556 (KCA 7.1.1.1/01)
<b>Applicant:</b>	Sumitomo Chemical Co. Ltd. Report No: TPM-0023
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>• US EPA Fate, Transport and Transformation Test Guidelines</li> <li>• OCSP 835.4100</li> <li>• OECD 307 Test Guideline</li> </ul>
<b>GLP:</b>	Yes
<b>Deviations:</b>	Yes (see below)

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Discrepancy between soil sampling location coordinates and town name provided	Clarification from applicant desired. The applicant has since clarified in a request for additional information (RAI) that the coordinates provided are accurate. HSE considers this as acceptable.
Details on transportation conditions not provided	Minor deviation. GLP assured, and therefore HSE, as the assessor, considers that these conditions are acceptable.
Sampling site pesticide history not provided	Major omission. HSE therefore requested clarification from the applicant on the pesticide history at the sample sites. The applicant has since stated in an RAI that "Due to the changing nature of the fields used for the study a pesticide history is unavailable. However, this a terrestrial soil

	where pesticides would not normally be applied, and the degradation properties of the soil are not outliers from the other soils used (see B.8.1.1.1.2). Therefore HSE considers the soil microbes as unlikely to be adapted to metabolise inpyrfluxam, and accepts its use here.
Sampling site weather history not mentioned	Minor omission. No study-affecting weather prior to sampling is assumed by HSE.
Soil maximum water holding capacity was held at 50 % $\pm$ 10 %. At 40 % MWHC, pF values were slightly above 2.5, and at 50-60 % pF values were slightly below pF 2.0.	Minor deviation. Soil was maintained within the recommended moisture content for optimal microbial activity of 40-60 % recommended by the OECD 307 guidelines.
<b>Deviations</b>	<b>HSE assessment of deviations</b>
Solvent evaporation in sample preparation step not mentioned, as required by OECD 307	Minor omission. Only 200 $\mu$ L solvent per 50 g soil sample used. Biomass measurements demonstrate solvent volume used does not decrease biomass.
LSC LOD and LOQ not provided in units of % AR, as required by OECD 307	Major omission, will require clarification from applicant. This has since been provided by the applicant in an RAI. . HSE considers that the LOD is suitably below the OECD 307 threshold of 1 % AR, and that LOQ has been specified.
<sup>14</sup> CO <sub>2</sub> trap NaOH concentration used (1M) differs from OECD 307 guideline (2M)	Minor deviation. Mass balances demonstrate NaOH concentration used is suitable in this study.
Microbial biomass measurement of solvent control at 1 DAT not provided	Minor omission. Other measurements demonstrate that the solvent used is suitable and does not impact microbial activity.
Volatile traps not checked at intervals given by OECD 307	Minor deviation. Volatile production is low. Not considered to have impacted study validity.

Decline phase of major metabolites not shown	HSE notes that separate metabolite dosed studies were provided by the applicant at KCA 7.1.2.1.2_01 TPM-0033 & KCA 7.1.2.1.2_02 TPM-0049
Analysis of soil samples took place ~ 1-2 weeks after removal according to chronology of events, as opposed to 'immediately' as stated by the applicant. No information on conditions of storage of extracts have been provided.	Minor deviation as GLP is assured, and a storage stability test (KCA 7.1.2.2.1_10) demonstrates that the compounds are stable over the period between extraction and analysis.

### HSE conclusion on deviations

Clarification of HPLC, LSC, and TLC LOD & LOQ, and sampling site pesticide history required from applicant. This has since been provided in an RAI, with LOD and LOQ in units of % AR. Other deviations are not considered by HSE to void the validity of the study, and therefore further clarification is not required.

## INTRODUCTION

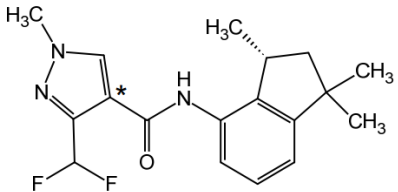
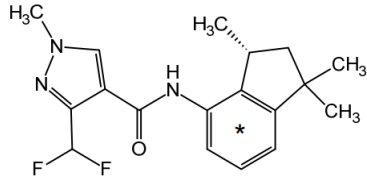
The route of the metabolism of the non-volatile active substance inpyrfluxam (S-2399) in Penn Series soil was investigated under aerobic conditions using two different labels of [ $^{14}\text{C}$ ] inpyrfluxam, [phenyl- $^{14}\text{C}$ ] inpyrfluxam (PHE-label) and [pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam (PYR-label) at a rate of 0.650 mg/kg and 0.634 mg/kg soil (dry weight basis) respectively. Treated soil samples were incubated at  $20 \pm 2^\circ\text{C}$  and  $50 \pm 10\%$  of the maximum water holding capacity (MWHC) in the dark for a maximum of 182 days and were periodically collected and extracted. The soil extracts were analysed by LSC (liquid scintillation counting) and 2D TLC (thin layer chromatography). Additionally, samples were occasionally analysed by HPLC (high performance liquid chromatography) to quantify and identify major radioactive components.

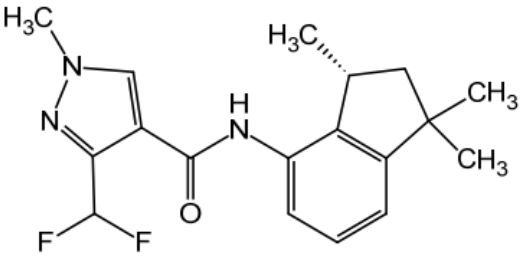
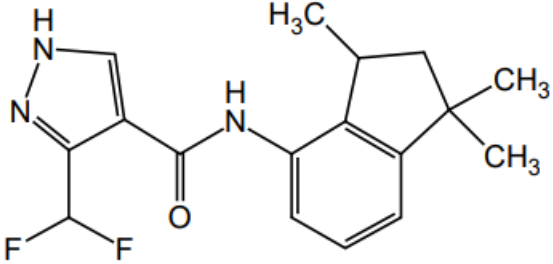
A number of metabolites of inpyrfluxam were identified, including the major metabolites 3'-OH-S-2840 and 1'-COOH-S-2840, as well as minor metabolites N-des-Me-S-2840 and N-des-Me-DFPA.

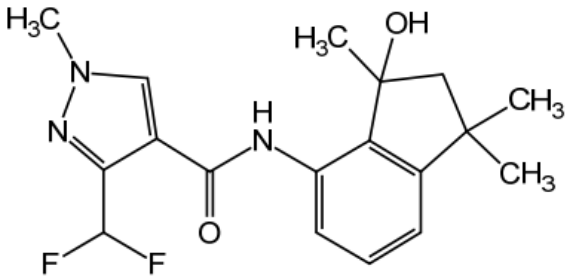
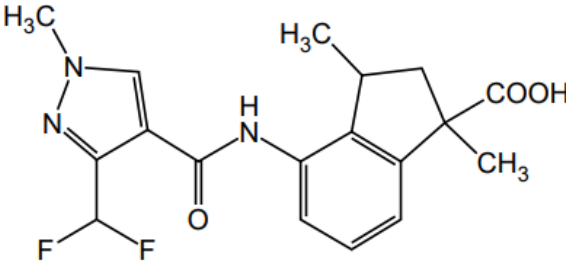
The applicant also provided a kinetic evaluation of inpyrfluxam aerobic degradation in Penn Series soil using PestDF. They did however state that the kinetic evaluation conducted within this original study does not meet the current FOCUS guidance. Therefore, a kinetic re-evaluation was conducted by the applicant and evaluated by HSE in a separate section (B.8.1.1.2.).

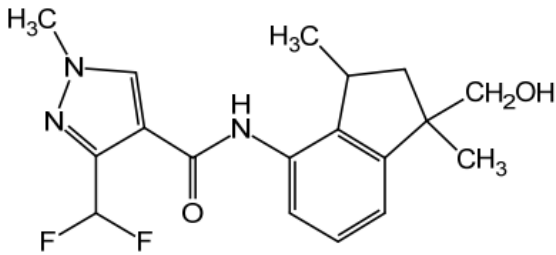
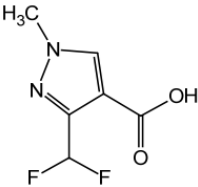
## MATERIALS AND METHODS

**Table B.8.1.1.1-01 Properties of the materials used in the aerobic soil degradation study**

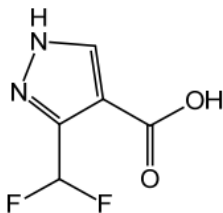
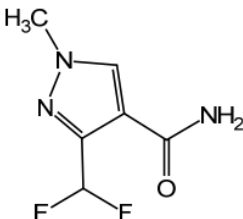
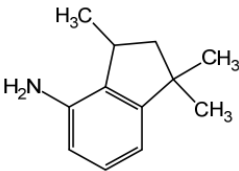
<b>1. Test Material</b>	[pyrazolyl-4- <sup>14</sup> C] inpyrfluxam (PYR-label)
<b>Lot/Batch:</b>	CFQ41802
<b>Specific activity:</b>	2.11 GBq/mmol
<b>Purity:</b>	Radiochemical purity 98.1 % (in the dosing solution)
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	Not stated
<b>Structure:</b>	 <p>Figure 1; * denotes <sup>14</sup>C label position</p>
<b>2. Test Material</b>	[phenyl- <sup>14</sup> C] inpyrfluxam (PHE-label)
<b>Lot/Batch:</b>	CFQ41803
<b>Specific activity:</b>	4.51 GBq/mmol
<b>Purity:</b>	Radiochemical purity 99.1 % (in the dosing solution)
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	Not stated
<b>Structure:</b>	 <p>Figure 2; * denotes <sup>14</sup>C label position</p>

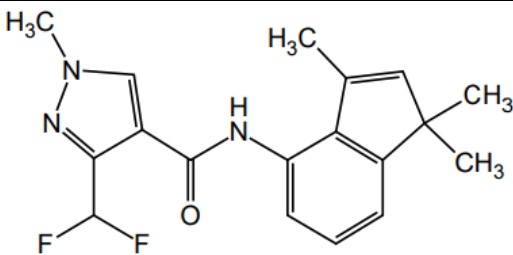
<b>3. Reference Material</b>	
<b>Code names:</b>	Inpyrfluxam (Pure 3'R isomer) S-2840 (Contains 2 enantiomers: 3'R & 3'S) S-2940 (Pure 3'S isomer)
<b>Chemical Name:</b>	N-[(3R)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2375a (inpyrfluxam Analytical Grade) (95.3 %) AS2375b (inpyrfluxam pure 3'R isomer) (99.8 %) AS2375c (inpyrfluxam Technical Grade) (95.4 %) AS2387a (S-2940 pure 3'S isomer) (99.8 %)
<b>Structure:</b>	
<b>4. Reference Material</b>	
<b>Code names:</b>	N-des-Me-S-2840 (Contains 2 enantiomers: 3'R & 3'S)
<b>Chemical Name:</b>	N-[(3RS)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2380a (97.5 %)
<b>Structure:</b>	
<b>5. Reference Material</b>	

<b>Code names:</b>	3'-OH-S-2840 (Contains 2 enantiomers: 3'R & 3'S)
<b>Chemical Name:</b>	N-[(3RS)-3-hydroxy-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamid
<b>Valent Lot Nos, Purity (%):</b>	AS2379a (99.7 %)
<b>Structure:</b>	
<b>6. Reference Material</b>	
<b>Code names:</b>	1'-COOH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-COOH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)
<b>Chemical Name:</b>	(1RS,3RS)-(1RS,3SR)-2,3-dihydro-1,3-dimethyl-4-[[1-methyl-3-(difluoromethyl)-1H-pyrazole-4-ylcarbonyl]amino}-1H-indene-1-carboxylic acid
<b>Valent Lot Nos, Purity (%):</b>	AS2393a (1'-COOH-S-2840A) (100 %) AS2394a (1'-COOH-S-2840B) (99.6 %)
<b>Structure:</b>	
<b>7. Reference Material</b>	

<b>Code names:</b>	1'-CH <sub>2</sub> OH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-CH <sub>2</sub> OH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)
<b>Chemical Name:</b>	N-[(1RS,3RS)-(1RS,3SR)--2,3-dihydro-1-(hydroxymethyl)-1,3-dimethyl-1H-inden-4-yl)]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2395a (1'-CH <sub>2</sub> OH-S-2840A) (100 %) AS2396a (1'-CH <sub>2</sub> OH-S-2840B) (99.5 %)
<b>Structure:</b>	
<b>8. Reference Material</b>	
<b>Code names:</b>	DFPA
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid
<b>Valent Lot Nos, Purity (%):</b>	AS2378a (99.2 %)
<b>Structure:</b>	
<b>9. Reference Material</b>	
<b>Code names:</b>	N-des-Me-DFPA
<b>Chemical Name:</b>	3-difluoromethyl-1H-pyrazole-4-carboxylic acid



<b>Valent Lot Nos, Purity (%)</b> :	AS2381a (97.8 %)
<b>Structure:</b>	
<b>10. Reference Material</b>	
<b>Code names:</b>	DFPA-CONH <sub>2</sub>
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%)</b> :	AS2382a (99.2 %)
<b>Structure:</b>	
<b>11. Reference Material</b>	
<b>Code names:</b>	ATMI
<b>Chemical Name:</b>	(3RS)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-amine
<b>Valent Lot Nos, Purity (%)</b> :	AS2383a (99.7 %)
<b>Structure:</b>	
<b>12. Reference Material</b>	
<b>Code names:</b>	3'-OH-S-2840-dehydrate
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-N-[1,1,3-trimethyl-1H-inden-4-yl]-1H-pyrazole-4-carboxamide

<b>Valent Lot Nos, Purity (%)</b> :	AS2413a (98.2 %)
<b>Structure:</b>	

HSE notes that the radiolabelling of both ring structures of the inpyrfluxam test material on either side of the likely cleavage point, the amide bridge, is recommended by the OECD 307 guidelines and is therefore deemed acceptable by HSE.

Several reference standards listed in Table B.8.1.1.1-01 were used which represent the active substance and most of the degradation products in this study were analysed, and all had a chemical or radiochemical purity > 95 %. HSE considers the range of reference compounds used to be acceptable for identifying the transformation products.

#### I. Soil:

The Penn series soil, collected from the top 3-inch layer of an agricultural area (GPS: [REDACTED]) located in Baptistown, NJ, was used in the study.

HSE notes that the coordinates provided by the applicant describe an area approximately 20 miles from Baptistown, NJ. This discrepancy is at odds with the accurate sample location desired by OECD 307. HSE views this as a minor deviation, as an approximate location is known. HSE has therefore assumed that the coordinates rather than the named town accurately describe the sampling location. The applicant has since confirmed in an RAI that the coordinates provided are accurate.

About 5 Kg of the soil was collected on 30 October 2013, using a shovel, and shipped to Valent Technical Center (VTC) on 1 November 2013, under ambient conditions. The soil was received by the center on 4 November 2013. HSE notes that no further notes describing the storage are given, such as light conditions or the storage containers used, as is required by OECD 307. However, HSE considers this as a minor deviation as biomass measurements demonstrate that the soil is biologically active throughout the study.

Upon receipt, the soil was designated as VTC-5-13, mixed thoroughly and sieved through a 2-mm mesh screen with a minimum of air-drying. After sieving, the soil

was stored in an incubator at approximately 10°C during the period between receipt and uses. The soil characteristics are summarised in Table B.8.1.1.1-02 and are within the normal range for European soils.

HSE notes that no details of vegetation cover has been provided for the site which the soil was collected. However, this is considered to be a minor deviation as the procedure of sieving and pre-incubating the soils would remove some of the effects of the vegetation cover on the soils.

HSE also notes that no details of treatments with chemicals, treatments with organic and inorganic fertilisers, additions of biological materials or other contamination regarding the soil collection site have been made available, as is required in OECD 307. HSE considers this a major deviation which potentially jeopardises the validity of the test. Although inpyrfluxam is a new active substance and is unlikely to have been used commercially in previous years at the test site, there are a number of structurally related compounds in the pyrazole carboxamide class that could have been used. Therefore there is the potential that soil microbes at this site may have been exposed to structural analogues which may impact degradation rates. HSE has therefore requested this information from the applicant to proceed with the study validation. The applicant has since stated in a response to an RAI that “Due to the changing nature of the fields used for the study a pesticide history is unavailable.” Whilst HSE would not normally accept this justification in isolation, it is noted that pesticide use histories were available from the other laboratory soil collection sites (see table B.8.1.1.1.2-02). The metabolite profile and parent degradation in this US soil was considered comparable to the behaviour seen at other sites (where pesticide histories were available). Furthermore, the applicant has stated in the anaerobic soil study (B.8.1.1.1.2) that used the same soil, that it was sampled from a terrestrial location which would not normally have pesticide applied. Overall HSE concluded that the results from this study could be considered valid in this case.

HSE notes that no mention of weather conditions preceding the soil collection are made by the applicant, as suggested by OECD 307 guidelines if long periods of drought, freezing or flooding occurred. It is therefore assumed by HSE that these conditions did not occur preceding soil collection.

**Table B.8.1.1.1-02 Chemical and Physical characteristics of test soil**

<b>Soil characteristic</b>	<b>Penn Series soil</b>
USDA Particle size distribution	
% sand (50 µm - 2 mm)	29
% silt (2 µm - 50 µm)	49
% clay <2 µm	22

pH (H <sub>2</sub> O)	6.8
% Moisture at 1/10 Bar (pF 2)	23.8
% Moisture at 1/3 Bar	19.3
Maximum water holding capacity (%)	48.1 ± 1.7
Cation exchange capacity (meq/100g)	7.6
% Organic carbon (Walkley Black)	1.1
% Organic Matter (Walkley Black)	1.9
Bulk density (g / cm <sup>3</sup> )	1.44
USDA Textural class	Loam
Microbial Biomass Carbon (mg/kg dry weight)	454.2 (Day-0) 487.4 (Day-182; inpyrfluxam control)
Microbial Biomass Carbon (% of Total Organic Carbon)	4.13 % (Day-0) 4.43 % (Day-182; inpyrfluxam control)

HSE is satisfied that the soil selected meets the pH 5.5 – 8.0 , organic carbon between 0.5 – 2.5 %, microbial biomass > 1.0 % total organic carbon, and textural class requirements stipulated in OECD 307. HSE notes that Commission Regulation (EU) No 283/2013 requires oxygen levels to be maintained that do not restrict micro-organisms ability to metabolise aerobically. While the applicant made no reference to oxygen levels HSE is content that these were sufficient as the microbial mass balances remained high throughout the test, reported in Table B.8.1.1.1.1-04 below.

## II. Experimental conditions

The degradation of [<sup>14</sup>C] inpyrfluxam was studied in the Penn Series soil for 182 days. HSE notes that the study duration exceeds the 120 days recommended by OECD 307 guidelines. The applicant has, however, provided measurements demonstrating that microbial biomass has not decreased during the study, which are given in Table B.8.1.1.1.1-04. HSE therefore deems the results obtained throughout the study duration acceptable with respect to microbial biomass concentration.

PYR- and PHE-labelled test item, dissolved in acetonitrile, were applied to 50 g dry weight soil at 0.634 mg/kg and 0.650 mg/kg respectively, equivalent to an application rate of 228 and 234 g a.s./ha (assuming that the active substance is homogeneously distributed in 2.5 cm soil of density 1.44 g/cm<sup>3</sup>). After dosing, soil samples were mixed by spatula by hand to ensure distribution of the active substance.

As stated in the OECD 307 Guidelines, the treatment rate should correspond to the highest application rate of a crop protection product recommended in the use instructions and uniform incorporation to an appropriate depth in the field. The

applicant has proposed a representative maximum application rate of 90 g/ha per crop/year (according to Document M-CP, Table 9.1.3-1: Summary of the proposed uses of inpyrfluxam 60 g/L EC) which corresponds to 0.250 mg/kg of inpyrfluxam, over 2.5 cm soil depth and 1.44 g/cm<sup>3</sup> soil density. HSE notes that the applicant used a soil depth of 2.5cm in their conversion calculations to determine the field application rate. The standard soil depth used in pesticide soil exposure calculations is 5 cm. However, HSE accepts that the concentration used is likely to be appropriate for evaluating the aerobic route of degradation of inpyrfluxam in soil, as the greater study application rate will help with the identification of relevant degradation products and the slightly higher rate is unlikely to adversely impact either the route or rate of degradation. Therefore, this is not expected to impact the outcomes of the study.

HSE notes that no mention of solvent evaporation is made by the applicant, as is recommended in the OECD 307 guidelines. HSE does not view this omission to impact the study validity as only 200 µL of acetonitrile solvent is used per 50g soil sample. HSE expects that such a small volume of high volatility solvent would mostly evaporate in the time taken to mix the soil. Furthermore, suitable control samples have been provided showing the solvent has not affected microbial biomass, in Table B.8.1.1.1.1-04.

It is also noted, based on the table of events in Annex A, that HPLC and TLC analysis of the soil samples at each sampling interval took place ~ 1-2 weeks after removal, rather than 'immediately' as stated by the applicant. HSE assumes that the applicant means that work-up and LSC analysis was performed immediately, and HPLC and TLC analysis later. No information on conditions of storage of the extracts has been provided in the report. However, a storage stability test (KCA 7.1.2.2.1\_10) demonstrates that the compounds are stable over the period between extraction and analysis.

Approximately 58.70 g fresh soil sample (corresponding to ca 50 g dry weight) was measured into each of 79 glass beakers (soil units). To bring the soil to ca 60 % of the maximum water holding capacity, 5.73 mL of water was added to each soil unit (final soil weight = 64.43 g). The beakers were placed into 4 Plexiglass incubation chambers and incubated at 20 ± 2 °C in darkness with a constant humidified air flow of approximately 10 mL/min. A pre-incubation period of 14 days was performed to allow germination and removal of seeds, and to re-establish equilibrium of microbial metabolism following the change from sampling or storage conditions to incubation conditions. The soil units were weighed at 'appropriate intervals' and any water loss was replaced with sterile HPLC grade water as needed throughout the study. Whilst HSE would prefer that the intervals be defined, this is not considered to be essential.

HSE notes that for Penn series, maintaining the soil at 50-60 % MWHC resulted in the soil moisture content being maintained slightly below the guideline value of pF 2.0 - 2.5. Furthermore, maintaining the soil at 40% resulted in soil moisture content

being maintained slightly above pF 2.5. Nevertheless, the soil was maintained within the recommended moisture content for optimal microbial activity of 40-60 % recommended by the OECD 307 guidelines. The biomass measurements performed at study start and end indicates there was little inhibition of microbial activity. Therefore, HSE considers this a minor deviation that does not impact the acceptability of the study.

1M NaOH traps were included to collect  $^{14}\text{CO}_2$  and tetraethylene glycol dimethyl ether (tetraglyme) traps were included to trap radioactive organic volatiles. HSE notes that OECD 307 guidelines recommend the use of 2M NaOH traps. As acceptable mass balances have been achieved, HSE believes that this deviation does not impact the validity of the study. A deviation of this type however may affect studies where a larger volume of volatile  $^{14}\text{CO}_2$  is produced, as fewer hydroxide ions in the trap will reduce the ability of the trap to form and solubilise the  $^{14}\text{C}$  as carbonate ions for LSC analysis.

### III. Sampling

Duplicate soil samples were taken at 0, 7, 14, 30, 63, 93, 120, 150 and 182 days after treatment. The trap solutions were analysed at the same points. The samples and controls used in the study are described below in Table B.8.1.1.1.1-03.

**Table B.8.1.1.1-03 Sample treatment groups**

<b>Treatment Group</b>	<b>Unit Nos.</b>	<b>No. of units</b>	<b>Incubator number</b>	<b>Sample type</b>
<b>A</b>	1-9	9	10	Untreated control
	68-75	8	16	Untreated control
	79	1	16	Untreated control
<b>B</b>	10-15	6	10	Organic solvent control
<b>C</b>	16-21	6	10	Non-radiolabelled inpyrfluxam Control
<b>D</b>	24-32	22	12	[Phenyl- $^{14}\text{C}$ ] inpyrfluxam
	34-35			Treated Sample
<b>E</b>	78			
<b>E</b>	46-67	22	13	[Pyrazolyl- $^{14}\text{C}$ ] inpyrfluxam
				Treated Sample

In addition to the above treatment groups, each of four soil units (#22 – 23 and #76-77) was dosed with 200  $\mu\text{L}$  of  $10\times$  non-radiolabelled inpyrfluxam dosing solution

in acetonitrile (AS2375A10×). These soil units provided additional soil sample material for metabolite isolation and identification.

#### IV. Description of analytical procedures

Soil was extracted with acetone, twice with acetone:water (3:2, v/v) and once with acetone:water:HCl (60:40:1, v/v/v). Aliquots of the extracts were analysed by LSC. Post-extracted soils (PES) were subjected to oxidative combustion and LSC to quantify bound or unextracted radioactivity. Limit of detection and quantification (LOD and LOQ) for LSC were determined as 13dpm and 50dpm, respectively. HSE noted that the applicant had not provided LSC LOD and LOQ values in the units of % AR (applied radioactivity) and had requested these to allow for the comparison of mass balances to the LOD and LOQ values.

This has since been provided by the applicant in an RAI. LOD for LSC is  $\ll 1$  % AR, and the maximum LOQ is 0.02 % AR. HSE considers that the LOD is suitably below the OECD 307 threshold of 1 % AR, and that LOQ has been specified, as required by OECD 307.

The PES sample containing significant radioactivity was fractionated into fulvic acid, humic acid and humin, and the residue in each fraction was analysed by LSC and/or combustion analysis, and when appropriate, by TLC.

The metabolite profile of the concentrated neutral and acidic extract was determined by 2D TLC analysis. The identification of the metabolites in the neutral and acidic extract was confirmed by HPLC analysis, equipped with an ultraviolet (UV) detector and a radioactivity monitor (RAM).

The  $^{14}\text{CO}_2$  collected in the NaOH trapping solution and organic volatiles collected in tetraglyme traps was quantified by LSC.

The structural assignments for [ $^{14}\text{C}$ ] inpyrfluxam and metabolites were based on co-chromatography with reference standards upon HPLC and TLC analysis.

The potential isomerisation from [ $^{14}\text{C}$ ] inpyrfluxam (both labels) was evaluated using chiral HPLC analysis on the extracts obtained from the 182 DAT samples and on the test substances prior to application.

Dates of sample collection and analytical procedures are recorded in Table B.8.1.1.1.1-08, Annex A.

HSE notes that no LOD or LOQ are provided for TLC, as is required by OECD 307 guidelines. This is regarded as a major deviation for TLC as it has been used quantitatively. The applicant should provide LOD and LOQ values of the Storm 820 PhosphorImager used, expressed in % AR. This has since been provided

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in an RAI. LOD is  $\ll 1$  % AR, and the maximum LOQ is 0.36 %. HSE considers that this is acceptable by the OECD 307 threshold of 1 % for LOD.

HSE notes that no LOD or LOQ are provided for the UV and RAM detectors linked to the HPLC. This is regarded as a major deviation as they are used quantitatively to determine metabolite fractions. HPLC LOD & LOQ for both the UV and RAM detectors will be required from the applicant to complete this study validation. This has since been provided by the applicant in an RAI. LOD for HPLC-RAM is  $\ll 1$  % AR, and the maximum LOQ is 0.3 % AR. The applicant has clarified that HPLC-UV has only been used qualitatively, and as such LOD and LOQ values are not required. HSE considers that this is acceptable according to OECD 307 guidelines.

## **RESULTS AND DISCUSSION**

### **I. Data**

No significant decrease of soil biomass carbon was observed between the initiation and termination of the incubation (Table B.8.1.1.1.1-04). Thus, HSE is satisfied that microbial viability was maintained during the incubation period.



**Table B.8.1.1.1-04 Microbial Biomass in Soil Samples at Various Stages of the Study**

<b>Study Stage</b>	<b>Sample ID</b>	<b>Sampling Date</b>	<b>Microbial Biomass Carbon µg/g dry wt. basis</b>
Soil sample after sieving	VTC-5-13	11/07/2013	454.2
1 day after application (Treatment A)	A-1D-2,6,7	12/10/2013	503.7
64 days after application (Treatment A)(soil Control)	A-64D-3,5,9	02/11/2014	573.7
64 days after application (Treatment B)(solvent Control)	B-64D-10,14,15	02/11/2014	555.7
64 days after application (Treatment C)(inpyrfluxam control)	C-64D-16,17,18	02/11/2014	576.5
120 days after application (Treatment A)(soil Control)	A-120D-68,70,71	04/08/2014	553.2
182 days after application (Treatment A)(soil Control)	A-182D-73,74,75	06/09/2014	474.4
182 days after application (Treatment B)(solvent Control)	B-182D-11,12,13	06/09/2014	467
182 days after application (Treatment C)(inpyrfluxam control)	C-182D-19,20,21	06/09/2014	487.4

HSE notes that no microbial biomass measurement of the solvent control sample was taken at 1 day after application, as is required by OECD 307. HSE considers this as a minor deviation, as HSE considers that measurements at DAT 0, 64 and

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182 sufficiently demonstrate that the solvent used has not affected microbial biomass.

It was confirmed that no isomerisation of [ $^{14}\text{C}$ ] inpyrfluxam occurred during incubation period based on chiral HPLC analysis.

The distribution and mass balance of applied radioactivity of [ $^{14}\text{C}$ ] inpyrfluxam in extractable, soil-bound and volatile fractions of soil samples are summarised in Table B.8.1.1.1.1-05. The quantification of inpyrfluxam and the metabolites is summarised in Table B.8.1.1.1.1-06. The distribution of bound radioactivity in PES (182 DAT soil samples) is summarised in B.8.1.1.1.1-07.

**Table B.8.1.1.1-05 Mean distribution and Mass Balance (Percent) of Applied Radioactivity of [Phenyl-<sup>14</sup>C] inpyrfluxam and [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam in Extractable, Soil-Bound and Volatile Fractions of Soil Samples**

Test Substance	Fraction	Days After Treatment (DAT)								
		0	7	14	30	63	93	120	150	182
[Phenyl- <sup>14</sup> C] inpyrfluxam	Neutral Extract	94.6	90.6	86.3	85.0	75.3	71.1	68.2	63.5	69.2
	Acidic Extract	1.0	5.3	8.5	11.1	17.9	18.3	19.5	20.6	18.8
	Total Extractable	95.6	95.9	94.8	96.2	93.3	89.4	87.8	84.1	88.0
	Soil-bound	0.1	1.6	2.8	3.5	6.6	7.1	7.9	8.8	8.9
	Volatiles ( <sup>14</sup> CO <sub>2</sub> )	NA	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
	<b>Total</b>	95.7	97.6	97.7	99.8	100.2	96.8	96.2	93.4	97.5
[Pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	Neutral Extract	96.5	89.7	85.8	83.2	74.2	71.1	68.3	62.7	66.9
	Acidic Extract	1.2	5.7	8.7	11.5	18.1	19.5	20.1	21.6	20.8
	Total Extractable	97.7	95.5	94.6	94.7	92.3	90.6	88.4	84.3	87.7
	Soil-bound	0.1	1.7	2.6	3.3	6.0	7.0	8.3	9.2	9.5
	Volatiles ( <sup>14</sup> CO <sub>2</sub> )	NA	0.0	0.0	0.1	0.1	0.2	0.2	0.3	0.3
	<b>Total</b>	97.8	97.2	97.2	98.0	98.4	97.8	96.9	93.8	97.5

Each value represents an average of two soil units, NA: not analysed. Organic volatiles were not detected in tetraglyme traps at any timepoints and are therefore excluded from the table.

HSE notes that by OECD 307 guidelines, volatile traps should be checked and changed once a week for the first month, and once every two weeks thereafter. This has not been performed here; samples were only checked on days 7 & 14, then 30, 60, 93, 120, 150, & 182. While a deviation, HSE does not consider this to have impacted study validity, as volatile production is low, and it is not expected that the volatile traps will have filled within the interval periods.

**Table B.8.1.1.1-06 Distribution (Percent) of Applied Radioactivity in [Phenyl-<sup>14</sup>C] inpyrfluxam, [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam and Their Metabolites at Various Sampling Times**

Test Substance	Fraction	Days After Treatment (DAT)								
		0	7	14	30	63	93	120	150	182
[Phenyl- <sup>14</sup> C] inpyrfluxam	inpyrfluxam	93.2	89.4	87.2	83.1	72.0	64.5	62.6	55.4	53.5
	3'-OH-S-2840-dehydrate	0.0	0.1	0.2	0.2	1.8	4.2	1.4	1.6	5.4
	3'-OH-S-2840	1.0	1.9	2.2	5.1	9.0	7.2	11.2	12.6	14.4
	3'-OH-S-2840 (incl. dehydrate)	1.0	2.0	2.4	5.4	10.8	11.4	12.6	14.2	19.7
	N-des-Me-S-2840	0.0	0.0	0.2	0.1	0.3	0.3	0.5	0.7	0.8
	1'-COOH-S-2840 (total)*	0.0	2.5	3.9	5.1	5.9	6.2	6.0	6.6	6.7
	Total Other Unknowns*	1.4	2.0	1.3	2.4	4.3	6.9	6.0	7.3	7.4
	Total Extractable	95.6	95.9	94.8	96.2	93.3	89.4	87.8	84.1	88.0
[Pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	inpyrfluxam	93.9	88.4	85.8	82.9	72.3	65.3	62.1	56.4	51.7
	3'-OH-S-2840-dehydrate	0.0	0.0	0.1	0.5	1.2	1.6	0.9	1.3	3.1
	3'-OH-S-2840	1.9	1.8	2.3	3.7	7.8	11.3	11.6	12.2	15.5

Test Substance	Fraction	Days After Treatment (DAT)								
		1.9	1.9	2.4	4.2	9.0	12.9	12.5	13.6	18.6
[Pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	3'-OH-S-2840 (incl. dehydrate)	1.9	1.9	2.4	4.2	9.0	12.9	12.5	13.6	18.6
	N-des-Me-S-2840	0.0	0.0	0.0	0.0	0.1	0.2	0.4	0.6	0.6
	1'-COOH-S-2840 (total)*	0.0	1.9	3.9	4.6	5.2	5.3	5.4	5.5	5.9
	DFPA	0.0	1.0	0.8	1.0	0.9	0.5	0.6	0.4	0.4
	N-des-Me-DFPA	0.0	0.0	0.0	0.3	0.6	0.9	0.9	1.2	1.6
	Total Other Unknowns*	1.9	2.2	1.6	1.8	4.3	5.6	6.5	6.8	8.9
	Total Extractable	97.7	95.5	94.6	94.7	92.3	90.6	88.4	84.3	87.7

Each percentage value represents an average of two determinations from replicate soil units; at the last sampling interval, one replicate was sampled at 182 DAT, and the other at 183 DAT

\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

\*\* Individual peaks represent <2 % of applied radioactivity at any sampling interval

HSE notes that while the decline phases of major metabolites have not been shown, as is required by OECD 307, separate metabolite dosing studies have been submitted (see B.8.1.1.1.3 and B.8.1.1.1.4). This lack of decline phase is therefore not considered as a significant deviation by HSE.

**Table B.8.1.1.1-07 Distribution of Bound Radioactivity in PES Fractions of 182 DAT Soil Samples**

<b>Test Substance</b>	<b>Fraction</b>	<b>% Distribution</b>	<b>% of Applied</b>
<b>[Phenyl-<sup>14</sup>C] inpyrfluxam</b>	Dismembrator Extract	47.9	4.4
	Fulvic Acid	20.5	1.9
	<i>Organic<sup>1</sup></i>	82.3	1.5
	<i>Aqueous</i>	17.7	0.3
	Humic Acid	13.4	1.2
	Humin	18.3	1.7
	Total Bound	100.0	9.1
<b>[Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam</b>	Dismembrator Extract	48.6	4.7
	Fulvic Acid	23.9	2.3
	<i>Organic</i>	89.2	2.1
	<i>Aqueous</i>	10.8	0.2
	Humic Acid	11.6	1.1
	Humin	15.9	1.5
	Total Bound	100.0	9.7

<sup>1</sup>Italicised values represent subsets of Fulvic acid

## **II. Mass balance**

The mass balance for the PYR-label ranged from 94 % to 98 % AR and for the PHE-label from 93 % to 100 % AR, both shown in Table B.8.1.1.1-05. These mass balances lie within the OECD 307 acceptable guideline of 90-110 % and are therefore deemed acceptable by HSE.

## **III. Bound residues**

Unextracted radioactivity reached a maximum of 9.5 % and 8.9 % AR for the PYR-label and PHE-label respectively at the end of the study.

## **IV. Volatilisation**

Very little <sup>14</sup>CO<sub>2</sub> was formed (maximum 0.3 % and 0.7 % AR for the PYR-label and PHE-label respectively at the end of the study). (Table B.8.1.1.1-05). The volatile radioactivity in the tetraglyme traps was 0 % AR at all sampling times, indicating that the formation of organic volatiles was negligible during the study period.

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## V. Metabolites

The degradation product with the largest proportion observed in the study was 3'-OH-S-2840 (maximum 18.6 % AR for PYR-label, and 19.7 % AR for PHE-label). These values include small quantities of 3'-OH-S-2840-dehydrate, which formed from 3'-OH-S-2840 via acid-catalysed dehydration, mainly in the acidic extraction step. As the formation of the dehydrate is an artefact of the extraction method, HSE agrees with the applicant that 3'-OH-S-2840-dehydrate should be considered as 3'-OH-S-2840 when presenting mass balances. Furthermore, HSE agrees with the applicant's position that the maximum amount of this metabolite should be expressed as the sum of 3'-OH-S-2840 and its dehydrate derivative.

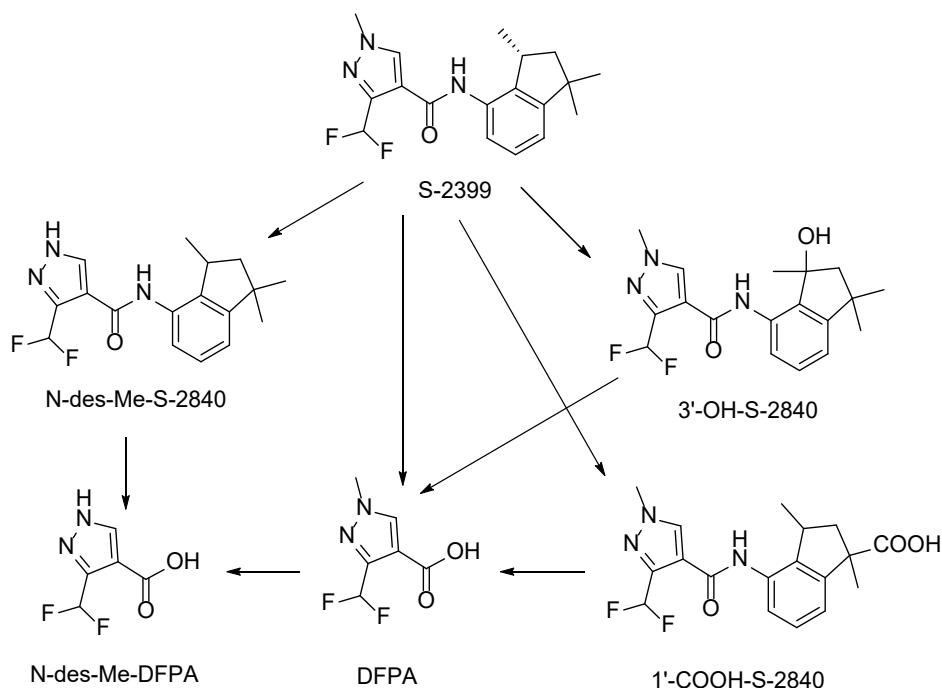
1'-COOH-S-2840 (maximum 5.9 % AR for PYR-label, and 6.6 % AR for PHE-label) also exceeded 5 % in at least two consecutive timepoints, and was still increasing at study termination. All other radioactive peaks were  $\leq 1.6$  % AR.

The primary degradation of inpyrfluxam appeared to occur in soil via oxidation of the 3'-position in the indenyl ring to produce 3'-OH-S-2840. This metabolite increased gradually during the incubation period, exceeded 9 % of applied radioactivity at 63 DAT, and reached a maximum of 19 – 20 % of applied radioactivity at the end of the study at 182 DAT. This metabolite was unstable under acidic conditions and formed its dehydrate via loss of H<sub>2</sub>O.

Inpyrfluxam degraded through a second metabolic pathway, which involved oxidation of one of the 1'-CH<sub>3</sub> groups of the indenyl ring into a 1'-COOH group.

Formation of small amounts of N-des-Me-S-2840 and N-des-Me-DFPA indicated that an additional pathway of degradation of inpyrfluxam existed in soil under aerobic conditions, which involved cleavage and loss of the N-methyl moiety in the pyrazolyl ring, followed by the cleavage of the amide bond. However, the concentration of N-des-Me-S-2840 and N-des-Me-DFPA was less than 0.8 % and 1.6 % of applied radioactivity, respectively, at all sampling time intervals, and are therefore deemed minor metabolites.

These compounds would be degraded and be finally mineralised to CO<sub>2</sub> or form un-extractable complexes with the soil. Figure B.8.1.1.1.1-01 shows the proposed metabolic pathway of inpyrfluxam in the soil under aerobic conditions.



**Figure B.8.1.1.1-01 Proposed metabolic pathways of inpyrfluxam in Penn soil under aerobic conditions**

## CONCLUSION

The degradation of inpyrfluxam in the aerobic soil involves conversion to 1'-COOH-S-2840 (max. 6.6 % AR), or 3'-OH-S-2840 (max. 19.7 % AR including dehydrate). These compounds may subsequently be degraded leading to the formation of CO<sub>2</sub> and non-extractable residues (noting that CO<sub>2</sub> formation within the 182 d study period was very low - < 1% AR). Non-extractable residues reached a maximum of 8.9 % and 9.5 % AR for the PHE- and PYR-labels respectively, in the study duration. HSE agrees with the applicant's analysis and the degradation pathway provided.



**Annex A**Table B.8.1.1.1-08 Chronology of major events in the study

<b>Date</b>	<b>Study Event Started or Conducted</b>
30/10/2013	Soil sample was collected
04/11/2013	Soil sample was received at VTC
25/11/2013	Soil sample units were prepared and placed in incubators
05/12/2013	Protocol was signed by the Study Director
06/12/2013	Preparation of dosing solutions
09/12/2013	Treatment of soil samples with dosing solutions
09/12/2013	Sampling and extraction of 0 DAT soil units
11/12/2013	LSC and HPLC analysis of dosing solution aliquots
16/12/2013	Sampling and extraction of 7 DAT soil units
23/12/2013	Sampling and extraction of 14 DAT soil units
08/01/2014	Sampling and extraction of 30 DAT soil units
22/01/2014	2D TLC analysis of 0 and 7 DAT extracts
23/01/2014	HPLC analysis of 0 and 7 DAT extracts
03/02/2014	HPLC analysis of 14 DAT extracts
10/02/2014	Sampling and extraction of 63 DAT soil units
20/02/2014	HPLC analysis of 30 and 63 DAT extracts
12/03/2014	Sampling and extraction of 93 DAT soil units
21/03/2014	HPLC analysis of 93 DAT extracts
26/03/2014	2D TLC analysis of 93 DAT extracts
08/04/2014	Sampling and extraction of 120 DAT soil units
16/04/2014	2D TLC analysis of 120 DAT extracts
21/04/2014	HPLC analysis of 120 DAT extracts
05/05/2014	2D TLC analysis of 14 DAT extracts
08/05/2014	Sampling and extraction of 150 DAT soil units
12/05/2014	2D TLC analysis of 30 DAT extracts
15/05/2014	HPLC analysis of 150 DAT extracts

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<b>Date</b>	<b>Study Event Started or Conducted</b>
19/05/2014	2D TLC analysis of 150 DAT extracts
19/05/2014	Preparative HPLC fractionation of 120 DAT extract
28/05/2014	2D TLC analysis of Metabolite fractions of 120 DAT extract
03/06/2014	HPLC analysis of Metabolite fractions of 120 DAT extract
04/06/2014	2D TLC analysis of 63 DAT extracts
09/06/2014	Sampling and extraction of 182 DAT soil units
17/06/2014	Analysis of 182 DAT Post-extraction soil (PES)
19/06/2014	2D TLC analysis of 182 DAT extracts
23/06/2014	HPLC analysis of 182 DAT extracts
24/06/2014	Chiral HPLC analysis of 182 DAT extracts
14/11/2014	TLC and HPLC confirmation of 3'-OH-S-2840-dehydrate

**B.8.1.1.1.2. Aerobic route of degradation of the active substance, 2  
UK soils & 1 US soil**

<b>Reference</b>	KCA 7.1.1.1/02
<b>Study Title:</b>	S-2399: Degradation under Aerobic Conditions in Soil Rate
<b>Author &amp; year:</b>	██████ (2017a)
<b>Address:</b>	Valent Technical Center (VTC), 6560 Trinity Court, Dublin, CA 94568
<b>Study No:</b>	VP-38898
<b>Applicant:</b>	Sumitomo Chemical Co. Ltd. Report No: TPM-0044
<b>Guidelines:</b>	OECD 307 Test Guideline
<b>GLP:</b>	Yes
<b>Deviations:</b>	Yes (see below)

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Test compound is only radiolabelled on one side of the likely cleavage point, the amide bridge (PYR-label)	Minor deviation. Another rate study, KCA 7.1.1.1_01, has been provided in which both sides of the amide bridge are labelled. This showed that metabolites only formed from the side that is labelled in this study.
Soil transport and storage containers not defined	Minor omission. Loose polyethylene bags are recommended by OECD 307 to minimise changes in soil water content. As the applicant has adjusted soil water content prior to test initiation, this omission is not considered to jeopardise study validity. Biomass measurements at the start and end of the incubation period suggest that the soils are microbially active throughout the study. Therefore, HSE assumes that the transport conditions are

	acceptable, and this has not affected the outcome of the study.
Atwater soil organic carbon is 0.3 % of soil mass and OECD 307 recommends a range of organic carbon contents from 0.5-2.5%	Minor deviation. Atwater soil represents the lower end of organic carbon for a European arable soil. Considered as a group across both studies (see also KCA 7.1.1.1/01), HSE deem that a suitable range of soil properties are covered within these studies.
Soils stored at ambient temperature before study initiation	Major deviation from OECD 307 guidelines, which requires refrigeration at $4 \pm 2$ °C. Likely to have resulted in a decrease in soil biomass. However, biomass measurements at study initiation and termination demonstrate that the soil was microbially active (biomass >1 % of organic carbon) at the start and end of the incubation period. Therefore, this deviation is not considered to adversely impact the study validity.
<sup>14</sup> CO <sub>2</sub> trap NaOH concentration used (0.5 M) differs from OECD 307 guideline (2M)	Minor deviation. Mass balances demonstrate NaOH concentration used is suitable in this study.
Applicant figure labelled as containing 2 M NaOH traps, whereas text specifies 0.5 M	Minor deviation. HSE believes that the figure has been mislabelled.
Biomass measurements during study not provided	Minor deviation. Biomass measurements have been provided at study initiation and termination that show soil is microbially active. Therefore, HSE considers that it is likely that the soil is microbially active during the study. Therefore, this omission is not deemed to void the study validity.

Oxygen level measurements not provided	Minor omission, required by OECD 307 guidelines. Applicant has however provided thorough description of flow-through apparatus. Therefore, HSE considers it likely that oxygen levels have been maintained sufficiently.
Decline phase of major metabolites not shown	HSE notes that separate metabolite dosed studies were provided by the applicant at B.8.1.1.1.3 & B.8.1.1.1.4. Therefore, this is not considered to impact study validity.

### **HSE conclusion on deviations**

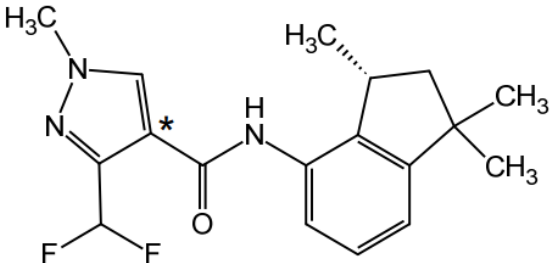
No deviations are considered by HSE to void the validity of the study, and therefore further clarification is not required.

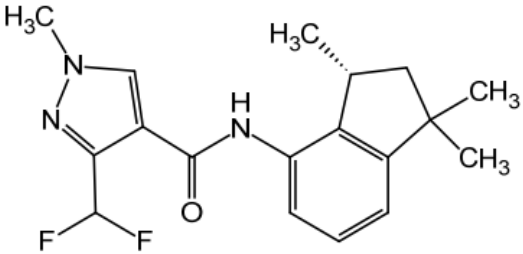
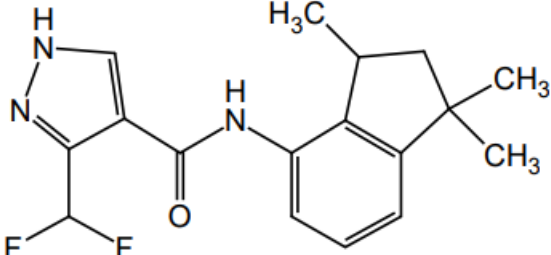
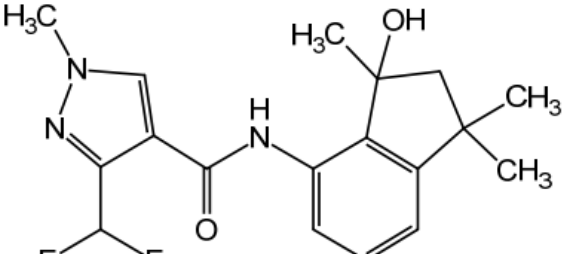
## **INTRODUCTION**

The route of the metabolism of the non-volatile active substance inpyrfluxam (S-2399) in three soils (Atwater, Newhaven, and Woodside farm) was investigated under aerobic conditions using [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PYR-label) at a rate of 0.603 mg/kg (dry weight basis). Treated soil samples were incubated at 20 ± 2 °C and approximately 50 % of the maximum water holding capacity (MWHC) in the dark for a maximum of 120 days and were periodically collected and extracted. The soil extracts were analysed by LSC (liquid scintillation counting) and 2D TLC (thin layer chromatography). Additionally, samples were occasionally analysed with HPLC (high performance liquid chromatography) to quantify and identify major radioactive components. The post-extraction solids (PES) were analysed by combustion analysis.

## MATERIALS AND METHODS

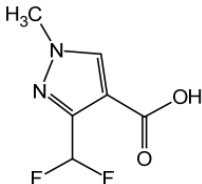
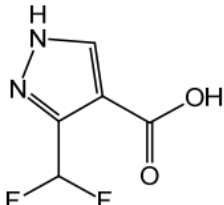
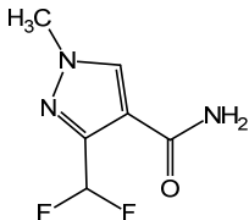
**Table B.8.1.1.1.2-01 Properties of the materials used in the aerobic soil degradation study**

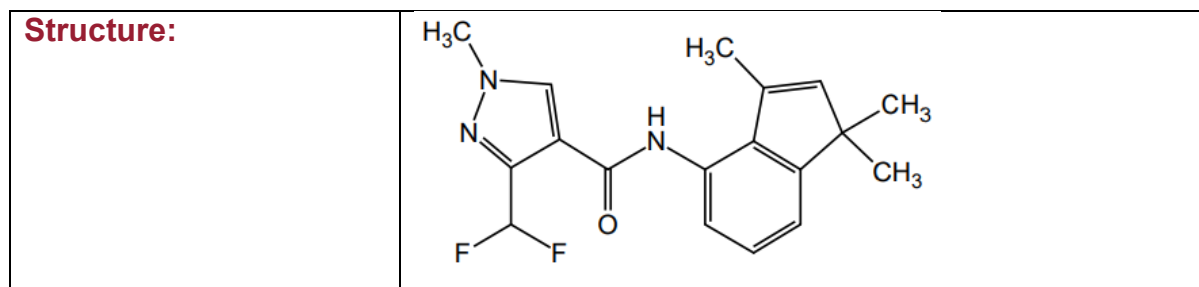
<b>1. Test Material</b>	[pyrazolyl-4- <sup>14</sup> C] inpyrfluxam (PYR-label)
<b>Lot/Batch:</b>	CFQ41802
<b>Specific activity:</b>	2.11 GBq/mmol
<b>Purity:</b>	Radiochemical purity 95.43 % (in the dosing solution). The chirality was determined to be 100 % R-isomer. Metabolite 3'-OH-S-2840 accounts for 1.79 % of the radioactivity.
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	Stable
<b>Structure:</b>	 <p><i>* Denotes <sup>14</sup>C label position</i></p>
<b>2. Reference Material</b>	
<b>Code names:</b>	inpyrfluxam (Pure 3'R isomer) inpyrfluxam (Pure 3'S isomer)
<b>Chemical Name:</b>	3-(difluoro methyl)-1-methyl-N-[(3'R)-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%)</b>	AS2375a (inpyrfluxam Analytical Grade) (96.0 %) AS2375b (inpyrfluxam pure 3'R isomer) (99.8 %) AS2387a (S-2940 pure 3'S isomer) (99.8 %)

<b>Structure:</b>	
<b>3. Reference Material</b>	
<b>Code names:</b>	N-des-Me-S-2840 (Contains 2 enantiomers: 3'R & 3'S)
<b>Chemical Name:</b>	N-[(3RS)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2380a (R enantiomer) (97.5 %) AS2380b (S enantiomer) (99.3 %) AS2389a (99.9 %)
<b>Structure:</b>	
<b>4. Reference Material</b>	
<b>Code names:</b>	3'-OH-S-2840 (Contains 2 enantiomers: 3'R & 3'S)
<b>Chemical Name:</b>	3-(difluoromethyl)-N-[3'-hydroxy-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1-methyl-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2379b (97.7 %)
<b>Structure:</b>	
<b>5. Reference Material</b>	

<b>Code names:</b>	1'-COOH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-COOH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)
<b>Chemical Name:</b>	(1RS,3RS)-(1RS,3SR)-2,3-dihydro-1,3-dimethyl-4-[[1-methyl-3-(difluoromethyl)-1H-pyrazole-4-ylcarbonyl]amino]-1H-indene-1-carboxylic acid
<b>Valent Lot Nos, Purity (%):</b>	AS2393a (1'-COOH-S-2840A) (100 %) AS2394a (1'-COOH-S-2840B) (99.6 %)
<b>Structure:</b>	
<b>6. Reference Material</b>	
<b>Code names:</b>	1'-CH <sub>2</sub> OH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-CH <sub>2</sub> OH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)
<b>Chemical Name:</b>	N-[(1RS,3RS)-(1RS,3SR)--2,3-dihydro-1-(hydroxymethyl)-1,3-dimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2395a (1'-CH <sub>2</sub> OH-S-2840A) (100 %) AS2396a (1'-CH <sub>2</sub> OH-S-2840B) (99.4 %)
<b>Structure:</b>	
<b>7. Reference Material</b>	
<b>Code names:</b>	DFPA



<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid
<b>Valent Lot Nos, Purity (%)</b> :	AS2378a (99.2 %)
<b>Structure:</b>	
<b>8. Reference Material</b>	
<b>Code names:</b>	N-des-Me-DFPA
<b>Chemical Name:</b>	3-difluoromethyl-1H-pyrazole-4-carboxylic acid
<b>Valent Lot Nos, Purity (%)</b> :	AS2381b (97.7 %)
<b>Structure:</b>	
<b>9. Reference Material</b>	
<b>Code names:</b>	DFPA-CONH <sub>2</sub>
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%)</b> :	AS2382a (99.2 %)
<b>Structure:</b>	
<b>10. Reference Material</b>	
<b>Code names:</b>	3'-OH-S-2840-dehydrate
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-N-[1,1,3-trimethyl-1H-inden-4-yl]-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%)</b> :	AS2413a (98.0 %)



HSE notes that inpyrfluxam test material has only been radiolabelled on one side of the likely cleavage point, the amide bridge. Labelling on both sides is recommended by the OECD 307 guidelines. However, the applicant has provided a second route study, at KCA 7.1.1.1\_01 which labels both sides of the bridge. As, in combination with this second study, a full description of the test material's degradation pathway is provided, the approach taken here is deemed acceptable by HSE. Furthermore, based on the results of the other study, formation of further metabolites from the unlabelled part of the molecule is not expected.

#### I. Soil:

The three soils, all recently collected from the top soil layer, were air-dried and sieved (2 mm). The soils characteristics are summarised in Table B.8.1.1.1.2-02. Properties relating to soil collection and storage are summarised in Table B.8.1.1.1.2-03.

**Table B.8.1.1.1.2-02 Chemical and Physical characteristics of test soils**

<b>Soil characteristic</b>		<b>Atwater</b>	<b>Newhaven</b>	<b>Woodside Farm</b>
Location		Madera, CA (USA)	Derbyshire, UK	Empingham, UK
Coordinates		[REDACTED]	[REDACTED]	[REDACTED]
Pesticide use history		No use for > 10 years	No use since 1991	No formal use for > 10 years
USDA Particle size distribution	% sand (50 µm - 2 mm)	72	35	41
	% silt (2 µm - 50 µm)	13	58	34
	% clay <2 µm	15	7	25
pH (H <sub>2</sub> O)		7.5	5.7	7.5

% moisture		3.91	34.19	16.50
% Moisture 1/3 bar		10.6	27.3	24.5
% Moisture 1/10 bar (pF 2)		14.3	33.0	28.6
Maximum water holding capacity ( %)		32.13	76.79	57.87
Cation exchange capacity (meq/100g)		10.3	13.1	24.7
% Organic carbon		0.30	3.8	3.2
% Organic Matter		0.51	6.5	5.5
Bulk density (disturbed) (g/cm <sup>3</sup> )		1.30	0.92	1.20
USDA Textural class		Sandy loam	Silt loam	Loam
Microbial Biomass Carbon, µg/g dry weight (% of organic carbon)	Day 0	499.1 (16.6)	960.2 (2.53)	1111.3 (3.47)
	Day 120; untreated control	265.1 (8.84)	573.9 (1.51)	1089.3 (3.40)
	Day 120; solvent control	383.4 (12.8)	560.8 (1.48)	1056.8 (3.30)
	Day 120; inpyrfluxam control	425.2 (14.2)	580.4 (1.53)	1098.9 (3.43)

**Table B.8.1.1.2-03 Properties of soil collection and storage**

<b>Soil characteristic</b>	<b>Atwater</b>	<b>Newhaven</b>	<b>Woodside Farm</b>
Soil inventory designation	VTC-3-15	VTC-1-15	VTC-2-15
Collection procedure	Collected with a shovel	Collected with a shovel	Collected with a shovel
Sampling depth (cm)	0-7.6	5-20	8-15
Collection date (DD/MM/YY)	16/04/15	26/03/15	26/03/15
Storage length: collection until arrival at Valent (days, inclusive)	6	8	8

Storage conditions: collection until arrival at Valent	Ambient temperature, in the dark	Ambient temperature, in the dark	Ambient temperature, in the dark
Storage length: arrival at Valent until test system preparation (days, inclusive)	17	37	37
<b>Soil characteristic</b>	<b>Atwater</b>	<b>Newhaven</b>	<b>Woodside Farm</b>
Storage conditions: arrival at Valent until test system preparation	Ambient temperature, in the dark	Ambient temperature, in the dark	Ambient temperature, in the dark

HSE notes that no further notes describing the transport or storage are given, such as the containers used, as is required by OECD 307. HSE considers this as a minor deviation, and does not consider it to void the study validity as biomass measurements at the study start (0 DAT) and study end (120 DAT; Table B.8.1.1.1.2-05) suggest that the soils were biologically active throughout the study. Furthermore, HSE does not consider possible changes in moisture content to be a potential deviation, as moisture was adjusted to 50 % of MWHC before study initiation.

HSE is satisfied that at least one soil selected meets the representative property requirements of pH 5.5 – 8.0 , organic carbon between 0.5 – 2.5 % , microbial biomass > 1.0 % total organic carbon, and textural class requirements stipulated in OECD 307 (including the first study reported at KCA 7.1.1.1/01). HSE notes that the Atwater soil has a low organic carbon of 0.3 %. The applicant has stated that the Atwater soil represents the worst-case range of characteristics for a European arable soil. HSE accepts the use of the Atwater soil on this basis, and because it meets the rest of the OECD 307 requirements. In combination with the other soils, HSE considers that a suitable range of soil properties are included across both studies.

HSE notes that soils were stored at ambient temperature prior to aerobic test system preparation. This is a deviation from OECD 307 guidelines, which requires that if soils are to be stored, it is done at  $4 \pm 2$  °C. HSE considers this a major deviation, which is likely to affect the soil biomass, given the considerable storage duration of 37 days. As no biomass measurements were taken immediately after collection, the effect of storage on biomass cannot be quantified. HSE has calculated the microbial biomass as a percentage of organic carbon at the study start and study end based on the measurements provided by the applicant, presented in Table B.8.1.1.1.2-02. As these calculations show that the microbial biomass present in the soils were above the OECD recommended minimum level of 1 % of organic carbon HSE deems the applicant has

demonstrated that biomass levels are sufficient to allow the soils to remain microbially active at the study start and end. Therefore, while the applicant has deviated from the OECD 307 guidelines in terms of soil storage, and not providing microbial biomass calculations throughout the study, HSE deems this deviation to be minor, and not to have had an adverse impact on the study outcomes.

Upon receipt, the soils were each mixed thoroughly and sieved through a 2-mm mesh screen with a minimum of air-drying.

HSE notes that Commission Regulation (EU) No 283/2013 requires oxygen levels to be maintained at a level that do not restrict micro-organism's ability to metabolise aerobically. While the applicant made no reference to oxygen levels HSE is content that these were sufficient due to the nature of the flow-through incubation apparatus used, which the applicant has thoroughly described, and is illustrated in Figure B.8.1.1.1.2-01.

## **II. Experimental Conditions**

The degradation of [ $^{14}\text{C}$ ] inpyrfluxam was studied in the soils for 120 days. A pre-incubation aerobic acclimation period of 12 - 13 days was performed. Soils were adjusted to  $50 \pm 10$  % of the MWHC by adding water as required (HPLC grade). To account for moisture weight at 50 % WHC, 58.03 g, 69.20 g, and 64.47 g of the Atwater, Newhaven, and Woodside Farm soil samples (equivalent to 50g dry weight) were used for the studies.

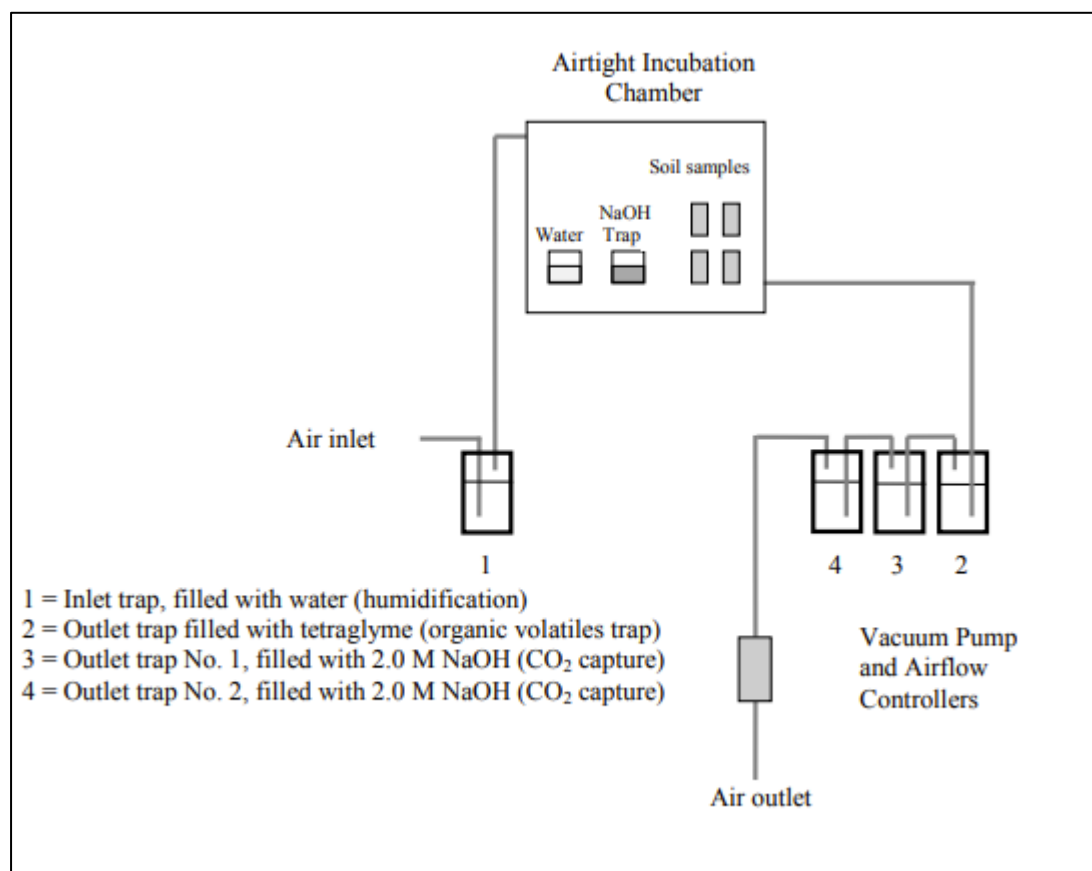
The target application rate of the test substance to soil was 0.662 mg/kg dry soil, equivalent to a field application rate of 215 g a.s./ha (assuming that the test substance is homogeneously distributed in 2.5 cm soil of density  $1.3 \text{ g/cm}^3$ ). As noted in the earlier study the application rate used is higher than the proposed maximum application rate of 90 g/ha for the representative use. However HSE accepts that the concentration used is likely to be appropriate for evaluating the aerobic route of degradation of inpyrfluxam in soil, as the greater study application rate will help with the identification of relevant degradation products and the slightly higher rate is unlikely to adversely impact either the route or rate of degradation.

[ $^{14}\text{C}$ ] inpyrfluxam dissolved in 220  $\mu\text{L}$  of acetonitrile was applied via syringe to the surface of the soils at an actual concentration of 0.603 mg/kg, equivalent to 11,388,00 dpm (detections per minute). Dosing was followed by five minutes of soil mixing with a metal spatula to ensure homogeneity. The applicant has stated that the acetonitrile solvent evaporated. HSE notes that solvent evaporation is required by OECD 307 guidelines and accepts the applicant's statement based on the high volatility of acetonitrile, and the small volume used.

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### III. Incubation apparatus

A schematic drawing of the incubation apparatus is presented in Figure B.8.1.1.1.2-01. The incubation apparatus consists of an airtight chamber with air inlet and outlet valves. Atwater, Newhaven, and Woodside Farm soil were placed inside separate chambers in open individual 100 mL labelled, glass beakers. The soil moisture was adjusted periodically to  $50 \pm 10$  % of the water holding capacity during the aerobic incubation (moisture was adjusted by adding HPLC grade water as needed). One open Petri dish, containing 50 mL of 0.5 M NaOH solution was placed inside the chamber to capture evolved  $^{14}\text{CO}_2$ . Open Petri dishes full of water were also placed inside the chamber to maintain high air humidity. Chamber air was continuously evacuated through the chamber air outlet, which was connected to a vacuum source, at a steady rate of approximately 10 mL/minute. The chamber inlet air was bubbled through a water trap to humidify the air before entering the chamber. The continuous airflow was used to maintain aerobic conditions as well as to evacuate any radiolabelled volatiles into the outlet traps. The evacuated air passed through a tetraglyme trap to capture any  $^{14}\text{C}$  volatiles other than  $^{14}\text{CO}_2$  and two consecutive 0.5 M NaOH traps to capture any  $^{14}\text{CO}_2$  present in the chamber's air. The incubation apparatus was incubated in the dark at  $20 \pm 2$  °C.



**Figure B.8.1.1.2-01 Schematic representation of the aerobic incubation apparatus showing locations of the soils samples and volatile traps**

HSE notes that in Figure B.8.1.1.2-01 provided by the applicant, the NaOH are labelled as 2 Molar, whereas in the study report, it is specified as 0.5 Molar. HSE considers the applicant figure to be mislabelled and therefore accepts the KCA text that the traps are 0.5 Molar.

HSE notes that OECD 307 guidelines recommend the use of 2M NaOH traps. As acceptable mass balances (90 - 110 %) have been achieved, HSE concludes that this deviation does not detract from the validity of the study.

#### IV. Sampling

Duplicate soil samples were taken at 0, 14, 30, 61, 90 and 120 days after treatment (DAT). The trap solutions were analysed at the same points, and at 84 DAT. The samples and controls used in the study are described below in Table B.8.1.1.2-04. HSE notes that the trap solutions were not sampled at 7-day intervals during the first month, as is recommended by OECD 307 guidelines. HSE does not consider this deviation to affect

study validity, as volatile production is minor, and trap solutions therefore do not need to be replenished on a weekly basis.

**Table B.8.1.1.1.2-01 Sample treatment groups**

<b>Treatment Group</b>	<b>Soil type</b>	<b>Dose rate</b>	<b>Sample type</b>
<b>A</b>	All soils	-	Untreated control
<b>B</b>	All soils	220 µL acetonitrile	Organic solvent control
<b>C</b>	All soils	46 µg inpyrfluxam per 50 g dry weight soil, in 250 µL acetonitrile	Non-radiolabelled inpyrfluxam Control
<b>D</b>	Atwater	30 µg inpyrfluxam per 50 g dry weight soil, in 220 µL acetonitrile	[Pyrazolyl- <sup>14</sup> C] inpyrfluxam Treated Sample
<b>E</b>	Newhaven	30 µg inpyrfluxam per 50 g dry weight soil, in 220 µL acetonitrile	[Pyrazolyl- <sup>14</sup> C] inpyrfluxam Treated Sample
<b>F</b>	Woodside Farm	30 µg inpyrfluxam per 50 g dry weight soil, in 220 µL acetonitrile	[Pyrazolyl- <sup>14</sup> C] inpyrfluxam Treated Sample

The purpose of the control substance treatment was to verify microbial activity under conditions similar to those of the test substance (Treatment C). A higher rate treatment (300 g a.s./ha) was initially added to the study and so the control substance was applied at approximately the same application rate as the higher rate treatment. The desire for a higher rate was later dropped by the applicant, but the higher rate was still used for Treatment C. HSE does not view this as a deviation as it considers the higher loading used in Treatment C to provide either the same or a more conservative assessment of the impact of the active substance and solvent on biomass than the active groups (Treatments A).

The dosed samples were placed in 5 separate incubation chambers (Treatments A-C: 2 chambers; Treatment D, Treatment E and Treatment F: 1 chamber each).



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## V. Sample processing and analysis

NaOH and tetraglyme traps were measured for  $^{14}\text{C}$  by LSC and replaced with fresh solvent at each sampling date. Radioactivity was extracted from soil samples by shaking for 20 minutes with 150 mL acetone:water (3:2, v:v) before centrifugation. This extraction was repeated twice more, and the liquid phases filtered and combined. The extract was rotary evaporated and redissolved in acetonitrile and water, and aliquots taken for LSC analysis.

The extraction method in this study was consistent with that of the first aerobic degradation in soil study. The soil pellet post-centrifugation was then re-suspended in 150 mL of acetone:water:HCl (60:40:1, v:v:v), shaken and centrifuged. The liquid phase was filtered and rotary evaporated and partitioned three times with ethyl acetate (~75 mL), and respective fractions combined. The aqueous and organic phases were then worked up for LSC to quantify radioactivity, and HPLC-RAM and/or TLC-autoradiography to identify metabolites.

The bound residues at 120 DAT were significant enough to warrant further extraction (9 - 12 % of the AR). One of the duplicate PES samples from each of the three soils (120 DAT) was extracted sequentially by shaking and centrifugation with a series of solvents of varying dielectric constant (ethyl acetate, dioxane and hexane). The extracts were collected separately and taken for LSC analysis. The remaining PES was fractionated into humin, humic acids and fulvic acids. Humin and humic acids were analysed by combustion/LSC, while the soluble fulvic acid was quantified in solution by LSC.

Limit of detection and quantification (LOD and LOQ) for LSC were determined as 11dpm and 43dpm respectively. HSE notes that this is well below the OECD 307 maximum guideline of 1 % applied radioactivity (AR).

The structural assignments for [ $^{14}\text{C}$ ] inpyrfluxam and degradants were based on co-chromatography with reference standard upon HPLC and TLC analysis.

The potential isomerisation from [ $^{14}\text{C}$ ] inpyrfluxam was evaluated using chiral HPLC analysis on the extracts obtained from the 120 DAT samples and on the test substances prior to application.

## RESULTS AND DISCUSSION

Soil biomass measurements are given in Table B.8.1.1.1.2-05.

## I. Data

**Table B.8.1.1.1.2-05 Microbial biomass carbon measurements (µg/g dry basis)**

Soil	Sample type	0 DAT	120 DAT	Difference ( %)
Woodside Farm	untreated	1,111.3	1,089.3	-2.0
	solvent	NA*	1,056.8	-4.9
	cold**	NA	1,098.9	-1.1
Atwater	untreated	499.1	265.1	-46.9
	solvent	NA	383.4	-23.2
	cold	NA	425.2	-14.8
Newhaven	untreated	960.2	573.9	-40.2
	solvent	NA	560.8	-41.6
	cold	NA	580.4	-39.6

\*Not Analysed

\*\*Unlabelled test material (inpyrfluxam)

HSE notes that a decrease in biomass is observed across all sample types over the study duration. HSE accepts the applicant's reasoning that this is typical behaviour for isolated soils in a laboratory environment. HSE agrees with the applicant that there is no correlation between sample type and decrease in soil microbial activity.

HSE notes that biomass measurements during the study have not been provided, as is required by OECD 307. HSE notes that the applicant has provided a thorough description of the incubation apparatus used, which HSE accepts as suitable for maintaining aerobic conditions. Furthermore, biomass measurements at study initiation and termination demonstrate that the soil is microbially active at both time points. HSE therefore consider it likely that the soil is microbially active within the study period. HSE therefore does not deem the omission of biomass measurements during the study duration to void the study validity

HSE notes that in KCA 7.1.1.1\_02, section 7.1.2, the applicant has stated that untreated samples (Treatment A) were analysed at 0 DAT. In Appendix G of the same KCA file, the applicant has stated that the treatment A samples were analysed at 1 DAT. As the samples are untreated, HSE does not view this discrepancy to invalidate the study as one day difference, under aerobic acclimation, is not expected to change the microbial biomass significantly.

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Mass balance data for the three radiolabelled soils are presented in Table B.8.1.1.1.2-06 to Table B.8.1.1.1.2-08. The radioactivity distribution by compound in total soil extracts are presented in Table B.8.1.1.1.2-09 to Table B.8.1.1.1.2-11. Radioactivity distribution in PES is given in Table B.8.1.1.1.2-12.

**Table B.8.1.1.2-06 Summary of the mass balance data for the Atwater soil as percent of Applied Radioactivity**

Fraction	Days After Treatment (DAT)																	
	0			14			30			61			90			120		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Neutral Extract	98.1	99.6	98.9	92.3	86.6	89.5	90.1	88.3	89.2	82.4	81.6	82.0	79.0	79.0	79.0	81.0	76.5	78.8
Acidic Extract	0.1	0.1	0.1	7.2	6.3	6.7	5.7	6.0	5.8	8.6	8.2	8.4	11.5	12.2	11.9	12.3	13.4	12.8
Total Extractable	98.3	99.7	99.0	99.5	92.9	96.2	95.8	94.3	95	91.1	89.9	90.5	90.5	91.2	90.8	93.2	89.9	91.6
Soil-bound	0	0	0	2.3	2.2	2.3	3.9	3.9	3.9	5.3	5.8	5.6	7.8	7.7	7.8	9.0	9.2	9.1
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
Total balance	98.3	99.8	99.0	101.8	95.2	98.5	99.8	98.2	99.0	96.5	95.8	96.2	98.5	99.1	98.8	102.6	99.4	101.0

NA: not analysed

**Table B.8.1.1.1.2-07 Summary of the mass balance data for the Newhaven soil as percent of Applied Radioactivity**

Fraction	Days After Treatment (DAT)																	
	0			14			30			61			90			120		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Neutral Extract	101.4	100.5	100.9	93.6	93.7	93.7	83.1	79.5	81.3	80.5	78.7	79.6	75.9	73.3	74.6	77.9	75.7	76.8
Acidic Extract	0.5	0.5	0.5	3.9	4.0	3.9	5.8	5.8	5.8	7.6	7.2	7.4	8.4	8.6	8.5	9.2	8.8	9.0
Total Extractable	101.9	101.0	101.4	97.5	97.8	97.6	88.9	85.3	87.1	88.1	85.9	87.0	84.3	81.8	83.1	87.1	84.6	85.9
Soil-bound	0.2	0.2	0.2	4.6	4.6	4.6	6.5	6.5	6.5	9.8	10.0	9.9	11.0	10.7	10.8	12.3	12.2	12.2
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.1	0.1	0.1	0.3	0.3	0.3	0.5	0.5	0.5	0.7	0.7	0.7	0.8	0.8	0.8
Total balance	102.1	101.1	101.6	102.1	102.4	102.3	95.7	92.1	93.9	98.4	96.4	97.4	96.0	93.2	94.6	100.2	97.6	98.9

NA: not analysed

**Table B.8.1.1.1.2-08 Summary of the mass balance data for the Woodside Farm soil as % of applied radioactivity**

Fraction	Days After Treatment (DAT)																	
	0			14			30			61			90			120		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Neutral Extract	99.2	99.4	99.3	97.5	97.6	97.6	94.1	91.7	92.9	88.5	89.3	88.9	87.4	88.3	87.8	86.8	85.7	86.3
Acidic Extract	0.3	0.3	0.3	1.3	1.3	1.3	1.8	1.8	1.8	2.7	2.6	2.6	3.0	3.1	3.0	3.2	3.1	3.2
Total Extractable	99.4	99.7	99.6	98.8	98.9	98.9	95.9	93.5	94.7	91.2	91.9	91.5	90.3	91.4	90.9	90.1	88.8	89.4
Soil-bound	0.2	0.2	0.2	3.0	3.0	3.0	4.0	3.8	3.9	6.3	6.4	6.3	7.6	7.3	7.4	9.0	9.7	9.3
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
Total balance	99.6	99.8	99.7	101.9	101.9	101.9	100.0	97.4	98.7	97.6	98.3	98.0	98.1	98.9	98.5	99.3	98.7	99.0

NA: not analysed

HSE notes that all soil samples display AR within the OECD 307 90-110 % guideline recommended limit. Therefore, HSE is satisfied with the mass balances provided.

**Table B.8.1.1.1.2-09 Radioactivity distribution in total soil extracts for the Atwater soil study as % of applied radioactivity**

Fraction	Days After Treatment (DAT)																	
	0			14			30			61			90			120		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
N-des-Me-DFPA	0.0	0.0	0.0	0.0	0.0	0.0	ND	0.0	0.0	0.8	1.2	1.0	1.3	1.6	1.4	2.4	1.6	2.0
DFPA-CONH <sub>2</sub>	0.0	0.0	0.0	0.0	0.0	0.0	ND	0.0	0.0	0.0	0.9	0.4	0.9	0.7	0.8	0.7	1.0	0.8
DFPA	0.0	0.0	0.0	1.5	1.0	1.3	ND	2.0	2.0	2.0	1.8	1.9	2.1	2.0	2.0	2.6	2.2	2.4
1'-CH <sub>2</sub> OH-S-2840 (total)*	0	0	0	0	0	0	ND	0	0	0	0.2	0.1	0	0	0	0	0	0
1'-COOH-S-2840 (total)**	0	0	0	4.7	4	4.3	ND	4.9	4.9	6.9	6.9	6.8	9.2	10	9.6	7.1	8	7.5
3'-OH-S-2840	1.8	2.3	2.1	4.3	4.0	4.2	ND	6.3	6.3	11.6	11.1	11.4	16.2	16.3	16.2	22.5	18.8	20.7
N-des-Me-S-2840	0.0	0.0	0.0	0.0	0.0	0.0	ND	0.0	0.0	0.0	0.5	0.2	0.0	0.0	0.0	2.7	3.9	3.3
inpyrfluxam	94.9	95.3	95.1	87.4	83.0	85.2	ND	80.1	80.1	69.0	65.7	67.3	56.5	54.1	55.3	48.0	47.4	47.7
Total other unknowns	1.4	2.0	1.7	0.7	0.7	0.7	ND	0.6	0.6	1.1	1.5	1.3	3.4	5.8	4.6	5.8	6.3	6.0
Total	98.1	99.6	98.9	98.6	92.7	95.7	ND	94.0	94.0	91.3	89.8	90.5	89.5	90.4	90.0	91.8	89.1	90.5

ND = no data (sample lost during workup)

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

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\* As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B



**Table B.8.1.1.2-10 Radioactivity distribution in total soil extracts for the Newhaven soil study as % of applied radioactivity**

Fraction	Days After Treatment (DAT)																	
	0			14			30			61			90			120		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
N-des-Me-DFPA	0.0	0.0	0.0	1.4	0.0	0.7	1.1	ND	1.1	1.2	1.6	1.4	1.2	1.3	1.3	0.9	1.6	1.2
DFPA-CONH <sub>2</sub>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	ND	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DFPA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	ND	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1'-CH <sub>2</sub> OH-S-2840 (total)*	0	0	0	0.2	0	0.1	0	ND	0	0	0	0	0	0	0	1.4	0	0.7
1'-COOH-S-2840 (total)**	0	0	0	15.4	15.4	15.4	18.1	ND	18.1	23.9	21.6	22.8	17.8	21.8	19.7	20.4	19.2	19.8
3'-OH-S-2840	2.0	1.9	2.0	6.8	7.8	7.3	7.0	ND	7.0	9.8	8.9	9.4	9.6	9.0	9.3	10.0	11.1	10.5
N-des-Me-S-2840	0.0	0.0	0.0	0.4	0.0	0.2	0.2	ND	0.2	0.4	0.3	0.3	0.7	0.4	0.5	1.9	1.2	1.5
Inpyrfluxam	96.3	96.4	96.3	67.1	71.7	69.4	57.3	ND	57.3	46.8	49.6	48.2	50.6	44.0	47.3	47.1	45.0	46.0
Total other unknowns	3.1	0.0	1.5	5.9	2.5	4.2	4.7	ND	1.6	5.9	4.0	4.9	3.9	4.8	4.4	5.1	6.0	5.6
Total	101.4	98.3	99.8	97.3	97.5	97.4	88.4	ND	88.4	88.0	85.9	87.0	83.7	81.3	82.5	86.7	84.1	85.4

ND = no data (sample lost during workup)

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

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\* As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.1.1.2-11 Radioactivity distribution in total soil extracts for Woodside Farm soil as % of applied radioactivity**

Fraction	Days After Treatment (DAT)																	
	0			14			30			61			90			120		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
N-des-Me-DFPA	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.3	1.5	1.5	1.5	2.0	1.9	2.0	0.8	2.2	1.5
DFPA-CONH <sub>2</sub>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DFPA	0.0	0.0	0.0	1.4	1.7	1.6	1.7	1.5	1.6	1.2	1.5	1.4	1.4	1.5	1.4	1.5	1.3	1.4
1'-CH <sub>2</sub> OH-S-2840 (total)*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1'-COOH-S-2840 (total)**	0	0	0	10.8	9.2	10	14.7	15.3	15	24	24.7	24.4	27.5	27.9	27.7	31.6	28.5	30.1
3'-OH-S-2840	1.9	2.1	2.0	5.7	5.6	5.6	6.4	6.1	6.3	7.8	6.9	7.4	8.1	7.7	7.9	8.4	8.4	8.4
N-des-Me-S-2840	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Inpyrflumax	94.8	95.0	94.9	78.9	80.3	79.6	69.9	68.3	69.1	53.8	53.7	53.7	46.6	47.5	47.0	39.6	44.0	41.8

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Total other unknowns	2.5	2.2	2.4	0.8	0.8	0.8	0.8	0.6	0.7	2.7	3.5	3.1	4.3	4.4	4.4	7.7	3.8	5.8
Total	99.2	99.4	99.3	97.5	97.6	97.5	94.1	91.7	92.9	91.1	91.8	91.4	89.9	90.9	90.4	89.6	88.3	88.9

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

\* As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840

**Table B.8.1.1.2-12 Distribution of Bound Radioactivity in PES Fractions of 120 DAT Soil Samples after exhaustive extraction**

	<b>Atwater</b>	<b>Newhaven</b>	<b>Woodside farm</b>
% TRR in PES <sup>1</sup>	9.22	12.29	9.66
% TRR in extracted <sup>2</sup>	-	-	-
Ethyl Acetate	4.87	4.36	1.56
Dioxane	0.57	0.66	0.23
Hexane	0.03	0.04	0.01
% TRR in extracted <sup>3</sup>	-	-	-
Dismembrator	1.02	1.45	0.98
% TRR <sup>4</sup>			
Humic Acid	0.55	1.46	0.92
Humin	1.00	1.45	2.76
Fulvic Acid <sup>5</sup>	1.49	2.71	2.49
<b>Total Recovery ( % )<sup>6</sup></b>	<b>103.5</b>	<b>98.7</b>	<b>92.7</b>

TRR = total radioactivity residue

<sup>1</sup>PES after neutral and acidic extraction.

<sup>2</sup> PES exhaustive extraction sequentially with 150 mL of ethyl acetate, dioxane and hexane.

<sup>3</sup> PES subjected to a dismembrator extraction (5/1 MeOH/0.5 M HCl, ca. 1hr.) after the ethyl acetate, dioxane and hexane exhaustive extraction.

<sup>4</sup> PES subjected to a final Humin, Humic acid, Fulvic acid fractionation after the dismembrator extraction.

<sup>5</sup> The majority of the fulvic acid fractions were partitioned into ethyl acetate. The ethyl acetate fraction was concentrated and analysed by HPLC. The fulvic acid fraction contained minor amounts of inpyrfluxam (0.3 % AR), DFPA (1.1 % AR), DFPA-CONH<sub>2</sub> (0.3 % AR), 1'-COOHS-2840 (1.0 % AR).

<sup>6</sup> Total recovery = Exhaustive Extraction + Dismembrator Extract + Humin + Humic acids + Fulvic acids ( %TRR).

It was confirmed that no isomerisation of [<sup>14</sup>C] inpyrfluxam occurred during incubation period based on chiral HPLC analysis.

## **II. Mass balance**

The average material balance for the study was 98.7 ± 2.2 %, 98.1 ± 3.5 %, and 99.3 ± 1.5 % of the AR, for Atwater, Newhaven, and Woodside farm soils, respectively. These mass balances lie within the OECD 307 acceptable guideline of 90-110 % and are therefore deemed acceptable by HSE.

## **III. Bound residues**

Unextracted radioactivity reached a maximum of 9.1 %, 12.2 % and 9.3 % of the AR by the last sampling point for the Atwater, Newhaven, and Woodside farm soils, respectively.

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#### IV. Volatilisation

The cumulative production of  $^{14}\text{CO}_2$  in all three soils was negligible, never exceeding 0.8 % of the AR during the study. The volatile radioactivity in the tetraglyme traps was 0 % AR at all sampling times, indicating that the formation of organic volatiles was negligible during the study period.

#### V. Metabolites

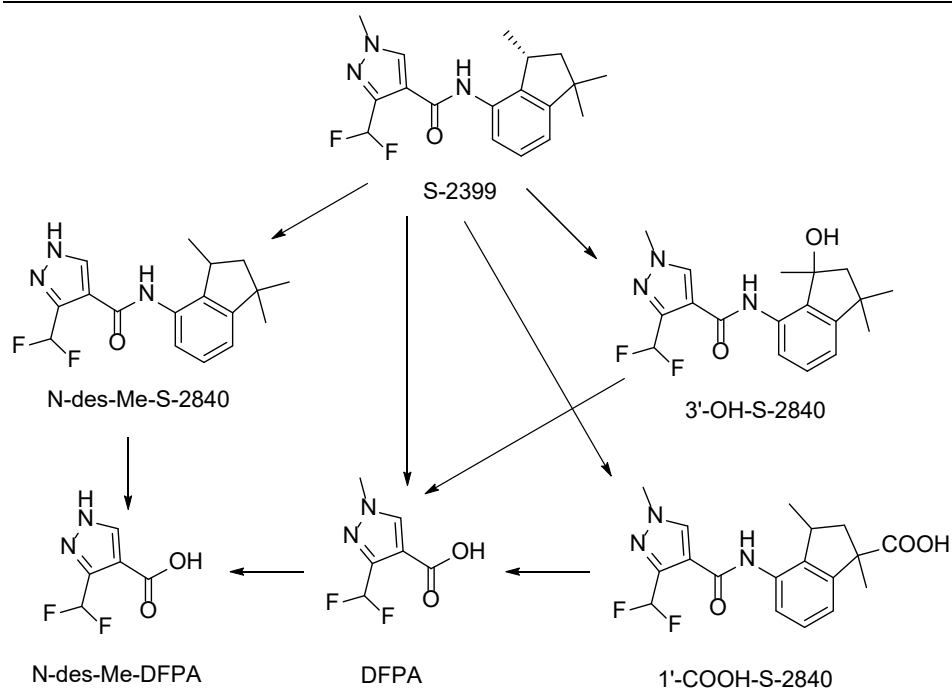
The principal degradation routes were oxidation of the 1' methyl group and at the 3-carbon on the indenyl ring to form 1'-COOH-S-2840 (max 9.6 % of the AR at 90 DAT in the Atwater soil, max 22.8 % of the AR at 61 DAT in the Newhaven soil and max 30.1 % of the AR at 120 DAT in the Woodside farm soil) and 3'-OH-S-2840 (max 20.7 %, 10.5 % and 8.4 % of the AR at 120 DAT in the Atwater soil, Newhaven soil and Woodside farm soil respectively).

Hydrolysis of the central amide linkage or dealkylation of the N-methyl group on the pyrazolyl ring to yield DFPA, DFPA-CONH<sub>2</sub> and N-des-Me-DFPA or N-des-Me-S-2840 was observed in minor yields.

Minor amounts of the intermediate oxidation degradant 1'-CH<sub>2</sub>OH-S-2840 was observed. 2D-TLC analyses of neutral and acidic soil extracts confirmed inpyrfluxam, and degradants products.

The highest single unknowns were not reported in the study, however the applicant confirmed that the only metabolites to exceeded 10 % AR or 5 % AR for at least two consecutive intervals were 1'-COOH-S-2840A, 1'-COOH-S-2840B and 3'-OH-S-2840.

A schematic of the metabolism of inpyrfluxam in aerobic soils is provided as Figure B.8.1.1.1.2-02.



**Figure B.8.1.1.1.2-02 Proposed aerobic soil degradation pathways of inpyrfluxam**

## CONCLUSION

Inpyrfluxam declined to 48, 46 and 42 % of the AR by the end of the 120 DAT study in the Atwater, Newhaven, and Woodside farm soils, respectively. The degradation of inpyrfluxam in aerobic soil involves conversion to 1'-COOH-S-2840 (max 30.1 % AR at 120 DAT - Woodside) and 3'-OH-S-2840 (max 20.7 % AR at 120 DAT – Atwater). DFPA and N-des-Me-DFPA or N-des-Me-S-2840 metabolites were observed in minor yields (< 5 % AR). Minimal degradation of inpyrfluxam and its metabolites to CO<sub>2</sub> occurred, as very little <sup>14</sup>CO<sub>2</sub> was formed (never exceeding 0.8 % in all three soils). No organic volatiles were found. Levels of bound residues at the end of the study were 9.1 %, 12.2 % and 9.3 % for Atwater, Newhaven and Woodside soils, respectively.

HSE agrees with the applicant's analysis of the transformation processes, and the degradation pathway provided.

**B.8.1.1.1.3. Aerobic route of degradation of metabolite 3'-OH-S-2840**

<b>Reference:</b>	KCA 7.1.2.1.2/01
<b>Study Title:</b>	[ <sup>14</sup> C]3'-OH-S-2840: Aerobic Soil Metabolism Study
<b>Author &amp; year:</b>	██████████ (2017a)
<b>Study No:</b>	3201399
<b>Applicant:</b>	Sumitomo Chemical Co. Ltd. Report No: TPM-0033
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>• US EPA Fate, Transport and Transformation Test Guidelines</li> <li>• OCSP 835.4100</li> <li>• OECD 307 Test Guideline</li> </ul>
<b>GLP:</b>	Yes
<b>Deviations:</b>	None

<b>Deviations</b>	<b>HSE assessment of deviations</b>
None	N/A
<p align="center"><b>HSE conclusion on deviations</b></p> <p>There are no major deviations from the Guideline and the study is considered acceptable to derive endpoints for use in the exposure assessment.</p>	

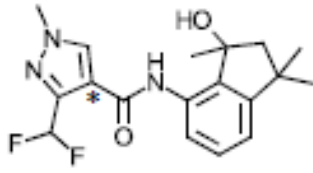
**INTRODUCTION**

The route of metabolism of 3'-OH-S-2840, a metabolite of the new active substance inpyrfluxam (S-2399) was investigated under aerobic conditions, with a radiolabel in the pyrazolyl ring. Soil was treated at rate of 14 µg per 50 g soil and incubated in the dark at 20 ± 2 °C and pH 2 for 120 days. Soil extracts were quantified by LSC and HPLC and the identity of 3'-OH-S-2840 and its metabolites confirmed by HPLC with TLC used as a confirmatory method.



## MATERIAL AND METHODS

**Table B.8.1.1.3-01 Properties of the materials used in the aerobic soil degradation study**

<b>1. Test Material</b>	[pyrazolyl-4- <sup>14</sup> C]3'-OH-S-2840
<b>Lot/Batch</b>	RIS2015-005
<b>Specific activity</b>	2.22 GBq/mmol (6.35 MBq/mg)
<b>Purity</b>	Radiochemical purity 98.3 % (in the dosing solution)
<b>CAS#</b>	Not assigned
<b>Stability of compound</b>	Not stated
<b>Structure</b>	 <p>Figure B.8.1.1.3-1 * denotes <sup>14</sup>C label position</p>

It is noted that only the pyrazolyl ring has been radiolabelled. The OECD 307 Guideline states that both ring structures should be radiolabelled in order that the degradation of the molecule can be fully described. The degradation behaviour of inpyrfluxam has however already been investigated in the study with parent by [REDACTED] (2017) presented under B.8.1.1.1.1 in which both rings were labelled. Five metabolites were observed in the parent applied study (including 3'-OH-S-2840). All metabolites in the parent study in which both rings were radiolabelled contained the pyrazolyl ring and no metabolite contained the phenyl ring alone. Mass balances in the current study ranged between 94 and 98 % AR for the pyrazolyl radiolabel, demonstrating that the degradation was adequately described using this radiolabel alone. This gives confidence that the radiolabelling strategy is adequate to describe the degradation of 3'-OH-S-2840. The data requirements also state that radiolabelling is not necessary with a metabolite applied study and analysis of subsequent metabolites is not required, as the study is submitted to quantify the degradation rate of the metabolite. The radiolabelling of the study therefore goes beyond the data requirements.

### I. Soil

Three soils were used in the study: Speyer 5M, Newhaven and Atwater. Land uses are described as meadow for Speyer 5M, grassland for Newhaven and fallow for Atwater. It is stated that there was no pesticide use at any of the sites in the last 5 years. Soils were sampled from the top 20 cm. All 3 soils were collected during March 2016 and arrived at the test facility in the same month. Conditions during shipping are not stated. After arrival at the test facility, soils were sieved (2 mm),

thoroughly mixed and stored in the dark in an environmental chamber routinely maintained at  $4 \pm 2$  °C in loosely tied plastic bags in accordance with ISO 10831-6. Microbial biomass was determined using the fumigation-extraction method. Characteristics of the soils used in the study are given in Table B.8.1.1.1.3-02.

**Table B.8.1.1.1.3-02 Chemical and Physical characteristics of test soil**

<b>Soil characteristic</b>	<b>Speyer 5M</b>	<b>Newhaven</b>	<b>Atwater</b>
USDA Particle size distribution			
% sand (50 µm - 2 mm)	59	23	85
% silt (2 µm - 50 µm)	30	60	6
% clay <2 µm	11	17	9
pH (H <sub>2</sub> O)	8.3	6.2	7.1
pH (0.01 M CaCl <sub>2</sub> )	7.3	5.5	6.3
% Moisture at 1/10 Bar (pF 2)	23.8	36.4	9.5
% Moisture at 1/3 Bar (pF 2.5)	24.3	30.2	5.9
Maximum water holding capacity (%)	40.1	68.1	33.5
Cation exchange capacity (meq/100g)	17.7	26.4	11.4
% Organic carbon	1.0	3.4	0.3
% Organic Matter	1.8	5.9	0.5
Bulk density (g / cm <sup>3</sup> )	1.3	1.0	1.2
USDA Textural class	Sandy loam	Silt loam	Loamy sand
Microbial biomass from certificate of analysis	212 (2.1 %)	675 (2.0 %)	89 (3.0)
Microbial Biomass Carbon (µg C per g soil); taken as subsample	243 (Day-0) 176 (Day-120)	524 Day-0) 325 (Day-120)	65 Day-0) 40 (Day-120)
Microbial Biomass Carbon (% of Total Organic Carbon); taken as subsample	2.4 % (Day-0) 1.8 % (Day-120)	2.0 (Day-0) 1.0 % (Day-120)	2.2 (Day-0) 1.3 % (Day-120)

HSE has considered the properties of the soils used. The selected soils include acidic and basic soils, with pH (in CaCl<sub>2</sub>) ranging between 5.5 and 7.3, differing microbial biomass contents ranging between 89 to 675 mg/kg and both low and higher organic carbon contents ranging between 0.3 and 3.4 %. The clay content of

the soils is somewhat similar, ranging between 9 and 17 %. Overall, HSE is satisfied that soils covering a range of soil properties have been selected.

## **STUDY DESIGN AND METHODS**

### **I. Experimental conditions**

A stock solution of 3'-OH-S-2840 with concentration of 1.6 mg/mL was made and the concentration verified by LSC. Stock solution (2 mL) was used to create application solution 1 with concentration 0.15 mg 3'-OH-S-2840; this was used in the method development test. Application solution 2 was prepared by dissolving stock solution (6 mL) to give a concentration of 0.15 mg/mL; application solution 2 was used in the definitive test. The concentration of both application solutions was verified by LSC.

Degradation of 3'-OH-S-2840 was studied in all 3 soils over 120 days. Soil samples (50 g) were acclimatised to test conditions (dark, pH 2,  $20 \pm 2$  °C) for 14 days prior to application of the test item. Moisture levels were maintained by weighing the test vessels every 2 to 9 days and replacing lost moisture with reverse osmosis water. Test item (14 µg) was applied to soil (50 g) placed in glass vessels. The application rate was calculated from a parent application rate of 200 g a.s./ha, assuming an incorporation depth of 5 cm, soil density of 1.5 g/cm<sup>3</sup> and 100 % conversion of parent to metabolite. It is noted that this application rate is higher than the 90 g a.s./ha proposed for the representative use but is accepted by HSE as it allows subsequent metabolites to be more easily identified and is unlikely to significantly impact degradation rates. The application solution was dispensed dropwise over the soil surface and the solvent allowed to evaporate; it is not stated if the test item was mixed into the soil, but there are no outliers in the data which indicate that soils and test item had not been homogenised. An equivalent volume of acetonitrile (94 µL) was applied to samples incubated alongside study samples and used for biomass determination at the final sampling time. The concentration of radioactivity in the application solutions was determined both pre- and post-application by diluting a known volume of application solution in triplicate (90 µL pre-treatment and 94 µL post treatment) and diluting to volume (10 mL) with acetonitrile. Triplicate aliquots (100 µL) were then analysed with LSC.

### **II. Sampling**

Duplicate samples were removed for analysis immediately after treatment and at 7, 14, 30, 59 and 120 days after treatment.

### **III. Description of analytical procedure**

Soil was transferred to a new container and the original container washed with primary extraction solvent acetone (100 mL). The soil was shaken (10 mins) and centrifuged (2000 g, 10 min) and the supernatant transferred to a new container and labelled extract 1. The soil was extracted again with acetone (x2, 100 mL) and the

supernatants combined and labelled extract 2. The supernatant was quantified with LSC. A subsequent extraction was conducted with the secondary extraction solvent, acetone: 0.5 M HCL (4:1 v/v, 100 mL, x3) and the extracts combined and quantified by LSC.

Extract 1 was concentrated to c.1 mL, reconstituted with acetone (5 mL) and labelled extract 3. Extract 2 samples (for extracts containing  $\geq 5$  % AR) were neutralised with sodium hydroxide (2 M), concentrated and reconstituted with acetone (4 mL) and water (2 mL).

The soil pellet was air dried, ground and combusted.

Radioactivity in the volatile traps was quantified by LSC as soon as possible after each sampling point, or after 30 days when reagents were replaced.

Extracts were analysed by HPLC with UV detection at 230 nm and non-radiolabelled reference standards used to confirm the identity of radioactive peaks. TLC was used for determination of radiochemical purity and as a confirmatory method. Extracts were co-chromatographed with non-radiolabelled standards.

The radioactivity in samples and traps was determined by measuring the total sample weight. Samples were extracted and air-dried, then ground and sub-sampled (c. 0.2 g) in triplicate. Samples were combusted and analysed in duplicate by LSC.

#### **IV. LOD values**

For LSC, the limit of detection was taken as 1.5 times the background radioactivity determined by counting blank samples. The LOD for LSC varied depending on the sample and aliquot size, but was generally  $<0.5$  % AR.

For HPLC, the applicant stated that peaks distinguishable from background were considered to be peaks. A peak representing 0.1 % AR was considered to be the minimum peak size that could be detected and therefore the LOD was set at c. 0.1 % AR.

### **RESULTS AND DISCUSSION**

#### **I. Data**

The microbial biomass was measured in a subsample of soil at both the start and end of incubation. Microbial biomass was 1.5 to 2.4 % in soil at the start of the incubation and declined in all soils to 1.0 to 1.8 % at the end of the incubation. The requirement for microbial biomass to be at least 1.0 % total organic carbon (TOC) for the duration of the study was therefore met and soils remained viable throughout the study.

The distribution of radioactivity and mass balance in each soil are summarised in Tables B.8.1.1.1.3-03 to B.8.1.1.1.3-08 below.

**Table B.8.1.1.1.3-03 Percent recovery of applied radioactivity from Speyer 5M soil treated with [<sup>14</sup>C]3'-OH-S-2840**

		Days after treatment (DAT)					
		0	7	14	30	59	120
<b>Primary soil extract</b>	Rep 1	98.6	92.0	83.5	81.0	76.7	66.3
	Rep 2	96.5	90.1	85.4	82.4	72.5	69.4
	<b>Mean</b>	<b>97.6</b>	<b>91.1</b>	<b>84.5</b>	<b>81.7</b>	<b>74.6</b>	<b>67.9</b>
<b>Secondary soil extract</b>	Rep 1	1.0	7.8	10.7	16.1	16.5	23.9
	Rep 2	1.2	8.4	11.1	15.6	18.5	22.8
	<b>Mean</b>	<b>1.1</b>	<b>8.1</b>	<b>10.9</b>	<b>15.9</b>	<b>17.5</b>	<b>23.4</b>
<b>Unextracted from soil</b>	Rep 1	0.1	1.6	3.1	3.3	5.3	8.3
	Rep 2	ND	1.8	3.3	3.7	5.7	6.5
	<b>Mean</b>	<b>0.1</b>	<b>1.7</b>	<b>3.2</b>	<b>3.5</b>	<b>5.5</b>	<b>7.4</b>
<b>Total in soil</b>	Rep 1	99.7	101.4	97.3	100.4	98.5	98.5
	Rep 2	97.7	100.3	99.8	101.7	96.7	98.7
	<b>Mean</b>	<b>98.7</b>	<b>100.9</b>	<b>98.6</b>	<b>101.1</b>	<b>97.6</b>	<b>98.6</b>
<b>Sodium hydroxide traps</b>	Rep 1	NA	ND	ND	0.1	0.2	0.5
	Rep 2	NA	ND	ND	0.1	0.3	0.2
	<b>Mean</b>	<b>NA</b>	<b>ND</b>	<b>ND</b>	<b>0.1</b>	<b>0.3</b>	<b>0.4</b>
<b>Mass balance</b>	Rep 1	99.7	101.4	97.3	100.5	98.7	99.0
	Rep 2	97.7	100.3	99.8	101.8	97.0	98.9
	<b>Mean</b>	<b>98.7</b>	<b>100.9</b>	<b>98.6</b>	<b>101.2</b>	<b>97.9</b>	<b>99.0</b>

ND = Not detected (or <0.1 % AR)

**Table B.8.1.1.1.3-04 Percent recovery of applied radioactivity from Newhaven soil treated with [<sup>14</sup>C]3'-OH-S-2840**

		Days after treatment (DAT)					
		0	7	14	30	59	120
<b>Primary soil extract</b>	Rep 1	94.4	89.7	84.8	83.5	75.6	67.8
	Rep 2	98.9	88.8	86.9	83.9	74.4	67.0
	<b>Mean</b>	<b>96.7</b>	<b>89.3</b>	<b>85.9</b>	<b>83.7</b>	<b>75.0</b>	<b>67.4</b>
<b>Secondary soil extract</b>	Rep 1	2.2	8.3	10.5	13.3	15.9	22.7
	Rep 2	2.1	8.0	9.9	13.1	17.4	22.9
	<b>Mean</b>	<b>2.2</b>	<b>8.2</b>	<b>10.2</b>	<b>13.2</b>	<b>16.7</b>	<b>22.8</b>
<b>Unextracted from soil</b>	Rep 1	0.1	1.4	3.1	3.2	4.3	7.1
	Rep 2	0.1	1.3	2.4	3.1	5.4	7.2
	<b>Mean</b>	<b>0.1</b>	<b>1.4</b>	<b>2.8</b>	<b>3.2</b>	<b>4.9</b>	<b>7.2</b>

<b>Total in soil</b>	Rep 1	96.7	99.4	98.4	100.0	95.8	97.6
	Rep 2	101.1	98.1	99.2	100.1	97.2	97.1
	<b>Mean</b>	<b>98.9</b>	<b>98.8</b>	<b>98.8</b>	<b>100.1</b>	<b>96.5</b>	<b>97.4</b>
		<b>Days after treatment (DAT)</b>					
		<b>0</b>	<b>7</b>	<b>14</b>	<b>30</b>	<b>59</b>	<b>120</b>
<b>Sodium hydroxide traps</b>	Rep 1	NA	ND	0.1	0.2	0.3	0.6
	Rep 2	NA	ND	0.1	0.2	0.4	0.6
	<b>Mean</b>	<b>NA</b>	<b>ND</b>	<b>0.1</b>	<b>0.2</b>	<b>0.4</b>	<b>0.6</b>
<b>Mass balance</b>	Rep 1	96.7	99.4	98.5	100.2	96.1	98.2
	Rep 2	101.1	98.1	99.3	100.3	97.6	97.7
	<b>Mean</b>	<b>98.9</b>	<b>98.8</b>	<b>98.9</b>	<b>100.3</b>	<b>96.9</b>	<b>98.0</b>

ND = Not detected (or <0.1 % AR)

**Table B.8.1.1.3-05 Percent recovery of applied radioactivity from Atwater soil treated with [<sup>14</sup>C]3'-OH-S-2840**

		<b>Days after treatment (DAT)</b>					
		<b>0</b>	<b>7</b>	<b>14</b>	<b>30</b>	<b>59</b>	<b>120</b>
<b>Primary soil extract</b>	Rep 1	98.6	91.7	89.4	86.3	82.5	76.5
	Rep 2	95.0	92.9	89.6	84.9	80.8	77.4
	<b>Mean</b>	<b>96.8</b>	<b>92.3</b>	<b>89.5</b>	<b>85.6</b>	<b>81.7</b>	<b>77.0</b>
<b>Secondary soil extract</b>	Rep 1	1.0	6.4	8.0	12.2	14.1	17.3
	Rep 2	2.7	5.8	7.8	11.7	14.8	17.2
	<b>Mean</b>	<b>1.9</b>	<b>6.1</b>	<b>7.9</b>	<b>12.0</b>	<b>14.5</b>	<b>17.3</b>
<b>Unextracted from soil</b>	Rep 1	ND	0.3	0.5	1.2	2.5	3.9
	Rep 2	ND	0.3	0.5	1.2	2.2	3.6
	<b>Mean</b>	<b>ND</b>	<b>0.3</b>	<b>0.5</b>	<b>1.2</b>	<b>2.4</b>	<b>3.8</b>
<b>Total in soil</b>	Rep 1	99.6	98.4	97.9	99.7	99.1	97.7
	Rep 2	97.7	99.0	97.9	97.8	97.8	98.2
	<b>Mean</b>	<b>98.7</b>	<b>98.7</b>	<b>97.9</b>	<b>98.8</b>	<b>98.5</b>	<b>98.0</b>
<b>Sodium hydroxide traps</b>	Rep 1	NA	ND	ND	0.1	0.2	0.7
	Rep 2	NA	ND	ND	0.1	0.3	0.7
	<b>Mean</b>	<b>NA</b>	<b>ND</b>	<b>ND</b>	<b>0.1</b>	<b>0.3</b>	<b>0.7</b>
<b>Mass balance</b>	Rep 1	99.6	98.4	97.9	99.8	99.3	98.4
	Rep 2	97.7	99.0	97.9	97.9	98.1	98.9
	<b>Mean</b>	<b>98.7</b>	<b>98.7</b>	<b>97.9</b>	<b>98.9</b>	<b>98.7</b>	<b>98.7</b>

ND = Not detected (or <0.1 % AR)

**Table B.8.1.1.3-06 Percent of applied radioactivity present as [<sup>14</sup>C]3'-OH-S-2840 and metabolites in Speyer 5M soil**

		Days after treatment (DAT)					
		0	7	14	30	59	120
<b>3'OH-S-2840</b>	Rep 1	98.9	96.6	91.7	92.9	83.6	78.3
	Rep 2	97.6	96.6	93.6	94.9	78.7	81.4
	<b>Mean</b>	<b>98.6</b>	<b>96.6</b>	<b>92.7</b>	<b>93.9</b>	<b>81.1</b>	<b>79.8</b>
<b>DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>N-des-Me-DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>Total Unknowns*</b>	Rep 1	ND	2.8	2.2	4.1	9.4	11.4
	Rep 2	ND	1.4	2.5	3.1	12.3	10.5
	<b>Mean</b>	<b>ND</b>	<b>2.1</b>	<b>2.4</b>	<b>3.6</b>	<b>10.9</b>	<b>10.9</b>
<b>Unresolved Background</b>	Rep 1	0.7	0.5	0.2	0.1	0.1	0.6
	Rep 2	0.1	0.5	0.4	0.1	0.1	0.3
	<b>Mean</b>	<b>0.4</b>	<b>0.5</b>	<b>0.3</b>	<b>0.1</b>	<b>0.1</b>	<b>0.4</b>
<b>Mass balance</b>	Rep 1	99.6	99.8	94.2	97.1	93.2	90.2
	Rep 2	97.7	98.5	96.5	98.0	91.0	92.2
	<b>Mean</b>	<b>98.7</b>	<b>99.1</b>	<b>95.4</b>	<b>97.6</b>	<b>92.1</b>	<b>91.2</b>

ND = Not detected (or &lt;0.1 % AR)

\*Each of which did not exceed an average of 3.8 % AR

**Table B.8.1.1.3-07 Percent of applied radioactivity present as [<sup>14</sup>C]3'-OH-S-2840 and metabolites Newhaven soil**

		Days after treatment (DAT)					
		0	7	14	30	59	120
<b>3'OH-S-2840</b>	Rep 1	96.5	92.7	88.0	89.4	76.0	78.8
	Rep 2	100.7	93.0	91.0	88.9	77.6	71.7
	<b>Mean</b>	<b>98.6</b>	<b>92.8</b>	<b>89.5</b>	<b>89.1</b>	<b>76.8</b>	<b>75.2</b>
<b>DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>N-des-Me-DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>Total Unknowns*</b>	Rep 1	ND	4.8	7.1	7.4	15.2	11.6
	Rep 2	ND	3.5	4.3	7.9	13.9	18.1
	<b>Mean</b>	<b>ND</b>	<b>4.1</b>	<b>5.7</b>	<b>7.7</b>	<b>14.5</b>	<b>14.8</b>

		Days after treatment (DAT)					
		0	7	14	30	59	120
<b>Unresolved Background</b>	Rep 1	0.1	0.5	0.2	0.0	0.3	0.1
	Rep 2	0.3	0.3	1.5	0.2	0.2	0.2
	<b>Mean</b>	<b>0.2</b>	<b>0.4</b>	<b>0.8</b>	<b>0.1</b>	<b>0.3</b>	<b>0.1</b>
<b>Mass balance</b>	Rep 1	96.6	98.0	95.3	96.8	91.5	90.5
	Rep 2	101.0	96.8	96.8	97.0	91.8	89.9
	<b>Mean</b>	<b>98.8</b>	<b>97.4</b>	<b>96.1</b>	<b>96.9</b>	<b>91.6</b>	<b>90.2</b>

ND = Not detected (or <0.1 % AR)

\*Each of which did not exceed an average of 4.0 % AR

**Table B.8.1.1.3-08 Percent of applied radioactivity present as [<sup>14</sup>C]3'-OH-S-2840 and metabolites in Atwater soil**

		Days after treatment (DAT)					
		0	7	14	30	59	120
<b>3'OH-S-2840</b>	Rep 1	99.4	95.0	94.5	89.0	82.8	75.7
	Rep 2	97.0	96.4	94.5	89.8	81.9	70.6
	<b>Mean</b>	<b>98.2</b>	<b>95.7</b>	<b>94.5</b>	<b>89.4</b>	<b>82.3</b>	<b>73.2</b>
<b>DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>N-des-Me-DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>Total Unknowns*</b>	Rep 1	ND	2.2	2.9	9.3	13.7	18.0
	Rep 2	ND	1.5	2.7	6.7	13.2	23.7
	<b>Mean</b>	<b>ND</b>	<b>1.8</b>	<b>2.8</b>	<b>8.0</b>	<b>13.4</b>	<b>20.9</b>
<b>Unresolved Background</b>	Rep 1	0.2	1.0	0.1	0.2	0.2	0.1
	Rep 2	0.7	0.8	0.2	0.1	0.1	0.3
	<b>Mean</b>	<b>0.5</b>	<b>0.9</b>	<b>0.2</b>	<b>0.1</b>	<b>0.1</b>	<b>0.2</b>
<b>Mass balance</b>	Rep 1	99.6	98.1	97.4	98.5	96.6	93.8
	Rep 2	97.7	98.7	97.4	96.6	95.2	94.6
	<b>Mean</b>	<b>98.7</b>	<b>98.4</b>	<b>97.4</b>	<b>97.6</b>	<b>95.9</b>	<b>94.2</b>

ND = Not detected (or <0.1 % AR)

\*Each of which did not exceed an average of 3.6 % AR

## II. Mass balance

Mean mass balance ranged between 98.7 and 100.9 % AR for the Speyer 5M soil, between 96.9 and 100.3 % AR for the Newhaven soil and between 97.9 and 98.9 % for the Atwater soil. For individual replicates, no mass balance was outside of the range 90-110 % AR, ranging between 96.1 and 101.4 % AR.



The primary soil extract contained most of the extracted radioactivity, but radioactivity extracted in the primary extraction declined over time, from a mean of 96.7 to 97.6 % AR on day 0 to a range of 67.4 to 77.0 % AR at study end. In contrast, the radioactivity in the secondary soil extract increased over time from a mean of 1.1 to 1.9 % AR at day 0 to 17.3 to 23.4 % AR at study end.

### **III. Bound residues**

Unextracted residues increased during the course of the study in all soils. Mean unextracted residues accounted for 3.8 to 7.4 % AR in soil at study end. Unextracted residues were lowest in the Atwater soil, while similar levels were observed in Newhaven and Speyer 5M soils.

### **IV. Volatilisation**

Low amounts of radioactivity were present in the sodium hydroxide traps, showing that little CO<sub>2</sub> was formed. Mean values at study end ranged between 0.4 and 0.7 % AR.

### **V. Metabolites**

Two degradation products were analysed for, DFPA and N-des-Me-DFPA, but neither was observed in any soil at any time point. This confirms the results of the study with the parent substance (see data point B.8.1.1.1.1) in which N-des-Me-DFPA was present at < 1.6 % AR at all time intervals in all soils. Total unknowns (mean value) were 10.9 % AR in the Speyer 5M soil, 14.8 % in the Newhaven soil and 20.9 % AR in the Atwater soil. The applicant states that all unknown peaks were ≤ 4.0 % AR. HSE has examined the sample chromatograms and can confirm that no additional significant peaks were observed.

Unresolved background was low in all soils, ranging between 0.1 and 0.4 % AR (mean values).

## **CONCLUSIONS**

The test item 3'OH-S-2840 was slowly degraded under test conditions, with undegraded parent material representing >70% applied radioactivity in all soils at the end of the 120 d study period. Unextracted residues reached a maximum of 7.4 % in soil, while mineralisation to CO<sub>2</sub> was low. There were no major metabolites observed in soil in the study. It is also noted that 73.2 to 79.8 % AR of 3'OH-S-2840 remains at study end, giving the potential for the further formation of metabolites. In particular, the amide bridge is not cleaved. As a result, route and rate of degradation of inpyrfluxam and 3'OH-S-2840 residues over longer timescales remains uncertain.

#### B.8.1.1.1.4. Aerobic route of degradation of metabolite 1'-COOH-S-2840

<b>Reference</b>	KCA 7.1.2.1.2/02
<b>Study Title:</b>	[ <sup>14</sup> C]1'-COOH-S-2840: Aerobic Soil Metabolism Study
<b>Author &amp; year:</b>	██████ (2017b)
<b>Study No:</b>	3201400
<b>Applicant:</b>	Sumitomo Chemical Co. Ltd. Report No: TPM-0049
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>• US EPA Fate, Transport and Transformation Test Guidelines</li> <li>• OCSPP 835.4100</li> <li>• OECD 307 Test Guideline</li> </ul>
<b>GLP:</b>	Yes
<b>Deviations:</b>	None

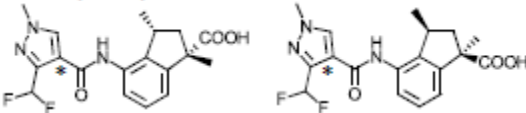
<b>Deviations</b>	<b>HSE assessment of deviations</b>
None	N/A
<p style="text-align: center;"><b>HSE conclusion on deviations</b></p> <p>There are no major deviations from the Guideline and the study is considered acceptable to derive endpoints for use in the exposure assessment.</p>	

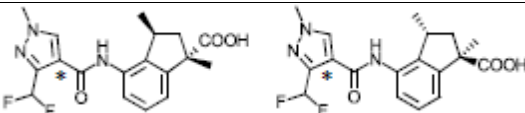
## INTRODUCTION

The route of metabolism of 1'-COOH-S-2840, a metabolite of the new active substance inpyrfluxam (S-2399) was investigated under aerobic conditions, with a radiolabel in the pyrazolyl ring. The test item is available as two isomers, 1'-COOH-S-2840A and B and each isomer exists as a pair of stereoisomers. Soils were dosed with a 50:50 mix of each set of isomers (A and B), but it was not possible to separate the individual pairs of isomers within the A and B groups. Soil was treated at rate of 14.5 µg per 50 g soil and incubated in the dark at 20 ± 2 °C and pH 2 for 120 days. Soil extracts were quantified by LSC and HPLC and the identity of 1'-COOH-S-2840 and its metabolites confirmed by HPLC with TLC used as a confirmatory method.

## MATERIAL AND METHODS

**Table B.8.1.1.1.4-01 Properties of the materials used in the aerobic soil degradation study**

<b>1. Test Material</b>	[pyrazolyl-4- <sup>14</sup> C]1'-COOH-S-2840A
<b>Lot/Batch</b>	RIS2015-003
<b>Specific activity</b>	2.11 GBq/mmol (5.81 MBq/mg)
<b>Purity</b>	Radiochemical purity ≥98.2 % (in the dosing solution)
<b>CAS#</b>	Not assigned
<b>Stability of compound</b>	Not stated
<b>Structure</b>	 <p>Figure B. 8.1.1.1.4-1* denotes <sup>14</sup>C label position</p>

<b>1. Test Material</b>	[pyrazolyl-4- <sup>14</sup> C]1'-COOH-S-2840A
<b>Lot/Batch</b>	RIS2015-004
<b>Specific activity</b>	2.11 GBq/mmol (5.81 MBq/mg)
<b>Purity</b>	Radiochemical purity ≥98.2 % (in the dosing solution)
<b>CAS#</b>	Not assigned
<b>Stability of compound</b>	Not stated
<b>Structure</b>	 <p>Figure B. 8.1.1.1.4-2 * denotes <sup>14</sup>C label position</p>

It is noted that only the pyrazolyl ring has been radiolabelled. The OECD 307 Guideline states that both ring structures should be radiolabelled in order that the degradation of the molecule can be fully described. The degradation behaviour of inpyrfluxam has however already been investigated in the study with parent by (2017) presented under B.8.1.1.1.1. Five metabolites were observed in this study (including 1'-COOH-S-2840). All metabolites contained the pyrazolyl ring and no metabolite contained the phenyl ring alone. Mass balances in this study ranged between 94 and 98 % AR for the pyrazolyl radiolabel, demonstrating that the degradation of inpyrfluxam was adequately described using this radiolabel alone. This gives confidence that the radiolabelling strategy is adequate to describe the degradation of 1'-COOH-S-2840. The data requirements also state that radiolabelling is not necessary with a metabolite applied study and analysis of subsequent metabolites is not required, as the study is submitted to quantify the degradation rate of the metabolite. The radiolabelling of the study therefore goes beyond the data requirements.

## I. Soil

Three soils were used in the study: Speyer 5M, Newhaven and Atwater. Land uses are described as meadow for Speyer 5M, grassland for Newhaven and fallow for Atwater. It is stated that there was no pesticide use at any of the sites in the last 5 years. Soils were sampled from the top 20 cm. All 3 soils were collected during March 2016 (Newhaven was sampled on 09 January from the source site and on 10 March from the soil nursery) and arrived at the test facility in the same month. Conditions during shipping are not stated. After arrival at the test facility, soils were sieved (2 mm), thoroughly mixed and stored in the dark in an environmental chamber routinely maintained at  $4 \pm 2$  °C in loosely tied plastic bags in accordance with ISO 10831-6. Soils were stored for <2 months (55-56 days) before being dispensed for use in the study. Microbial biomass was determined using the fumigation-extraction method. Characteristics of the soils used in the study are given in Table B.8.1.1.1.4-02.

**Table B.8.1.1.1.4-02 Chemical and Physical characteristics of test soil**

<b>Soil characteristic</b>	<b>Speyer 5M</b>	<b>Newhaven</b>	<b>Atwater</b>
USDA Particle size distribution			
% sand (50 µm - 2 mm)	59	23	85
% silt (2 µm - 50 µm)	30	60	6
% clay <2 µm	11	17	9
pH (H <sub>2</sub> O)	8.3	6.2	7.1
pH (0.01 M CaCl <sub>2</sub> )	7.3	5.5	6.3
% Moisture at 1/10 Bar (pF 2)	23.8	36.4	9.5
% Moisture at 1/3 Bar (pF 2.5)	24.1	30.2	5.9
Maximum water holding capacity (%)	40.1	68.1	33.5
Cation exchange capacity (meq/100g)	17.7	26.4	11.4
% Organic carbon	1.0	3.4	0.3
% Organic Matter	1.8	5.9	0.5
Bulk density (g / cm <sup>3</sup> )	1.3	1.0	1.2
USDA Textural class	Sandy loam	Silt loam	Loamy sand
Microbial biomass from certificate of analysis	212 (2.1 %)	675 (2.0 %)	89 (3.0 %)
Microbial Biomass Carbon (µg C per g soil);	222 (Day-0)	641 Day-0)	66 Day-0) 48 (Day-120)

	155 (Day-120)	350 (Day-120)	
<b>Soil characteristic</b>	<b>Speyer 5M</b>	<b>Newhaven</b>	<b>Atwater</b>
Microbial Biomass Carbon (% of Total Organic Carbon)	2.2 % (Day-0) 1.6 % (Day-120)	1.9 (Day-0) 1.0 % (Day-120)	2.2 (Day-0) 1.6 % (Day-120)

HSE has considered the properties of the soils used. The selected soils include acidic and basic soils, with pH (in CaCl<sub>2</sub>) ranging between 5.5 and 7.3, differing microbial biomass contents ranging between 89 to 675 mg/kg and both low and higher organic carbon contents ranging between 0.3 and 3.4 %. The clay content of the soils is somewhat similar, ranging between 9 and 17 %. Overall, HSE is satisfied that soils covering a range of soil properties have been selected.

## STUDY DESIGN AND METHODS

### I. Experimental conditions

All non-radiolabelled test substances and reference standards were stored frozen (nominally -20 °C) in the dark following arrival at the test facility.

Separate stock solutions of 1'-COOH-S-2840A and B, each with concentrations of 0.16 mg/mL were made, and the concentrations verified by LSC. Stock solutions 1A and 1B (1 mL of each) were used to create application solution 1 with concentration 0.16 mg 1'-COOH-S-2840; this was used in the method development test.

Application solution 2 was prepared by combining stock solution 1A and 1B (3 mL of each) to give a concentration of 0.16 mg/mL; application solution 2 was used in the definitive test. The concentration of both application solutions was verified by LSC.

Degradation of 1'-COOH-S-2840 was studied in all 3 soils over 120 days. Soil samples (50 g) were acclimatised to test conditions (dark, pH 2, 20 ± 2 °C) for 14 days prior to application of the test item. Moisture levels were maintained by weighing the test vessels every 2 to 9 days and replacing lost moisture with reverse osmosis water. Test item (14.6 µg) was applied to soil (50 g) placed in glass vessels. The application rate was calculated from a parent application rate of 200 g a.s./ha, assuming an incorporation depth of 5 cm, soil density of 1.5 g/cm<sup>3</sup> and 100 % conversion of parent to metabolite. It is noted that this application rate is higher than the 90 g a.s./ha proposed for the representative use but is accepted by HSE as it allows subsequent metabolites to be more easily identified and is unlikely to significantly impact degradation rates. The application solution was dispensed dropwise over the soil surface and the solvent allowed to evaporate. The test item was then mixed into the soil. An equivalent volume of acetonitrile (94 µL) was

applied to samples incubated alongside study samples and used for biomass determination at the final sampling time. The concentration of radioactivity in the application solutions was determined both pre- and post-application by diluting a known volume of application solution in triplicate (90  $\mu$ L pre-treatment and 94  $\mu$ L post treatment) and diluting to volume (10 mL) with acetonitrile. Triplicate aliquots (100  $\mu$ L) were then analysed with LSC.

Sixteen vessels containing test item and six control vessels were prepared per soil type. The control vessels were used for biomass determination with three vessels tested before treatment with the test item occurred and at study end. Incubation conditions for test samples and control samples were the same.

Moistened air was drawn through the vessels and through a series of traps, including 2 traps containing sodium hydroxide to collect carbon dioxide.

## **II. Sampling**

Duplicate samples were removed for analysis immediately after treatment and at 7, 14, 30, 61 and 120 days after treatment.

## **III. Description of analytical procedure**

Soil was transferred to a new container and the original container washed with primary extraction solvent acetone (100 mL). The soil was shaken (10 mins) and centrifuged (2000 g, 10 min) and the supernatant transferred to a new container and labelled extract 1. The soil was extracted again with acetone (x2, 100 mL) and the three supernatants combined. The supernatant was quantified with LSC. A subsequent extraction was conducted with the secondary extraction solvent, acetone: 0.5 M HCL (4:1 v/v, 100 mL, x3) and the extracts combined, labelled extract 2 and quantified by LSC. Individual estimates of concentration derived from duplicate or triplicate LSC aliquots were averaged to provide a mean concentration of radioactivity in the sample.

Extract 1 was concentrated to c.1 mL, reconstituted with acetone (5 mL) and labelled extract 3. Extract 2 samples (for extracts containing  $\geq 5$  % AR) were concentrated and reconstituted with acetone (4 mL) and water (1-2 mL).

The soil pellet was air dried, ground and combusted.

Radioactivity in the volatile traps was quantified by LSC at the soonest of each sampling point or after 30 days when reagents were replaced.

Extracts were analysed by HPLC with UV detection at 230 nm and non-radiolabelled reference standards used to confirm the identity of radioactive peaks. TLC was used for determination of radiochemical purity and as a confirmatory method. Extracts were co-chromatographed with non-radiolabelled standards.

Stability of the test item and its metabolites in the soil extracts stored frozen was assessed for a storage time of between 3 and 4 months; this exceeds the maximum storage time of the study sample extracts which was one month and 18 days.

#### IV. LOD values

For LSC, the limit of detection was taken as 1.5 times the background radioactivity determined by counting blank samples. The LOD for LSC varied depending on the sample and aliquot size, but was generally <0.5 % AR.

For HPLC, the applicant stated that peaks distinguishable from background were considered to be peaks. A peak representing 0.1 % AR was considered to be the minimum peak size that could be detected and therefore the LOD was set at c. 0.1 % AR.

### RESULTS AND DISCUSSION

#### I. DATA

The microbial biomass was measured in a subsample of soil at both the start and end of incubation. Microbial biomass was 1.9 to 2.2 % in soil at the start of the incubation and declined in all soils to 1.0 to 1.6 % at the end of the incubation. The requirement for microbial biomass to be at least 1.0 % TOC for the duration of the study was therefore met and soils remained viable throughout the study.

The distribution of radioactivity and mass balance in each soil are summarised in Tables B.8.1.1.1.4-03 to B.8.1.1.1.4-08 below.

**Table B.8.1.1.1.4-03 Percent recovery of applied radioactivity from Speyer 5M soil treated with [<sup>14</sup>C]1'-COOH-S-2840**

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Primary soil extract</b>	Rep 1	82.8	64.4	70.5	67.3	61.4	43.7
	Rep 2	81.9	65.9	68.9	68.5	59.1	45.1
	<b>Mean</b>	<b>82.4</b>	<b>65.2</b>	<b>69.7</b>	<b>67.9</b>	<b>60.3</b>	<b>44.4</b>
<b>Secondary soil extract</b>	Rep 1	21.2	34.1	28.0	28.8	30.1	35.7
	Rep 2	21.8	35.1	28.8	27.0	31.6	36.1
	<b>Mean</b>	<b>21.5</b>	<b>34.6</b>	<b>28.4</b>	<b>27.9</b>	<b>30.9</b>	<b>35.9</b>
<b>Unextracted from soil</b>	Rep 1	0.3	2.3	4.0	5.4	8.3	20.1
	Rep 2	0.3	2.4	3.5	5.1	11.3	18.2
	<b>Mean</b>	<b>0.3</b>	<b>2.4</b>	<b>3.8</b>	<b>5.3</b>	<b>9.8</b>	<b>19.2</b>
<b>Total in soil</b>	Rep 1	104.3	100.8	102.5	101.5	99.8	99.5
	Rep 2	104.0	103.4	101.2	100.6	102.0	99.4
	<b>Mean</b>	<b>104.2</b>	<b>102.1</b>	<b>101.9</b>	<b>100.1</b>	<b>100.9</b>	<b>99.5</b>

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Sodium hydroxide traps</b>	Rep 1	NA	0.0	0.0	0.1	0.4	1.7
	Rep 2	NA	0.0	0.0	0.1	0.5	1.5
	<b>Mean</b>	<b>NA</b>	<b>ND</b>	<b>ND</b>	<b>0.1</b>	<b>0.5</b>	<b>1.6</b>
<b>Mass balance</b>	Rep 1	104.3	100.8	102.5	101.6	100.2	101.2
	Rep 2	104.0	103.4	101.2	100.7	102.5	100.9
	<b>Mean</b>	<b>104.2</b>	<b>102.1</b>	<b>101.9</b>	<b>101.2</b>	<b>101.4</b>	<b>101.1</b>

NA = not applicable; ND = not detected (or <0.1 % AR)

**Table B.8.1.1.4-04 Percent recovery of applied radioactivity from Newhaven soil treated with [<sup>14</sup>C]1'-COOH-S-2840**

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Primary soil extract</b>	Rep 1	79.8	55.5	52.5	48.5	43.9	35.5
	Rep 2	78.0	54.4	51.7	49.2	44.5	35.9
	<b>Mean</b>	<b>78.9</b>	<b>55.0</b>	<b>52.1</b>	<b>48.9</b>	<b>44.2</b>	<b>35.7</b>
<b>Secondary soil extract</b>	Rep 1	23.8	39.3	38.8	37.4	38.3	45.2
	Rep 2	24.5	39.7	37.9	37.9	37.9	44.9
	<b>Mean</b>	<b>24.2</b>	<b>39.5</b>	<b>38.4</b>	<b>37.7</b>	<b>38.1</b>	<b>45.1</b>
<b>Unextracted from soil</b>	Rep 1	0.9	7.2	10.4	12.9	14.4	18.8
	Rep 2	1.0	7.6	10.7	12.6	14.8	18.3
	<b>Mean</b>	<b>1.0</b>	<b>7.4</b>	<b>10.6</b>	<b>12.8</b>	<b>14.6</b>	<b>18.6</b>
<b>Total in soil</b>	Rep 1	104.5	102.0	101.7	98.8	96.6	99.5
	Rep 2	103.5	101.7	100.3	99.7	97.2	99.1
	<b>Mean</b>	<b>104.0</b>	<b>101.9</b>	<b>101.0</b>	<b>99.3</b>	<b>96.9</b>	<b>99.3</b>
<b>Sodium hydroxide traps</b>	Rep 1	NA	0.0	0.1	0.3	0.6	1.0
	Rep 2	NA	0.1	0.1	0.3	0.6	1.0
	<b>Mean</b>	<b>NA</b>	<b>0.1</b>	<b>0.1</b>	<b>0.3</b>	<b>0.6</b>	<b>1.0</b>
<b>Mass balance</b>	Rep 1	104.5	102.0	101.8	99.1	97.2	100.5
	Rep 2	103.5	101.8	100.4	100.0	97.8	100.1
	<b>Mean</b>	<b>104.0</b>	<b>101.9</b>	<b>101.1</b>	<b>99.6</b>	<b>97.5</b>	<b>100.3</b>

NA = not applicable; ND = not detected (or <0.1 % AR)

**Table B.8.1.1.4-05 Percent recovery of applied radioactivity from Atwater soil treated with [<sup>14</sup>C]1'-COOH-S-2840**

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Primary soil extract</b>	Rep 1	87.6	75.8	76.4	78.5	71.5	56.9
	Rep 2	82.6	60.2	76.2	70.6	63.1	55.8
	<b>Mean</b>	<b>85.1</b>	<b>68.0</b>	<b>76.3</b>	<b>74.6</b>	<b>67.3</b>	<b>56.4</b>



		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Secondary soil extract</b>	Rep 1	16.2	25.5	23.3	22.1	29.0	35.0
	Rep 2	15.6	39.6	22.5	27.5	32.2	36.0
	<b>Mean</b>	<b>15.9</b>	<b>32.6</b>	<b>22.9</b>	<b>24.8</b>	<b>30.6</b>	<b>35.5</b>
<b>Unextracted from soil</b>	Rep 1	0.1	1.0	1.7	1.5	4.2	9.3
	Rep 2	0.1	0.9	1.6	3.8	5.9	9.0
	<b>Mean</b>	<b>0.1</b>	<b>1.0</b>	<b>1.7</b>	<b>2.7</b>	<b>5.1</b>	<b>9.2</b>
<b>Total in soil</b>	Rep 1	103.9	102.3	101.4	102.1	104.7	101.2
	Rep 2	98.3	100.7	100.3	101.9	101.2	100.8
	<b>Mean</b>	<b>101.1</b>	<b>101.5</b>	<b>100.9</b>	<b>102.0</b>	<b>103.0</b>	<b>101.0</b>
<b>Sodium hydroxide traps</b>	Rep 1	NA	0.0	0.1	0.0	0.2	1.8
	Rep 2	NA	0.0	0.0	0.2	0.6	1.8
	<b>Mean</b>	<b>NA</b>	<b>ND</b>	<b>0.1</b>	<b>0.1</b>	<b>0.4</b>	<b>1.8</b>
<b>Mass balance</b>	Rep 1	103.9	102.3	101.5	102.1	104.9	103.0
	Rep 2	98.3	100.7	100.3	102.1	101.8	102.6
	<b>Mean</b>	<b>101.1</b>	<b>101.5</b>	<b>100.9</b>	<b>102.1</b>	<b>103.4</b>	<b>102.8</b>

NA = not applicable; ND = not detected (or <0.1 % AR)

**Table B.8.1.1.4-06 Percent of applied radioactivity present as [<sup>14</sup>C]1'-COOH-S-2840 and metabolites in Speyer 5M soil**

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>1'-COOH-S-2840A</b>	Rep 1	51.9	47.8	47.8	43.2	35.0	15.0
	Rep 2	53.5	47.6	47.3	43.0	33.4	19.3
	<b>Mean</b>	<b>52.7</b>	<b>47.7</b>	<b>47.5</b>	<b>43.1</b>	<b>34.2</b>	<b>17.2</b>
<b>1'-COOH-S-2840B</b>	Rep 1	50.2	45.3	45.5	41.0	36.2	18.1
	Rep 2	49.8	45.6	46.9	41.7	34.9	20.4
	<b>Mean</b>	<b>50.0</b>	<b>45.4</b>	<b>46.2</b>	<b>41.4</b>	<b>35.5</b>	<b>19.3</b>
<b>Total 1'-COOH-S-2840</b>	Rep 1	102.1	93.1	93.3	84.2	71.2	33.1
	Rep 2	103.3	93.2	94.2	84.7	68.3	39.7
	<b>Mean</b>	<b>102.7</b>	<b>93.1</b>	<b>93.7</b>	<b>84.5</b>	<b>69.7</b>	<b>36.5</b>
<b>DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>N-des-Me-DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>1'-keto-S-2840</b>	Rep 1	ND	1.9	2.8	4.1	7.1	13.4
	Rep 2	ND	1.8	1.9	2.8	10.5	17.5
	<b>Mean</b>	<b>ND</b>	<b>1.9</b>	<b>2.4</b>	<b>3.5</b>	<b>8.8</b>	<b>15.5</b>

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Total Unknowns*</b>	Rep 1	ND	3.4	1.7	6.8	12.8	24.9
	Rep 2	ND	5.3	1.0	7.2	11.4	45.2
	<b>Mean</b>	<b>ND</b>	<b>4.3</b>	<b>1.4</b>	<b>7.0</b>	<b>12.1</b>	<b>35.0</b>
<b>Unresolved Background</b>	Rep 1	1.8	0.1	0.7	1.0	0.4	1.7
	Rep 2	0.5	0.7	0.6	0.8	0.5	1.6
	<b>Mean</b>	<b>1.2</b>	<b>0.4</b>	<b>0.6</b>	<b>0.9</b>	<b>0.5</b>	<b>1.6</b>
<b>Mass balance</b>	Rep 1	104.0	98.5	98.5	96.1	91.5	73.2
	Rep 2	103.7	101.0	97.7	95.5	90.7	104.0
	<b>Mean</b>	<b>103.9</b>	<b>99.8</b>	<b>98.1</b>	<b>95.8</b>	<b>91.1</b>	<b>88.6</b>

ND = Not detected (or <0.1 % AR)

\*Each of which did not exceed an average of 4.2 % AR

From reflux extraction of the residue, an additional 1.3 % AR each of 1'-COOH-S-2840A, 1'-COOH-S-2840B and 1'-keto-S-2840 were identified

**Table B.8.1.1.4-07 Percent of applied radioactivity present as [<sup>14</sup>C]1'-COOH-S-2840 and metabolites Newhaven soil**

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>1'-COOH-S-2840A</b>	Rep 1	49.6	32.6	28.0	21.1	19.1	16.3
	Rep 2	50.2	32.2	28.0	20.2	19.5	17.8
	<b>Mean</b>	<b>49.9</b>	<b>32.4</b>	<b>28.0</b>	<b>20.6</b>	<b>19.3</b>	<b>17.1</b>
<b>1'-COOH-S-2840B</b>	Rep 1	53.3	37.1	33.8	26.6	26.7	20.2
	Rep 2	50.5	36.2	34.2	25.1	24.3	20.7
	<b>Mean</b>	<b>51.9</b>	<b>36.6</b>	<b>34.0</b>	<b>25.8</b>	<b>25.5</b>	<b>20.5</b>
<b>Total 1'-COOH-S-2840</b>	Rep 1	102.9	69.7	61.8	47.7	45.8	36.5
	Rep 2	100.7	68.4	62.2	45.3	43.8	38.5
	<b>Mean</b>	<b>101.8</b>	<b>69.0</b>	<b>62.0</b>	<b>47.0</b>	<b>44.8</b>	<b>37.6</b>
<b>DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>N-des-Me-DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>1'-keto-S-2840</b>	Rep 1	ND	10.3	16.6	20.4	20.8	22.7
	Rep 2	ND	14.3	16.9	19.6	22.7	20.5
	<b>Mean</b>	<b>ND</b>	<b>12.3</b>	<b>16.8</b>	<b>20.0</b>	<b>21.7</b>	<b>21.6</b>
<b>Total Unknowns*</b>	Rep 1	ND	14.0	12.7	17.3	14.9	20.2
	Rep 2	1.0	10.6	10.1	21.9	15.1	20.4
	<b>Mean</b>	<b>0.5</b>	<b>12.3</b>	<b>11.4</b>	<b>19.6</b>	<b>15.0</b>	<b>20.3</b>

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>Unresolved Background</b>	Rep 1	0.6	0.9	0.3	0.6	0.7	1.3
	Rep 2	0.8	0.8	0.3	0.4	0.8	1.4
	<b>Mean</b>	<b>0.7</b>	<b>0.9</b>	<b>0.3</b>	<b>0.5</b>	<b>0.7</b>	<b>1.3</b>
<b>Mass balance</b>	Rep 1	103.6	94.8	91.3	85.9	82.2	80.7
	Rep 2	102.5	94.1	89.6	87.1	82.4	80.8
	<b>Mean</b>	<b>103.0</b>	<b>94.5</b>	<b>90.5</b>	<b>86.5</b>	<b>82.3</b>	<b>80.7</b>

ND = Not detected (or <0.1 % AR)

\*Each of which did not exceed an average of 2.8 % AR

From reflux extraction of the residue, an additional 1.1 % AR 1'-COOH-S-2840A, 12.1 % AR 1'-COOH-S-2840B and 1.6 % AR 1'-keto-S-2840 were identified

**Table B.8.1.1.4-08 Percent of applied radioactivity present as [<sup>14</sup>C]1'-COOH-S-2840 and metabolites in Atwater soil**

		Days after treatment (DAT)					
		0	7	14	30	61	120
<b>1'-COOH-S-2840A</b>	Rep 1	51.1	46.7	45.0	46.0	41.5	26.1
	Rep 2	49.9	47.2	45.2	42.8	35.7	27.4
	<b>Mean</b>	<b>50.5</b>	<b>46.9</b>	<b>45.1</b>	<b>44.4</b>	<b>38.6</b>	<b>26.7</b>
<b>1'-COOH-S-2840B</b>	Rep 1	52.1	45.0	47.7	43.0	43.5	27.2
	Rep 2	48.3	47.0	43.9	42.2	37.3	26.4
	<b>Mean</b>	<b>50.2</b>	<b>46.0</b>	<b>45.8</b>	<b>42.6</b>	<b>40.4</b>	<b>26.8</b>
<b>Total 1'-COOH-S-2840</b>	Rep 1	103.2	91.7	92.7	89.0	85.0	53.3
	Rep 2	98.2	94.2	89.1	85.0	73.0	53.8
	<b>Mean</b>	<b>100.7</b>	<b>92.9</b>	<b>91.0</b>	<b>87.0</b>	<b>79.0</b>	<b>53.5</b>
<b>DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>N-des-Me-DFPA</b>	Rep 1	ND	ND	ND	ND	ND	ND
	Rep 2	ND	ND	ND	ND	ND	ND
	<b>Mean</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
<b>1'-keto-S-2840</b>	Rep 1	ND	0.8	1.6	0.9	3.7	9.4
	Rep 2	ND	1.2	1.2	3.4	6.8	10.0
	<b>Mean</b>	<b>ND</b>	<b>1.0</b>	<b>1.4</b>	<b>2.1</b>	<b>5.2</b>	<b>9.7</b>
<b>Total Unknowns*</b>	Rep 1	ND	7.9	5.2	10.3	3.7	9.4
	Rep 2	ND	3.7	8.2	9.7	6.8	10.0
	<b>Mean</b>	<b>ND</b>	<b>5.8</b>	<b>6.7</b>	<b>10.0</b>	<b>5.2</b>	<b>9.7</b>
<b>Unresolved Background</b>	Rep 1	0.6	1.0	0.1	0.5	0.2	1.3
	Rep 2	0.1	0.7	0.2	0.1	0.6	0.6
	<b>Mean</b>	<b>0.3</b>	<b>0.8</b>	<b>0.2</b>	<b>0.3</b>	<b>0.4</b>	<b>0.9</b>
<b>Mass balance</b>	Rep 1	103.8	101.3	99.6	100.6	100.5	91.9

Mass balance		Days after treatment (DAT)					
		0	7	14	30	61	120
	Rep 2	98.2	99.8	98.7	98.1	95.3	91.8
	Mean	101.0	100.5	99.2	99.4	97.9	91.9

ND = Not detected (or <0.1 % AR)

\*Each of which did not exceed an average of 3.7 % AR

**Table B.8.1.1.1.4-09 Changes in enantiomeric excess**

	1'-COOH-S- 2840A	1'-COOH-S- 2840B	Enantiomeric excess	Change in enantiomeric excess
<b>Speyer 5M</b>				
<b>0</b>	51.3	48.7	2.6	-
<b>7</b>	51.2	48.8	2.4	-0.2
<b>14</b>	50.7	49.3	1.4	-1.2
<b>30</b>	51.0	49.0	2.0	-0.6
<b>61</b>	49.1	50.9	-1.8	-4.4
<b>120</b>	47.1	52.9	-5.8	-8.4
<b>Newhaven</b>				
<b>0</b>	49.0	51.0	-2.0	-
<b>7</b>	47.0	53.0	-6.0	-4.0
<b>14</b>	45.2	54.8	-9.6	-7.6
<b>30</b>	43.8	54.9	-11.2	-9.2
<b>61</b>	43.1	56.9	-13.8	-11.8
<b>120</b>	45.5	54.5	-9.0	-7.0
<b>Atwater</b>				
<b>0</b>	50.1	49.9	0.2	-
<b>7</b>	50.5	49.5	1.0	0.8
<b>14</b>	49.6	50.4	-0.8	-1.0
<b>30</b>	51.0	49.0	2.0	1.8
<b>61</b>	48.9	51.1	-2.2	-2.4
<b>120</b>	49.9	50.1	-0.2	-0.4

## II. Storage stability

**Table B.8.1.1.4-10 The % AR of 1'-COOH-S-2840 in samples before and after frozen storage for between 3 and 4 months**

	<b>Before storage (05 Aug 2016)</b>	<b>After storage (17 Nov 2016)</b>	<b>Change</b>
<b>Sample A10 Ext 3</b>	47.6 % AR	46.6 % AR	-2.1 % AR
<b>Sample C7 Ext 4</b>	13.7 % AR	16.1 % AR	+17.1 % AR

The applicant observes that the frozen storage time in the storage stability study exceeded the sample storage time of one month and 18 days and considers that the chromatograms (i.e. the % AR analyte present) was very similar before and after storage. The applicant has therefore concluded that 1'-COOH-S-2840 and its metabolites were stable during frozen storage. HSE notes that only two samples were tested, which is a limited sample size. Also, only the peak size for the test item is specified and therefore it is not possible to ascertain whether its metabolites were also stable during storage. It is agreed that for sample A10 Ext 3, the 2.1 % decrease in % AR during storage is small. An increase of 17.1 % AR is however observed for sample C7 Ext 4 during storage. HSE considers that this is within the range of analytical variability and therefore agrees that, based on the limited information available, 1'-COOH-S-2840 is stable during storage periods exceeding those used for samples in the study.

## III. Mass balance

Mean mass balance ranged between 101.1 and 104.2 % AR for the Speyer 5M soil, between 97.5 and 104.0 % AR for the Newhaven soil and between 100.9 and 103.4 % for the Atwater soil. For individual replicates, no mass balance was outside of the range 90-110 % AR, ranging between 97.2 and 104.9 % AR.

The primary soil extract contained most of the extracted radioactivity, but radioactivity extracted in the primary extraction declined over time, from a mean of 78.9 to 82.6 % AR on day 0 to a range of 35.7 to 56.4 % AR at study end. In contrast, the radioactivity in the secondary soil extract increased over time from a mean of 15.9 to 24.2 % AR at day 0 to 35.5 to 45.1 % AR at study end.

## IV. Bound residues

Unextracted residues increased during the course of the study in all soils. Mean unextracted residues accounted for 9.2 to 19.2 % AR in soil at study end.

Unextracted residues were lowest in the Atwater soil, while similar levels were observed in Newhaven and Speyer 5M soils.

Two 120 DAT samples containing >10 % AR as bound residues were further extracted (ethyl acetate, hexane and acetone:HCl (0.5 M), 1:1 v/v) to determine the nature of the bound residues.

**Table B.8.1.1.4-11 Characterisation of bound residues**

Sample	% AR					
	Ethyl acetate	Hexane	Acetone:0.5 M HCl (1:1 v/v)	Fulvic acid	Humic acid	Humin
<b>A11</b>	0.8	ND	6.3	5.8	1.7	6.0
<b>B11</b>	1.1	ND	6.8	5.4	2.7	3.2

The acetone:HCl solvents extracted the most radioactivity, this being 6.3 to 6.8 % AR in the two samples tested. In the A11 sample, the humin fraction contained the most radioactivity at 6.0 % AR, while 5.4 to 5.8 % AR was associated with the fulvic acid fraction. Only low amounts of radioactivity (1.7 to 2.7 % AR) were associated with the humic acid fraction.

## V. Volatilisation

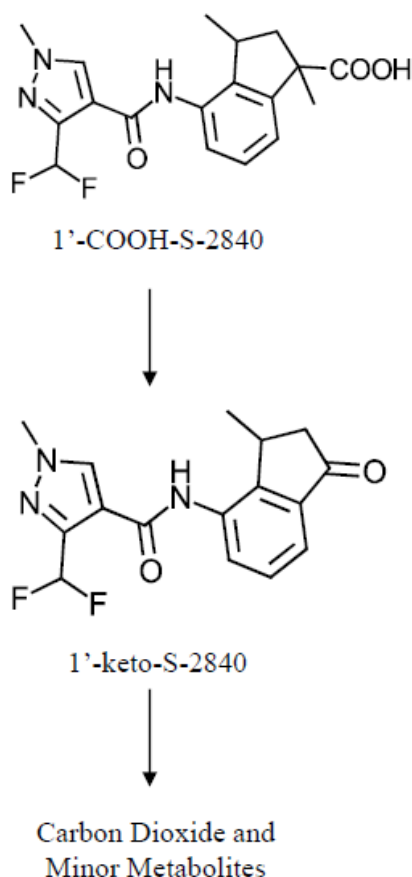
Low amounts of radioactivity were present in the sodium hydroxide traps, showing that little CO<sub>2</sub> was formed. Mean values at study end ranged between 1.0 and 1.8 % AR.

## VI. Metabolites

Total 1'-COOH-S-2840 decreased from a mean of 100.7 to 102.7 % AR on day 0 to 53.5 to 36.5 % AR on day 120.

Three degradation products were analysed for, DFPA, N-des-Me-DFPA and 1'-Keto-S-2840. Metabolite 1'-keto-S-2840 increased during the course of the study and was present at a mean of 9.7 to 21.6 % AR at study end (there was a marginally higher mean value of 21.7 % AR at day 61).

A proposed degradation pathway is shown below.



**Figure B.8.1.1.4-03 Degradation pathway of 1'-COOH-S-2840 in soil**

Potential for 1'-keto-S-2840 to be a major soil metabolite and included in exposure assessment

As metabolite 1'-keto-S-2840 may be a major metabolite exceeding the trigger of 5 % at two consecutive timepoints, the need for this metabolite to be included in the exposure assessment has been given further consideration. The case presented was put together by HSE with additional input from the applicant and covers the potential for formation above the trigger values, mobility of the metabolite and potential for leaching to groundwater.

Formation of 1'-keto-S-2840 in soil studies

Metabolite 1'-keto-S-2840 increased during the course of the study and was present at a maximum of 21.7 % AR and a mean of 9.7 to 21.6 % AR at study end. Based on the maximum observed level of 1'-COOH-S-2840 in the parent applied study of 30.1 % AR (Woodside Farm, sum of A and B isomers in the total soil extract) and a maximum of 21.7 % AR in the present study for 1'-keto-S-2840, 1'-keto-S-2840 might form at a maximum of 6.5 % AR when related back to applied parent inpyrfluxam.

Furthermore, parent degradation was not complete in the parent applied studies, with 41.8 to 53.5% AR remaining in soil after 182 days, indicating the potential for continued formation of 1'-keto-S-2840 in soil after the end of the study.

The formation fractions of 1'-keto-S-2840 from 1'-COOH-S-2840 range between 0.23 and 0.39. The formation fractions for Speyer 2.3 and Atwater soils are based on a conservative default formation fraction of 1 for formation of 1'-COOH-S-2840 from parent and so the overall formation fraction for 1'-keto-S-2840 is likely to be overestimated in these soils. Nevertheless, the data indicate that there is potential for substantial formation of 1'-keto-S-2840 in the soils studied.

**Table B.8.1.1.4-12 Summary of estimated formation fractions for 1'-keto-S-2840 in laboratory degradation studies**

Soil	Formation fraction (from 1'-COOH-S-2840)	Formation fraction (from inpyrfluxam)*
Speyer 2.3	0.25	0.25
Newhaven	0.39	0.21
Atwater	0.23	0.23

\*Formation fractions from inpyrfluxam to 1'-COOH-S-2840 are 1 for Speyer 2.3 and Atwater soils and 0.5348 from the Newhaven soil

Metabolite 1'-keto-S-2840 was not specifically searched for in any of the parent applied laboratory studies. After up to 182 days, unknowns reached a maximum of 8.9 % in one soil with individual peaks representing <2 % AR at all sampling intervals. In the other three soils, total unknowns ranged between 5.6 and 6.0 % AR, with the maximum peak size not stated. The submitted chromatograms do not indicate the potential for substantial formation of 1'-keto-S-2840 in the parent applied studies.

The applicant considered that the above based on a maximum formation of 1'-keto-S-2840 of 21.7 % AR in the 1'-COOH-S-2840 applied study combined with maximum formation of precursor 1'-COOH-S-2840 in the parent applied study of 30.1 % AR was a worst case approach which simplified the overall degradation process. The applicant estimated expected formation of 1'-keto-S-2840 from only the Newhaven and Atwater soils, as these were the only soils which appeared in both the parent applied and 1'-COOH-S-2840 applied datasets. In the Newhaven soil, a maximum of 22.8 % AR of 1'-COOH-S-2840 was formed following inpyrfluxam incubation 61 days after treatment with 21.7 % AR of 1'-keto-S-2840 in the 1'-COOH-S-2840 applied study, leading to 4.9 % AR 1'-keto-S-2840. In the Atwater soil, 9.6% AR of 1'-COOH-S-2840 was formed following inpyrfluxam incubation 90 days after treatment, and 9.7% AR of 1'-keto-S-2840 following 1'-COOH-S-2840 incubation 120 days after



treatment (leading to maximum of 1% 1'-keto-S-2840 being expected from parent degradation). The applicant therefore observed that neither of these soils show estimated formation > 5%, although it is close to the trigger in the Newhaven soil.

The applicant also referred to the field dissipation studies. Nine field dissipation studies were submitted, with 4 sites (Mississippi, California, North Dakota and Washington) rejected as not being relevant to GB conditions based on an ENGASIPS analysis. The applicant's references to the rejected sites are included here as part of a weight of evidence approach to show expected formation of 1'-COOH-S-2840, as 1'-COOH-S-2840 was monitored for at all sites. The levels observed are presented below, with those sites where 1'-COOH-S-2840 was observed at >LOD highlighted in bold.

**Table B.8.1.1.4-13 Residues of inpyrfluxam and 1'-COOH-S-2840 observed in field studies (taken from section B.8.1.2.1.1)**

<b>Study</b>	<b>Site</b>	<b>% of applied inpyrfluxam degraded at the end of the study<sup>a</sup>)</b>	<b>1'-COOH-S-2840A</b>	<b>1'-COOH-S-2840B</b>
██████ (2017a) TPR-0031	Mississippi	> 90% (488 days)	Not observed > LOD	Not observed > LOD
██████ (2017b) TPR-0032	California	> 90% (270 days)	Not observed > LOD	Not observed > LOD
██████ (2017a) TPR-0034	North Dakota	> 90% (478 days)	Not observed > LOD	Not observed > LOD
██████ (2017c) TPR-0053	Washington	> 90% (730 days)	Not observed > LOD	Not observed > LOD
██████ (2017b) TPR-0033	Ontario	~ 88.3% (489 days)	Not observed > LOD	Not observed > LOD
██████ (2018a) TPR-0085	Germany	<b>&gt; 90% (730 days)</b>	<b>Observed &gt; LOD</b>	<b>Observed &gt; LOD</b>

	<b>Czech Republic</b>	<b>&gt; 90% (730 days)</b>	<b>Observed &gt; LOD</b>	<b>Observed &gt; LOD</b>
	<b>Italy</b>	<b>~ 83.8% (730 days)</b>	<b>Observed &gt; LOD</b>	<b>Observed &gt; LOD</b>
	<b>Spain</b>	<b>~ 89.6% (730 days)</b>	<b>Observed &gt; LOD</b>	<b>Observed &gt; LOD</b>

<sup>a</sup> The stated percentage degradation values assume that the mean residue values for inpyrfluxam following the final application (0 DALA) are 100% of applied parent with the exception that for the Czech Republic site percentage degradation was calculated considering the residues at 3 DALA as 100 % because reported parent residues at 0 DALA at this site were lower than those reported for the following three samples. Percentage degradation values are calculated based on mean residues

The applicant therefore observes that at 5 of the 9 sites, residues of 1'-COOH-S-2840A and 1'-COOH-S-2840B were not detected at any timepoint. They then give further consideration to the sites at which these metabolites were detected. These sites are also amongst those considered to be relevant to the soils and climatic conditions of GB.

**Table B.8.1.1.4-14 Residues of 1'-COOH-S-2840 as a percentage of inpyrfluxam at field studies in which 1'-COOH-S-2840 was observed (taken from section B.8.1.2.1.1)**

<b>Site</b>	<b>Inpyrfluxam (mg/kg)</b>	<b>1'-COOH-S-2840A (mg/kg)</b>	<b>1'-COOH-S-2840B (mg/kg)</b>	<b>Maximum fraction of 1'-COOH-S-2840 formed from inpyrfluxam<sup>c</sup></b>
<b>Germany</b>	0.154 <sup>a</sup>	0.00332 <sup>b</sup>	0.00652 <sup>b</sup>	5.9% (14 DAA)
<b>Czech Republic</b>	0.101 <sup>a</sup>	0.00419 <sup>b</sup>	0.00437 <sup>b</sup>	7.8% (60 DAA)
<b>Italy</b>	0.054 <sup>a</sup>	0.0005 <sup>d</sup>	0.00134 <sup>b</sup>	3.1% (180 DAA)
<b>Spain</b>	0.168 <sup>a</sup>	0.00654 <sup>b</sup>	0.01320 <sup>b</sup>	10.8% (455 DAA)

<sup>a</sup> Mean initial value of inpyrfluxam (0 DAA)

<sup>b</sup> Maximum value from the individual replicates. Note that LOD values were reached at the end of the study (730 DAA) for all sites except Spain (values decrease from 270 DAA).

<sup>c</sup> Fraction formed from inpyrfluxam (MW<sub>inpyrfluxam</sub> = 333.38 g/mol; MW<sub>1'-COOH-S-2840</sub> = 363.36 g/mol).

Max. fraction = Maximum [1'-COOH-S-2840A + 1'-COOH-S-2840B] \* 100 / Initial inpyrfluxam \* MW<sub>inpyrfluxam</sub> / MW<sub>1'-COOH-S-2840</sub>

<sup>d</sup> Highest value reported as <LOQ; ½ LOQ was used for the calculation of the maximum formation.

Under field conditions, based on a maximum formation of 1'-COOH-S-2840 (total A + B) and maximum formation of 1'-keto-S-2840 of 21.7 % AR (as observed in laboratory studies), the maximum formation of 1'-keto-S-2840 is 2.3 %. Under field conditions therefore, formation of 1'-keto-S-2840 at >5 % is unlikely.

#### Sorption behaviour and potential for leaching to groundwater

The structure of 1'-keto-S-2840 has also been considered for indications of its potential sorption behaviour relative to the primary metabolites 1'-COOH-S-2840 and 3'-OH-S-2840. The structure of 1'-keto-S-2840 is similar to 1'-COOH-S-2840, with the COOH and CH<sub>3</sub> groups in 1'-COOH-S-2840 having been replaced with a ketone. Generally, for otherwise similar molecules, a carboxylic acid would be expected to be more soluble in water than a ketone. It is therefore likely that 1'-keto-S-2840 would be more strongly sorbed and less mobile than 1'-COOH-S-2840. Similarly, metabolite 3'-OH-S-2840 contains an -OH group and so would also be expected to be more mobile than 1'-keto-S-2840.

QSAR estimates of adsorption parameters have been derived to try to confirm the relative mobilities indicated by the molecular structures.

**Table B.8.1.1.4-15 QSAR estimates of  $K_{foc}$  for inpyrfluxam metabolites (derived by HSE)**

	<b>Experimentally derived range <math>K_{foc}</math> (L/kg)</b>	<b>Experimentally derived geometric mean <math>K_{foc}</math> (L/kg)</b>	<b>QSAR estimated <math>K_{oc}</math> (L/kg)</b>
<b>1'-COOH-S-2840</b>	11-45	24	36
<b>1'-keto-S-2840</b>	-	-	150

The comparison of the experimentally derived  $K_{foc}$  values for 1'-COOH-S-2840 with the QSAR estimate indicates that the QSAR estimate is likely to be a reasonable estimate of leaching potential. The QSAR estimates also indicate that 1'-keto-S-2840 may be significantly less mobile than 1'-COOH-S-2840. This is consistent with the relative structures of the two metabolites. This suggests that the leaching potential of 1'-keto-S-2840 is likely to be within that for the primary metabolite.

#### Summary

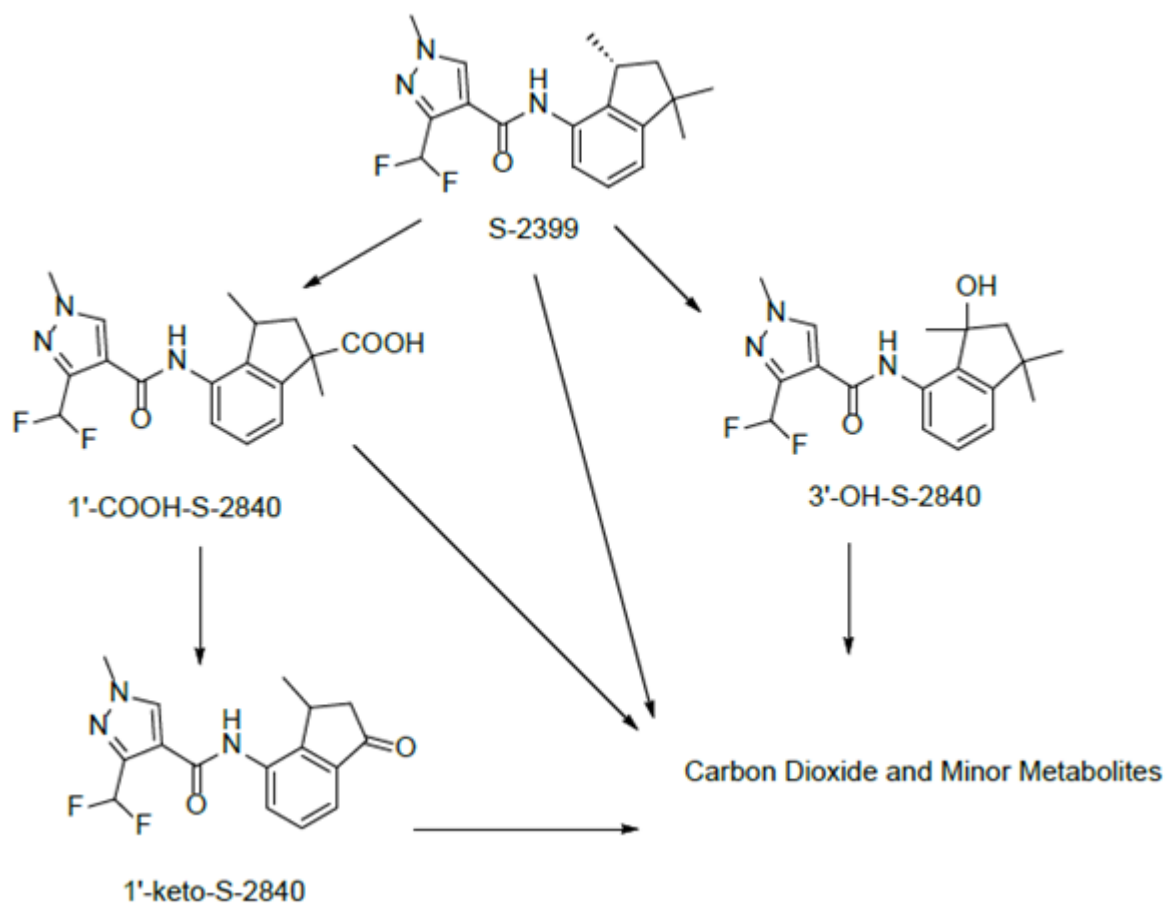
From an Environmental Fate perspective, the evidence indicates that 1'-keto-S-2840 is likely to be less mobile than 1'-COOH-S-2840. HSE therefore proposes that exposure to groundwater for 1'-keto-S-2840 is covered by the exposure assessment for 1'-COOH-S-2840. Based on the levels of 1'-COOH-S-2840 detected in field studies, 1'-keto-S-2840 is also considered unlikely to form at >5 % under field

conditions. It is therefore considered that the environmental exposure from 1'-keto-S-2840 is covered by the exposure assessments for parent and metabolites 1'-COOH-S-2840 and 3'-OH-S-2840.

Furthermore, toxicological data demonstrates that 1'-keto-S-2840 is of lower toxicity than the parent substance and is not toxicologically relevant (see B.6.8.1.1 in section 3CA - B.6 (Toxicology)).

An exposure assessment for 1'-keto-S-2840 is not required and no further consideration is necessary.

The applicant has provided a new degradation scheme for inpyrfluxam in soil including 1'-keto-S-2840, as shown in the figure below.



**Figure B.8.1.1.4-04 Degradation scheme of inpyrfluxam including 1'-keto-S-2840 in Speyer 2.3 soil**

## Degradation kinetics for 1'-keto-S-2840

**Figure B.8.1.1.1.4-05 Degradation kinetics of 1'-COOH-S-2840 and 1'-keto-S-2840 in Speyer 2.3 soil**

<b>1'-COOH-S-2840 and 1'-keto-S-2840, Speyer 2.3 (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>1'-COOH-S-2840 SFO</b>	<b>Good</b>	<b>3.56</b>	<b>M<sub>0</sub>: 103.3 k: 0.007665</b>	<b>k: &lt;0.05</b>	<b>90.4</b>	<b>300</b>
<b>1'-keto-S-2840 SFO-SFO</b>	<b>Good</b>	<b>10.5</b>	<b>k: 6.68E<sup>-9</sup> ff: 0.237</b>	<b>k: 0.5</b>	<b>&gt;1000</b>	<b>&gt;1000</b>
1'-COOH-S-2840 DFOP	Good	4.48	M <sub>0</sub> : 103.3 k <sub>1</sub> : 0.0 007654 k <sub>2</sub> : 0.007654 g: 0.001828	k <sub>1</sub> : 0.3829 k <sub>2</sub> : <0.05	90.6	301
1'-keto-S-2840 DFOP-SFO	Good	10.5	k: 7.08E <sup>-9</sup> ff: 0.2373	k: 0.5	>1000	>1000
<p>The visual fit of the SFO model to the data set is good, with the starting concentrations and the decline phase well represented for the parent. The increase in the metabolite concentration and the peak concentration are also well represented. This is also shown in the plot of the residuals, which shows small errors. The <math>\chi^2</math> value is 3.56 % for the parent and 10.5 % for the metabolite (overall 5.14 %). It is noted however, that the k parameter for the metabolite fails the t-test as there is no clear decline phase.</p> <p>The DFOP model gives a good visual fit which is almost identical to the SFO fit, but the covariance matrix could not be calculated, while the estimated values for</p>						

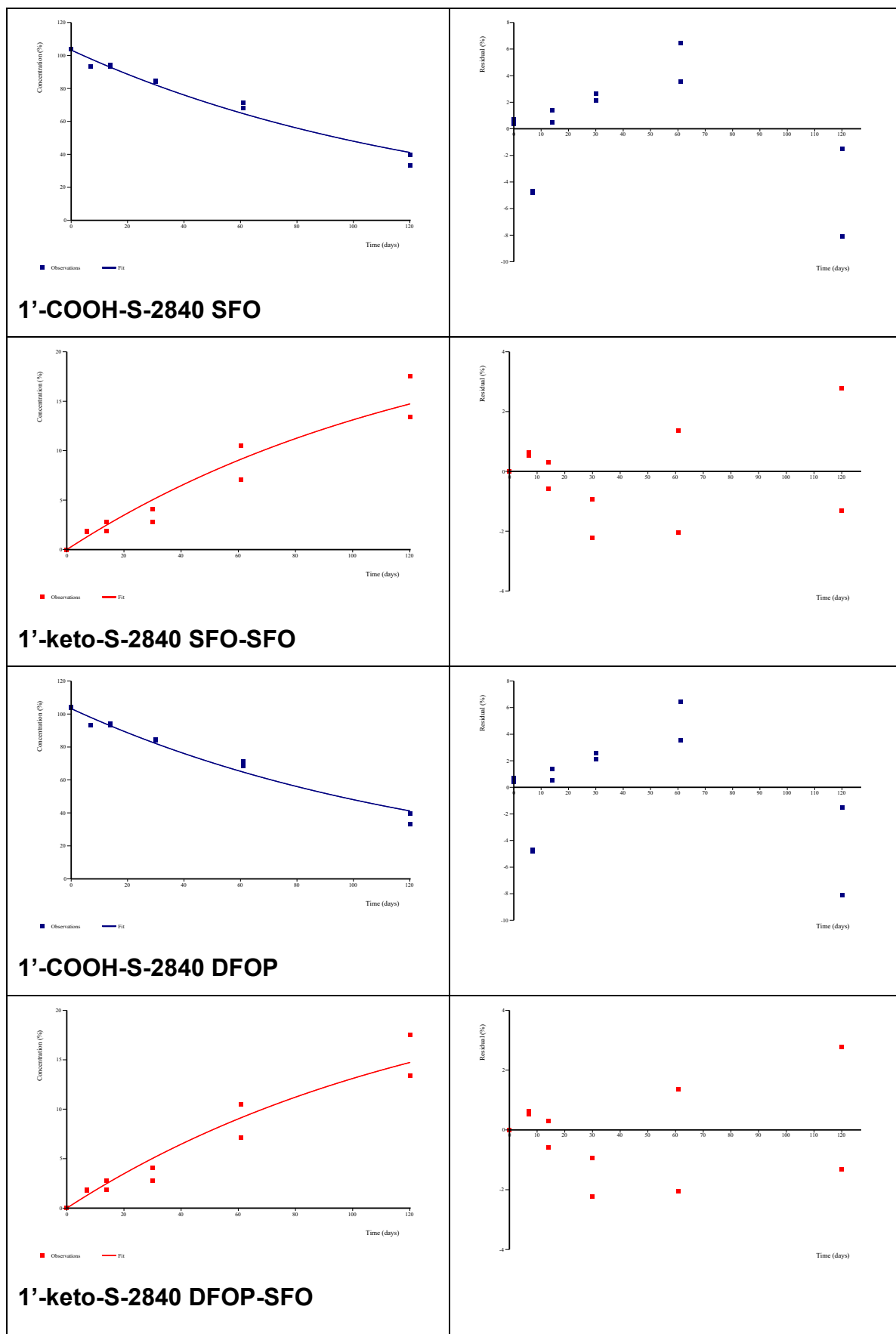
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K1 and K2 are identical. This means that DFOP is not an appropriate model to use for the data set.

**Conclusion: SFO is the best fit model**

**Try fixing  $DT_{50}$  for the metabolite to 1000 d**

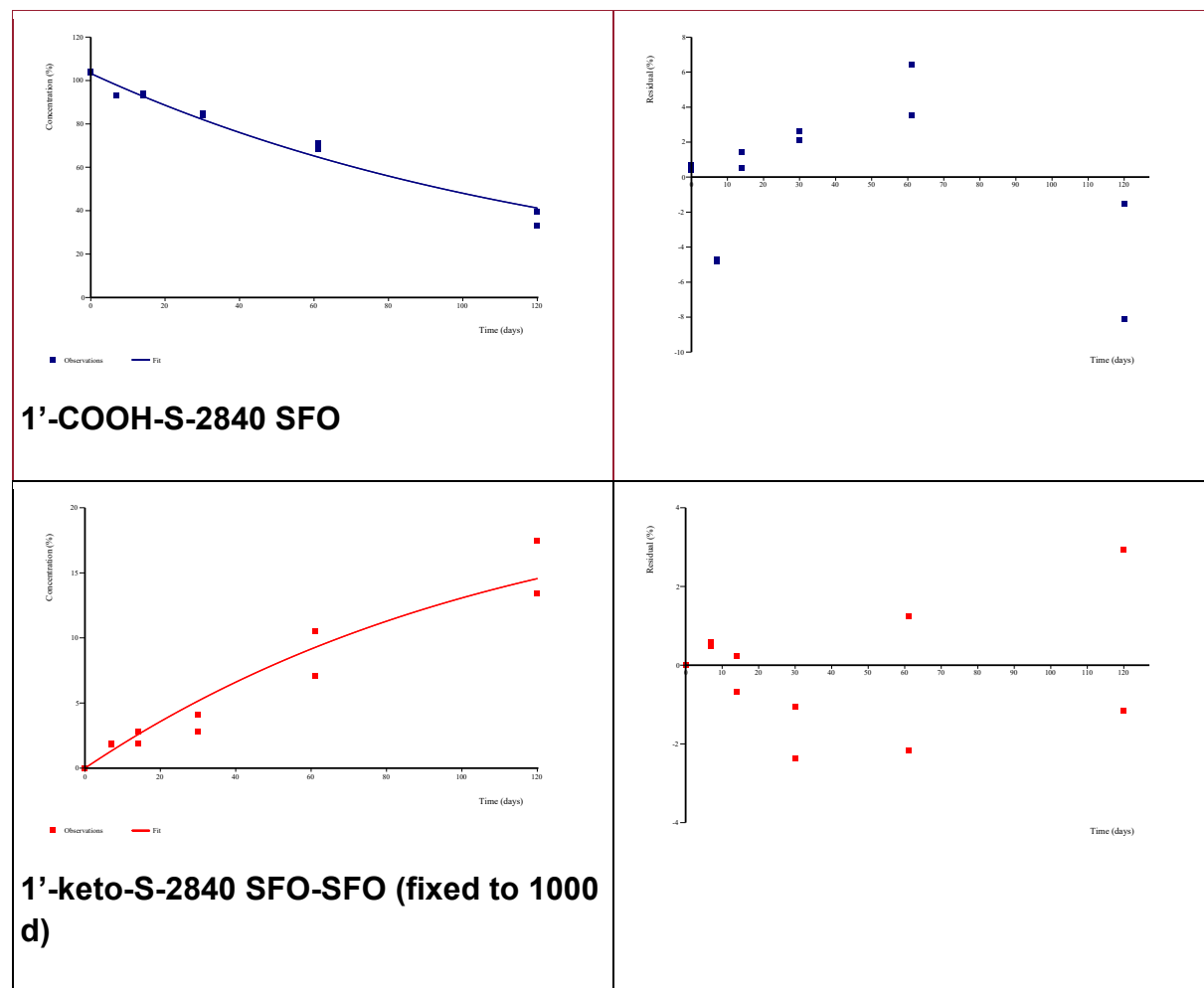
**Selected kinetics in bold**



**Figure B.8.1.1.1.4-06 Degradation kinetics of 1'-COOH-S-2840 and 1'-keto-S-2840 in Speyer 2.3 soil, metabolite DT<sub>50</sub> fixed to 1000 d**

<b>1'-COOH-S-2840 and 1'-keto-S-2840, Speyer 2.3 (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>1'-COOH-S-2840 SFO</b>	<b>Good</b>	<b>3.56</b>	<b>M<sub>0</sub>: 103.3 k: 0.007652</b>	<b>k: &lt;0.05</b>	<b>90.6</b>	<b>301</b>
<b>1'-keto-S-2840 SFO-SFO</b>	<b>Good</b>	<b>10.5</b>	<b>k: 0.0006931 (fixed) ff: 0.246</b>	<b>-</b>	<b>1000</b>	<b>3320</b>
<p>The parent and metabolite fit are almost identical to the fit above in which the metabolite k value was allowed to freely optimise. The <math>\chi^2</math> value is 3.56 % for the parent and 10.5 % for the metabolite (which are both identical to the freely optimised fit). This demonstrates that 1000 d is a reasonable and conservative estimate of the DT<sub>50</sub> in this soil.</p> <p><b>Conclusion: SFO is the best fit model (DT<sub>50</sub> = 90.6 days, DT<sub>90</sub> = 301 days (1'-COOH-S-2840; DT<sub>50</sub> = 1000 days, DT<sub>90</sub> = 3320 days (1'-keto-S-2840), formation fraction 0.25). Metabolite DT<sub>50</sub> can be fixed to 1000 d.</b></p> <p><b>Selected kinetics in bold</b></p>						





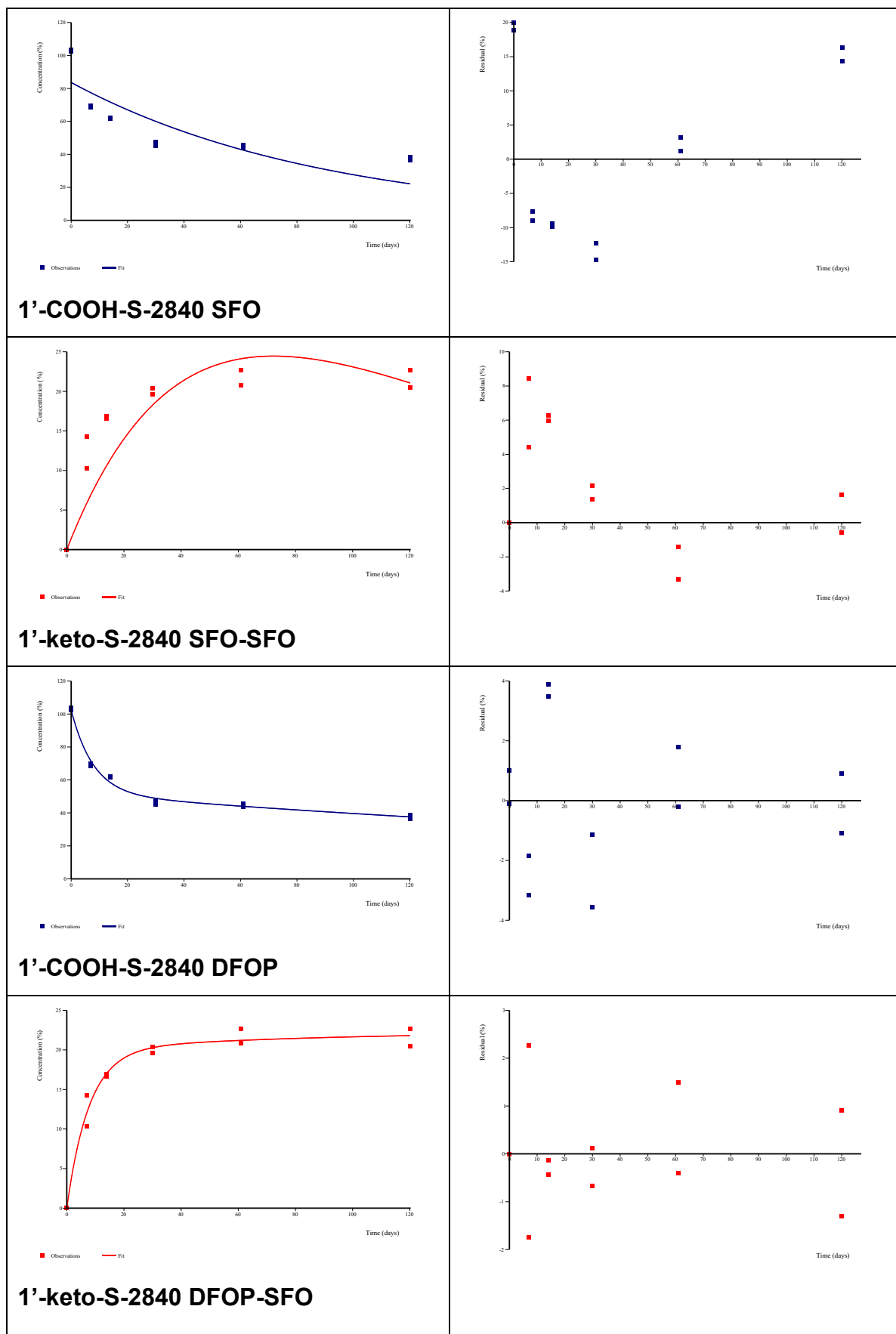
**Figure B.8.1.1.4-07 Degradation kinetics of 1'-COOH-S-2840 and 1'-keto-S-2840 in Newhaven soil**

<b>1'-COOH-S-2840 and 1'-keto-S-2840, Newhaven (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
1'-COOH-S-2840 SFO	Poor	16.7	M <sub>0</sub> : 83.63 k: 0.01106	k: <0.05	62.7	208
1'-keto-S-2840 SFO-SFO	Fair	18.1	k: 0.01699 ff: 1	k: 0.1	40.8	136
<b>1'-COOH-S-2840 DFOP</b>	<b>Good</b>	<b>3.46</b>	<b>M<sub>0</sub>: 102.6</b> <b>k<sub>1</sub>: 0.128</b> <b>k<sub>2</sub>: 0.002658</b> <b>g: 0.4959</b>	<b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b>	<b>23.1</b>	<b>609</b>
<b>1'-keto-S-2840 SFO</b>	<b>Poor</b>	<b>1.46</b>	<b>k: 0.001497</b> <b>ff: 0.39</b>	<b>k: 0.09</b>	<b>463</b>	<b>1540</b>
<p>The visual fit of the SFO model to the data set is poor, with the starting concentrations underestimated, the decline poorly represented and the rate of decline overestimated at study end. This is also shown in the plot of the residuals, which shows large errors. The <math>\chi^2</math> value is 16.7 % for 1'-COOH-S-2840. For the metabolite 1'-keto-S-2840 the model underestimates the rate of decline, but gives a reasonable representation of the peak concentrations. This is demonstrated in the plot of the residuals where there is systematic underestimation of the data points early in the study. The <math>\chi^2</math> value is 18.1 %, which is high.</p> <p>The DFOP model gives a better visual fit. The starting concentrations and decline phase for the parent and increase in concentration and peak concentration for the 1'-keto-S-2840 metabolite are well represented and the plot of the residuals shows small, random errors. The <math>\chi^2</math> values of 3.46 % for 1'-COOH-S-2840 and 1.46 for 1'-keto-S-2840 are lower than for the SFO model.</p>						

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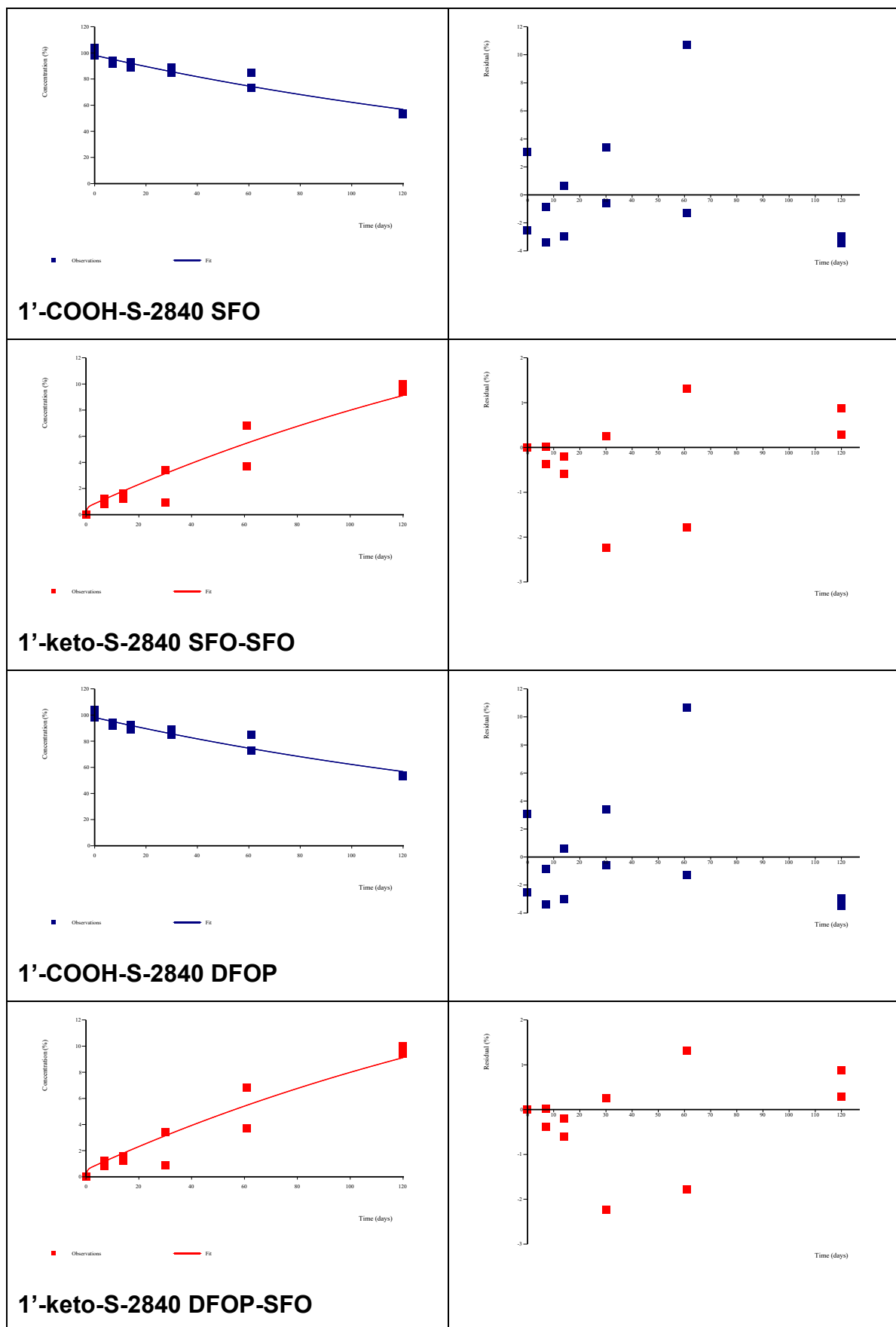
**Conclusion: DFOP is the best fit model (overall  $DT_{50} = 23.1$  days,  $DT_{90} = 609$  days (1'-COOH-S-2840;  $DT_{50} = 463$  days,  $DT_{90} = 1540$  days (1'-keto-S-2840), formation fraction 0.39)**

**Selected kinetics in bold**



**Figure B.8.1.1.4-08 Degradation kinetics of 1'-COOH-S-2840 and 1'-keto-S-2840 in Atwater soil**

<b>1'-COOH-S-2840 and 1'-keto-S-2840, Atwater (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>1'-COOH-S-2840 SFO</b>	<b>Good</b>	<b>2.62</b>	<b>M<sub>0</sub>: 99.09 k: 0.00470</b>	<b>k: &lt;0.05</b>	<b>148</b>	<b>490</b>
<b>1'-keto-S-2840 SFO-SFO</b>	<b>Good</b>	<b>7.82</b>	<b>k: 0 ff: 0.22</b>	<b>k: 0.5</b>	<b>&gt;1000</b>	<b>&gt;1000</b>
1'-COOH-S-2840 DFOP	Good	3.09	M <sub>0</sub> : 100.7 k <sub>1</sub> : 2.443 k <sub>2</sub> : 0.004562 g: 0.02573	k <sub>1</sub> : <0.05 k <sub>2</sub> : <0.05	416	499
1'-keto-S-2840 DFOP-SFO	Good	11.5	k: 3.82E-010 ff: 0.2074	k: 0.09	>1000	>1000
<p>The visual fit of the SFO model to the data set is good, with the starting concentrations and the decline phase well represented for the parent. The increase in the metabolite concentration and the peak concentration are also well represented. This is also shown in the plot of the residuals, which shows small errors. The <math>\chi^2</math> value is 3.82 % for the parent and 2.62 % for the metabolite (overall 7.82 %). It is noted however, that the k parameter for the metabolite fails the t-test, as there is no clear decline phase.</p> <p>The DFOP model gives a good visual fit which is almost identical to the SFO fit, but the covariance matrix could not be calculated. This means that DFOP is not an appropriate model to use for the data set.</p> <p><b>Conclusion: SFO is the best fit model</b></p> <p><b>Try fixing DT<sub>50</sub> for the metabolite to 1000 d</b></p> <p><b>Selected kinetics in bold</b></p>						



**Figure B.8.1.1.4-09 Degradation kinetics of 1'-COOH-S-2840 and 1'-keto-S-2840 in Atwater soil, metabolite DT<sub>50</sub> fixed to 1000 d**

<b>1'-COOH-S-2840 and 1'-keto-S-2840, Atwater (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>1'-COOH-S-2840 SFO</b>	<b>Good</b>	<b>2.62</b>	<b>M<sub>0</sub>: 99.09 k: 0.0047</b>	<b>k: &lt;0.05</b>	<b>148</b>	<b>490</b>
<b>1'-keto-S-2840 SFO-SFO</b>	<b>Good</b>	<b>7.98</b>	<b>k: 0.0006931 (fixed) ff: 0.228</b>	<b>-</b>	<b>1000</b>	<b>3320</b>
<p>The parent and metabolite fit are almost identical to the fit above in which the metabolite k value was allowed to freely optimise. The <math>\chi^2</math> value is 2.62 % for the parent (which is identical to the freely optimised fit) and 7.98 % for the metabolite (which is only marginally higher than the 7.82 % calculated for the freely optimised fit). This demonstrates that 1000 d is a reasonable and conservative estimate of the DT<sub>50</sub> in this soil.</p> <p><b>Conclusion: SFO is the best fit model (DT<sub>50</sub> = 148 days, DT<sub>90</sub> = 490 days (1'-COOH-S-2840; DT<sub>50</sub> = 1000 days, DT<sub>90</sub> = &gt;1000 days (1'-keto-S-2840), formation fraction 0.23). Metabolite DT<sub>50</sub> can be fixed to 1000 d.</b></p> <p><b>Selected kinetics in bold</b></p>						

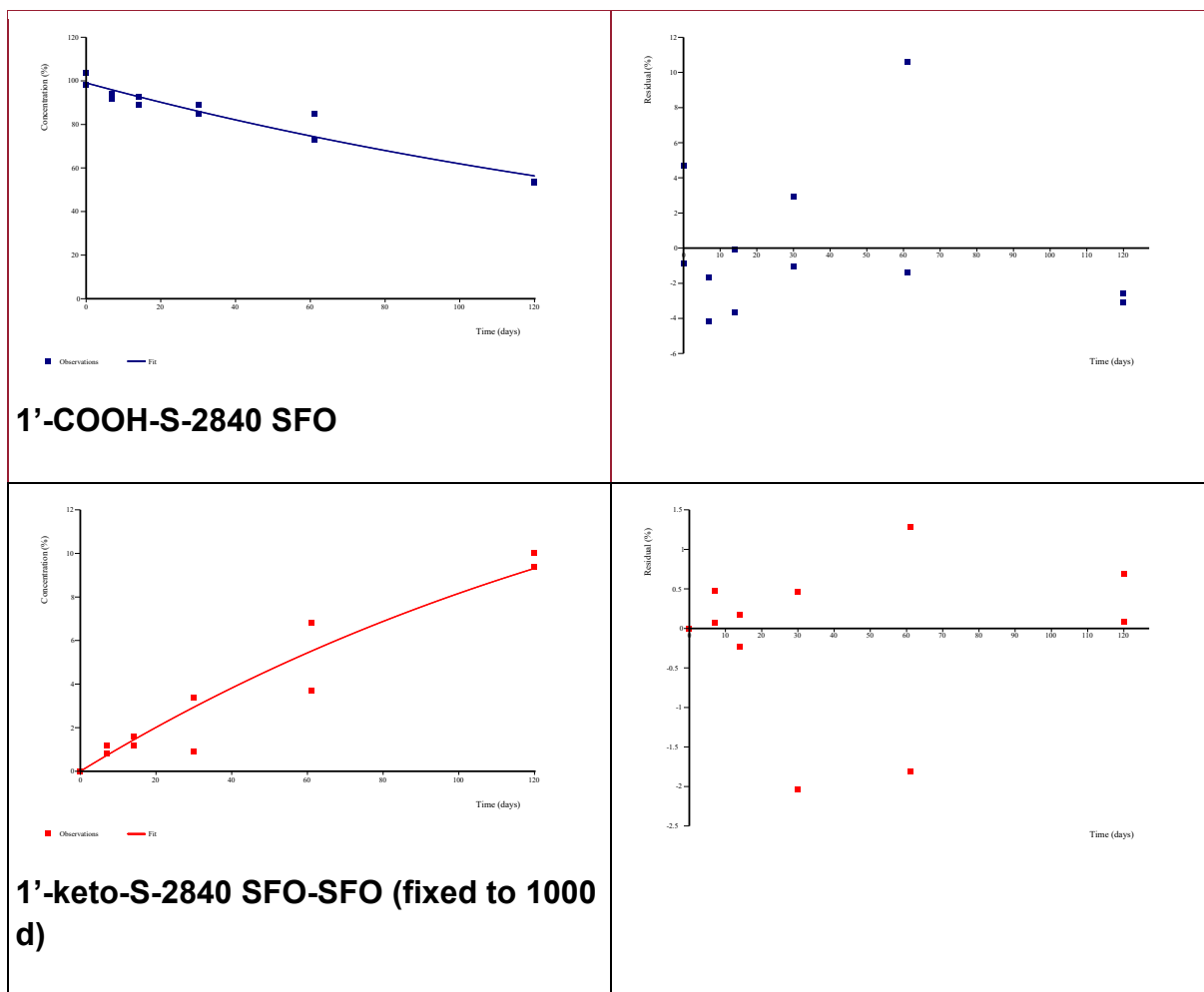


Table B.8.1.1.4-16 Summary of kinetic parameters for 1'-keto-S-2840

Soil	Best fit model	Overall DT <sub>50</sub> (days)	Overall DT <sub>90</sub> (days)	Formation fraction (from 1'-COOH-S-2840)	Formation fraction (from inpyrfluxam)
<b>Speyer 2.3</b>	SFO-SFO	1000	3320	0.25	0.25
<b>Newhaven</b>	DFOP-SFO	463	1540	0.39	0.21
<b>Atwater</b>	SFO-SFO	1000	3320	0.23	0.23

\* Metabolite DT<sub>50</sub> fixed to 1000 d.



The overall DT<sub>50</sub> for metabolite 1'-keto-S-2840 ranges between 463 and 1000 d and the formation fraction from 1'-COOH-S-2840 ranges between 0.23 and 0.39.

Formation fractions for 1'-COOH-S-2840 from inpyrfluxam could be derived from the Newhaven and Woodside soils only and were 0.5348 and 0.612 respectively. For Newhaven soil, the formation fraction for 1'-keto-S-2840 is therefore calculated by multiplying the formation fractions together for both metabolites from their precursor, giving an overall formation fraction from inpyrfluxam of 0.21. As no endpoints for 1'-COOH-S-2840 could be derived from the parent applied study for other soils, a formation fraction of 1 is proposed for 1'-COOH-S-2840 from inpyrfluxam. Overall formation fractions for 1'-keto-S-2840 therefore range between 0.21 and 0.25.

Neither DFPA or N-des-Me-DFPA were observed in any soil at any time point. This confirms the results of the study with the parent substance (see data point B.7.1.1.1/01) in which N-des-Me-DFPA was present at <1.6 % AR at all time intervals in all soils. Several unknown metabolites were also detected. Total unknowns (mean value) were 35.0 % AR in the Speyer 5M soil, 20.3 % in the Newhaven soil and 27.7 % AR in the Atwater soil. The applicant states that all unknown peaks were ≤4.2 % AR. HSE has examined the sample chromatograms and can confirm that no additional significant peaks were observed.

Unresolved background was low in all soils, ranging between 0.2 and 1.6 % AR (mean values).

## VII. Isomers

Changes in enantiomeric excess have been considered in accordance with the principles outlined in the EFSA Guidance document, 'Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers' (2019). This guidance document considers changes >10 % to be potentially significant with respect to the environmental risk assessment. The amounts of 1'-COOH-S-2840A and 1'-COOH-S-2840B were recorded at each timepoint in all 3 soils and the change in the enantiomeric excess calculated. The change in the enantiomeric excess was <10 % in both the Speyer 5M and the Atwater soil. For the Newhaven soil, the change in the enantiomeric excess was <10 % at almost all timepoints, but was >10 % at 61 d, reaching -11.8 %. The value was <10 % at study end with a value of -7.0 %. At this time point, more than 50 % (but less than 90 %) degradation of the total substance has occurred and according to the EFSA Guidance document is considered relevant for risk assessment, triggering isomer-specific exposure assessments. There are no experimental artefacts indicated in the data and mass balance was acceptable.

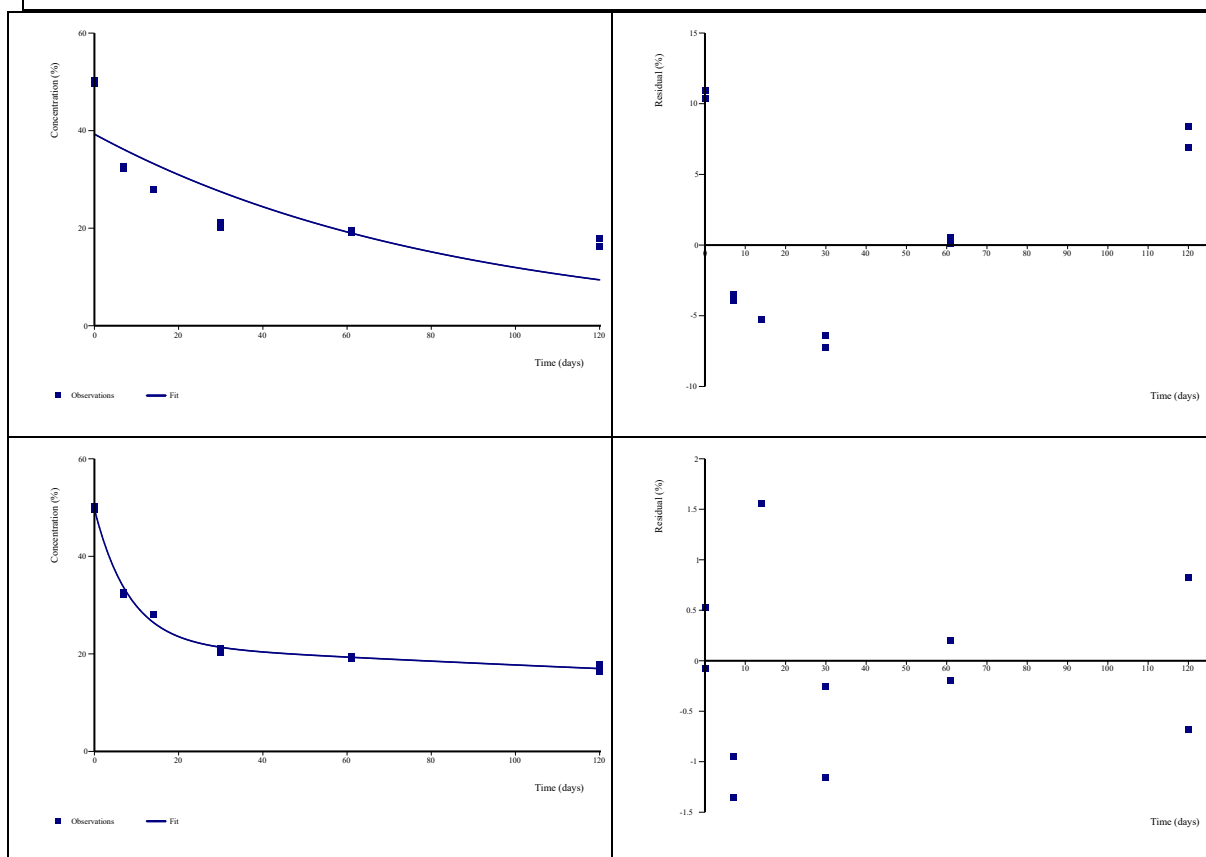
The GB guidance, ‘Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers’ recommends that the DFOP model would provide a fully mechanistic description of preferential transformation of a pair of stereoisomers, as the model consists of separate first order rate constants to describe preferential transformation rates. The g parameter would be analogous to the isomer ratio of the original active substance and for a racemic mixture, the g value could be fixed to 0.5 to reflect the 50:50 ratio of the original material. The degradation kinetics for the Newhaven soil are best described by DFOP kinetics. This was in contrast to the other soils, which are best described by SFO kinetics. In addition, the g value was 0.496 and therefore fixing the g value to 0.5 resulted in a good kinetic fit. It was therefore considered appropriate to investigate the behaviour of the different stereoisomers in this soil further.

FOCUS kinetic analysis was therefore conducted for each isomer, A and B, separately using the percent AR values given in Table B.8.1.1.1.4-06 to B.8.1.1.1.4-08. The kinetics for the individual isomers are shown below.

**Figure B.8.1.1.1.4-10 Degradation kinetics of 1'-COOH-S-2840A in Newhaven soil**

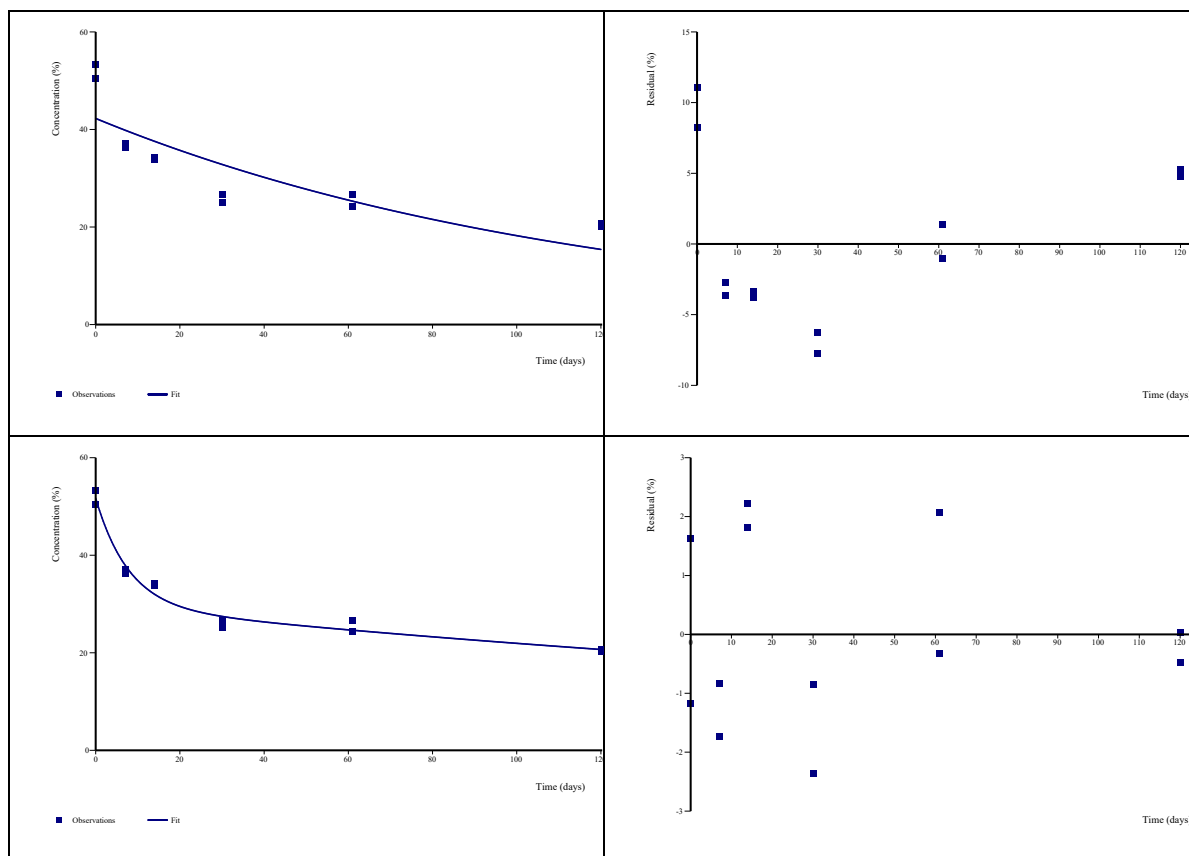
<b>1'-COOH-S-2840A, Newhaven (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	Poor	18.8	M <sub>0</sub> : 39.26 k: 0.01191	k: <0.05	58.2	193
<b>DFOP</b>	<b>Good</b>	<b>3.04</b>	<b>M<sub>0</sub>: 49.67</b> <b>k<sub>1</sub>: 0.1207</b> <b>k<sub>2</sub>: 0.002154</b> <b>g: 0.5574</b>	<b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b>	<b>16.8</b>	<b>691</b>
<p>The visual fit of the SFO model to the data set is poor, with the starting concentrations underestimated and the decline poorly represented. This is also shown in the plot of the residuals, which shows large errors. The <math>\chi^2</math> value is 18.8 %.</p> <p>The DFOP model gives a better visual fit. The starting concentrations and decline phase are well represented and the plot of the residuals shows small, random errors. The <math>\chi^2</math> value of 3.04 % is lower than for the SFO model.</p>						

**Conclusion: DFOP is the best fit model ( $DT_{50} = 16.8$  days,  $DT_{90} = 691$  days)**  
**Selected kinetics in bold**



**Figure B.8.1.1.2-11 Degradation kinetics of 1'-COOH-S-2840B in Newhaven soil**

<b>1'-COOH-S-2840B, Newhaven (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	Poor	13.8	M <sub>0</sub> : 42.26 k: 0.008411	k: <0.05	82.4	274
<b>DFOP</b>	<b>Good</b>	<b>3.82</b>	<b>M<sub>0</sub>: 51.86</b> <b>k<sub>1</sub>: 0.128</b> <b>k<sub>2</sub>: 0.002958</b> <b>g: 0.4295</b>	<b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b>	<b>45.8</b>	<b>589</b>
<p>The visual fit of the SFO model to the data set is poor, with the starting concentrations underestimated and the decline poorly represented. This is also shown in the plot of the residuals, which shows large errors. The <math>\chi^2</math> value is 13.8 %.</p> <p>The DFOP model gives a better visual fit. The starting concentrations and decline phase are well represented and the plot of the residuals shows small, random errors. The <math>\chi^2</math> value of 3.82 % is lower than for the SFO model.</p> <p><b>Conclusion: DFOP is the best fit model (DT<sub>50</sub> = 45.8 days, DT<sub>90</sub> = 589 days)</b>  <b>Selected kinetics in bold</b></p>						



Both isomers clearly decline according to DFOP kinetics. This demonstrates that the behaviour of both isomers is biphasic and that this is due to other reasons than the behaviour of individual stereoisomers.

The  $k_1$  and  $k_2$  values for the stereoisomers are compared below:

**Table B.8.1.1.4-17 Comparison of  $k$  values for 1'-COOH-S-2840A and B**

<b>K1</b>	<b>K2</b>
<b>1'-COOH-S-2840A</b>	
0.1207	0.002154
<b>1'-COOH-S-2840B</b>	
0.128	0.002958

The  $K$  values are very similar for the stereoisomers for both the fast and slow phases. This would not be the case if the stereoisomers were degrading at different rates.

The overall degradation behaviour of 1'-COOH-S-2840 (A and B combined) has also been considered in section B.8.1.3. It was determined that overall, this metabolite follows SFO kinetics which would not be the case if the stereoisomers showed

different degradation behaviour in soil. This indicates that a single exposure assessment for the sum of the isomers is appropriate.

In summary, the change in enantiomeric excess >10 %, is transient and only indicated in this one soil, while there is no evidence that the isomers are degrading in at different rates in this soil. Overall, the degradation behaviour of 1'-COOH-S-2840A and B can be described by SFO kinetics. It is not therefore considered that the change in the enantiomeric excess in this one soil is significant and HSE considers that it is appropriate to conduct a single exposure assessment for the combined stereoisomers.

## CONCLUSIONS

The test item 1'-COOH-S-2840 was ultimately degraded to bound residues with small amounts of CO<sub>2</sub> in all soils. Unextracted residues reached a maximum of 19.2 % in soil, while mineralisation to CO<sub>2</sub> was low. There was one major metabolite, 1'-keto-S-2840 which reached a maximum of 21.7 % AR (day 61, Newhaven soil) but generally increased throughout the study. Based on the maximum level of 1'-COOH-S-2840 formed in the parent applied study of 30.1 % AR, there is potential for 1'-keto-S-2840 to form at up to 6.5 % AR overall. Formation of 1'-keto-S-2840 is discussed in detail above and it was concluded that 1'-keto-S-2840 is likely to be less mobile than 1'-COOH-S-2840 and is unlikely to form at >5 % under field conditions. It is therefore considered that the environmental exposure from 1'-keto-S-2840 is covered by the exposure assessments for parent and metabolites 1'-COOH-S-2840 and 3'-OH-S-2840 and an exposure assessment for this metabolite is not required.

Between 36.5 and 53.5 % AR total 1'-COOH-S-2840 remains at study end in the metabolite applied study. Further formation of metabolites may therefore have occurred had the study been continued. In particular, metabolites N-des-Me-DFPA and DFPA were not detected, indicating that there was limited cleaving of the amide bridge. The extent to which these metabolites would form over longer timescales is therefore unclear.

### B.8.1.1.1.5. Anaerobic route of degradation of the active substance, 1 US soil

<b>Data point</b>	KCA 7.1.1.2/01
<b>Study Title:</b>	S-2399: Anaerobic Soil Metabolism
<b>Author &amp; year:</b>	██████████ (2017a)
<b>Address:</b>	Valent Technical Center (VTC), 6560 Trinity Court, Dublin, CA 94568

<b>Study No:</b>	VP-39081
<b>Applicant:</b>	Sumitomo Chemical Co. Ltd. Report No: TPM-0040
<b>Guidelines:</b>	OECD 307 Test Guideline
<b>GLP:</b>	Yes
<b>Deviations:</b>	Yes

<b>Deviations from OECD 307 guidelines</b>	<b>HSE assessment of deviations</b>
Discrepancy between soil sampling location coordinates and town name provided	Town name given is 20 miles from coordinate location. Clarification from applicant desired, however coordinates are assumed to be the accurate location. Regardless, an approximate location is known.
Soil transport containers not defined	Minor omission. Loose polyethylene bags are recommended by OECD 307 to minimise changes in soil water content. As the applicant has adjusted soil water content prior to test initiation, HSE does not consider this omission to jeopardise the study validity. Biomass measurements demonstrate that the soils are viable throughout the study, therefore HSE considers that the transport conditions are acceptable.
Soil stored at ambient temperature for three days between collection and arrival at Valent, not necessarily transported in a manner which minimises changes in soil water content, as per OECD 307 guidelines	Minor deviation. Soil was held at ambient temperatures for a short time. Furthermore, biomass measurements demonstrate that the soil is biologically viable throughout the study.
Sampling site pesticide history not provided	Major omission, however this is the same soil as used in the aerobic laboratory studies (See B.8.1.1.1.1 & 2). As degradation was comparable to the other soils, the Penn soil

	was assumed to not be metabolically adjusted to inpyrfluxam and its metabolites. Furthermore, the applicant has stated that this is a terrestrial soil where pesticides would not normally be applied.
Storage temperature at Valent test centre not provided	Minor omission. Soil is recorded as having been refrigerated, which HSE considers to be at approximately the $4 \pm 2^{\circ}\text{C}$ required by OECD 307 guidelines.
$^{14}\text{CO}_2$ trap NaOH concentration used (1 M) differs from OECD 307 guideline (2 M) during aerobic phase	Minor deviation. Mass balances demonstrate that the NaOH concentration used is suitable in this study, as mass is suitably accounted for.
28 day aerobic onset period used rather than OECD 307 guideline of 30 days, or one half-life. The shortest $\text{DT}_{50}$ is 49 days (Newhaven soil, aerobic conditions).	Minor deviation. Relatively small difference in duration of 2 days from the guideline recommendation of 30 days.
No mention of solvent evaporation, as is required by OECD 307 guideline	Minor deviation. A small volume of volatile solvent employed. Microbial biomass measurements do not show an adverse effect, and evaporation is assumed.
Dates provided are anachronous to the study method provided	Minor deviation. Applicant appears to have made a typo error with the date given for the day after dosing of the active substance. HSE was able to confer the correct timeline based on other event dates provided.
LSC LOD & LOQ not provided in same units as mass balances	LOD & LOQ expressed as % AR will be required from the applicant. This has since been provided in an RAI HSE accepts that the limits are in line with OECD 307.
No LOD or LOQ for HLPC-UV, HPLC-RAM or TLC-autoradiography	Major omission. Requires clarification from the applicant. These values have since been provided in an RAI, and the applicant has clarified that TLC has only been used



	qualitatively, and as such LOD and LOQ values have not been provided.
R <sup>2</sup> metric only used for kinetic evaluation	Minor deviation. Unsuitable, but does not affect study validity as kinetic evaluation is a separate process.
Only ranges of replicates and controls provided	Minor deviation. Preferably a table summarising treatment groups, sample numbers, and sample DAT's to be provided by the applicant.
Study exceeds OECD 307 guideline maximum duration of 120 days	Minor deviation. Anaerobic period lasts for 126 days, a minor exceedance. Furthermore, microbial biomass measurements demonstrate that the soil is still biologically active at study termination.
No organic carbon % of soil at start of study (0 DAT) provided	Minor deviation. Calculated by HSE using OM = 1.724 * %OC conversion factor.
<b>HSE conclusion on deviations</b>	
HSE considers that there are no deviations affecting study validity.	

## INTRODUCTION

An anaerobic soil metabolism study was conducted according to OECD guidelines (OECD 307: Aerobic and anaerobic transformation in soil). The transformation of radiolabelled inpyrfluxam was studied in one terrestrial US soil (Penn) under aerobic conditions for 28 days. Samples were adjusted to 50% water holding capacity (WHC) and were maintained at 50 ± 10% of the WHC throughout the aerobic phase and then anaerobic conditions for a further 127 days at 20 ± 2 °C in the dark.

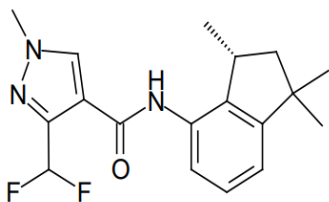
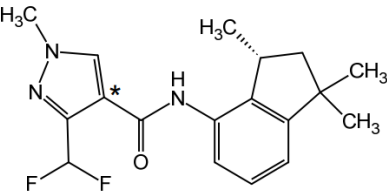
HSE notes that by OECD 307, study duration should not usually exceed 120 days. HSE notes however, that the applicant has provided biomass measurements at 122 DAT, and at study termination (155 DAT) demonstrating that biomass has not declined below 1 % of organic carbon. HSE therefore considers that biomass has been satisfactorily maintained as to support the extended study duration.

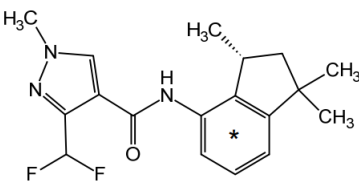
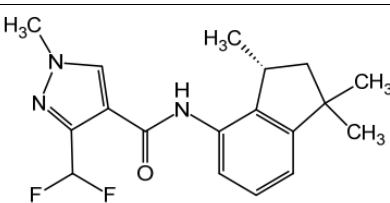
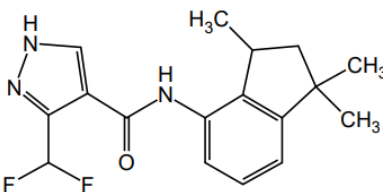
Samples were periodically collected and extracted. The soil was also used in the inpyrfluxam aerobic soil metabolism studies. Two different radiolabelled compounds of

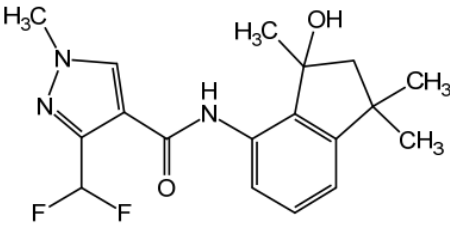
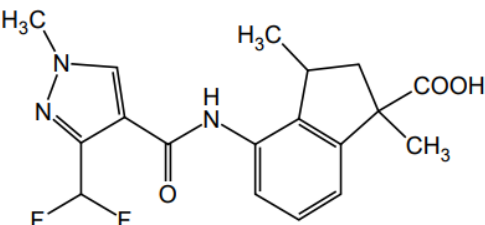
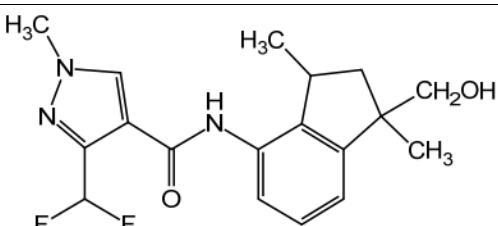
inpyrfluxam – [phenyl- $^{14}\text{C}$ ] inpyrfluxam and [pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam were used. The soil extracts were analysed by liquid scintillation counting (LSC) and 2D thin layer chromatography (TLC). In addition the samples were analysed occasionally by high-performance liquid chromatography (HPLC) to quantify and identify major radioactive components and the radioactive volatiles from the soil were quantified. The post-extraction soil (PES) was analysed by combustion analysis. The PES sample containing significant radioactivity was fractionated into fulvic acid, humic acid and humin, and the residue in each fraction was analysed by LSC and/or combustion analysis, and when appropriate, by TLC.

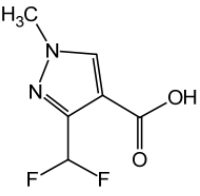
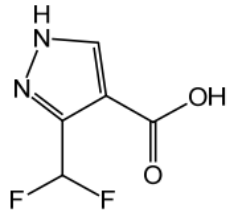
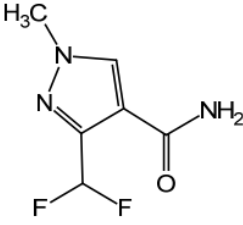
## MATERIALS

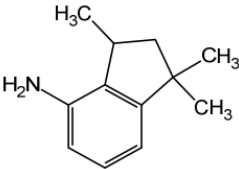
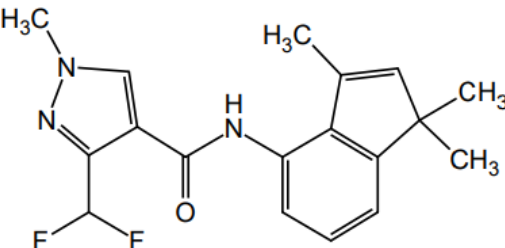
**Table B.8.1.1.5-01 Properties of the materials used in the anaerobic soil degradation study**

<b>Code name:</b>	Inpyrfluxam
<b>Chemical Purity:</b>	95.3 %
<b>Structure:</b>	
<b>1. Test Material</b>	[pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam (PYR-label)
<b>Lot/Batch:</b>	CFQ41802
<b>Specific activity:</b>	2.11 GBq/mmol
<b>Purity:</b>	Radiochemical purity 98.1 % (in the dosing solution)
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	Not stated
<b>Structure:</b>	 <p><i>* denotes <math>^{14}\text{C}</math> label position</i></p>
<b>2. Test Material</b>	[phenyl- $^{14}\text{C}$ ] inpyrfluxam (PHE-label)
<b>Lot/Batch:</b>	CFQ41803
<b>Specific activity:</b>	4.51 GBq/mmol
<b>Purity:</b>	Radiochemical purity 99.1 % (in the dosing solution)

<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	Not stated
<b>Structure:</b>	 <p><i>* denotes <sup>14</sup>C label position</i></p>
<b>3. Reference Material</b>	
<b>Code names:</b>	Inpyrfluxam (Pure 3'R isomer) S-2840 (Contains 2 enantiomers: 3'R & 3'S) S-2940 (Pure 3'S isomer)
<b>Chemical Name:</b>	N-[(3R)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2375a (inpyrfluxam Analytical Grade) (95.3 %) AS2375b (inpyrfluxam pure 3'R isomer) (99.8 %) AS2375c (inpyrfluxam Technical Grade) (95.4 %) AS2387a (S-2940 pure 3'S isomer) (99.8 %)
<b>Structure:</b>	
<b>4. Reference Material</b>	
<b>Code names:</b>	N-des-Me-S-2840 (Contains 2 enantiomers: 3'R & 3'S)
<b>Chemical Name:</b>	N-[(3RS)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2380a (97.5 %)
<b>Structure:</b>	
<b>5. Reference Material</b>	
<b>Code names:</b>	3'-OH-S-2840 (Contains 2 enantiomers: 3'R & 3'S)

<b>Chemical Name:</b>	N-[(3RS)-3-hydroxy-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamid
<b>Valent Lot Nos, Purity (%):</b>	AS2379a (99.7 %)
<b>Structure:</b>	
<b>6. Reference Material</b>	
<b>Code names:</b>	1'-COOH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-COOH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)
<b>Chemical Name:</b>	(1RS,3RS)-(1RS,3SR)-2,3-dihydro-1,3-dimethyl-4-[[1-methyl-3-(difluoromethyl)-1H-pyrazole-4-ylcarbonyl]amino]-1H-indene-1-carboxylic acid
<b>Valent Lot Nos, Purity (%):</b>	AS2393a (1'-COOH-S-2840A) (100 %) AS2394a (1'-COOH-S-2840B) (99.6 %)
<b>Structure:</b>	
<b>7. Reference Material</b>	
<b>Code names:</b>	1'-CH2OH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-CH2OH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)
<b>Chemical Name:</b>	N-[(1RS,3RS)-(1RS,3SR)-2,3-dihydro-1-(hydroxymethyl)-1,3-dimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%):</b>	AS2395a (1'-CH2OH-S-2840A) (100 %) AS2396a (1'-CH2OH-S-2840B) (99.5 %)
<b>Structure:</b>	

<b>8. Reference Material</b>	
<b>Code names:</b>	DFPA
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid
<b>Valent Lot Nos, Purity (%)</b>	AS2378a (99.2 %)
<b>Structure:</b>	
<b>9. Reference Material</b>	
<b>Code names:</b>	N-des-Me-DFPA
<b>Chemical Name:</b>	3-difluoromethyl-1H-pyrazole-4-carboxylic acid
<b>Valent Lot Nos, Purity (%)</b>	AS2381a (97.8 %)
<b>Structure:</b>	
<b>10. Reference Material</b>	
<b>Code names:</b>	DFPA-CONH <sub>2</sub>
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos, Purity (%)</b>	AS2382a (99.2 %)
<b>Structure:</b>	
<b>11. Reference Material</b>	
<b>Code names:</b>	ATMI
<b>Chemical Name:</b>	(3RS)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-amine
<b>Valent Lot Nos, Purity (%)</b>	AS2383a (99.7 %)

<b>Structure:</b>	
<b>12. Reference Material</b>	
<b>Code names:</b>	3'-OH-S-2840-dehydrate
<b>Chemical Name:</b>	3-difluoromethyl-1-methyl-N-[1,1,3-trimethyl-1H-inden-4-yl]-1H-pyrazole-4-carboxamide
<b>Valent Lot Nos,Purity (%)</b>	AS2413a (98.2 %)
<b>Structure:</b>	

HSE notes that the radiolabelling of both ring structures of the inpyrfluxam test material on either side of the likely cleavage point, the amide bridge, is recommended by the OECD 307 guidelines, therefore HSE agrees with the label positions selected by the applicant.

## METHODS

### Test Systems

The Penn series soil, collected from the top 3 inch layer of an agricultural area (GPS: [REDACTED]) was used in the study. The applicant stated that these coordinates describe the location Baptistown, NJ,. HSE notes however, that the coordinates provided describe an area approximately 20 miles from this location. This discrepancy is at odds with the accurate sample location desired by OECD 307. HSE views this as a minor deviation, as an approximate location is known.

The soils were thoroughly mixed and passed through a 2-mm mesh sieve with a minimum of air-drying. Soil collection properties are summarised in Table B.8.1.1.1.5-02, and details of the soil analysis are presented in Table B.8.1.1.1.5-03.

**Table B.8.1.1.5-02 Soil collection and storage details**

<b>Description</b>	<b>Details</b>
Geographic locations:	Penn: Baptistown, NJ: GPS [REDACTED]
Pesticide use history at the collection site:	Not available. The site was a terrestrial soil where pesticides would normally not be applied.
Collection procedures for soil:	Collected with a shovel.
Sampling depth for soil:	Top 0-3 inches of soil.
Storage conditions:	Stored at ambient temperature from the date of collection (24/8/15) until arrival at Valent (27/8/15). Sieved at Valent through 2 mm (27/8/15). Stored in a refrigerator until aerobic test system preparation (14/9/15).
Storage length:	23 days from soil collection to aerobic test system preparation.
Preparation of soil samples	The soil was sieved through a 2 mm sieve with a minimum of air drying
Soil moisture (%):	16.20
Water holding capacity (%):	49.14

Samples were adjusted to 50% WHC and were maintained at  $50 \pm 10\%$  of the WHC throughout the aerobic phase of the study. Following moisture adjustment, and prior to treatment with the test substance, all soil samples for this study were acclimated in the aerobic test system for 7 days prior to dosing. Soil microbial biomass was determined after sieving and on control samples around dosing, during, and at the end of the anaerobic phase.

HSE notes that no details of vegetation cover, treatments with chemicals, treatments with organic and inorganic fertilisers, additions of biological materials or other contamination regarding the soil collection site have been made available, as is required in OECD 307. HSE considers this a major deviation which potentially jeopardises the validity of the test, as the soil microbes may be adapted to metabolise inpyrfluxam through previous exposure to similar compounds. This will require clarification from the applicant. The applicant has since addressed this request in an RAI, stating that “Due to the changing nature of the fields used for the study a pesticide history is unavailable.” HSE considers that this soil was also used in the aerobic route and rate studies, and did not produce outlying results when

compared to other soils. This suggests that the soil microbes are not specifically adapted to degrade inpyrfluxam and its metabolites through previous exposure. HSE also notes that the applicant has stated that this is a terrestrial soil, where pesticides would not normally be applied. HSE therefore accepts this soil even though a pesticide history is not provided.

HSE notes that no mention of weather conditions preceding the soil collection are made, as should be done so by OECD 307 guidelines if long periods of drought, freezing or flooding occurred. It is therefore assumed that these conditions did not occur preceding soil collection.

HSE notes that the applicant has not provided information on the containers used to transport the soil. OECD 307 recommends the use of loosely tied polythene bags to prevent changes in soil moisture content. HSE considers this as a minor deviation, as the applicant has adjusted the soil moisture content prior to test initiation.

HSE notes that the applicant has stated that the soil was stored in a refrigerator from study initiation until aerobic test system preparation, however the applicant has not stated the temperature of storage. By OECD 307 guidelines this should be at  $4 \pm 2^{\circ}\text{C}$ . The HSE views this omission as only a minor deviation, as refrigeration implies this temperature, and GLP is assured.

**Table B.8.1.1.1.5-03 Soil properties**

<b>Soil characteristic</b>	<b>Penn Series soil</b>
USDA Particle size distribution	
% sand (50 µm - 2 mm)	28
% silt (2 µm - 50 µm)	48
% clay <2 µm	24
pH (H <sub>2</sub> O)	6.1
% Moisture at 1/3 Bar	26.0
Maximum water holding capacity ( %)	48.1 ± 1.7
Cation exchange capacity (meq/100g)	8.8
% Organic carbon (Walkley Black)	Not provided
% Organic Matter (Walkley Black)	2.3
Disturbed bulk density (g/cm <sup>3</sup> )	1.04
USDA Textural class	Loam
Microbial Biomass Carbon (mg/kg dry weight)	732



HSE notes that the applicant has not provided a value for organic carbon % at 0 DAT, as is required by OECD 307 guidelines. HSE notes however that  $OC = OM / 1.724$ , and therefore OC is calculated to be 1.3 % at 0 DAT.

### **Incubation apparatus**

The test system simulates the circumstances where the test substance is applied to a terrestrial soil that becomes waterlogged and in which anaerobic conditions therefore develop. Initially, the individual soil samples (Treatments A-E as described in Table B.8.1.1.1.5-05) were incubated in an aerobic test system as described. The aerobic soil study with Penn soil produced a half-life greater than 30 days. 28 days was used as the maximum period of aerobic incubation. At 28 days the soil samples were transferred to biometers and waterlogged, the systems purged with nitrogen and anaerobic conditions allowed to develop. Samples were analysed during the 28 day aerobic phase and the 127 day anaerobic phase (total study: 155 days)

HSE notes that OECD 307 guidelines recommend that an aerobic period of 30 days should be maintained before anaerobic conditions are initiated when the test substance  $DT_{50}$  is longer than 30 days (as is the case here). The applicant has used a 28 day onset period, and has not provided reasoning for this deviation. HSE views this as a minor deviation due to the relatively small difference in duration from the guideline 30 days and so is accepted by HSE on this occasion.

### **Aerobic test system**

The incubation apparatus consists of an airtight chamber with air inlet and outlet valves. Soil samples (wet weight equivalent to 50 g dry weight, adjusted to ca. 50% WHC) were placed inside the chamber in open individual 100 mL glass beakers. The soil moisture was adjusted periodically to  $50 \pm 10\%$  of the water holding capacity during the aerobic incubation by adding HPLC grade water as needed. One open Petri dish, containing 50 mL of 1.0 M NaOH solution was placed inside the chamber to capture evolved  $^{14}CO_2$ . Several open Petri dishes full of water were also placed inside the chamber to maintain high air humidity. Chamber air was continuously evacuated through the chamber air outlet, which was connected to a vacuum source, at a steady rate of approximately 10 mL per minute. The chamber inlet air was bubbled through a water trap to humidify the air before entering the chamber. The continuous airflow was used to maintain aerobic conditions as well as to evacuate any radiolabelled volatiles into the outlet traps. The evacuated air passed through a tetraglyme trap to capture any organic  $^{14}C$  volatiles present in the chamber's air followed by two consecutive 1.0 M NaOH traps to capture any  $^{14}CO_2$  present in the chamber's air. Before opening the chamber for each sampling, airflow was increased for a length of time sufficient to evacuate any lingering volatile degradation products through the traps. The incubation apparatus was incubated in the dark at  $20 \pm 2^\circ C$ .

HSE notes that the applicant has used 1.0 M NaOH in the internal and external traps in the aerobic test system. This is a deviation from the OECD 307 guideline of 2.0 M. This is considered as a minor deviation as the applicant has provided suitable mass balances, suggesting that the use of 1.0 M NaOH has not affected the study validity.

### Anaerobic test system

After the aerobic phase, each individual soil was quantitatively transferred (with 80 mL of N<sub>2</sub> purged HPLC water) into each of the biometer flasks, this gave waterlogged soils with ca. 2 - 3 cm water layer. Dried ground alfalfa was added to each biometer at a rate of 0.1% (0.05 g) of the soil dry weight to speed the formation of anaerobic conditions and to ensure the survival of the microbial anaerobes. The <sup>14</sup>CO<sub>2</sub> side-arm trap was charged with 25 mL of 2.0 M NaOH solution (N<sub>2</sub> purged). The biometers were assembled and sparged with a stream of nitrogen for approximately 0.25 hours to facilitate the formation of anaerobic conditions in the test system. Following sparging, the flasks were sealed and incubated in the dark at 20 ± 2 °C. The amount of evolved <sup>14</sup>CH<sub>4</sub> was based on the oxidation of a known volume of the headspace gas and the total headspace.

**Table B.8.1.1.1.5-04 Experimental parameters**

<b>Parameter</b>		<b>Experimental details</b>
Duration of the test:		28 days aerobic, 127 days anaerobic (total study 155 days)
Soil condition: (Air dried/fresh)		Fresh
Soil: (g/replicate)		50 g (dry weight)
Target test concentrations:		0.696/ 0.676 ppm (µg per g of dry soil) Based on use rate of 0. 192 lb a.i./ acre, equivalent to 215 g a.s/ha, 1.238 mg/kg assuming 2.5 cm soil depth and 1.44 g/cm <sup>3</sup> soil density
Experimental conditions:	Incubation conditions	Incubated in the dark at 20 ± 2°C
	Moisture content	50 ± 10 % of the water holding capacity
	Moisture maintenance method	Weigh sample to verify moisture levels and add required water
	Any indication of the test material adsorbing to the walls of the test apparatus:	No

No. of Replication:	Controls, if used	3-4
	Treatments	1-2
Aerobic test apparatus:		50 g of soil (dry) incubated in a 100mL glass beaker with metal spatula. Incubated in closed, continuous flow, negative pressure incubation apparatus
Traps for CO <sub>2</sub> :		CO <sub>2</sub> : 1 trap of 50 mL 1.0 M NaOH in Petri dishes inside apparatus, 2 traps each 50 mL 1.0 M NaOH outside apparatus.
Anaerobic Test apparatus:		50 g of soil (dry) plus 80 mL of HPLC water incubated in a closed custom glass biometer.
Identity and concentration of co-solvent:		Acetonitrile, neat (~19.10 M)
Test material application	Volume of test solution used for treatment	250 µL
	Application method	applied on surface with syringe then 5 minutes of homogeneous mixing
	Co-solvent	Evaporated

HSE notes that while a range for ‘number of control’ and ‘number of treatment’ have been provided, actual values for each group have not been provided. HSE regards this as a minor deviation from OECD 307 guidelines. HSE requests that the applicant provides a table summarising treatment group, sample numbers, and sample DAT’s.

### Treatment groups

**Treatment A:** Untreated Controls. Samples were used to measure the microbial biomass. Control samples were analysed around the time of test substance application (i.e., aerobic phase time zero). Other samples were analysed around the end of sampling (i.e., at the last anaerobic phase sampling interval).

**Treatment B:** Organic solvent controls. Samples were used to measure the effect of the administered solvent on microbial biomass at the end of sampling (i.e., at the last anaerobic phase sampling interval).

**Treatment C:** Non-radiolabelled inpyrfluxam samples (treated with non-radiolabelled S2399 (AS2375a)) were dosed at 0.662 ppm (33.1 µg/50 g dry weight soil). Samples were used to measure the effect of non-radiolabelled inpyrfluxam and solvent on microbial biomass at the end of sampling (i.e., at the last anaerobic phase sampling interval).

**Treatment D:** Penn soil. Consists of the test substance [phenyl-<sup>14</sup>C] inpyrfluxam dosed at 0.696 ppm (34.8 µg/50 g dry weight soil, 27,937,900 dpm). This treatment was used to determine the test substance dissipation rate during the aerobic and anaerobic phases, and its [<sup>14</sup>C]-metabolite formation. Volatile production of <sup>14</sup>CO<sub>2</sub> was evaluated during the aerobic phase. Volatile production of <sup>14</sup>CH<sub>4</sub> and <sup>14</sup>CO<sub>2</sub> were evaluated during the anaerobic phase. Two 10X dosing samples were included with the 18 samples with 6.66 ppm (333 µg/50 g dry weight soil, 28,069,100 dpm).

**Treatment E:** Penn soil. Consists of the test substance [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam dosed at 0.676 ppm (33.8 µg/50 g dry weight soil, 12,769,400 dpm). This treatment was used to determine the test substance dissipation rate during the aerobic and anaerobic phases, and its [<sup>14</sup>C]-metabolite formation. Volatile production of <sup>14</sup>CO<sub>2</sub> was evaluated during the aerobic phase. Volatile production of <sup>14</sup>CH<sub>4</sub> and <sup>14</sup>CO<sub>2</sub> were evaluated during the anaerobic phase. Two 10X dosing samples were included with the 18 samples with 6.6 ppm (332 µg/50 g dry weight soil, 13,016,200 dpm).

### Test substance application rate

Beakers containing 50 g (dry weight) of soil were dosed with about 34.8 µg of [phenyl-<sup>14</sup>C] inpyrfluxam or 33.8 µg of [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam. This corresponds to an application rate of approximately 215 g a.s / hectare, representing two 100 g / ha foliar treatments, and one 15 g / ha seed treatment.

The application groups and rates are summarised below in Table B.8.1.1.1.5-05.

**Table B.8.1.1.1.5-05 Summary of treatment groups**

<b>Treatment group</b>	<b>Dosing substance</b>	<b>Dosing rate (ppm)</b>	<b>Sample type</b>	<b>Purpose</b>
A	NA	NA	Untreated control	Measure microbial biomass throughout study
B	Acetonitrile	NA	Organic solvent control	Measure effect of solvent on microbial biomass
C	Non-radiolabelled inpyrfluxam samples	0.662	Active substance in organic solvent control	Measure effect of solvent and inpyrfluxam on microbial biomass
D	[phenyl- <sup>14</sup> C] inpyrfluxam	0.696	Active substance	Route determination
E	[pyrazolyl- <sup>14</sup> C] inpyrfluxam	0.676	Active substance	Route determination

**Control substance application** (treatment C), since the purpose of the control substance treatment was to verify microbial activity under conditions similar to those of the test substance, the control substance was applied at approximately the same molecular rate as the <sup>14</sup>C-labeled test substance. Beakers containing 50 g (dry weight) of soil were dosed with about 33.1 µg of inpyrfluxam. The dosing solution was made to deliver 33.1 µg of inpyrfluxam to each test sample in 250 µL of acetonitrile co-solvent.

#### **Test substance application**

After aerobic acclimation (7 days), the test substances were added to the aerobic test systems at the concentrations specified in Table B.8.1.1.1.5-04. The test substances were delivered in 250 µL of acetonitrile then applied drop wise to the soil surface with a syringe, and the soil mixed with a metal spatula for 5 minutes . HSE notes that no

mention of solvent evaporation is made by the applicant, as suggested by OECD 307 guidelines. HSE notes however, that end of study microbial analysis has demonstrated that the solvent has not had a detrimental effect on the microbial biomass, compared to the blank control. Therefore HSE considers this a minor deviation which does not void the study validity.

The control samples (A, B and C) were placed in common incubation chambers. The treatment samples (D and E) were placed in separate incubation chambers.

### Test dates

HSE has prepared a chronological table below, of study dates collated from the KCA 7.1.1.2/01 submission.

**Table B.8.1.1.5-06 Chronological study dates from KCA**

<b>Date (DD/MM/YYYY)</b>	<b>Event number</b>	<b>Event</b>	<b>KCA page</b>
24/08/2015	1	Soil collection date	29
27/08/2015	2	Arrived at Valent	29
27/08/2015	3	Seived at 2mm at Valent	29
31/08/2015	4	Dosing (inferred from 7)	48
01/09/2015	5	Study initiation date	6
01/09/2015	6	Experimental start date	6
01/09/2015	7	On 9/1/15, one day after dosing [applicant used US date format], replicate untreated controls (Treatment A) were combined and sent to Agvise for microbial analysis	48
14/09/2015	8	Aerobic test systems prepared	29

<b>Date (DD/MM/YYYY)</b>	<b>Event number</b>	<b>Event</b>	<b>KCA page</b>
25/01/2016	9	(126 DAT, after the redox potential of the soil/water had turned negative) replicate untreated controls (Treatment A) were sent to Smithers Viscient for anaerobic soil microbial biomass carbon	48
24/02/2016	10	(156 DAT, after the last sampling) replicate untreated controls (Treatment A) and treated controls (Treatment B - solvent controls; Treatment C - cold inpyrfluxam treated controls) were combined in three separate containers and were sent to Smithers Viscient for anaerobic soil microbial biomass carbon.	48
17/11/2017	11	Experimental termination date	6
22/03/2017	12	Study completion date	6

HSE notes that the dates provided do not appear to match the method provided. Notably event (8) is recorded as occurring after event (4). This is anachronous to the applicants statement that “soil samples for this study were acclimated in the aerobic test system for 7 days prior to dosing”. HSE considers the correct date of dosing to be 21/09/2015 as is inferred from subsequent events (9) and (10) (126 DAT and 156 DAT, respectively). This dosing date also aligns with a 7 day aerobic acclimation period up of the soils, which starts on 14/09/2015 (event 8). Therefore, HSE considers the date of the event given for (7) as a typo.

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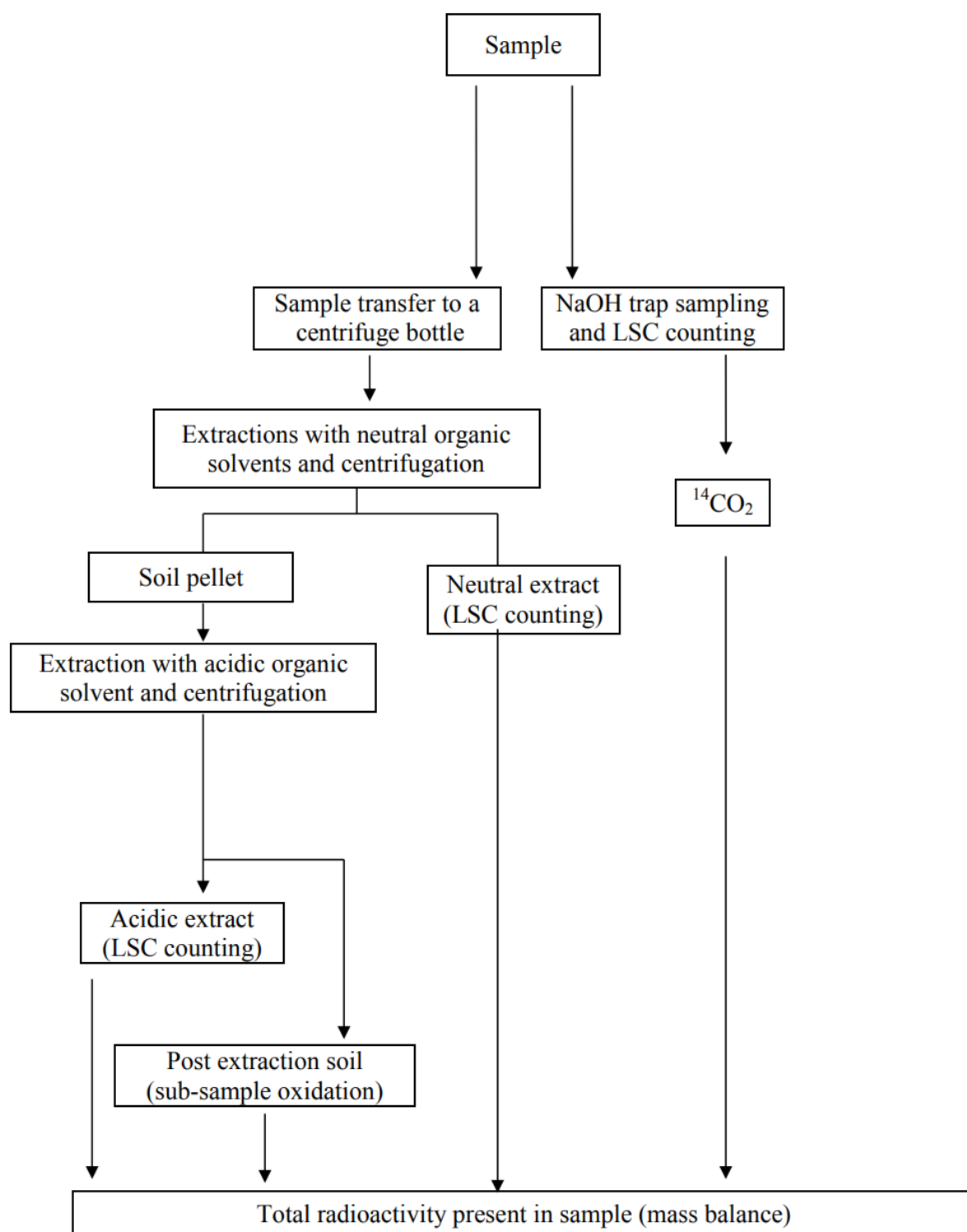
**Preparation of [ $^{14}\text{C}$ ] inpyrfluxam dosing solutions**

The radiochemical purity of the dosing solutions were determined to be 98.27 % and 97.70 % for the [phenyl- $^{14}\text{C}$ ] inpyrfluxam and 95.82 % and 96.05 % for the [pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam test systems respectively, before and after dosing using HPLC.

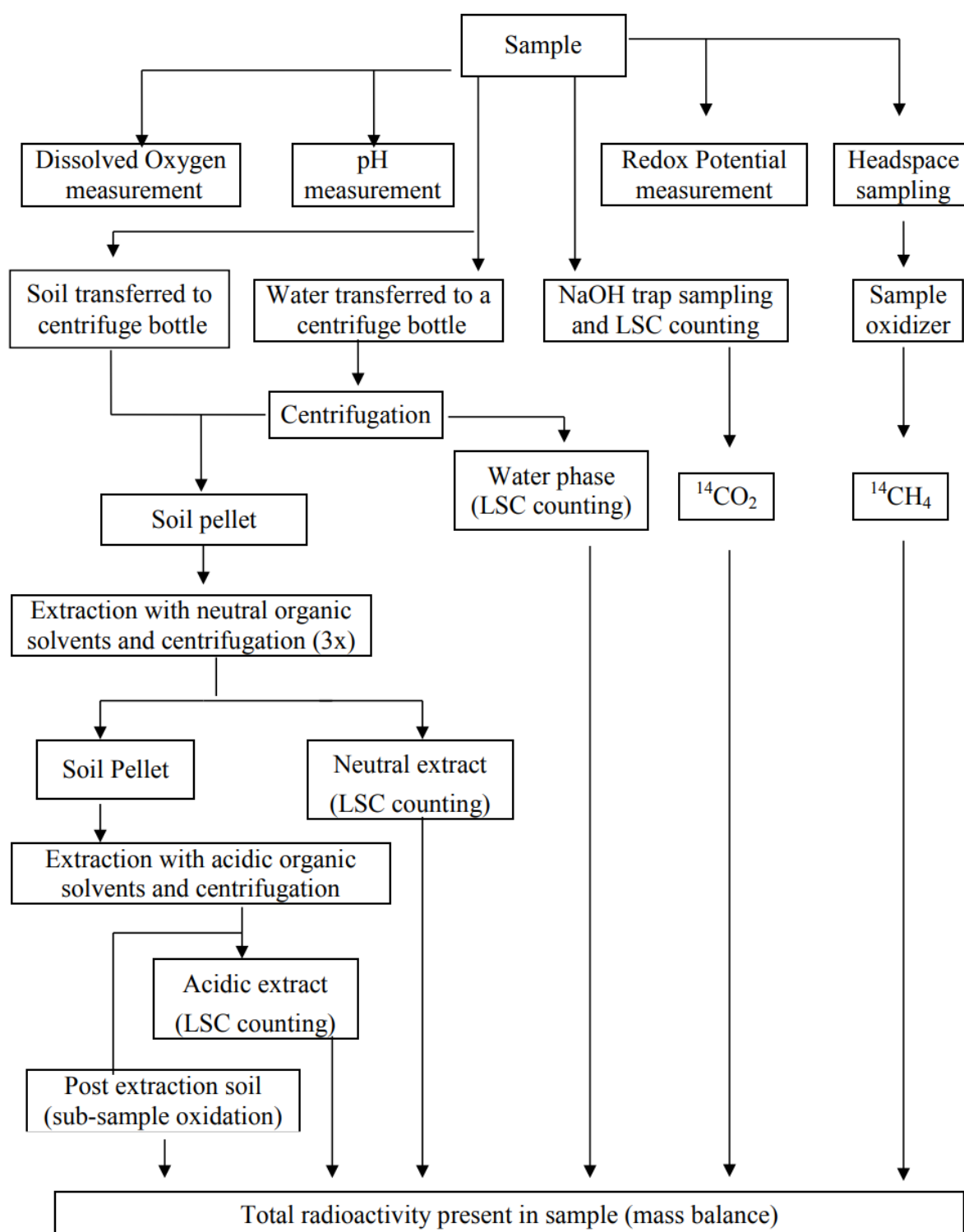
**Sampling, sample processing and analysis**

Duplicate samples were removed for analysis according to the following schedule: Treatment D and E samples were analysed at 0, 28, 42, 57, 85, 122 and 155 days after dosing (aerobic phase – 0 to 28 DAT; anaerobic phase – 28 to 155 DAT); aerobic samples were measured for  $^{14}\text{CO}_2$  production and then extracted with organic solvents. Anaerobic samples were measured for  $^{14}\text{CH}_4$  production, dissolved oxygen concentration, redox potential, and pH in that order at the time of analysis. Samples were analysed immediately after removal from the incubator and were not stored prior to any of the physical-chemical property or chemical analyses. Flowchart summaries of the aerobic and anaerobic sample processing procedures are presented below in Figures B.8.1.1.1.5-01 and B.8.1.1.1.5-02.





**Figure B.8.1.1.5-01 Summary of aerobic sample processing procedure**



**Figure B.8.1.1.5-02 Summary of anaerobic sample processing procedure**

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**Headspace sampling and analysis ( $^{14}\text{CH}_4$  quantification, anaerobic stage only)**

The biometer flask was removed from the incubator and a graduated Gastight™ syringe (50 mL) was used to withdraw a headspace sample through the sampling valve. The headspace sample was immediately injected into a Harvey Biological Oxidizer and the radioactivity was quantified using LSC. Total radioactivity in each biometer headspace was calculated based on the radioactivity in the oxidized sample and the total headspace volume of the flask. Measured radioactivity (dpm) was converted to percent of applied radioactivity and ppm inpyrfluxam equivalents.

**Anaerobic test system - verification of anaerobic conditions**

Anaerobic conditions for each sample were verified by measuring dissolved oxygen and redox potential. Following headspace sampling, the biometer flask was opened and a dissolved oxygen electrode was inserted into the water phase of the soil/water mixture and the dissolved oxygen reading was recorded. Following dissolved oxygen measurement, a redox electrode was placed into the water phase and then the soil phase of the soil/water mixture and the readings of the electrical potential recorded. A combination pH electrode was used to measure the pH of the water and soil, separately.

**Anaerobic test system - NaOH traps**

The volume of each NaOH solution in the side-arm trap was measured and three aliquots removed for LSC. Radioactivity present in each trap was determined and evolved  $^{14}\text{CO}_2$  was calculated as a percentage of applied radioactivity and ppm inpyrfluxam equivalents.

**Anaerobic test system - separation of water and soil phases**

The water from each biometer was carefully decanted into a pre-weighed 250 mL centrifuge bottle (pore water stays with the soil). The bottle was capped and centrifuged at 7,000 RPM for 10 min. The water phase was carefully decanted into a pre-weighed glass bottle. The bottle was weighed to determine the water volume and aliquots taken for LSC. Water and soil phases were analysed separately

**Anaerobic test system - water analysis**

The water phase was analysed directly by HPLC-RAM and/or TLC-autoradiography to determine radioactivity.

**Anaerobic test system - soil analysis**

The soil remaining in the biometer was transferred (with solvent) into the pre-weighed 250 mL centrifuge bottle that the water phase was previously decanted out of. The

remaining soil was extracted with a neutral organic solution to yield extract #1 (acetone once and 3:2 acetone:water (v/v) twice) and then extracted once with an acidic organic solution to yield extract #2 (60:40:1 acetone:water:HCl (v/v/v)).

### **Neutral extract**

The soil sample in the centrifuge bottle was extracted with 150 mL of acetone. The centrifuge bottle was shaken for 20 minutes (on a reciprocal shaker) and sonicated for 5 minutes. The phases were separated by centrifugation (15 minutes at 7,000 RPM). The supernatant was decanted into a graduated cylinder through a glass funnel loosely packed with glass wool. The extraction was repeated two more times with 3:2 acetone:water (v/v) and centrifuged for 15 - 25 minutes. The three supernatants combined in the graduated cylinder (neutral extract). The volume of the combined neutral extracts was measured and aliquots were removed from the extracts for LSC. The neutral extracts were rotary evaporated at 35 °C to dryness. The remaining residues were dissolved in acetonitrile/water (1/1, v/v) and transferred into a small vial. An ultrasonic bath was used to help dislodge and solubilise the residues. The final volumes were measured and an aliquot was removed for LSC. The extracts were analysed by HPLC-RAM and/or TLC-autoradiography.

### **Acidic extract**

The remaining soil pellet was re-suspended in 150 mL of 60:40:1 acetone:water:HCl (v/v/v) and the sample was shaken for 20 minutes (reciprocal shaker) and sonicated for 5 minutes. The phases were separated by centrifugation (15 to 25 minutes at 7,000 RPM). The supernatant was decanted into a graduated cylinder through a glass funnel loosely packed with glass wool (acidic extract). The volume of each extract was measured and aliquots were removed from each extract for LSC.

The acidic extracts were transferred into round bottom flasks and organic portion removed using rotary evaporation under vacuum and at  $\leq 35$  °C. The remaining aqueous portion was partitioned with 75 mL ethyl acetate three times. The volume of the aqueous and organic phases was measured in graduated cylinders and aliquots taken for LSC. The ethyl acetate was rotary evaporated to dryness at  $\leq 35$  °C. The remaining residues were dissolved in acetonitrile/water (1/1, v/v) and transferred into a small vial. The concentrated extract volumes were measured and their radioactivity determined by LSC. The extracts were analysed by HPLC-RAM and/or TLC-autoradiography. The PES was weighed and sub-samples were combusted using a sample oxidizer and its content of radioactivity was determined by LSC.

### **Potential for isomerisation**

The potential isomerisation from [ $^{14}\text{C}$ ] inpyrfluxam (both labels) was evaluated using chiral HPLC analysis on the extracts obtained from the 155 DAT samples and on the

test substances prior to application. Only inpyrfluxam *R-isomer* was determined at 155 DAT, meaning that no isomerisation took place.

### Detection limits

Limits of detection and quantification (LOD and LOQ) for LSC were determined as 10 dpm and 42 dpm respectively. The applicant did not previously provide limits expressed as % AR, however this has now been addressed by the applicant following an RAI.

The LOD for LSC is reported as << 1% AR, and for LOQ; 0.02 % AR for acidic extracts, 0.06 % AR for neutral extracts and 0.01 % AR for water extracts (pyrazolyl label); 0.01 % AR for acidic extracts, 0.03 % AR for neutral extracts and 0.01 % AR for water extracts (phenyl label).

The LOD and LOQ for TLC-radio chromatography and HPLC were not previously provided and requested in the RAI. This has now been addressed by the applicant. For HPLC-RAM, the applicant stated that LOD varied by matrix and analyst judgement was used. However, what was detectable was generally clearly distinguishable from background, in this case << 1 % AR. For LOQ, these were reported as 0.45 % AR for acidic extracts, 0.37 % AR for neutral extracts and 0.74 % AR for water extracts in the phenyl label. In the pyrazolyl label, these were 0.20 % AR for acidic extracts, 0.40 % AR for neutral extracts and 0.60 % AR for water extracts. No LOD and LOQ values were reported for HPLC-UV and TLC as the applicant stated these were used for qualitative confirmation of the compound identities only. HSE accepts that the limits are in line with OECD 307, along with the applicant's justification that no limits are required for HPLC-UV.

## RESULTS AND DISCUSSION

**Table B.8.1.1.1.5-07 Microbial biomass throughout the study**

Soil	Microbial Analysis				
Penn	1 DAT <sup>1</sup>	122 DAT <sup>2</sup>	156 DAT <sup>2</sup>		
	Treatment A	Treatment A	Treatment A	Treatment B	Treatment C
	732 (5.5% <sup>3</sup> )	1.81%	1.00%	1.25%	1.46%

<sup>1</sup>Microbial Biomass Carbon Analysis (µg carbon/g soil, dry basis).

<sup>2</sup>Microbial Biomass as % organic carbon at 122 DAT and Study Termination by Fumigation/Extraction

<sup>3</sup>Calculated by HSE

HSE notes that the result at 1 DAT was not expressed by the applicant as a percentage of OC, and that a value for OC % in soil has not been given either. This value is required to be presented according to the OECD 307 guidelines, however, HSE has calculated a value from information provided by the applicant.

HSE does note that OC is equal to OM divided by 1.724 . Therefore, HSE has calculated OC from the OM value of 2.3 % given by the applicant, giving OC as 1.3 % of soil. Microbial biomass can then be calculated as a % of OC for 1 DAT, giving 5.5 %. HSE notes that this value suggests a decline in biomass over study duration, however, biomass does not decrease below 1 % of OC. Therefore HSE considers that the soils have remained microbially active throughout the study.

**Table B.8.1.1.1.5-08 Dissolved oxygen, redox potential, and pH measurement averages - Penn - phenyl label**

<b>DAT</b>	<b>0</b>	<b>28</b>	<b>42</b>	<b>57</b>	<b>85</b>	<b>122</b>	<b>155</b>
<b>Days after anaerobic changeover</b>	<b>-</b>	<b>0<sup>1</sup></b>	<b>14</b>	<b>29</b>	<b>57</b>	<b>94</b>	<b>127</b>
pH (water phase)	NA	NA	7.05	7.21	7.75	8.64	8.09
pH (soil phase)	NA	NA	6.69	7.06	7.54	8.14	7.93
Dissolved Oxygen (ppm)	NA	NA	1.20	0.58	0.34	0.28	0.30
Redox potential H <sub>2</sub> O (mV)	NA	NA	204	94	161	-108	-57
Redox potential Soil (mV)	NA	NA	192	70	91	-161	-115

<sup>1</sup>The changeover from aerobic to anaerobic was made at 28 DAT. NA = Not analysed. (Aerobic phase)

**Table B.8.1.1.1.5-09 Dissolved oxygen, redox potential, and pH measurement averages - Penn pyrazolyl label**

<b>DAT</b>	<b>0</b>	<b>28</b>	<b>42</b>	<b>57</b>	<b>85</b>	<b>122</b>	<b>155</b>
<b>Days after anaerobic changeover</b>	<b>-</b>	<b>0<sup>1</sup></b>	<b>14</b>	<b>29</b>	<b>57</b>	<b>94</b>	<b>127</b>
pH (water phase)	NA	NA	6.97	7.28	7.24	8.24	8.34
pH (soil phase)	NA	NA	6.55	6.90	7.33	7.85	7.95
Dissolved Oxygen (ppm)	NA	NA	0.37	0.55	0.28	0.35	0.14
Redox potential H <sub>2</sub> O (mV)	NA	NA	222	125	163	-51	-129
Redox potential Soil (mV)	NA	NA	196	126	111	-85	-198

<sup>1</sup>The changeover from aerobic to anaerobic was made at 28 DAT. NA = Not analysed. (Aerobic phase)

**Table B.8.1.1.5-10 Distribution and mass balance (%) of applied radioactivity of [Phenyl-<sup>14</sup>C] inpyrfluxam in extractable, soil-bound and volatile fractions of soil sample (anaerobic conditions initiated after 28 DAT)**

Fraction	Days After Treatment (DAT)											
	0			28			42			57		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg	1	2	Avg
Water Phase	NA	NA	NA	NA	NA	NA	10.3	9.7	10	10.7	9.7	10.2
<b>Soil extracts</b>												
Neutral Extract	99.5	100.8	100.1	87	91.2	89.1	74.6	78.1	76.3	69.6	72.6	71.1
Acidic Extract	0.6	0.6	0.6	7.8	8.3	8.1	10.1	9.8	10	12.9	12.3	12.6
Total Extractable	100.1	101.3	100.7	94.7	99.5	97.1	84.7	88	86.3	82.5	84.9	83.7
PES (soil-bound)	0.1	0.1	0.1	5.6	4.6	5.1	4.9	4.6	4.8	5.8	5.9	5.8
<b>Volatiles</b>												
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Biometer/beaker rinse	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0

Fraction	Days After Treatment (DAT)											
	0			28			42			57		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg	1	2	Avg
Total (Mass balance; %)	100.2	101.5	100.8	100.6	104.3	102.5	100.2	102.7	101.4	99.3	100.8	100.1

NA: not analysed

Table B.8.1.1.1.5-10 continued

Fraction	Days After Treatment (DAT)								
	85			122			155		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg
Water Phase	11.2	11	11.1	11.3	11.8	11.5	12	11.2	11.6
<b>Soil extracts</b>									
Neutral Extract	60.8	59.6	60.2	63	64.6	63.8	59.9	66.6	63.3
Acidic Extract	14.9	15.3	15.1	19.7	16.6	18.2	19.3	15.6	17.4
Total Extractable	75.8	74.9	75.4	82.8	81.2	82	79.2	82.2	80.7



PES (soil-bound)	5.6	5.7	5.6	7.2	6.8	7	7	6.5	6.7
<b>Volatiles</b>									
$^{14}\text{CO}_2$	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
$^{14}\text{CH}_4$	0	0	0	0	0	0	0	0	0
Biometer/beaker rinse	0	0	0	0	0	0	0	0	0
Total (Mass balance; %)	93	92	92.5	101.6	100.2	100.9	98.6	100.4	99.5

**Table B.8.1.1.5-11 Distribution and mass balance as percent of applied radioactivity of [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam in extractable, soil-bound and volatile fractions of soil sample (anaerobic conditions initiated after 28 DAT)**

Fraction	Days After Treatment (DAT)											
	0			28			42			57		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg	1	2	Avg
Water Phase	NA	NA	NA	NA	NA	NA	10.9	10.7	10.8	11	9.7	10.3
<b>Soil extracts</b>												
Neutral Extract	97.5	100.7	99.1	86.1	86.7	86.4	76.1	76.1	76.1	69.6	72.6	71.1
Acidic Extract	0.6	0.6	0.6	8.4	8.3	8.3	10.8	10.1	10.5	12.9	12.3	12.6
Total Extractable	98.1	101.3	99.7	94.5	95	94.8	86.9	86.2	86.5	82.5	84.9	83.7
PES (soil-bound)	0.1	0.1	0.1	4.4	4.7	4.5	4.9	4.5	4.7	5.5	5.6	5.5
<b>Volatiles</b>												
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Biometer/beaker rinse	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0

Total (Mass balance)	98.1	101.3	99.7	99	99.8	99.4	102.7	101.5	102.1	99.3	103.1	101.2
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NA: not analysed

Table B.8.1.1.5-11 continued

Fraction	Days After Treatment (DAT)								
	85			122			155		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg
Water Phase	9.3	13.1	11.2	12.5	13.2	12.8	12.5	12.1	12.3
<b>Soil extracts</b>									
Neutral Extract	69.2	65	67.1	66.6	64.7	65.7	62.1	59.2	60.7
Acidic Extract	15.2	15.8	15.5	14	16.5	15.2	18.8	19.4	19.1
Total Extractable	84.4	80.8	82.6	80.6	81.2	80.9	80.9	78.7	79.8
PES (soil-bound)	5.2	5.3	5.3	5.3	6.1	5.7	6.4	6.3	6.4
<b>Volatiles</b>									
<sup>14</sup> CO <sub>2</sub>	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<sup>14</sup> CH <sub>4</sub>	0	0	0	0	0	0	0	0	0

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Biometer/beaker rinse	0	0	0	0	0	0	0	0	0
Total (Mass balance)	99	99.3	99.2	98.5	100.6	99.6	99.9	97.3	98.6

**Table B.8.1.1.5-12 Radioactivity distribution of the soil samples treated with [Phenyl-<sup>14</sup>C] inpyrfluxam (anaerobic conditions initiated after 28 DAT) as percent of applied radioactivity (neutral and acidic soil and water extracts combined)**

Fraction	Days After Treatment (DAT)											
	0			28			42			57		
	1	2	Avg	1	2	Avg	1	2	Avg	1	2	Avg
ATMI	0	0	0	0	0	0	0	0	0	0	0	0
1'-CH <sub>2</sub> OH-S-2840 (total)*	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840 (total)**	0	0	0	10.6	12	11.2	10.3	10.1	10.2	10	10.6	10.3
3'-OH-S-2840***	2.6	3.9	3.2	9.1	9.8	9.4	9	8.3	8.6	8.1	8.5	8.3
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0
Inpyrfluxam	96.9	96.9	96.9	74.7	76.6	75.6	75	78.4	76.7	73.9	74.4	74.1
Unknown A	0	0	0	0	0	0	0.2	0.3	0.3	0.4	0.4	0.4
Other unknowns	0	0	0	0	0	0	0	0	0	0	0	0

Total	99.5	100.8	100.1	94.3	98.4	96.4	94.5	97	95.8	92.4	94	93.2
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Table B.8.1.1.1.5-12 continued

Fraction	Days After Treatment (DAT)								
	85			122			155		
	1	2	Avg	1	2	Avg	1	2	Avg
ATMI	0	0	0	0	0	0	0	0	0
1'-CH <sub>2</sub> OH-S-2840 (total)*	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840 (total)**	9.9	9.4	9.7	8.9	9.3	9.1	8	8.1	8.1
3'-OH-S-2840***	7.5	6.8	7.1	6.8	7.4	7.1	8	6.9	7.4
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0
Inpyrfluxam	68.2	68.8	68.5	76.8	75.1	75.9	74.6	78.7	76.7
Unknown A	0.5	0.3	0.4	0.3	0.5	0.4	0.4	0.2	0.3

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Other unknowns	0	0	0	0	0	0	0	0	0
Total	86	85.4	85.7	92.8	92.3	92.5	91	93.9	92.4

\* As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

\*\*\*3'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840-dehydrate, which co-elutes with N-des-Me-S-2840 on HPLC and is identified on TLC. Total 3'-OH-S-2840 includes both forms in the acidic extract.

**Table B.8.1.1.5-13 Radioactivity distribution of the soil samples treated with [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (anaerobic conditions initiated after 28 DAT) as percent of applied radioactivity (neutral and acidic soil and water extracts combined)**

Fraction	Days After Treatment (DAT)											
	0			28			42			57		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg	1	2	Avg
N-des-Me-DFPA	0	0	0	2.1	2.4	2.3	3	2.5	2.7	2.7	2.9	2.8
DFPA-CONH <sub>2</sub>	0	0	0	0	0	0	0	0	0	0	0	0
DFPA	0	0	0	0	0	0	0	0	0	0	0	0
1'-CH <sub>2</sub> OH-S-2840 (total)*	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840 (total)**	0	0	0	5.7	6.3	6	6.9	7.9	7.4	7.6	8.7	8.1
3'-OH-S-2840***	4.8	4.4	4.6	9.2	9.6	9.4	9.6	9.5	9.5	8.6	9.8	9.2
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0
Inpyrfluxam	92.7	95.7	94.2	67.9	67.6	67.8	74.4	74.3	74.4	72.9	72.3	72.6



Unknown A	0	0.6	0.3	1.1	0.8	0.9	0.9	1.3	1.1	1.2	0.9	1.1
Other unknowns	0	0	0	0	0	0	0	0	0	0	0	0
Total	97.5	100.7	99.1	86.1	86.7	86.4	95.2	95.5	95.3	92.9	94.5	93.7

Table B.8.1.1.1.5-13 continued

Fraction	Days After Treatment (DAT)								
	85			122			155		
Replicates	1	2	Avg	1	2	Avg	1	2	Avg
N-des-Me-DFPA	1.7	2	1.8	2.6	2.2	2.4	2.7	1.1	1.9
DFPA-CONH <sub>2</sub>	0	0	0	0	0	0	0	0	0
DFPA	0	0	0	0	0	0	1.3	1	1.1
1'-CH <sub>2</sub> OH-S-2840 (total)*	0	0	0	0	0	0	0	0	0

1'-COOH-S-2840 (total)**	6.8	5.1	5.9	7.6	7.5	7.6	7.4	7	7.3
3'-OH-S-2840***	7.5	0.6	4.1	7.8	7.8	7.8	6.9	7.3	7.1
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0
Inpyrfluxam	75	74	74.5	72.1	72.9	72.5	70.8	69.1	70
Unknown A	0.5	0	0.3	0.9	1.4	1.2	0.8	1.1	1
Other unknowns	0	0	0	0	0	0	0	0.7	0.4
Total	91.5	81.7	86.6	91.1	91.8	91.4	89.9	87.3	88.6

\* As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

\*\*\*3'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840-dehydrate. The 3'-OH-S-2840-dehydrate in acidic extract was added to 3'-OH-S-2840 total soil extractable above.

**Table B.8.1.1.5-14 Average radioactivity distribution of the Penn soil phenyl label (Treatment D) anaerobic soil study (neutral and acidic soil and water extracts combined) as percent of applied radioactivity**

<b>DAT</b>	<b>0</b>	<b>28</b>	<b>42</b>	<b>57</b>	<b>85</b>	<b>122</b>	<b>155</b>
<b>Days after anaerobic changeover</b>	<b>-</b>	<b>0<sup>1</sup></b>	<b>14</b>	<b>29</b>	<b>57</b>	<b>94</b>	<b>127</b>
ATMI	0	0	0	0	0	0	0
1'-CH <sub>2</sub> OH-S-2840A	0	0	0	0	0	0	0
1'-COOH-S-2840A	0	4.4	3.9	4.2	3.5	3.3	3.1
1'-CH <sub>2</sub> OH-S-2840B	0	0	0	0	0	0	0
1'-COOH-S-2840B	0	6.8	6.3	6.1	6.2	5.8	5.0
3'-OH-S-2840 <sup>2</sup>	3.2	9.4	8.6	8.3	7.1	7.1	7.4
N-des-Me-S-2840	0	0	0	0	0	0	0
Inpyrfluxam	96.9	75.6	76.7	74.1	68.5	75.9	76.7
Unknown A	0	0	0.3	0.4	0.4	0.4	0.3
Other unknowns	0	0	0.0	0.0	0.0	0.0	0.0
Total	100.1	96.4	95.8	93.2	85.7	92.5	92.4

<sup>1</sup>The changeover from aerobic to anaerobic was made at 28DAT

<sup>2</sup>3'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840 dehydrate. The 3'-OH-S-2840 dehydrate in acidic extract was added to 3'-OH-S-2840 total soil extra

**Table B.8.1.1.5-15 Average radioactivity distribution of the Penn soil pyrazolyl label (Treatment E) anaerobic soil study (neutral soil and acidic soil and water extracts combined) as percent of applied radioactivity**

<b>DAT</b>	<b>0</b>	<b>28</b>	<b>42</b>	<b>57</b>	<b>85</b>	<b>122</b>	<b>155</b>
<b>Days after anaerobic changeover</b>	<b>-</b>	<b>0<sup>1</sup></b>	<b>14</b>	<b>29</b>	<b>57</b>	<b>94</b>	<b>127</b>
N-des-Me-DFPA	0	2.3	2.7	2.8	1.8	2.4	1.9
DFPA-CONH <sub>2</sub>	0	0	0	0	0	0	0
DFPA	0	0	0	0	0	0	1.1
1'-CH <sub>2</sub> OH-S-2840A	0	0	0	0	0	0	0
1'-COOH-S-2840A	0	2.6	3.1	3.6	2.5	3.8	2.8
1'-CH <sub>2</sub> OH-S-2840B	0	0	0	0	0	0	0
1'-COOH-S-2840B	0	3.4	4.3	4.5	3.4	3.8	4.5
3'-OH-S-2840 <sup>2</sup>	4.6	9.4	9.5	9.2	4.1	7.8	7.1
N-des-Me-S-2840	0	0	0.2	0	0	0	0
Inpyrfluxam	94.2	67.8	74.4	72.6	74.5	72.5	70.0
Unknown A	0.3	0.9	1.1	1.1	0.3	1.2	1.0
Other unknowns	0.0	0.0	0.0	0.0	0.0	0	0.4
Total	99.1	86.7	95.3	93.7	86.6	91.4	88.6

<sup>1</sup>The changeover from aerobic to anaerobic was made at 28DAT

<sup>2</sup>3'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840 dehydrate. The 3'-OH-S-2840 dehydrate in acidic extract was added to 3'-OH-S-2840 total soil extract

Neither labelled inpyrfluxam was found to isomerise during the study, using chiral HPLC.

**Table B.8.1.1.1.5-16 Distribution of Bound Radioactivity in PES Fractions of 155 DAT Soil Samples after exhaustive extraction (Phenyl and Pyrazolyl label)**

<b>Sample</b>	<b>Phenyl</b>	<b>Pyrazolyl</b>
%TRR in PES <sup>1</sup>	6.80	6.21
Ethyl Acetate	3.07	3.20
Acetone:Water:HCl (60:40:1)	3.30	3.63
%TRR in extracted <sup>2</sup>	3.74	3.01
Humic Acid	0.45	0.43
Humin	2.59	1.82
Fulvic Acid	0.71	0.78
<b>Total Recovery ( %)<sup>3</sup></b>	<b>100.45</b>	<b>100.73</b>

TRR = total radioactivity residue

<sup>1</sup>PES after neutral and acidic extraction.

<sup>2</sup> PES was extracted once with ethyl acetate after the combustion analysis of the PES and before fractionation.

<sup>3</sup> Total recovery = Humin + Humic acids + Fulvic acids (comparing against %TRR in extract).

The average material balance for the study was  $99.7 \pm 3.3 \%$  and  $100.0 \pm 1.7 \%$  AR, for the phenyl and pyrazolyl labels, respectively. All mass balances are within the 90-110 % range outlined by OECD 307 and therefore considered acceptable by the HSE.

### Bound residues

Radioactive residues were incorporated into the PES during the aerobic phase to 5 % AR (both labels). The activity in the PES from the aerobic phase did not rise sufficiently enough to require further extraction or fractionation.

PES residues rose slightly from ca. 5 % in both labels from the 0 DAT of the anaerobic phase to about 7 % and 6 % in the phenyl and pyrazolyl labels respectively.

The majority of the bound residues remained in the humin (phenyl 2.6 % AR, pyrazolyl 1.8 % AR). The humic acid fraction contained 0.5 % AR both labels and the fulvic acid fractions 0.7 % phenyl and 0.8 % AR, pyrazolyl.

### Volatilisation

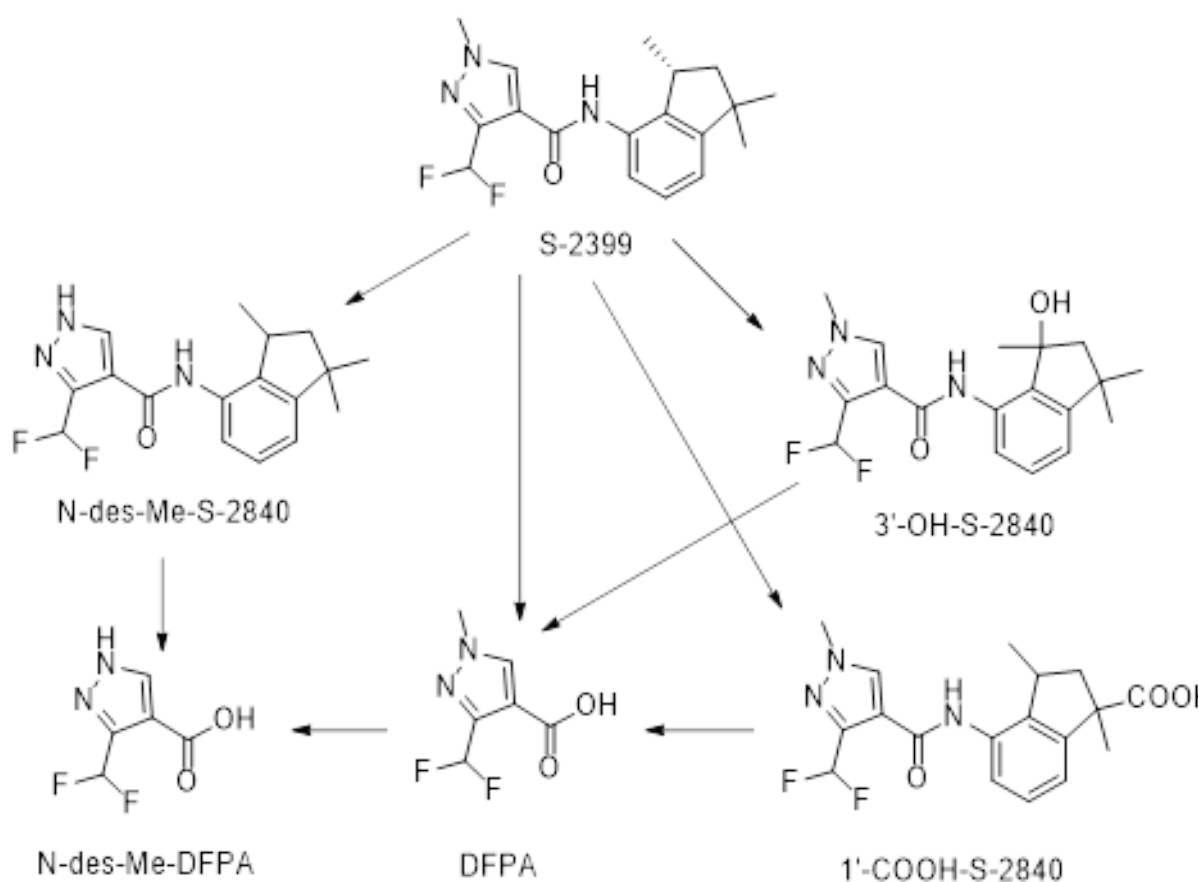
Insignificant amounts of  $^{14}\text{C}$ -volatile activity ( $^{14}\text{CH}_4/^{14}\text{C}$ -volatiles) were observed. The cumulative production of  $^{14}\text{CO}_2$  reached 0.5 % and 0.1 % AR at 155 DAT for the phenyl and pyrazolyl labels, respectively, which occurred mainly during the aerobic phase.

### Metabolites

Inpyrfluxam did not degrade significantly under anaerobic conditions. The concentrations of the two principal metabolites in the phenyl label (1'-COOH-S-2840 and 3'-OH-S-2840) decreased during the anaerobic phase of the study. The concentration changes of the two principal metabolites in the pyrazolyl label varied, with 1'-COOH-S-2840 staying the same, and 3'-OH-S-2840 decreasing during the anaerobic phase of the study. The maximum average detected values were 8.6 % & 9.5 % AR for 3'-OH-S-2840 (phenyl and pyrazolyl), 10.3 % & 8.1 % AR for 1'-COOH-S-2840 (phenyl and pyrazolyl) and N-des-Me-DFPA (2.8 % AR, pyrazolyl). 3'-OH-S-2840 is only slightly more water soluble than inpyrfluxam and remains primarily in the soil extractable activity and little is found in the water phase (1 % both labels). Minor amounts of 1'-COOH-S-2840 (6 % & 5 % AR, phenyl and pyrazolyl) and N-des-Me-DFPA (2 % AR, pyrazolyl) was found in the water phase.

### CONCLUSION

Figure B.8.1.1.5-05 shows the proposed metabolic pathways of inpyrfluxam in the soil under anaerobic conditions. However, the compounds were also found in the aerobic phase of the study.



**Figure B.8.1.1.1.5-05 Proposed anaerobic soil degradation pathway of inpyrfluxam (Compounds were primarily formed during the aerobic phase)**

The degradation of inpyrfluxam occurred mainly during the aerobic phase and remained largely stable under the anaerobic conditions. Inpyrfluxam declined to 68 - 76 % AR by the end of the aerobic phase (28 DAT), and changed little during the anaerobic phase to 70 - 77 % AR by the end of the study (155 DAT). For this reason accurate  $DT_{50}$  and  $DT_{90}$  values were not determined (HSE modelling using CAKE v3.7 resulted in an SFO  $DT_{50} > 10,000$  d). The principal degradation routes were oxidation of the 3'-position in the indenyl ring to produce 3'-OH-S-2840 (<10 % AR) and the oxidation of one of the 1'-CH<sub>3</sub> groups of the indenyl ring to form 1'-COOH-S-2840 (11 % AR phenyl, 8 % AR pyrazolyl). Minor amount of N-des-Me-DFPA was observed (<5 % AR). Neither labelled inpyrfluxam was found to isomerise during the study.

**B.8.1.1.1.6. Soil photolysis**

<b>Data Point:</b>	KCA 7.1.1.3/01
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2014
<b>Report Title:</b>	Photodegradation of [ <sup>14</sup> C] S-2399 in/on Soil by Artificial Sunlight
<b>Study number</b>	2454W-1
<b>Guideline(s) followed in study:</b>	US EPA OPPTS Guideline 835.2410  OECD Guideline for the Testing of Chemicals: Phototransformation of Chemicals on Soil Surfaces (Draft Document, January 2002)
<b>GLP?</b>	Yes

<b>Deviations from guideline</b>	<b>HSE assessment of deviations</b>
TLC analysis indicates that the HPLC peak assigned to 3'OH-S-2840 consists of two components. The other component has not been identified.	As this component is predicted to occur at a maximum of 3.9 % AR, this is not predicted to be a major metabolite. While both this metabolite and 3'OH-S-2840 are still increasing at study end and approximately 90 % AR parent still remains, photolysis is not expected to be a major degradation process in soil. This is not considered to be a major deviation.
<p style="text-align: center;"><b>HSE conclusion</b></p> <p>The study is acceptable to derive endpoints for use in the exposure assessment.</p>	



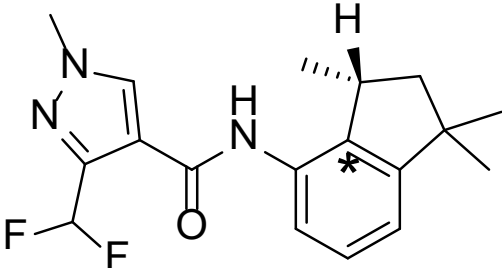
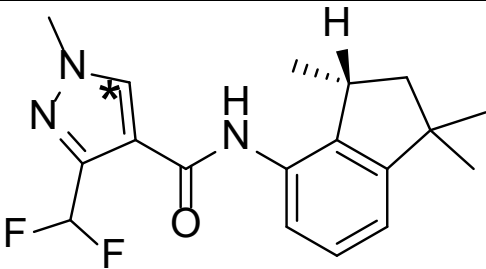
## Introduction

The photolytic degradation of the new active substance inpyrfluxam was studied in one soil. The study followed the OECD draft Guideline on photo transformation of chemicals in soil and the US EPA OPPTS Guideline 835.2410, and was conducted to Good Laboratory Practice (GLP) standards.

## MATERIALS AND METHODS

### I. Test item

**Table B.8.1.1.6-01 Radiolabelled test substance**

<p><b>[phenyl-U-<sup>14</sup>C]</b> <b>Inpyrfluxam</b></p>	 <p>Radiolabel position denoted by *</p>
<p><b>Specific activity</b></p>	<p>4.51 GBq/mmol</p>
<p><b>Radiochemical purity</b></p>	<p>97.26 % (analysed)</p>
<p><b>[pyrazolyl-4-<sup>14</sup>C]</b> <b>inpyrfluxam</b></p>	 <p>Radiolabel position denoted by *</p>
<p><b>Specific activity</b></p>	<p>2.11 GBq/mmol</p>
<p><b>Radiochemical purity</b></p>	<p>98.01 % (analysed)</p>

**Table B.8.1.1.1.6-02 Non-radiolabelled test substances**

<b>Name</b>	Inpyrfluxam ( <i>R</i> -isomer only)
<b>Chemical purity</b>	99.9 %

<b>Name</b>	Inpyrfluxam TG (Mix of <i>R</i> - and <i>S</i> -isomers)
<b>Chemical purity</b>	99.8 %

<b>Name</b>	3'OH-S-2840
<b>Chemical purity</b>	99.9 %

<b>Name</b>	DFPA-CONH <sub>2</sub>
<b>Chemical purity</b>	99.5 %

<b>Name</b>	ATMI
<b>Chemical purity</b>	100.0 %

<b>Name</b>	DFPA
<b>Chemical purity</b>	99.3 %

## II. Test soils

One soil was used in the test.

**Table B.8.1.1.1.6-03: Characteristics of the test soil used in the photolysis study of [<sup>14</sup>C] inpyrfluxam**

<b>Name</b>	Penn Soil Baptistown, NJ
<b>Date of collection</b>	30 October 2013
<b>Country of Origin</b>	United States
<b>Storage conditions</b>	Refrigerated
<b>Received at PTRL</b>	15 January 2014
<b>Particle size:</b>	
<b>Percent Sand</b>	29
<b>Percent Silt</b>	49
<b>Percent Clay</b>	22
<b>Texture (USDA)</b>	Loam
<b>Bulk Density (gm/cc)</b>	1.00
<b>CEC (meq/100 g soil)</b>	7.6
<b>% Moisture at 1/10 bar</b>	23.8
<b>% Moisture at 1/3 bar</b>	19.3
<b>% Moisture at 15 Bar</b>	10.8
<b>Organic matter (%)</b>	1.9
<b>Organic carbon (%)<sup>1</sup></b>	1.1
<b>pH 1:1 soil:water ratio</b>	6.8
<b>Microbial analysis prior to experimental start (CFU/g)</b>	
<b>Actinomycetes</b>	372,000
<b>Fungi</b>	4,360
<b>Bacteria</b>	726,000

The draft OECD Guideline recommends that a silty loam or a clay loam rather than a sandy soil should be selected to ensure the soil is less affected by the heat from the light source. A loam soil with 29 % sandy has been selected for the study which is acceptable. The Guideline states that detailed information on the history of the field sites from where the soil was collected should be available, including vegetation cover, treatments with crop protection chemicals, fertilizers and biological materials and accidental contaminations should be available. No information is provided in the study report, but this is less important than for a microbial degradation study and so this is not considered to be a major deviation. The soil was collected from the top 3 cm of the soil profile and stored refrigerated in the dark before use. The soil was

analysed for aerobic microbial viability prior to the test start. No other details of soil treatment are given.

The wet/dry ratio of the soil was analysed and found to be 1.179. Soil aliquots (3.655 g, 3.1 g dry weight equivalent) was weighed into 50 mm quartz and Pyrex soil dishes and deionised water added to create a slurry. The soil was dried with a flow of air to create an even layer of soil before being maintained at 75 % field moisture capacity at 1/3 bar (equivalent to a net moist soil weight of 3.549 g) with deionised water. Moisture content of soil samples was then monitored throughout the study with moisture replenished using deionised water as required.

## STUDY DESIGN

### Experimental Conditions

#### Solutions

Dosing solutions were prepared by diluting [ $^{14}\text{C}$ ] inpyrfluxam with acetonitrile as shown below. The target dose rate was 2.15  $\mu\text{g/g}$  soil, based on the maximum proposed field application rate of 215 g a.s./ha and the theoretical soil depth and density of 1 cm and 1  $\text{g/cm}^3$ . Based on the standard assumptions used in PECsoil calculations of 5 cm soil depth and density of 1.5  $\text{g/cm}^3$ , the equivalent field rate is 1612.5 g a.s./ha, which is somewhat higher than the proposed rate. The rate used is not however expected to affect the rate of photolytic degradation in soil and this is therefore considered to be a minor deviation.

**Table B.8.1.1.6-04 Volumes of test solution and acetonitrile solvent used to make up dose solutions**

Test Substance	Volume of Solutions	
	$^{14}\text{C}$ Material	ACN
[PYR- $^{14}\text{C}$ ] inpyrfluxam (Preliminary study)	114 $\mu\text{L}$	886 $\mu\text{L}$
[PH- $^{14}\text{C}$ ] inpyrfluxam (Definitive study)	480 $\mu\text{L}$	1570 $\mu\text{L}$
[PYR- $^{14}\text{C}$ ] inpyrfluxam (Definitive study)	235 $\mu\text{L}$	1815 $\mu\text{L}$

Reference standard solutions were prepared by diluting reference standards with acetonitrile to obtain a concentration of approximately 5  $\text{mg/mL}$ . All standard solutions were stored frozen when not in use (temperature and duration of storage not stated).

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## Test system

A Heraeus Suntest CPS+ unit equipped with a Xenon arc lamp was used as an artificial light source. Wavelengths <290 nm were blocked with a quartz glass filter, while the device was set at a light intensity of 600 W/m<sup>2</sup>, giving an average intensity of 457 W/m<sup>2</sup> for the 290-800 nm range. The light intensity and spectral distribution of the light source were checked both before and after the test. One solar day was equivalent to either 9.9 h (U.S. 40 °N summer, 290-800 nm) or 10.6 hours (OECD global day, 30-50 °N, 290-400 nm) of continuous irradiation, respectively.

Quartz soil dishes were used for irradiated samples and Pyrex soil dishes for dark control samples. All dishes were equipped with Teflon® lined silicon septum screw caps. Dark control samples were additionally covered with aluminium foil. Irradiated samples were placed in a water bath with the temperature recorded continuously using a thermocouple placed in a surrogate vessel in the water bath (20 ± 3 °C; range 16.7 to 22.1 °C). Dark control samples were placed in an incubator maintained at 20 ± 2 °C and the temperature continuously monitored. The draft test guideline states that a constant temperature of 20 ± 2 °C should be maintained. The temperatures in the study are therefore slightly outside of this range. Temperatures were mostly outside the range in the first 36 h after test item application and at study termination and stable for the majority of the study. The Guideline is only a draft rather than an agreed document and, as temperatures generally remained consistent during the course of the study and little variation was shown between dark, irradiated and the two radiolabels allowing them to be directly compared, this is not considered to be a major deviation.

With the exception of time 0 samples, the samples were connected to trapping vessels for continuous trapping during the study and air moisturised with deionised water was pumped through the system. Volatiles were collected in a foam plug trap, an ethylene glycol trap to collect organic volatiles and two 10 % aqueous sodium hydroxide traps to trap CO<sub>2</sub>.

## Preliminary study

A preliminary study was conducted to determine the approximate degradation rate of the test item in the test soil. Eight samples dosed with [pyrazolyl-<sup>14</sup>C] inpyrfluxam. Duplicate samples were sacrificed immediately following dosing and the remaining samples connected to the trapping system. Duplicate samples were taken at 2 and 6 days for irradiated samples and after 6 days for dark control samples. Soil was extracted with acetone/0.5 N HCl (8/2, v/v, x3), the extracts combined and extracts and volatiles assayed by LSC. Recoveries were 92.0 to 101.1 %AR.

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**Definitive test**

Twenty-six samples were prepared, of which 2 were used for time zero and 12 were used for each of the irradiated and dark control samples. Aliquots (50 µL) of dosing solution were added directly onto the soil surface using a glass syringe. Two dosings (of separate samples) were conducted during each experimental set to allow for sampling at different intervals. Aliquots (50 µL) of application solutions were taken prior to and following the dosing and radio-assayed to determine the dose rate and confirm homogeneity. Following application, the dosing solution was also analysed by HPLC (following dilution with acetonitrile) to ensure stability under the conditions of administration. Time 0 samples were processed immediately after dosing.

Sampling occurred at time 0 and/or 16-17 h and 1, 3, 6, 8 and 12-13 days after application. The draft Guideline recommends a duration of 10 days but the study continuing for an additional 2-3 days is not considered a major deviation as no changes in degradation rate were observed which might have indicated that this had affected the outcome.

It is noted that, although the test items were applied in acetonitrile, it is not stated if the solvent was evaporated from the soil surface prior to the start of the irradiation process.

**Analytical procedures**

Soil samples were removed from the Suntest apparatus, weighed and extracted with acetone/0.5 N HCl (8/2, v/v; 10 mL; x3). Samples were shaken (30 min) and centrifuged (5 min; 4000 rpm) and the extracts combined. Triplicate aliquots (200 µL) were analysed by LSC. Aliquots of the extracts were evaporated under nitrogen to remove the acetone, diluted with acetonitrile, microfuged and analysed by reverse phase HPLC and TLC was used as a confirmatory method on selected samples. Selected samples were also analysed by chiral HPLC. Quantitation was based on HPLC analysis, while structural assignments were based on HPLC and TLC co-chromatography with reference standards. Reference standards were co-chromatographed with all samples. After the final extract was decanted, the post-extracted soil weight was determined. Volumes of trap solutions were measured and aliquots (3 x 0.5 mL) assayed by LSC.

The extracted soil pellet was weighed and aliquots (4 x 0.2 g) combusted and assayed by LSC to determine unextracted residues. The limits of detection for combustion samples were calculated relative to the background levels and were 0.00043 ppm for the Ph label and 0.00092 ppm for the Pyr label.

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## Sample storage and storage stability

All samples were extracted on the day of collection. Initial HPLC analysis was conducted within 3 days of collection. Samples were combusted within 5 days of sampling. All samples and standard solutions were stored frozen (<0 °C) when not in use. Repeated HPLC analysis of mixed reference standard solutions (prepared in acetonitrile) showed no degradation of the reference standards throughout the study. HSE has examined sample chromatograms supplied by the applicant and confirmed that this is the case.

## RESULTS AND DISCUSSION

- Extraction and analysis of at least duplicate soil samples at time 0 to indicate repeatability of the analytical method and uniformity of application of test substance.
- Repeatability of the analytical method (excluding extraction efficiency) to quantify test substance and transformation products by duplicate analysis of the same soil extract incubated long enough for formation of metabolites.

HPLC analysis demonstrated that the radiopurities of [<sup>14</sup>C] inpyrfluxam were >97 %. HPLC also confirmed that the test substances were stable in the dose solution under the conditions of administration. HPLC has examined the sample chromatograms supplied by the applicant and confirmed that this is the case.

The definitive treatment rate was confirmed from aliquots of the dosing solution taken throughout the dosing process. The actual dosing rate was between 2.107 and 2.227 µg/g with a relative standard deviation of 2.5 % (n ≥ 2), relative to a target dose rate of 2.15 µg. The draft Guideline recommends testing the dosed soil at time 0 to ensure correct dosing of the system and homogeneity of application, which is not the same as the homogeneity of the dosing solution.

The preliminary study demonstrated that after 6 days of exposure to artificial light, inpyrfluxam represented 93.4 to 93.6 % AR in light exposed samples with mass balance 96.7 ± 3.2 % AR.

### I. Mass balance

The mass balance determined in the irradiated and dark control samples for the Ph and Pyr labelled inpyrfluxam are shown below.

**Table B.8.1.1.1.6-05 Material balance for irradiated samples, [Ph-<sup>14</sup>C] inpyrfluxam on soil**

			<b>[<sup>14</sup>C-PH]- inpyrfluxam</b>			
<b>Light exposed</b>						
		<b>Volatile traps</b>				
<b>Sample</b>	<b>Soil Extract</b>	<b>EG trap</b>	<b>NaOH</b>	<b>Foam Plug</b>	<b>Combustions</b>	<b>Total Recovery</b>
T0A	101.0	NA	NA	NA	0.5	101.5
T0B	93.0	NA	NA	NA	0.4	93.4
<b>Average</b>	<b>97.0</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>0.5</b>	<b>97.5</b>
T16hrA	92.5	0.0	0.0	0.0	0.7	93.2
T16hrB	93.1	0.0	0.0	0.0	0.8	93.9
<b>Average</b>	<b>92.8</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>93.6</b>
T1dA	92.4	0.0	0.0	0.0	0.8	93.2
T1dB	96.3	0.0	0.0	0.0	1.0	97.3
<b>Average</b>	<b>94.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.9</b>	<b>95.3</b>
T3dA	99.0	0.0	0.1	0.0	1.2	100.3
T3dB	97.5	0.0	0.0	0.0	1.3	98.8
<b>Average</b>	<b>98.3</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.3</b>	<b>99.6</b>
T6dA	83.2	0.0	0.2	0.0	1.1	84.5
T6dB	91.4	0.0	0.2	0.0	1.4	93.0
<b>Average</b>	<b>87.3</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.3</b>	<b>88.8</b>
T8dA	97.5	0.0	0.3	0.0	1.5	99.3
T8dB	98.5	0.0	0.3	0.0	1.2	100.0
<b>Average</b>	<b>98.0</b>	<b>0.0</b>	<b>0.3</b>	<b>0.0</b>	<b>1.4</b>	<b>99.7</b>
T12dA	89.9	0.0	0.5	0.0	2.4	92.8
T12dB	95.1	0.0	0.5	0.0	1.5	97.1
<b>Average</b>	<b>92.5</b>	<b>0.0</b>	<b>0.5</b>	<b>0.0</b>	<b>2.0</b>	<b>95.0</b>
					Average	95.6
					Standard deviation	4.5

NA = not applicable



**Table B.8.1.1.1.6-06 Material balance for dark control samples, [Ph-<sup>14</sup>C] inpyrfluxam on soil**

			<b>[<sup>14</sup>C-PH]- inpyrfluxam</b>			
<b>Dark control</b>						
		<b>Volatile traps</b>				
<b>Sample</b>	<b>Soil Extra ct</b>	<b>EG trap</b>	<b>NaOH</b>	<b>Foam Plug</b>	<b>Combustions</b>	<b>Total Recovery</b>
T0A	101.0	NA	NA	NA	0.5	101.5
T0B	93.0	NA	NA	NA	0.4	93.4
<b>Average</b>	<b>97.0</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>0.5</b>	<b>97.5</b>
T16hrA	95.6	0.0	0.0	0.0	0.6	96.2
T16hrB	94.4	0.0	0.0	0.0	0.8	95.2
<b>Average</b>	<b>95.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.7</b>	<b>95.7</b>
T1dA	96.3	0.0	0.0	0.0	0.9	97.2
T1dB	96.0	0.0	0.0	0.0	0.7	96.7
<b>Average</b>	<b>96.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>97.0</b>
T3dA	98.7	0.0	0.0	0.0	1.1	99.8
T3dB	95.8	0.0	0.0	0.0	0.8	96.6
<b>Average</b>	<b>97.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>98.2</b>
T6dA	93.9	0.0	0.1	0.0	1.2	95.2
T6dB	96.1	0.0	0.1	0.0	1.2	97.4
<b>Average</b>	<b>95.0</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.2</b>	<b>96.3</b>
T8dA	103.3	0.0	0.1	0.0	1.0	104.4
T8dB	99.6	0.0	0.1	0.0	1.1	100.8
<b>Average</b>	<b>101.5</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.1</b>	<b>102.6</b>
T12dA	100.4	0.0	0.1	0.0	1.1	101.6
T12dB	93.3	0.0	0.2	0.0	1.4	94.9
<b>Average</b>	<b>96.9</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.3</b>	<b>98.3</b>
					Average	97.9
					Standard deviation	3.2
					<b>Average for all samples</b>	<b>96.7</b>
					<b>Standard deviation</b>	<b>4</b>

NA = not applicable

**Table B.8.1.1.1.6-07 Material balance for irradiated samples, [Pyr-<sup>14</sup>C] inpyrfluxam on soil**

			<b>[<sup>14</sup>C-PYR]- inpyrfluxam</b>			
<b>Light exposed</b>						
		<b>Volatile traps</b>				
<b>Sample</b>	<b>Soil Extract</b>	<b>EG</b>	<b>NaOH</b>	<b>Foam Plug</b>	<b>Combustions</b>	<b>Total Recovery</b>
T0A	95.2	NA	NA	NA	0.4	95.6
T0B	95.7	NA	NA	NA	0.4	96.1
<b>Average</b>	<b>95.5</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>0.4</b>	<b>95.9</b>
T17hrA	98.1	0.0	0.0	0.0	0.5	98.6
T17hrB	98.8	0.0	0.0	0.0	0.7	99.5
<b>Average</b>	<b>98.5</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.6</b>	<b>99.1</b>
T1dA	97.2	0.0	0.0	0.0	0.5	97.7
T1dB	96.1	0.0	0.0	0.0	0.5	96.6
<b>Average</b>	<b>96.7</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.5</b>	<b>97.2</b>
T3dA	95.0	0.0	0.0	0.0	1.1	96.1
T3dB	97.4	0.0	0.0	0.0	1.1	98.5
<b>Average</b>	<b>96.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.1</b>	<b>97.3</b>
T6dA	95.5	0.0	0.1	0.0	1.0	96.6
T6dB	95.7	0.0	0.1	0.0	1.1	96.9
<b>Average</b>	<b>95.6</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.1</b>	<b>96.8</b>
T8dA	95.8	0.0	0.1	0.0	1.6	97.5
T8dB	97.3	0.0	0.2	0.0	1.7	99.2
<b>Average</b>	<b>96.6</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.7</b>	<b>98.4</b>
T13dA	98.5	0.0	0.1	0.0	1.6	100.2
T13dB	95.2	0.0	0.2	0.0	2.2	97.6
<b>Average</b>	<b>96.9</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.9</b>	<b>98.9</b>
					Average	97.6
					Standard deviation	1.4

NA = not applicable

**Table B.8.1.1.1.6-08 Material balance for dark control samples, [Pyr-<sup>14</sup>C] inpyrfluxam on soil**

			<b>[<sup>14</sup>C-PYR]- inpyrfluxam</b>			
<b>Dark control</b>						
		<b>Volatile traps</b>				
<b>Sample</b>	<b>Soil Extract</b>	<b>EG</b>	<b>NaOH</b>	<b>Foam Plug</b>	<b>Combustions</b>	<b>Total Recovery</b>
T0A	95.2	NA	NA	NA	0.4	95.6
T0B	95.7	NA	NA	NA	0.4	96.1
<b>Average</b>	<b>95.5</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>0.4</b>	<b>95.9</b>
T17hrA	102.6	0.0	0.0	0.0	0.6	103.2
<b>Average</b>	<b>102.6</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.6</b>	<b>103.2</b>
T1dA	98.7	0.0	0.0	0.0	0.5	99.2
T1dB	98.1	0.0	0.0	0.0	0.5	98.6
<b>Average</b>	<b>98.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.5</b>	<b>98.9</b>
T3dA	98.4	0.0	0.0	0.0	1.1	99.5
T3dB	99.3	0.0	0.0	0.0	0.8	100.1
<b>Average</b>	<b>98.9</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>99.8</b>
T6dA	100.0	0.0	0.1	0.0	0.8	100.9
T6dB	98.1	0.0	0.0	0.0	0.8	98.9
<b>Average</b>	<b>99.1</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>0.8</b>	<b>99.9</b>
T8dA	97.1	0.0	0.0	0.0	0.7	97.8
T8dB	95.6	0.0	0.0	0.0	0.8	96.4
<b>Average</b>	<b>96.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>97.1</b>
T13dA	94.8	0.0	0.0	0.0	1.2	96.0
T13dB	96.5	0.0	0.0	0.0	0.8	97.3
<b>Average</b>	<b>95.7</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>96.7</b>
					Average	98.4
					Standard deviation	2.2
					<b>Average for all samples</b>	<b>98.2</b>
					<b>Standard deviation</b>	<b>1.8</b>

NA = not applicable

In the definitive test the mass balance, based on the sum of soil extracts, volatile traps and residual soil carbon, ranged between:

- 84.5 and 101.5 % AR for the Ph labelled irradiated samples
- 93.4 and 104.4 % AR for the Ph labelled dark control samples
- 95.6 to 100.2 % AR for the Pyr labelled irradiated samples

- 
- 95.6 to 103.2 % AR for the Pyr labelled dark control samples.

One mass balance was <90 % AR and therefore outside of the accepted 90-110 % range for a radiolabelled study. This was at 3 days after application in the irradiated samples for the Ph-labelled study, with one replicate was 84.5 % AR and the mean value 88.8 % AR. As this was one sample and the mean is only slightly <90 % AR, this is accepted and it is not considered necessary to exclude this data point.

For the Ph labelled test item, extractable residues decreased throughout the study from 97.0 % AR to 92.5 % AR in the light exposed samples or 96.9 % in dark control samples (all mean of two values). Unextracted residues increased but remained low, reaching 2.0 % AR in light exposed samples and 1.3 % AR in dark control samples at study end. CO<sub>2</sub> and other volatiles remained low.

For the Pyr labelled test item, there was no decline in extractable residues in either irradiated or dark control samples. Unextracted residues were detected by study end but remained at low levels of 1.9 % AR in irradiated samples and 1.0 % in dark control samples (all values mean of two replicates). CO<sub>2</sub> and other volatiles remained low in both irradiated and dark control samples.

**Table B.8.1.1.1.6-09 Identity of residues determined by HPLC for the Ph label in irradiated samples**

			% Applied Dose					
Sample ID	Inpyrfluxam	3'OH-S-2840	Other HPLC Peaks	EG Trap	NaOH Trap	Foam Plug Trap	PES	Total
Time 0 Rep A	97.7	1.9	1.4	NA	NA	NA	0.5	101.5
Time 0 Rep B	90.2	1.7	1.2	NA	NA	NA	0.4	93.5
<b>Average</b>	<b>94.0</b>	<b>1.8</b>	<b>1.3</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>0.5</b>	<b>97.5</b>
Light 16 hour Rep A	90.1	2.4	0.0	0.0	0.0	0.0	0.7	93.2
Light 16 hour Rep B	90.7	2.4	0.0	0.0	0.0	0.0	0.8	93.9
<b>Average</b>	<b>90.4</b>	<b>2.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>93.6</b>
Light 1 day Rep A	91.1	1.3	0.0	0.0	0.0	0.0	0.8	93.2
Light 1 day Rep B	94.8	1.5	0.0	0.0	0.0	0.0	1.0	97.3
<b>Average</b>	<b>93.0</b>	<b>1.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.9</b>	<b>95.3</b>
Light 3 day Rep A	95.6	3.4	0.0	0.0	0.1	0.0	1.2	100.3
Light 3 day Rep B	92.3	3.7	1.5	0.0	0.0	0.0	1.3	98.8
<b>Average</b>	<b>94.0</b>	<b>3.6</b>	<b>0.8</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.3</b>	<b>99.6</b>
Light 6 day Rep A	78.0	5.2	0.0	0.0	0.2	0.0	1.1	84.5
Light 6 day Rep B	85.6	5.8	0.0	0.0	0.2	0.0	1.4	93.0
<b>Average</b>	<b>81.8</b>	<b>5.5</b>	<b>0.0</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.3</b>	<b>88.8</b>
Light 8 day Rep A	92.0	5.5	0.0	0.0	0.3	0.0	1.5	99.3
Light 8 day Rep B	92.4	6.1	0.0	0.0	0.3	0.0	1.2	100.0
<b>Average</b>	<b>92.2</b>	<b>5.8</b>	<b>0.0</b>	<b>0.0</b>	<b>0.3</b>	<b>0.0</b>	<b>1.4</b>	<b>99.7</b>
Light 12 day Rep A	78.8	9.3	1.8	0.0	0.5	0.0	2.4	92.8
Light 12 day Rep B	89.1	6.0	0.0	0.0	0.5	0.0	1.5	97.1
<b>Average</b>	<b>84.0</b>	<b>7.7</b>	<b>0.9</b>	<b>0.0</b>	<b>0.5</b>	<b>0.0</b>	<b>2.0</b>	<b>95.0</b>

**Table B.8.1.1.1.6-10 Identity of residues determined by HPLC for the Ph label in dark control samples**

Sample ID	Inpyrfluxam	3'OH-S-2840	% Applied Dose		NaOH Trap	Foam Plug Trap	PES	Total
			Other HPLC Peaks	EG Trap				
Dark 16 hour Rep A	93.6	2.0	0.0	0.0	0.0	0.0	0.6	96.2
Dark 16 hour Rep B	90.8	2.9	0.7	0.0	0.0	0.0	0.8	95.2
<b>Average</b>	<b>92.2</b>	<b>2.5</b>	<b>0.4</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.7</b>	<b>95.7</b>
Dark 1 day Rep A	94.7	1.6	0.0	0.0	0.0	0.0	0.9	97.2
Dark 1 day Rep B	93.7	2.3	0.0	0.0	0.0	0.0	0.7	96.7
<b>Average</b>	<b>94.2</b>	<b>2.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>97.0</b>
Dark 3 day Rep A	95.8	2.3	0.5	0.0	0.0	0.0	1.1	99.7
Dark 3 day Rep B	92.9	2.9	0.0	0.0	0.0	0.0	0.8	96.6
<b>Average</b>	<b>94.4</b>	<b>2.6</b>	<b>0.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>98.2</b>
Dark 6 day Rep A	89.4	3.0	1.5	0.0	0.1	0.0	1.2	95.2
Dark 6 day Rep B	92.2	3.3	0.7	0.0	0.1	0.0	1.2	97.5
<b>Average</b>	<b>90.8</b>	<b>3.2</b>	<b>1.1</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.2</b>	<b>96.4</b>
Dark 8 day Rep A	97.9	3.8	1.6	0.0	0.1	0.0	1.0	104.4
Dark 8 day Rep B	94.6	3.7	1.3	0.0	0.1	0.0	1.1	100.8
<b>Average</b>	<b>96.3</b>	<b>3.8</b>	<b>1.5</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.1</b>	<b>102.6</b>
Dark 12 day Rep A	95.6	3.7	1.0	0.0	0.1	0.0	1.1	101.5
Dark 12 day Rep B	86.1	5.6	1.7	0.0	0.2	0.0	1.4	95.0
<b>Average</b>	<b>90.9</b>	<b>4.7</b>	<b>1.4</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.3</b>	<b>98.3</b>

**Table B.8.1.1.1.6-11 Identity of residues determined by HPLC for the Pyr label in irradiated samples**

Sample ID	Inpyrfluxam	3'OH-S-2840	% Applied Dose		EG Trap	NaOH Trap	Foam Plug Trap	PES	Total
			DFPA	Other HPLC Peaks					
Time 0 Rep A	92.8	2.4	0.0	0.0	NA	NA	NA	0.4	95.6
Time 0 Rep B	93.6	2.1	0.0	0.0	NA	NA	NA	0.4	96.1
<b>Average</b>	<b>93.2</b>	<b>2.3</b>	<b>0.0</b>	<b>0.0</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>0.4</b>	95.9
Light 17 hour Rep A	94.6	2.2	0.0	1.3	0.0	0.0	0.0	0.5	98.6
Light 17 hour Rep B	96.0	1.5	0.0	1.3	0.0	0.0	0.0	0.7	99.5
<b>Average</b>	<b>95.3</b>	<b>1.9</b>	<b>0.0</b>	<b>1.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.6</b>	99.1
Light 1 day Rep A	93.9	3.3	0.0	0.0	0.0	0.0	0.0	0.5	97.7
Light 1 day Rep B	92.8	3.3	0.0	0.0	0.0	0.0	0.0	0.5	96.6
<b>Average</b>	<b>93.4</b>	<b>3.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.5</b>	97.2
Light 3 day Rep A	91.8	3.2	0.0	0.0	0.0	0.0	0.0	1.1	96.1
Light 3 day Rep B	91.9	3.1	0.0	2.4	0.0	0.0	0.0	1.1	98.5
<b>Average</b>	<b>91.9</b>	<b>3.2</b>	<b>0.0</b>	<b>1.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.1</b>	97.3
Light 6 day Rep A	90.2	2.0	0.0	3.3	0.0	0.1	0.0	1.0	96.6
Light 6 day Rep B	89.2	2.1	0.0	4.4	0.0	0.1	0.0	1.1	96.9
<b>Average</b>	<b>89.7</b>	<b>2.1</b>	<b>0.0</b>	<b>3.9</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>1.1</b>	96.8
Light 8 day Rep A	89.2	3.8	0.0	2.9	0.0	0.1	0.0	1.6	97.6
Light 8 day Rep B	87.3	5.1	0.0	4.8	0.0	0.2	0.0	1.7	99.1
<b>Average</b>	<b>88.3</b>	<b>4.5</b>	<b>0.0</b>	<b>3.9</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.7</b>	98.4
Light 13 day Rep A	88.1	8.8	1.7	0.0	0.0	0.1	0.0	1.6	100.3
Light 13 day Rep B	87.5	7.7	0.0	0.0	0.0	0.2	0.0	2.2	97.6
<b>Average</b>	<b>87.8</b>	<b>8.3</b>	<b>0.9</b>	<b>0.0</b>	<b>0.0</b>	<b>0.2</b>	<b>0.0</b>	<b>1.9</b>	<b>99.0</b>

**Table B.8.1.1.1.6-12 Identity of residues determined by HPLC for the Pyr label in dark control samples**

Sample ID	Inpyrfluxam	3'OH-S-2840	% Applied Dose		EG Trap	NaOH Trap	Foam Plug Trap	PES	Total
			DFPA	Other HPLC Peaks					
Dark 17 hour Rep A	99.1	1.9	0.0	1.6	0.0	0.0	0.0	0.6	103.2
<b>Average</b>	<b>99.1</b>	<b>1.9</b>	<b>0.0</b>	<b>1.6</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.6</b>	<b>103.2</b>
Dark 1 day Rep A	96.3	2.4	0.0	0.0	0.0	0.0	0.0	0.5	99.2
Dark 1 day Rep B	95.1	3.0	0.0	0.0	0.0	0.0	0.0	0.5	98.6
<b>Average</b>	<b>95.7</b>	<b>2.7</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.5</b>	<b>98.9</b>
Dark 3 day Rep A	96.3	2.1	0.0	0.0	0.0	0.0	0.0	1.1	99.5
Dark 3 day Rep B	95.3	2.6	0.0	1.4	0.0	0.0	0.0	0.8	100.1
<b>Average</b>	<b>95.8</b>	<b>2.4</b>	<b>0.0</b>	<b>0.7</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>99.8</b>
Dark 6 day Rep A	97.1	2.9	0.0	0.0	0.0	0.1	0.0	0.8	100.9
Dark 6 day Rep B	95.9	2.2	0.0	0.0	0.0	0.0	0.0	0.8	98.9
<b>Average</b>	<b>96.5</b>	<b>2.6</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.1</b>	<b>0.0</b>	<b>0.8</b>	<b>99.9</b>
Dark 8 day Rep A	93.7	2.3	0.0	1.1	0.0	0.0	0.0	0.7	97.8
Dark 8 day Rep B	91.6	3.1	0.0	0.9	0.0	0.0	0.0	0.8	96.4
<b>Average</b>	<b>92.7</b>	<b>2.7</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.8</b>	<b>97.1</b>
Dark 13 day Rep A	86.9	3.2	0.0	4.6	0.0	0.0	0.0	1.2	95.9
Dark 13 day Rep B	89.3	3.4	0.0	3.8	0.0	0.0	0.0	0.8	97.3
<b>Average</b>	<b>88.1</b>	<b>3.3</b>	<b>0.0</b>	<b>4.2</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>96.6</b>



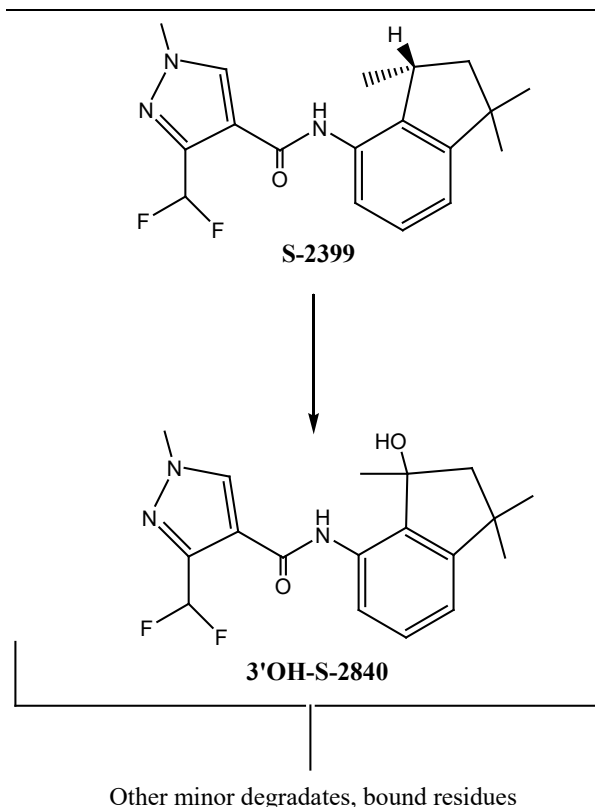
For the Ph label irradiated samples, inpyrfluxam declined during the course of the study from a mean of 94.0 % AR to a mean of 84.0 % AR by study end. There was a corresponding increase in the metabolite 3'OH-S-2840, reaching a mean of 7.7 % AR by study end. The applicant observes that the region in HPLC chromatograms assigned to 3'OH-S-2840 consists of one broad peak or two adjacent peaks. Further analysis by TLC showed that this region contained two components in approximately equal amounts, one of which was 3'OH-S-2840. The other peak has not been identified, but as it is predicted to occur at a maximum of 3.9 % AR, this is not predicted to be a major metabolite. While both this metabolite and 3'OH-S-2840 are still increasing at study end and approximately 90 % AR parent still remains, photolysis is not expected to be a major degradation process in soil. The metabolite 3'OH-S-2840 is included in exposure assessments due to being a major metabolite in microbial degradation studies (see Section 8.1.1.1) and therefore further consideration is not required under the current study. Other HPLC peaks reached a mean maximum of 1.3 % AR, with volatiles remaining at low levels, reaching a mean maximum of 0.5 % AR at study end. Unextracted residues were also low, reaching a mean maximum of 2.0 % AR at study end.

In the dark controls for the Ph label, there was a slight decline in the concentration of inpyrfluxam from a mean of 92.2 % AR to a mean of 90.9 % AR. The photodegradate 3'OH-inpyrfluxam reached a mean of 4.7 % AR by study end. Other peaks in the HPLC chromatograms did not exceed a mean of 1.4 % at any time point. Volatiles remained low at all time points, reaching a mean of 0.2 % AR at study end. Unextracted residues (PES) also remained low, reaching a mean of 1.3 % AR at study end.

Pyr-labelled inpyrfluxam in the irradiated samples declined from a mean of 93.2 % AR to 87.8 % AR at study end. This was accompanied by an increase in metabolite 3'OH-S-2840 which reached 8.3 % AR by study end. As for the other radiolabel, this broad peak was DFPA was also detected in low amounts reaching a mean average of 0.9 % AR by study end. Other metabolites remained low, reaching a maximum mean average of 3.9 % AR before declining to 0.0 % AR by study end. Similarly, volatiles remained at low levels reaching a mean of 0.2 % AR. Unextracted residues (PES) rose throughout the study to reach a mean of 1.9 % AR by study end.

In the dark controls, Pyr-labelled inpyrfluxam declined from a mean of 96.3 % AR to 88.1 % AR. Metabolite 3'OH-S-2840 increased to a mean of 3.3 % AR by study end, while metabolite DFPA remained undetected throughout the study. Other HPLC peaks reached a mean maximum of 4.2 % AR by study end. Volatiles remained low or undetected throughout the study, with CO<sub>2</sub> reaching a mean maximum of 0.1 % AR on day 6, but undetected at study end. Unextracted residues remained low throughout the study, peaking at a mean of 1.0 % AR at both day 3 and study end.

The proposed degradation pathway is shown below.



**Figure B.8.1.1.6-01 Proposed photolytic degradation pathway for inpyrfluxam in soil**

## II. Chiral HPLC analysis

The potential isomerisation of inpyrfluxam was assessed using selected standards and samples prepared in hexane/isopropanol (95/5) and analysed by normal phase HPLC. No isomerisation from *R*-inpyrfluxam to *S*-inpyrfluxam was observed in either the time 0 or final time point samples (95.8 % as *R* isomer at time 0 and 95.5 % as *R*-isomer at study end).

## III. TLC as confirmatory method

Two-dimensional TLC analysis of selected samples and reference standards was used to confirm the identification of inpyrfluxam and 3'OH-S-2840. The TLC analysis confirmed the two peaks observed in the HPLC chromatograms for 3'OH-S-2840.

## IV. Photodegradation Rate of [<sup>14</sup>C] inpyrfluxam in soil

The applicant has calculated the DT<sub>50</sub>, DT<sub>75</sub> and DT<sub>90</sub> values for both radiolabels for irradiated and dark control samples. These have been verified by HSE against the FOCUS Kinetics Guidance (2006) using CAKE version 3.7 and are accepted. No fits were supplied by the applicant, only the degradation rates. Fits displayed below are therefore those generated by HSE. For time 0 samples, the total AR was used with any metabolites, volatiles or unextracted residues added to the parent % AR. As no values approached the LOD or LOQ no consideration of these values according to

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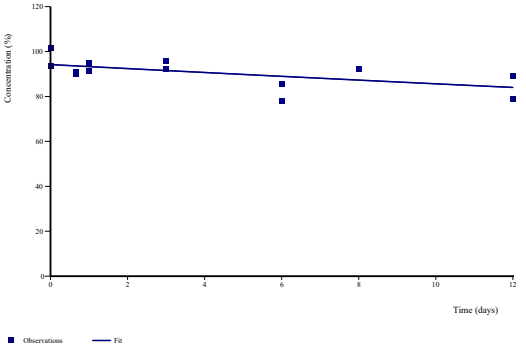
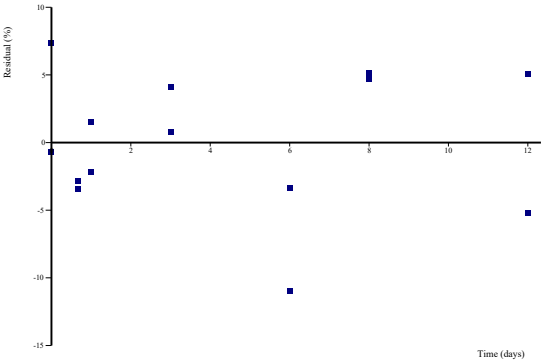
FOCUS Kinetics was required. All degradation data clearly followed SFO kinetics with low  $\chi^2$  values and therefore biphasic models were not considered.

### Ph label, irradiated

**Table B.8.1.1.1.6-13 Data used in the FOCUS Kinetics evaluation (Ph label, irradiated)**

<b>Time Point (days)</b>	<b>% AR</b>
0	101.5
0	93.5
0.67	90.1
0.67	90.7
1	91.1
1	94.8
3	95.6
3	92.3
6	78
6	85.6
8	92
8	92.4
12	78.8
12	89.1

**Table B.8.1.1.1.6-14 Rate of degradation for the Ph label in irradiated samples – HSE fitting**

Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	good	3.37	M <sub>0</sub> : 94.16 k: 0.009499	k: <0.05	73	242
<b>Conclusion: select SFO as best-fit for modelling endpoints (DT<sub>50</sub> = 73 days, DT<sub>90</sub> = 242 days).</b>						
<div style="display: flex; justify-content: space-around;">   </div>						

**Ph label, dark controls****Table B.8.1.1.1.6-15 Data used in the FOCUS Kinetics evaluation (Ph label, dark control)**

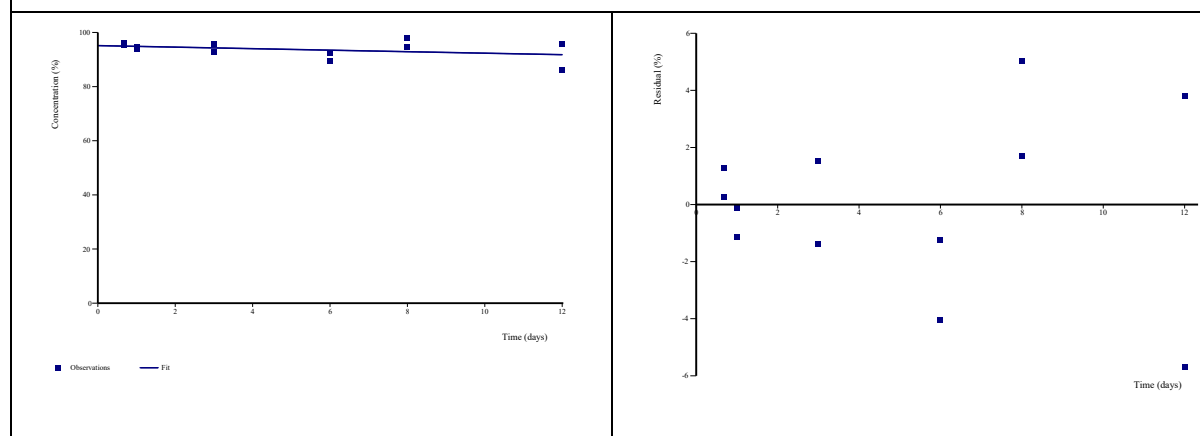
Time Point (days)	% AR
0.67	96.2
0.67	95.2
1	94.7
1	93.7
3	95.8
3	92.9

6	89.4
6	92.2
8	97.9
8	94.6
12	95.6
12	86.1

**Table B.8.1.1.6-16 Rate of degradation for the Ph label in dark control samples – HSE fitting**

Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	good	1.56	M <sub>0</sub> : 95.12 k: 0.002971	p = 0.1246	233	775

**Conclusion: select SFO as best-fit for modelling endpoints (DT<sub>50</sub> =233 days, DT<sub>90</sub> = 755 days).**

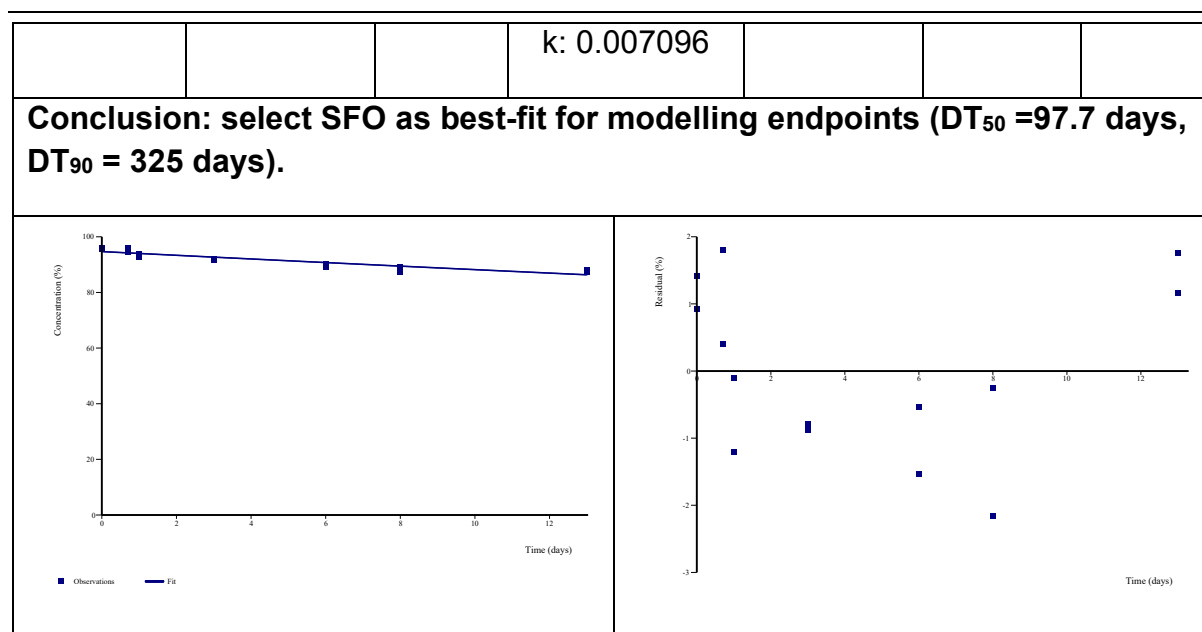


**Pyr label, irradiated****Table B.8.1.1.6-17 Data used in the FOCUS Kinetics evaluation (Pyr label, irradiated)**

<b>Time Point (days)</b>	<b>% AR</b>
0	95.6
0	96.1
0.71	94.6
0.71	96
1	93.9
1	92.8
3	91.8
3	91.9
6	90.2
6	89.2
8	89.2
8	87.3
13	88.1
13	87.5

**Table B.8.1.1.6-18 Rate of degradation for the Pyr label in irradiated samples – HSE fitting**

<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	good	0.948	M <sub>0</sub> : 94.68	k: <0.05	97.7	325

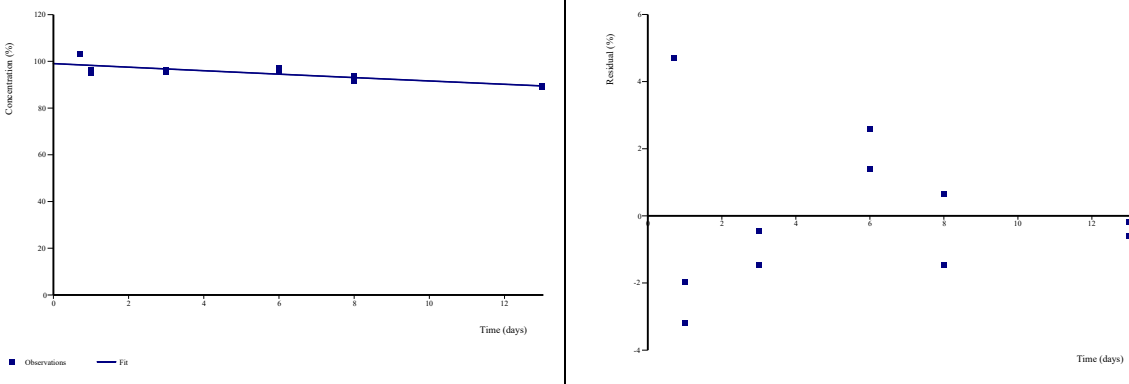


### Pyr label, dark controls

**Table B.8.1.1.1.6-19 Data used in the FOCUS Kinetics evaluation (Pyr label, dark control)**

Time Point (days)	% AR
0.71	103.2
1	96.3
1	95.1
3	96.3
3	95.3
6	97.1
6	95.9
8	93.7
8	91.6
13	88.9
13	89.3

**Table B.8.1.1.6-20 Rate of degradation for the Pyr label in dark control samples – HSE fitting**

Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	good	1.98	M <sub>0</sub> : 99.05 k: 0.007805	k: <0.05	88.8	295
<b>Conclusion: select SFO as best-fit for modelling endpoints (DT<sub>50</sub> = 88.8 days, DT<sub>90</sub> = 295 days).</b>						
						

## VI. Summary of degradation rates

The degradation rates for both labels in irradiated and dark controls are summarised below.

**Table B.8.1.1.6-21 Summary of degradation data calculated by HSE using CAKE 3.7**

	Visual fit	Model	$\chi^2$ (%)	Rate constant	DT <sub>50</sub> (days)	DT <sub>90</sub> (days)
Ph label, irradiated	Good	SFO	3.37	0.009499	73	242
Ph label, dark control	Good	SFO	1.56	0.002971	233	775
Ph label, photolysis				0.006528	106	352



Pyr label, irradiated	Good	SFO	0.948	0.007096	97.7	325
Pyr label dark control	Good	SFO	1.98	0.007805	88.8	295
Pyr label, photolysis				0	97.7	325

$$DT_{50} = \ln(2)/k; DT_{90} = \ln(10)/k$$

The degradation rates obtained by HSE are slightly different to those calculated by the applicant, although the broad trends are the same. For the Ph label, slow degradation in irradiated samples was observed, with a longer degradation rate in the dark controls. In the Pyr-labelled samples, slow degradation was observed in both the irradiated and dark control samples. While for the PYR label the  $DT_{50}$  and  $DT_{90}$  values in the dark control are slightly shorter than for the irradiated samples, it is noted that the  $DT_{50}$  values calculated by the applicant also indicated minimal photolytic degradation, with a  $DT_{50}$  value of 116 days for both irradiated and dark controls and  $DT_{90}$  values of 231 days in the irradiated and 233 days in dark controls. HSE therefore agrees with the applicant that photolytic degradation is a minor process in soil for inpyrfluxam.

The intensity of the Xenon lamp was used to convert the  $DT_{50}$  and  $DT_{90}$  values into equivalent solar days. This was done using the following equation:

$$d = h \times r / (0.75 \times 12)$$

Where:

d = days of summer sunlight

h = hours of irradiation by the Xenon lamp

r = ratio of irradiance of the Xenon radiation to that of summer sunlight

0.75 = correction for diurnal variation of natural sunlight

12 = conversion factor of hours to days

**Table B.8.1.1.6-22 Conversion of  $DT_{50}$  values observed under the Xenon lamp into equivalent  $DT_{50}$  values at 30°N, 40°N and 50°N in days**

	Days under Continuous Artificial Light	Summer Days at 30°N (290-800 nm)	Summer Days at 40°N (290-800 nm)	Summer Days at 50°N (290-800 nm)
Ph label	106	763	688	641
Pyr label	97.7	703	634	591

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## V. Chiral HPLC analysis

The enantiomer ratio was measured at time 0 and at the final time point. The isomer ratio remained roughly constant throughout the study. For both the Ph- and Pyr-label, HSE has verified from the supplied HPLC chromatograms that the S-isomer was present at roughly 4.5 % AR with the remainder present as the R-isomer.

## CONCLUSIONS

The photolytic degradation of inpyrfluxam was studied in one soil. Inpyrfluxam was radiolabelled in two ring positions and applied to the soil surface before being irradiated for 12 or 13 days alongside a control incubated under dark conditions.

At study end, 84 to 87.8 % AR remained as inpyrfluxam in irradiated samples and 88.1 to 90.1 % AR in dark controls. The main degradate was 3'OH-S-2840 reaching a maximum of 8.3 % AR; TLC analysis showed that this HPLC peak consisted of two components present in roughly equal amounts although the second component was not identified. As photodegradation in soil is not expected to be a major process and it was present at a maximum of approximately 3.9 % at study end, it is not considered necessary to consider this component further. Small amounts of unextracted residues and volatile components were also formed.

Photodegradation occurred slowly with minimal difference between the rate in irradiated and dark control samples. Degradation followed SFO kinetics according to FOCUS, with DT<sub>50</sub> values calculated to be 703-763 days at 30°N, 634-688 d at 40°N and 591-641 days at 50°N.

Photodegradation is not expected to be a major degradation process in soil for inpyrfluxam.

**B.8.1.1.2. Rate of degradation in soil****B.8.1.1.2.1. Aerobic rate of degradation of the active substance**

<b>Data Point:</b>	KCA 7.1.1.1/01, KCA 7.1.1.1/02
<b>Report Author:</b>	██████ & ██████████
<b>Report Year:</b>	2023
<b>Report Title:</b>	<p>KCA 7.1.1.1_01: Aerobic Soil Metabolism of [Phenyl-<sup>14</sup>C] S-2399 and [Pyrazolyl-4-<sup>14</sup>C] S-2399; Amended Report</p> <p>KCA 7.1.1.1_02: S-2399: Degradation under Aerobic Conditions in Soil Rate Studies</p> <p>Kinetic Modelling: Recalculation of the laboratory aerobic degradation rate of inpyrfluxam (S-2399) in soil according to FOCUS Kinetics Guidance</p>
<b>Guideline(s) followed in study:</b>	FOCUS (2006), FOCUS (2014)
<b>Previous evaluation:</b>	New data, submitted for purpose of review
<b>GLP:</b>	<p>Underlying aerobic study: Yes</p> <p>Kinetic Modelling: Not applicable</p>

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Metabolite 3'-OH-S-2840 concentration set to 0 at time zero, when metabolite was detected consistently in starting mixture	Minor deviation. As this study is ultimately only relied upon by the applicant for parent degradation, with reliable metabolite endpoints evaluated from separate metabolite dosed studies, this does not affect study outcome. In the independent HSE assessment of the pathway fit for parent + metabolites, the correct handling of the 3'-OH-S-2840 metabolite in the dosing solution was carried out.
Penn soil study duration exceeds 120 days	Biomass measurements provided by the applicant demonstrate that the soil is still viable at the end of the study. (KCA 7.1.1.1_01)
Penn soil replicate measurements were averaged before modelling	Minor deviation, from FOCUS recommendation. HSE has modelled the individual replicates.
<b>HSE conclusions on deviations</b>	
No deviations considered to void the study validity.	

## Introduction

A kinetic evaluation of the degradation behaviour of inpyrfluxam (S-2399) and metabolites in two UK and two US soils under laboratory conditions in the dark at  $20 \pm 2^\circ\text{C}$  was undertaken. The evaluation was conducted to derive kinetic parameters that are suitable to trigger additional studies (trigger endpoints) and for modelling and environmental risk assessments (modelling endpoints). An assessment of the route of degradation has been performed on one (Penn) soil using phenyl and pyrazolyl labels, in KCA 7.1.1.1\_01. The degradation rate of inpyrfluxam was determined in three further soils, using the pyrazolyl label only, in KCA 7.1.1.1\_02. The applicant's kinetic analysis was provided separately in appendix 1 of the submission folder, file 2006362.UK0-5243, title '*Recalculation of the laboratory aerobic degradation rate of inpyrfluxam (S-2399) in soil according to*

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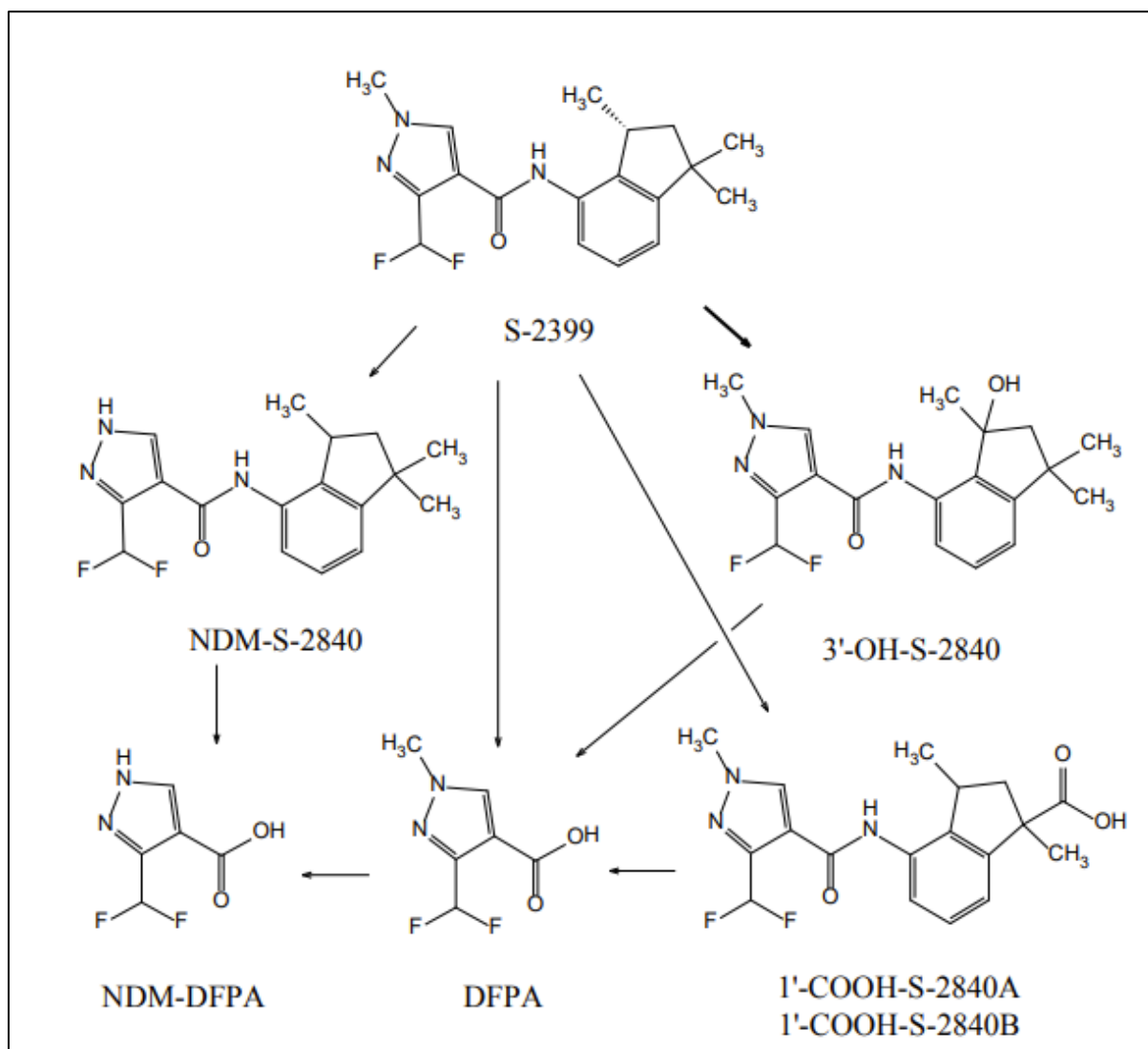
*FOCUS Kinetics Guidance*'. This file contains analysis of data determined in both KCA submissions, and is evaluated here.

Although the applicant proposed not to take any of the kinetic parameters for the metabolites from this study forward into the regulatory assessment (preferring to take metabolite endpoints from separate metabolite dosed studies instead), HSE has attempted to validate metabolite kinetics from the parent dosed study. HSE considers that useful information on metabolite behaviour when formed in situ from the parent dosed study may be gained from this assessment, and can be used alongside the information from the metabolite dosed studies in order to conclude on the overall persistence and degradation behaviour of the soil metabolites.

The route of degradation is shown in Figure B.8.1.1.2.1-1, and the KinGUI compartment model used for kinetic fitting is shown in Figure B.8.1.1.2.1-2. The KinGUI compartment model for fitting the parent with metabolites conducted as part of the independent HSE evaluation is shown in Figure B.8.1.1.2.1-3. A summary of the 'major' metabolites detected (and their max occurrences) in each parent-dosed study is presented in Table B.8.1.1.2.1-1. Mass balances for the degradation of

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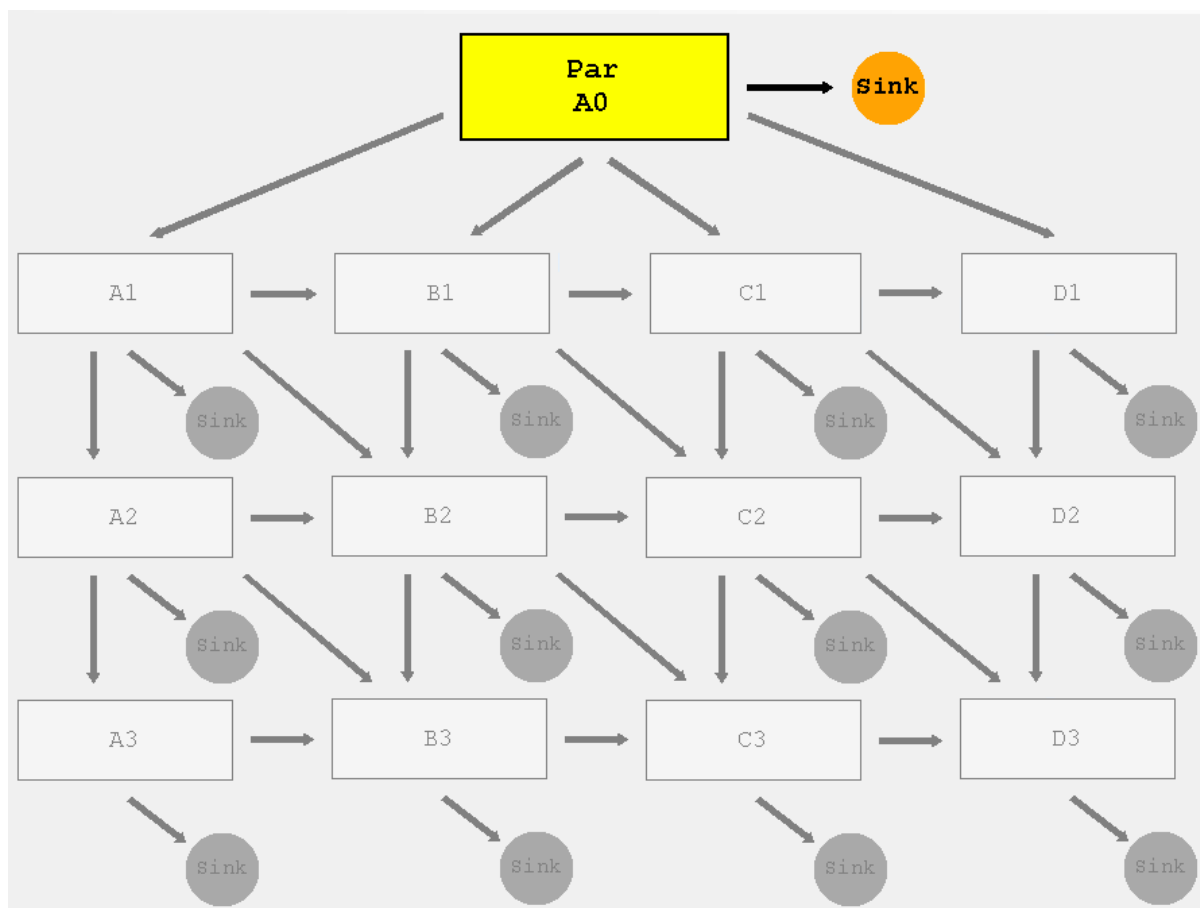
inpyrfluxam and production of major metabolites in the four soils are given in Table B.8.1.1.2.1-2 to Table B.8.1.1.2.1-6.



**Figure B.8.1.1.2.1-1 inpyrfluxam aerobic degradation in soil metabolic pathway**

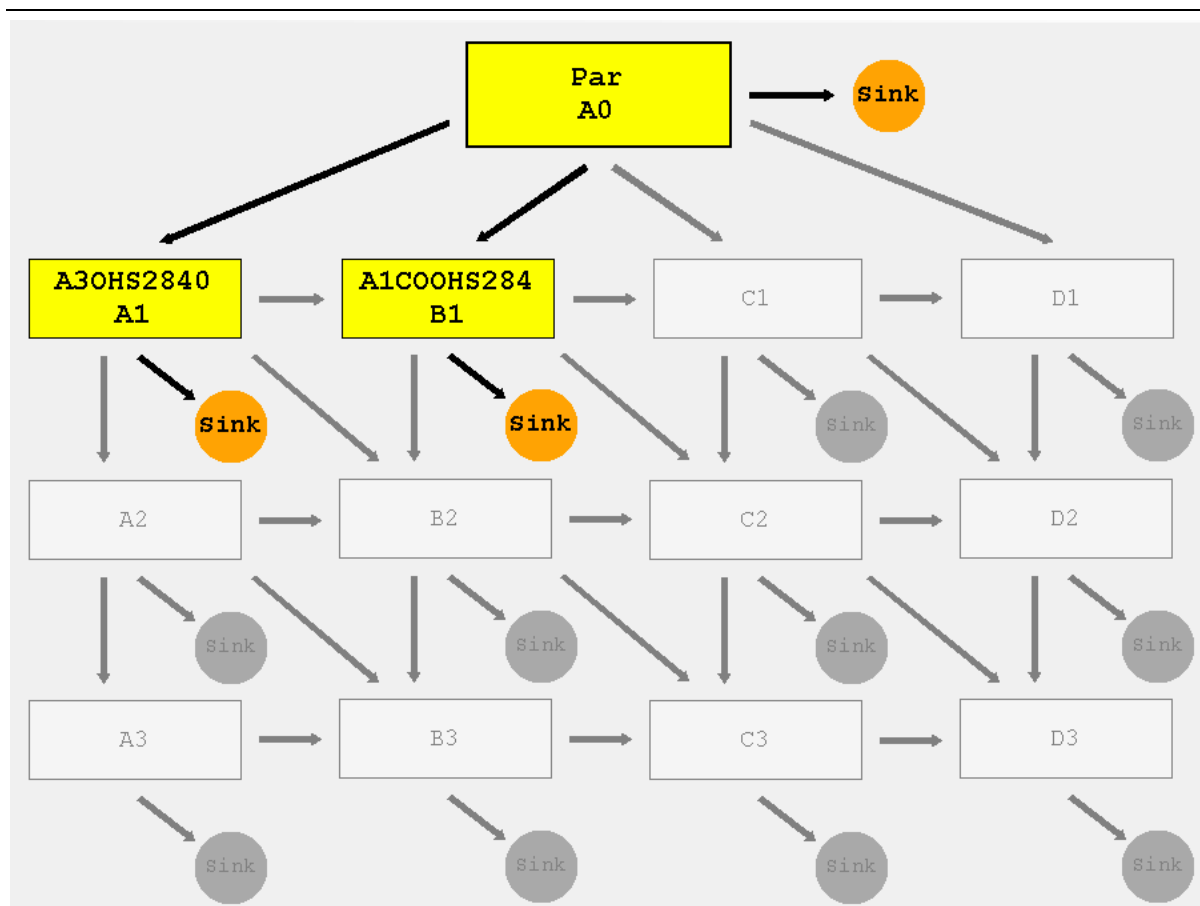
HSE notes that 1'-COOH-S-2840A & B each represent an enantiomeric pair, giving a total of four stereoisomers. For the purpose of kinetic degradation modelling, the applicant has considered these stereoisomers as one compound. HSE is satisfied that

this is appropriate, as no enantiomeric shift was noted during the aerobic soil metabolism study for 1'-COOH-S-2840, given at B.8.1.1.1.4.



**Figure B.8.1.1.2.1-2 Compartment model of aerobic soil degradation of inpyrfluxam degradation, parent only fits**





Par = inpyrfluxam

A3OHS2840 = 3'-OH-S-2840

A1COOHS284 = 1'-COOH-S-2840

**Figure B.8.1.1.2.1-3 Compartment model of aerobic soil degradation of inpyrfluxam degradation, parent with metabolites fit**

**Table B.8.1.1.2.1-1 Major metabolites detected in parent-dosed aerobic degradation in soil studies (levels <5% and not increasing by study end not included)**

<b>Soil</b>	<b>Test compound</b>	<b>[Phenyl-<sup>14</sup>C] inpyrfluxam*</b>	<b>[Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam</b>
Atwater	3'-OH-S-2840	-	22.5
	1'-COOH-S-2840	-	10
Newhaven	3'-OH-S-2840	-	11.1
	1'-COOH-S-2840	-	23.9
Penn	3'-OH-S-2840	19.7	18.6
	1'-COOH-S-2840	6.7	5.9
Woodside	3'-OH-S-2840	-	31.6
	1'-COOH-S-2840	-	8.4

\*The phenyl radio label was only assessed in the Penn soil

**Mass balances****Table B.8.1.1.2.1-2 Mass balances of parent and major metabolites in Atwater soil**

	<b>Days After Treatment (DAT)</b>											
<b>Fraction</b>	<b>0</b>		<b>14</b>		<b>30</b>		<b>61</b>		<b>90</b>		<b>120</b>	
<b>Sample rep</b>	1	2	1	2	1	2	1	2	1	2	1	2
Inpyrfluxam	94.9	95.3	87.4	83.0	ND	80.1	69.0	65.7	56.5	54.1	48.0	47.4
1'-COOH-S-2840 (total)*	0	0	4.7	4	ND	4.9	6.9	6.9	9.2	10	7.1	8
3'-OH-S-2840	1.8	2.3	4.3	4.0	ND	6.3	11.6	11.1	16.2	16.3	22.5	18.8

ND = no data (sample lost during workup)

\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

LOQ and LOD are &lt;&lt;0.01% AR

**Table B.8.1.1.2.1-3 Mass balances of parent and major metabolites in Newhaven soil**

	<b>Days After Treatment (DAT)</b>											
<b>Fraction</b>	<b>0</b>		<b>14</b>		<b>30</b>		<b>61</b>		<b>90</b>		<b>120</b>	
<b>Sample rep</b>	1	2	1	2	1	2	1	2	1	2	1	2
Inpyrfluxam	96.3	96.4	67.1	71.7	57.3	ND	46.8	49.6	50.6	44.0	47.1	45.0
1'-COOH-S-2840 (total)*	0	0	15.4	15.4	18.1	ND	23.9	21.6	17.8	21.8	20.4	19.2
3'-OH-S-2840	2.0	1.9	6.8	7.8	7.0	ND	9.8	8.9	9.6	9.0	10.0	11.1

ND = no data (sample lost during workup)

\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

LOQ and LOD are <<0.01% AR

**Table B.8.1.1.2.1-4 Mass balances of parent and major metabolites in Penn soil – phenyl label**

Test Substance	Fraction	Days After Treatment (DAT)									
		0		7		14		30		63	
[Phenyl <sup>14</sup> C] inpyrfluxam	Sample rep	1	2	1	2	1	2	1	2	1	2
	Inpyrfluxam	92.99	93.33	90.51	88.38	86.17	88.17	82.50	83.70	68.78	75.22
	3'-OH-S-2840 (incl. dehydrate)	0.95	1.12	1.96	2.04	2.42	2.32	5.28	5.43	10.95	10.60
	1'-COOH-S-2840 (total)*	0		2.5		3.9		5.1		5.9	
	Days After Treatment (DAT)										
	Fraction	93		120		150		182			
	Sample rep	1	2	1	2	1	2	1	2		
	Inpyrfluxam	64.35	64.71	62.18	63.07	57.09	53.64	51.73	55.20		
	3'-OH-S-2840 (incl. dehydrate)	12.01	10.84	12.32	12.86	14.99	13.41	20.88	18.57		
	1'-COOH-S-2840 (total)*	6.2		6.0		6.6		6.7			

\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B. The applicant has not tabulated individual sample determinations for 1'-COOH-S-2840 so an average of duplicate samples is provided.

LOQ and LOD are <<0.01% AR

**Table B.8.1.1.2.1-5 Mass balances of parent and major metabolites in Penn soil – pyrazolyl label**

Test Substance	Fraction	Days After Treatment (DAT)									
		0		7		14		30		63	
[Pyrazolyl <sup>14</sup> C] inpyrfluxam	Sample rep	1	2	1	2	1	2	1	2	1	2
	Inpyrfluxam	92.99	93.33	90.51	88.38	86.17	88.17	82.50	83.70	68.78	75.22
	3'-OH-S-2840 (incl. dehydrate)	0.95	1.12	1.96	2.04	2.42	2.32	5.28	5.43	10.95	10.60
	1'-COOH-S-2840 (total)*	0		2.5		3.9		5.1		5.9	
		Days After Treatment (DAT)									
	Fraction	93		120		150		182			
	Sample rep	1	2	1	2	1	2	1	2		
	Inpyrfluxam	64.90	65.63	64.03	60.25	54.83	58.05	51.66	51.73		
	3'-OH-S-2840 (incl. dehydrate)	13.39	12.34	11.42	13.60	14.83	12.29	19.31	17.90		
	1'-COOH-S-2840 (total)*	5.3		5.4		5.5		5.9			

\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B. The applicant has not tabulated individual sample determinations for 1'-COOH-S-2840 so an average of duplicate samples is provided.

LOQ and LOD are <<0.01% AR

**Table B.8.1.1.2.1-6 Mass balances of parent and major metabolites in Woodside soil**

	<b>Days After Treatment (DAT)</b>											
<b>Fraction</b>	<b>0</b>		<b>14</b>		<b>30</b>		<b>61</b>		<b>90</b>		<b>120</b>	
<b>Sample rep</b>	1	2	1	2	1	2	1	2	1	2	1	2
Inpyrfluxam	94.8	95.0	78.9	80.3	69.9	68.3	53.8	53.7	46.6	47.5	39.6	44.0
1'-COOH-S-2840 (total)*	0	0	10.8	9.2	14.7	15.3	24	24.7	27.5	27.9	31.6	28.5
3'-OH-S-2840	1.9	2.1	5.7	5.6	6.4	6.1	7.8	6.9	8.1	7.7	8.4	8.4

\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

LOQ and LOD are <<0.01% AR

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## Kinetic assessment

Metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 were detected in all four soils at either >10 % AR, or consecutively > 5% AR for two time points. In Atwater soil, the major route of degradation was via 3'-OH-S-2840, in the other three soils it was via 1'-COOH-S-2840.

The selection of the most appropriate kinetic model was based on a detailed statistical analysis including visual assessment,  $\chi^2$  error % statistic, randomness of residuals, and t-test significance following the FOCUS guidance (2006, 2014a).

The applicant used CAKE v3.7 to perform their kinetic analysis (with IRLS selected); HSE has validated the modelling using KinGUI (also with IRLS). In cases where HSE has performed modelling with fixed k values (i.e DFOP parent  $k_2$  and metabolite k values) , CAKE v3.7 has been used. While HSE notes that CAKE v3.7 is the program the applicant has performed their own modelling in, the applicant has not fixed rate constant parameters in any of their modelling provided. Therefore HSE is of the view that using CAKE v3.7 to perform only novel modelling of the data is not duplication, and is therefore an acceptable form of independent validation and testing.

## Kinetic modelling

The degradation behaviour of pyrazolyl and phenyl (Penn soil only) and pyrazolyl labelled inpyrfluxam was assessed in two US and two UK soils. For the Penn soil, both the phenyl and pyrazolyl labels are present in both major metabolites. Furthermore the two label studies were performed concurrently, at the same laboratory. Therefore HSE views the combining of the data from separate labels as true independent replicates for kinetic analysis as acceptable.

Once the label studies were combined, the applicant has averaged duplicate samples at each timepoint for each label before performing kinetic modelling. As the samples were incubated individually in separate flasks, they are considered true, independent replicates by HSE. By FOCUS guidance it is recommended that they should therefore not be averaged before modelling.

HSE notes that this is therefore a minor deviation from FOCUS guidance, though does not consider this to invalidate the applicant's kinetic modelling as this is simply a recommendation. HSE has, however, performed kinetic modelling on the individual sample replicates, giving four data points at each sampling date (duplicates samples for both labels) in order to evaluate the degradation of inpyrfluxam within the Penn soil as independently as possible.

Best-fit endpoints were determined for the parent-only degradation in order to assess whether soil dissipation studies are triggered, for use in the Persistence assessment and/or for PECsoil calculations. In line with the FOCUS guidance, the applicant ran



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SFO and FOMC initially and then proceeded to run DFOP and HS models when necessary.

Modelling endpoints were determined according to FOCUS guidance. In the independent HSE fitting, when a choice was to be made between biphasic models, DFOP was favoured by HSE due to the increased suitability for environmental modelling (eg for implementation in FOCUS models). Pseudo SFO DT<sub>50</sub> values and separate fast and slow phase DT<sub>50</sub>'s are given in Table B.8.1.1.2.1-19 in case higher tier refinement of modelling was required, and to facilitate comparison of laboratory and field derived modelling endpoints.

The applicant rated the fits using the following scale:

- Not acceptable: the fitted curve does not represent the trend of the data points and residuals show strong deviations from random distribution.
- Acceptable: the fitted curve describes the trend of the data points, residuals may show some deviations from random distribution but it is not significant.
- Good: the fitted curve closely follows all the data points (limited scatter of data points); residuals are randomly distributed (no bias of residuals).
- Very good: no bias of residuals or scatter of data points.

HSE has also followed these definitions in its own, independent assessment.

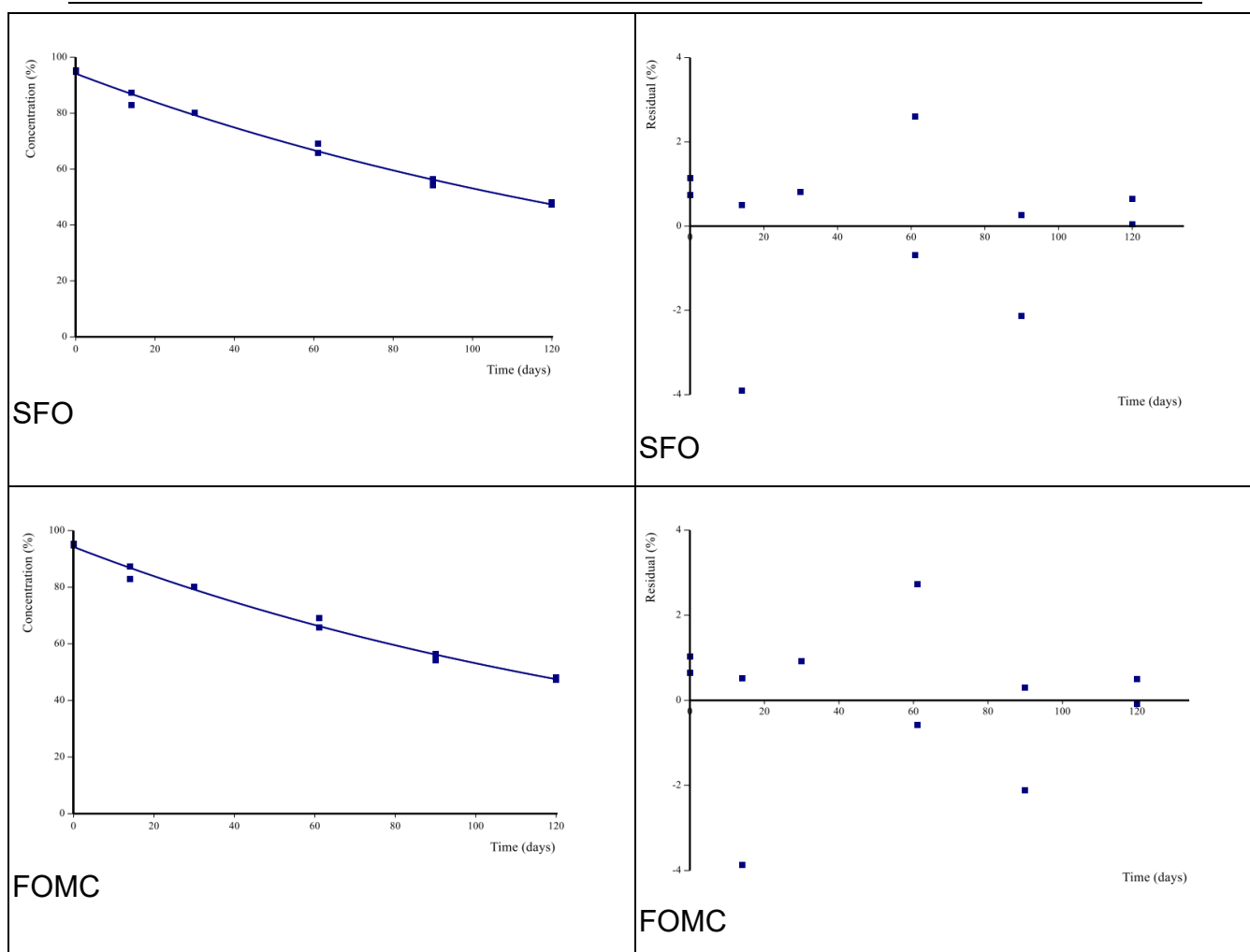
Summary tables of trigger and modelling endpoints for inpyrfluxam independently validated by HSE are given below in Table B.8.1.1.2.1-18 and Table B.8.1.1.2.1-19 respectively.

**Table B.8.1.1.2.1-7 Applicant statistics of degradation of inpyrfluxam in Atwater soil under aerobic conditions (parent only fit, not normalised)**

<b>Atwater</b> <b>(KCA 7.1.1.1_02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>very good</b>	<b>1.14</b>	<b>M<sub>0</sub>: 94.2</b> <b>k: 0.00573</b>	<b>&lt; 0.01</b>	<b>121</b>	<b>402</b>
FOMC	very good	1.26	M <sub>0</sub> : 94.3 alpha: 15.1 beta: 2583	not applicable	121	426
<b>Conclusion: The applicant has selected SFO for trigger and modelling endpoints (DT<sub>50</sub> = 121 days, DT<sub>90</sub> = 402 days, pseudo SFO DT<sub>50</sub> = 121 days)</b>						

**Table B.8.1.1.2.1-8 HSE statistics of degradation of inpyrfluxam in Atwater soil under aerobic conditions (parent only fit, not normalised)**

<b>Atwater</b> <b>(KCA 7.1.1.1_02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>good</b>	<b>1.14</b>	<b>M<sub>0</sub>: 94.2</b> <b>k: 0.00573</b>	<b>k: &lt; 0.05</b>	<b>121</b>	<b>402</b>
FOMC	good	1.26	M <sub>0</sub> : 94.3 alpha: 15.1 beta: 2580	not applicable	121	426
DFOP	good	1.28	M <sub>0</sub> : 95.1 k <sub>1</sub> : 1.46 k <sub>2</sub> : 0.00575 g: 0.022	k <sub>1</sub> : < 0.05 k <sub>2</sub> : < 0.05	121	410
<p><b>SFO:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are small and randomly distributed.</p> <p><b>FOMC:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are small and randomly distributed, though no improvement compared to SFO.</p> <p><b>DFOP:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are small and randomly distributed, as with FOMC, no improvement compared to SFO however.</p> <p><b>Conclusion:</b> select <b>SFO</b> for trigger and modelling endpoints (DT<sub>50</sub> = 121 days, DT<sub>90</sub> = 402 days, pseudo SFO DT<sub>50</sub> = 121 days)</p>						



**Figure B.8.1.1.2.2-4 Applicant visual-fits and residual plots of degradation of inpyrfluxam in Atwater soil under aerobic conditions (parent only fit, not normalised)**

### Trigger

The applicant has selected an SFO fit. HSE agrees that this is the best-fit model, and acceptable by FOCUS guidance.

### Modelling

The applicant concluded that for modelling endpoint selection the SFO fit was visually and statistically acceptable for inpyrfluxam. HSE agrees with this assessment. Therefore an SFO model was accepted by HSE for the parent

compound in parent-only fits for the Atwater soil. HSE agrees with the applicant DT<sub>50</sub> of 121 d for the Atwater soil.

**Table B.8.1.1.2.1-9 Applicant statistics of degradation of inpyrfluxam in Newhaven soil under aerobic conditions (parent only fit, not normalised)**

<b>Newhaven (KCA 7.1.1.1_02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	acceptable	11.5	M <sub>0</sub> : 84.6 k: 0.00683	k: < 0.01	102	337
<b>FOMC</b>	<b>very good</b>	<b>2.36</b>	<b>M<sub>0</sub>: 96.4</b> <b>alpha: 0.223</b> <b>beta: 3.7</b>	<b>not applicable</b>	<b>79.1</b>	<b>&gt; 1,000</b>
DFOP	very good	0.728	M <sub>0</sub> : 96.3 k <sub>1</sub> : 0.0571 k <sub>2</sub> : 0.000403 g: 0.498	k <sub>1</sub> : < 0.01 k <sub>2</sub> : 0.367	66.9	4004
<b>HS</b>	<b>very good</b>	<b>2.88</b>	<b>M<sub>0</sub>: 96.4</b> <b>k<sub>1</sub>: 0.0234</b> <b>k<sub>2</sub>: 0.00208</b> <b>t<sub>b</sub>: 24.2</b>	<b>k<sub>1</sub>: &lt; 0.01</b> <b>k<sub>2</sub>: 0.0146</b>	<b>85.2</b>	<b>859</b>
<b>Conclusion: The applicant has selected FOMC for trigger endpoints (DT<sub>50</sub> = 79.1 days, DT<sub>90</sub> = &gt; 1,000 days) and HS for modelling endpoints ( pseudo SFO DT<sub>50</sub> = 333 days)</b>						

**Table B.8.1.1.2.1-10 HSE statistics of degradation of inpyrfluxam in Newhaven soil under aerobic conditions (parent only fit, not normalised)**

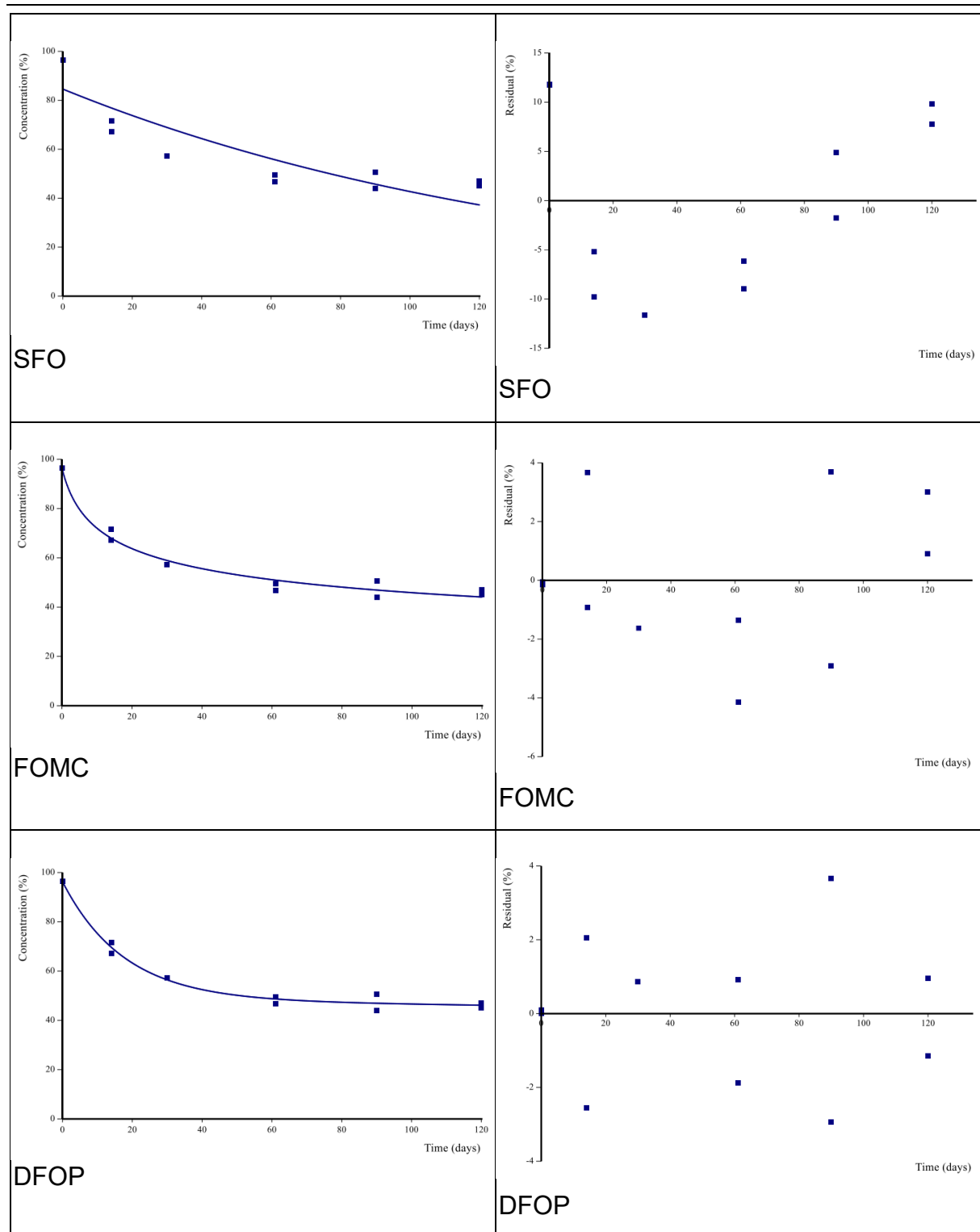
<b>Newhaven (KCA 7.1.1.1_02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	unacceptable	11.5	M <sub>0</sub> : 84.6 k: 0.00683	k: < 0.05	102	337
FOMC	acceptable	2.36	M <sub>0</sub> : 96.4 alpha: 0.223 beta: 3.70	not applicable	79.1	>1,000
DFOP	good	0.728	M <sub>0</sub> : 96.3 k <sub>1</sub> : 0.0571 k <sub>2</sub> : 4.03e-04 g: 0.498	k <sub>1</sub> : < 0.05 k <sub>2</sub> : 0.367	66.9	>1,000
<b>DFOP (k<sub>2</sub> fixed)</b>	<b>good</b>	<b>0.662</b>	<b>M<sub>0</sub>: 96.3</b> <b>k<sub>1</sub>: 0.0595</b> <b>k<sub>2</sub>: 0.000693</b> <b>g: 0.483</b>	<b>k<sub>1</sub>: &lt; 0.05</b>	<b>69.7</b>	<b>&gt;1,000</b>
HS	unacceptable	5.93	M <sub>0</sub> : 96.4 k <sub>1</sub> : 0.106 k <sub>2</sub> : 0.00412 t <sub>b</sub> : 3.26	k <sub>1</sub> : < 0.05 k <sub>2</sub> : < 0.05	87.6	478
<p><b>SFO:</b> Unacceptable fit visually. Degradation underestimated at lower timepoints, and overestimated towards study end, producing large and systematically distributed residuals.</p> <p><b>FOMC:</b> Acceptable fit visually and statistically. However, fit does not pass between all duplicate samplings. DT<sub>90</sub> extrapolated well beyond study duration, adding additional uncertainty to FOMC endpoints, and reduced reliability for PECsoil calculations.</p>						

**DFOP:** Good fit, visually and statistically, but  $k_2$  fails t-test;  $\chi^2$  error is very low and the residuals are very small and randomly distributed. Fitted curve bisects all duplicate sampling pairs.

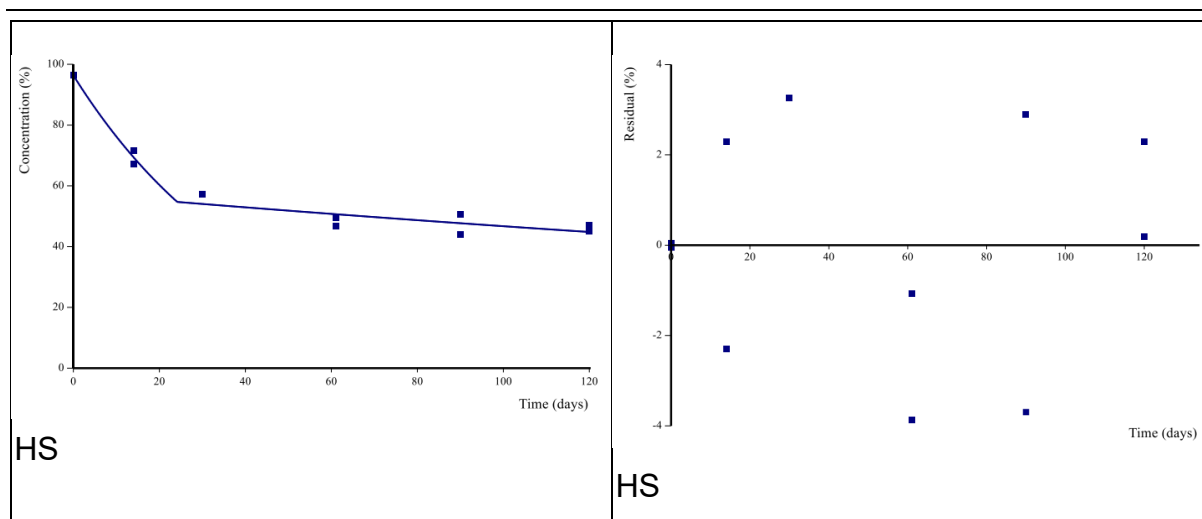
**DFOP ( $k_2$  fixed):** Good fit, visually and statistically. Similar  $M_0$  and  $g$  values to unfixed DFOP. Very low  $\chi^2$  value.

**HS:** Unacceptable fit visually, residuals are not randomly distributed and degradation is overestimated towards study end.

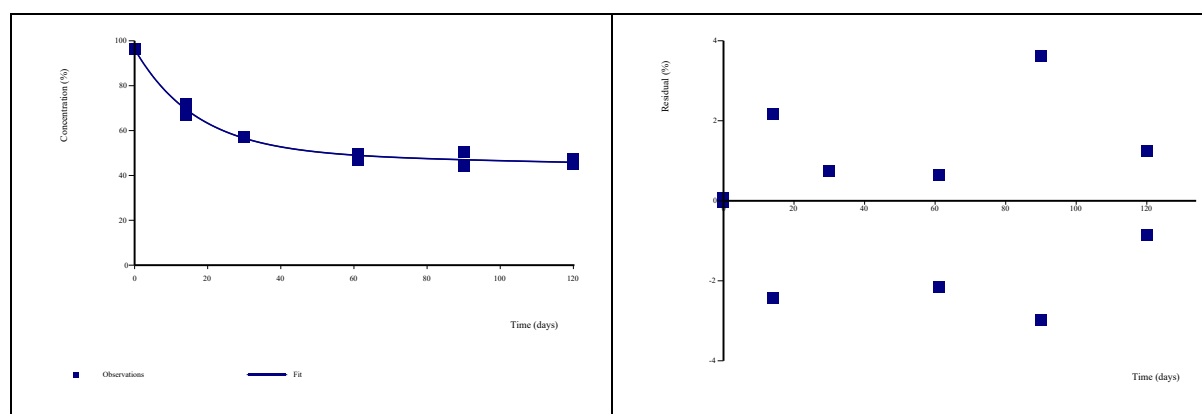
**Conclusion: select DFOP ( $k_2$  fixed) for trigger and modelling endpoints (pseudo SFO  $DT_{50} = 1,000$  days)**







**Figure B.8.1.1.2.1-5 Applicant visual-fits and residual plots of degradation of inpyrfluxam in Newhaven soil under aerobic conditions (parent only fit, not normalised)**



**Figure B.8.1.1.2.1-6 HSE Newhaven soil DFOP fit,  $k_2$   $DT_{50}$  fixed at 1000 d**

### Trigger

The applicant has selected an FOMC fit for the Newhaven parent-only system, on the basis of a better visual fit than SFO. Following FOCUS guidance, HSE finds that a DFOP fit is the most appropriate best fit model, as a lower  $\chi^2$  value was determined, and FOMC slightly underestimates inpyrfluxam concentration at the final timepoint, leading to a slightly better visual fit for the DFOP kinetic. In accepting the DFOP fit HSE has carried out an additional step of fixing the  $k_2$  rate constant to a value equivalent to a  $DT_{50}$  of 1000 d, noting that strictly the freely optimised  $k_2$  value failed the t-test. In reaching this decision HSE has put more weight on the quality of the visual fit, and the lower  $\chi^2$  value in particular when comparing DFOP with the

alternative biphasic models. In addition it is noted that the FOMC  $DT_{90}$  was reported to be >1,000 d, and also likely to be subject to a high degree of uncertainty.

## Modelling

The applicant selected a HS model for the Newhaven soil. HSE notes that by FOCUS guidance, because the  $DT_{90}$  was extrapolated beyond study duration, a choice between a HS or DFOP model needs to be made in this instance.

HSE notes that the DFOP model provides a better statistical fit than the HS model based on the lower  $\chi^2$  value. In addition, looking at the underlying data and choice of sample points, there was no obvious evidence of a clear break point indicative of hockey stick behaviour. HSE also notes that in the kinetic evaluation performed in KinGUI, a HS fit of the Newhaven system produced a different  $t_b$  (applicant = 24.2 d, HSE = 3.26 d). This might have been due to differences in the individual fitting tools, but may also be indicative of uncertainty in the hockey stick fit (ie where alternative sets of parameters are able to describe the data adequately).

HSE notes that the applicant's and HSE's DFOP model contain a  $k_2$  value that fails the t-test ( $p > 0.05$ ). This is expected, as the  $k$  value is small, and is therefore not significantly different from 0. However HSE does not consider the t-test result alone to be a reason to select HS over the DFOP model. Instead, to account for the possible uncertainty in the  $k_2$  value, has refit the DFOP fit for this soil, with the  $k_2$  value fixed to a value of 6.93E-04 (equivalent to a default  $DT_{50}$  of 1000 d). As can be seen from the visual fit, fixing the  $k_2$  value still resulted in an acceptable visual fit, and therefore HSE proposes to use this specific fit for modelling purposes for this soil.

Therefore, HSE does not accept the applicants selection of the HS fit with a pseudo SFO  $DT_{50}$  of 333 d. Instead, the DFOP fit is selected for the Newhaven soil, in

combination with a fixed  $k_2$  rate constant giving a  $DT_{50}$  of 69.7 d and  $DT_{90}$  of >1,000 d, and a pseudo SFO  $DT_{50}$  1000 d.

**Table B.8.1.1.2.1-11 Applicant statistics of degradation of inpyrfluxam in Penn soil under aerobic conditions (parent only fit, not normalised)**

<b>Penn (KCA 7.1.1.1_01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b><math>DT_{50}</math> (d)</b>	<b><math>DT_{90}</math> (d)</b>
SFO	very good	1.70	$M_0$ : 91.2 k: 0.00326	k: < 0.01	213	706
FOMC	very good	1.02	$M_0$ : 92.8 alpha: 0.769 beta: 166	not applicable	243	3145
DFOP	very good	1.09	$M_0$ : 92.8 $k_1$ : 0.0125 $k_2$ : 0.0171 g: 0.265	$k_1$ : 0.158 $k_2$ : 0.182	241	1166
<b>Conclusion: The applicant has selected FOMC for trigger endpoints (<math>DT_{50}</math> = 243 days, <math>DT_{90}</math> = &gt;1,000 days) and SFO for modelling endpoints ( pseudo SFO <math>DT_{50}</math> = 213 days)</b>						

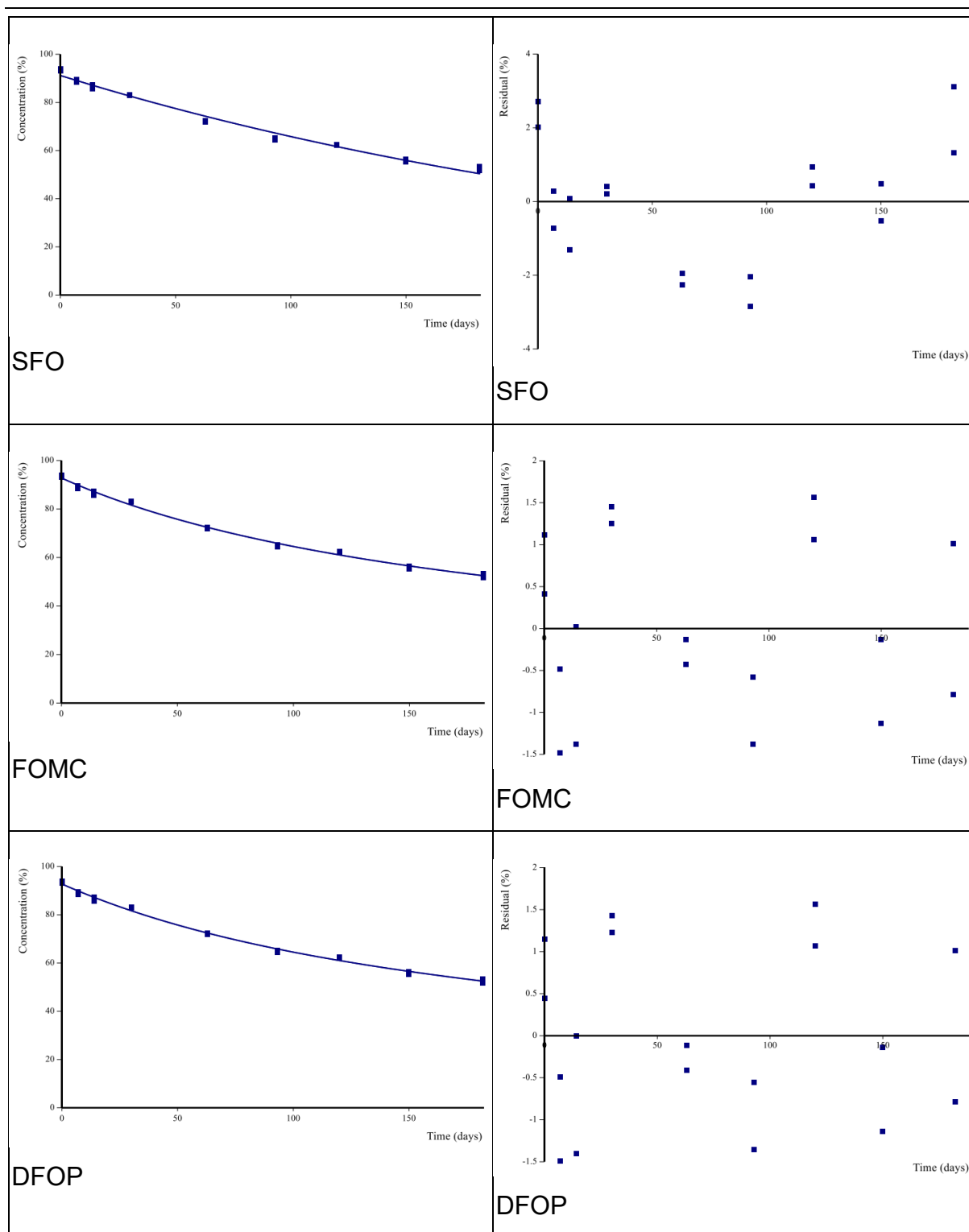
**Table B.8.1.1.2.1-12 HSE statistics of degradation of inpyrfluxam in Penn soil under aerobic conditions (parent only fit, not normalised)**

<b>Penn (KCA 7.1.1.1_01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	acceptable	1.70	M <sub>0</sub> : 91.2 k: 3.26e-03	k: < 0.05	213	707
FOMC	good	1.01	M <sub>0</sub> : 92.8 alpha: 0.775 beta: 167.7	not applicable	242	>1,000
DFOP	good	1.08	M <sub>0</sub> : 92.7 k <sub>1</sub> : 0.0126 k <sub>2</sub> : 0.00174 g: 0.258	k <sub>1</sub> : 0.183 k <sub>2</sub> : 0.196	240	>1,000
<b>DFOP (k<sub>2</sub> fixed)</b>	<b>good</b>	<b>1.02</b>	<b>M<sub>0</sub>: 92.6</b> <b>k<sub>1</sub>: 0.00861</b> <b>k<sub>2</sub>: 0.000693</b> <b>g: 0.465</b>	<b>k<sub>1</sub>: &lt; 0.05</b>	<b>254.1</b>	<b>&gt;1,000</b>
<p><b>SFO:</b> Acceptable fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are small, however they are not randomly distributed.</p> <p><b>FOMC:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are very small and randomly distributed. DT<sub>90</sub> extrapolated well beyond study duration, adding additional uncertainty to FOMC endpoints, and reduced reliability for PECsoil calculations.</p> <p><b>DFOP:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are very small and randomly distributed, however, both k values fail the t-test.</p> <p><b>DFOP (k<sub>2</sub> fixed):</b> Good fit, visually and statistically. Very low <math>\chi^2</math> value with randomly distributed residuals.</p>						

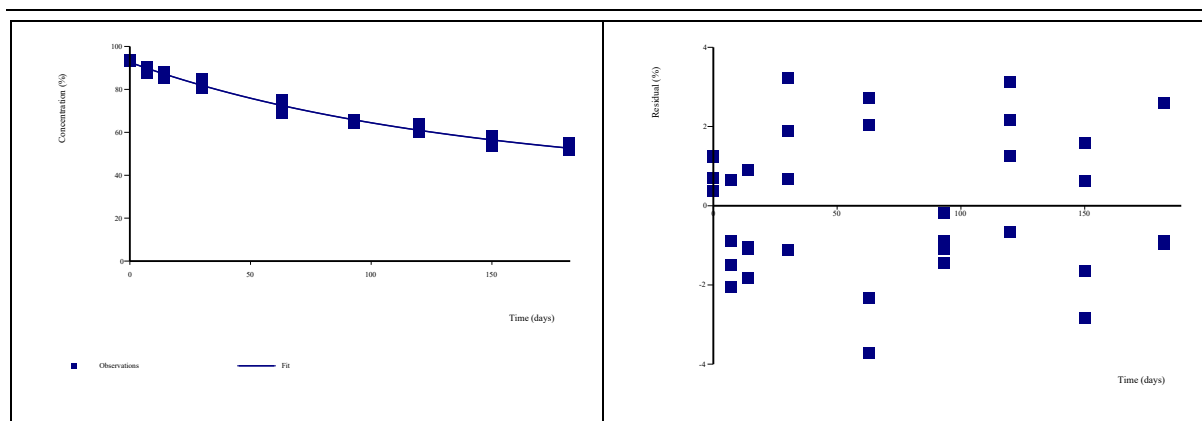
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**Conclusion: select HSE DFOP ( $k_2$  fixed) fit for trigger and modelling endpoints ( $DT_{50} = 254$  days,  $DT_{90} = 1,000$  days, pseudo SFO  $DT_{50} = 1,000$  days)**

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**Figure B.8.1.1.2.1-7 Applicant visual-fits and residual plots of degradation of inpyrfluxam in Penn soil under aerobic conditions (parent only fit, not normalised)**



**Figure B.8.1.1.2.1-8 HSE Penn soil DFOP fit,  $k_2$   $DT_{50}$  fixed at 1000 d**

### Trigger

The applicant has selected an FOMC fit for the Penn soil. HSE considers that a DFOP model with  $k_2$  fixed is more appropriate, as the  $DT_{90}$  value in this instance is extrapolated well beyond the study duration. HSE therefore proposes to accept the parent DFOP fit for this soil with the fixed  $k_2$  rate constant. This gives a  $DT_{50}$  of 254.1 d and  $DT_{90}$  of >1,000 d, and a pseudo SFO  $DT_{50}$  of 1000 d.

### Modelling

The applicant proposed that for modelling endpoint selection the SFO fit was visually acceptable for inpyrfluxam. Therefore, the applicant selected the SFO model to determine modelling endpoints for parent and for use in the metabolite calculations. HSE considers that although the SFO parent-only fit met the statistical criteria for fitting, and the difference in quality of visual fit between SFO and biphasic models was very marginal, the SFO model potentially underestimated the parent concentration at the final time points. In addition, more than 50% parent remained undegraded at the end of the study duration, and therefore the potential for the chosen kinetic model and the actual substance behaviour to deviate more significantly over longer time periods is greater in these circumstances. Furthermore, when HSE attempted to fit parent plus metabolites, it was noted that the DFOP model appeared to be necessary to achieve acceptable metabolite fits. For these reasons, HSE proposed to use the DFOP model for the Penn soil.

HSE notes that a DFOP fit gave t-test p values for  $k_1$  and  $k_2$  larger than 0.05 whereas an SFO fit did not. To account for the possible uncertainty in the DFOP rate constants, HSE first set the  $k_2$  to a representative value corresponding to a  $DT_{50}$  of 1000 d. The fit is presented in Figure B.8.1.1.2.2-8 and the statistics given in Table B.8.1.1.2.1-12. The resultant fit was visually acceptable, and the re-optimised  $k_1$  variable passed the t-test with a value of  $1.02e-06$ . This fit was performed in CAKE 3.7. HSE therefore proposes to accept the parent DFOP fit for this soil with the fixed

$k_2$  rate constant. This gives a  $DT_{50}$  of 254.1 d and  $DT_{90}$  of >1,000 d, and a pseudo SFO  $DT_{50}$  of 1000 d.

**Table B.8.1.1.2.1-13 Applicant statistics of degradation of inpyrfluxam in Woodside soil under aerobic conditions (parent only fit, not normalised)**

<b>Woodside (KCA 7.1.1.1_02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b><math>DT_{50}</math> (d)</b>	<b><math>DT_{90}</math> (d)</b>
SFO	acceptable	4.25	$M_0$ : 90.3 $k$ : 0.00738	$k$ : < 0.01	93.9	312
<b>FOMC</b>	<b>very good</b>	<b>0.762</b>	<b><math>M_0</math>: 95 <math>\alpha</math>: 0.626 <math>\beta</math>: 43.3</b>	<b>not applicable</b>	<b>87.8</b>	<b>1674</b>
DFOP	very good	0.714	$M_0$ : 94.7 $k_1$ : 0.0255 $k_2$ : 0.0021 $g$ : 0.46	$k_1$ : 0.0108 $k_2$ : 0.201	87.2	805
<b>HS</b>	<b>very good</b>	<b>1.18</b>	<b><math>M_0</math>: 94.2 <math>k_1</math>: 0.0107 <math>k_2</math>: 0.00429 <math>t_b</math>: 46.9</b>	<b><math>k_1</math>: &lt; 0.01 <math>k_2</math>: &lt; 0.01</b>	<b>91.2</b>	<b>467</b>
<b>Conclusion: The applicant has selected FOMC for trigger endpoints (<math>DT_{50}</math> = 87.8 days, <math>DT_{90}</math> = 1674 days) and HS for modelling endpoints ( pseudo SFO <math>DT_{50}</math> = 162 days)</b>						

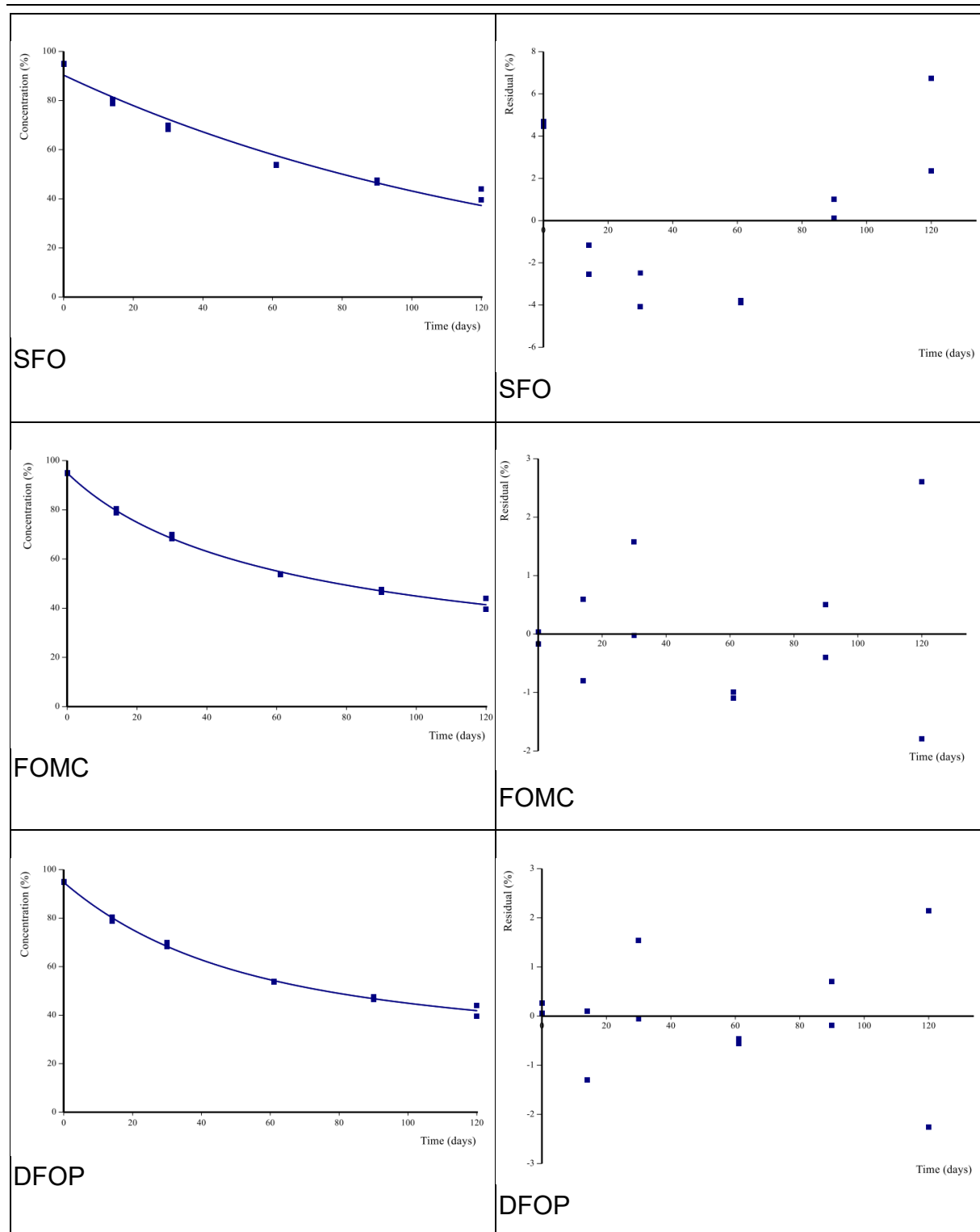


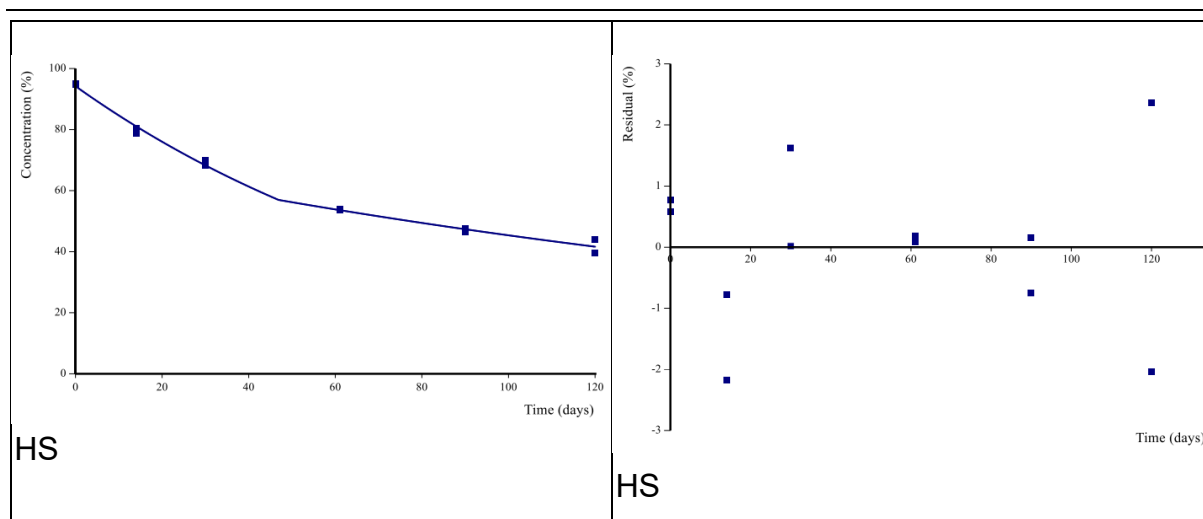
**Table B.8.1.1.2.1-14 HSE statistics of degradation of inpyrfluxam in Woodside soil under aerobic conditions (parent only fit, not normalised)**

<b>Woodside (KCA 7.1.1.1_02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	unacceptable	4.25	M <sub>0</sub> : 90.3 k: 7.83e-03	k: < 0.05	93.9	312
FOMC	good	0.762	M <sub>0</sub> : 95.0 alpha: 0.626 beta: 43.3	not applicable	87.8	>1,000
DFOP	good	0.714	M <sub>0</sub> : 94.7 k <sub>1</sub> : 0.0255 k <sub>2</sub> : 0.00210 g: 0.460	k <sub>1</sub> : < 0.05 k <sub>2</sub> : 0.201	87.2	805
<b>DFOP (k<sub>2</sub> fixed)</b>	<b>good</b>	<b>0.680</b>	<b>M<sub>0</sub>: 94.6</b> <b>k<sub>1</sub>: 0.02141</b> <b>k<sub>2</sub>: 0.000693</b> <b>g: 0.563</b>	<b>k<sub>1</sub>: &lt; 0.05</b>	<b>86.1</b>	<b>&gt;1,000</b>
HS	good	2.29	M <sub>0</sub> : 94.9 k <sub>1</sub> : 1.26e-02 k <sub>2</sub> : 5.81e-03 t <sub>b</sub> : 24.6	k <sub>1</sub> : < 0.05 k <sub>2</sub> : < 0.05	90.7	368
<p><b>SFO:</b> Good fit statistically; <math>\chi^2</math> error is low, however the residuals are considerable and systematically distributed, giving a poor visual fit.</p> <p><b>FOMC:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are small and randomly distributed. DT<sub>90</sub> extrapolated well beyond study duration, adding additional uncertainty to FOMC endpoints, and reduced reliability for PECsoil calculations.</p> <p><b>DFOP:</b> Good fit, visually and statistically; <math>\chi^2</math> error is very low and the residuals are very small and randomly distributed.</p>						

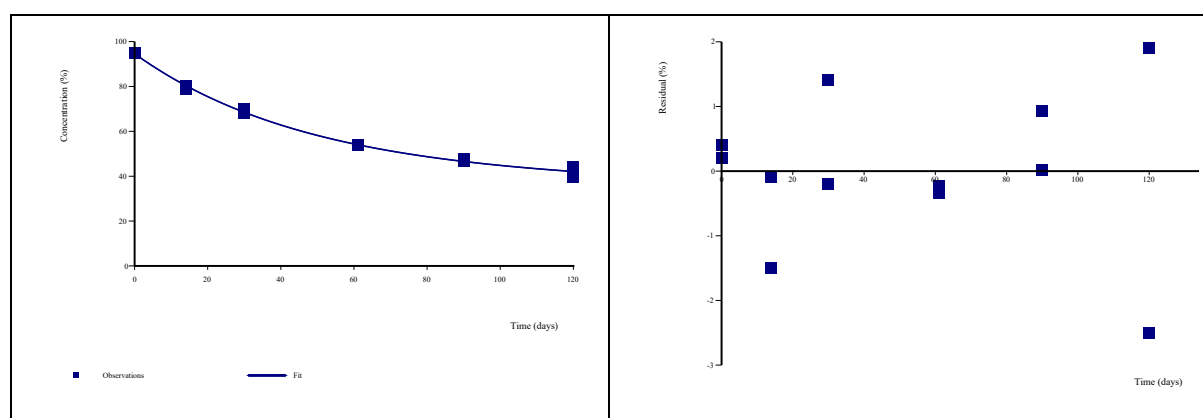
**DFOP ( $k_2$  fixed):** Good fit, visually and statistically. Similar  $M_0$  and  $g$  values to unfixed DFOP. Very low  $\chi^2$  value.

**Conclusion: select DFOP ( $k_2$  fixed) for trigger and modelling endpoints ( $DT_{50} = 86.1$  days,  $DT_{90} = 1,000$  days, pseudo SFO  $DT_{50} = 1,000$  days)**





**Figure B.8.1.1.2.1-9 Applicant visual-fits and residual plots of degradation of inpyrfluxam in Woodside soil under aerobic conditions (parent only fit, not normalised)**



**Figure B.8.1.1.2.1-10 HSE Woodside soil DFOP fit,  $k_2$   $DT_{50}$  fixed at 1000 d**

### Trigger

The applicant has selected an FOMC fit. HSE finds that a DFOP model produces a fit with a very slightly lower  $\chi^2$  value, even though the DFOP model has one more optimised parameter than FOMC, and one less degree of freedom as a result. HSE therefore does not accept the applicant's choice of FOMC as best fit model.

HSE notes that the applicant may have selected FOMC over DFOP as  $k_2$  for DFOP fails the t-test. However, as FOMC does not have associated  $k$  values to t-test, FOMC does not necessarily provide a more robust statistical fit. In this case, HSE also notes that the FOMC predicted  $DT_{90}$  is longer than that predicted by DFOP, and therefore also likely to be subject to a degree of uncertainty when extrapolated far beyond study duration. HSE therefore proposes to accept the parent DFOP fit for

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this soil with the fixed  $k_2$  rate constant. This gives a  $DT_{50}$  of 86.1 d and  $DT_{90}$  of >1,000 d, and a pseudo SFO  $DT_{50}$  of 1000 d.

## Modelling

The applicant selected a HS model for the Woodside soil. HSE notes that by FOCUS guidance, a choice between a HS or DFOP model needs to be made in this instance.

HSE notes that the DFOP model provides a better statistical fit than the HS model based on the lower  $\chi^2$  value. In addition, looking at the underlying data and choice of sample points, there was no obvious evidence of a clear break point indicative of hockey stick behaviour. HSE also notes that in the kinetic evaluation performed in KinGUI, a HS fit of the Woodside system produced a different  $t_b$ . (applicant = 46.9 d, HSE = 24.6 d). This might have been due to differences in the individual fitting tools, but may also be indicative of uncertainty in the hockey stick fit (ie where alternative sets of parameters are able to describe the data adequately). HSE further notes that a HS model of inpyrfluxam in Woodside soil provides a very poor fit for the 3'-OH-S-2840 metabolite, when metabolites are considered (Figure B.8.1.1.2.2- 17). HSE regards this as a further reason to select a DFOP model over HS for the parent model.

HSE notes that the applicant's and HSE's DFOP model contain a  $k_2$  value that fails the t-test ( $p > 0.05$ ). This is expected, as the  $k$  value is small, and is therefore not significantly different from 0. However HSE does not consider the t-test result alone to be a reason to select HS over the DFOP model.

Instead, to account for the possible uncertainty in the  $k_2$  value, has refit the DFOP fit for this soil, with the  $k_2$  value fixed to a value of 6.93E-04 (equivalent to a default  $DT_{50}$  of 1000 d). As can be seen from the visual fit, fixing the  $k_2$  value still resulted in an acceptable visual fit, and therefore HSE proposes to use this specific fit for modelling purposes for this soil. Therefore, HSE does not accept the applicant's selection of the HS fit with a pseudo SFO  $DT_{50}$  of 162 d. Instead, the DFOP fit is selected for the Woodside soil, in combination with a fixed  $k_2$  rate constant. This gives a  $DT_{50}$  of 86.1 d and  $DT_{90}$  of >1,000 d, and a pseudo SFO  $DT_{50}$  of 1000 d.

HSE finds that for Newhaven, Penn, and Woodside soils, a DFOP model with  $k_2$  fixed provides a better fit for the purposes of best-fit / trigger endpoint determination. HSE has also determined slightly different  $DT_{50}$  values in all soils apart from Atwater. However, HSE and the applicant are in agreement that all soils produce experimental  $DT_{50}$ 's > 60 days, therefore triggering field dissipation studies. These

have been provided by the applicant (KCA 7.1.2.2.1\_01 to KCA 7.1.2.2.1\_05). The longest laboratory soil DT<sub>50</sub> determined was 254 days, in the Penn soil.

HSE has used modelling of the parent run alone to select which parent model type metabolites should then be run with. Upon running models with the parent and metabolites, different parent endpoints can be determined from the parent-only models. Therefore discussion of parent endpoints taken for regulatory consideration is considered and discussed after parent and metabolite modelling.

The applicant notes that the kinetic DT<sub>50</sub> values determined for inpyrfluxam arise from laboratory studies undertaken at 20 °C and 50 % MWHC and hence need to be normalised to pF2 prior to input into EU models. However, since in each case the soil moisture content was slightly above the pF2 value, no correction ultimately needed to be made. HSE agrees with this assessment. Table B.8.1.1.2.1-15 below compares the soil moisture values during study conduct and at pF2.

**Table B.8.1.1.2.1-15 Comparison of soil moisture during study and at pF2**

<b>Soil</b>	<b>Study soil moisture (%)</b>	<b>pF2 soil moisture (%)</b>
Atwater	16.1	14.3
Newhaven	38.4	33
Penn	24.1	23.8
Woodside	28.9	28.6

For endpoints, the Kendall's test showed a weak positive relationship with pH for DT<sub>50</sub>, but a moderate negative correlation with pH for the DT<sub>90</sub>. Neither relationship was statistically significant and the opposite direction of the correlation is noted. The lack of a correlation with pH was corroborated by the linear regression with a maximum R<sup>2</sup> value of only 0.2403. HSE accepts that geometric mean averaging is acceptable for the determination of a modelling endpoint, in the absence of pH dependant degradation. pH dependence is discussed in more depth in the summary section (see B.8). Due to the biphasic nature of three out of four of the fits, HSE has calculated separate fast and slow-phase modelling endpoints for consideration in further modelling.

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## Modelling endpoints: Parent and Metabolite Modelling

As clear decline phases were not necessarily observed within the study duration for either major metabolite (3'-OH-S2840, 1'-COOH-S-2840) metabolite dosed studies were performed by the applicant. These have been evaluated separately by HSE (B.8.1.1.1.3 and B.8.1.1.1.4). These metabolite dosed studies have been used by the applicant to determine modelling endpoints for the metabolites. While the applicant has not proposed to use metabolite modelling endpoints from the parent dosed study being evaluated in this section, the results of the applicants fitting, and HSE's independent evaluation for the parent-dosed metabolite degradation study are evaluated here to verify the triggering of the additional metabolite studies. HSE also considers that useful information on metabolite behaviour when formed in situ from the parent dosed study may also be gained from this assessment.

The applicant has provided a parent-with-metabolite fit corresponding to their chosen kinetic model for modelling endpoint selection of the parent-only fits. These fits were also performed in CAKE 3.7 with IRLS selected. HSE has replicated this approach in KinGUI, with IRLS for the Atwater soil. For the other soils, HSE has also used CAKE 3.7 with IRLS selected. HSE notes that the applicant has set the  $M_0$  concentration of metabolite 3'-OH-S-2840 to 0, when HPLC chromatograms and AR measurements of the starting compound indicate it is present at an average of 1.7 % AR. By FOCUS guidance, HSE has not constrained  $M_0$  of 3'-OH-S-2840, but allowed it to be fit by the models as a separate parameter. In the HSE fitting, the parent kinetic model selected by HSE from the parent only fits was used for the pathway kinetic fit of the metabolites. In both the applicant and HSE fitting, all metabolites were assumed to degrade by SFO kinetics.

The degradation profiles of metabolites associated with the applicant's modelling endpoint selection for the parent compound are summarised in Table B.8.1.1.2.1-16. Due to differences in data handling for metabolite 3'-OH-S-2840, and differences in selection of parent kinetic model between the applicant and HSE kinetics assessment, the HSE fitting is considered most reliable for endpoint selection. The applicant values are therefore provided for information and completeness only. The degradation profiles of metabolites associated with HSE's modelling endpoint selection for the parent compound are summarised in Table B.8.1.1.2.1-17. When

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robust metabolite endpoints have been determined for regulatory consideration, they are summarised in Table B.8.1.1.2.1-20.

#### Atwater

The applicant has selected an SFO fit for the parent and both major metabolites. HSE is in agreement that this is the most appropriate model by FOCUS guidance.

Metabolite endpoints were not considered robust for the Atwater soil. For 3'-OH-S-2840 this is because the t-test is failed, and for 1'-COOH-S-2840 it is because the metabolite formation fraction is too small, with a poorly defined formation and decline phase.

#### Newhaven

The applicant has selected a HS model for the parent and both major metabolites. HSE finds that a DFOP-parent (with  $k_2$  fixed), and SFO-metabolites model produces a better statistical fit.

The endpoint for 3'-OH-S-2840 is not considered robust as the t-test is failed, however HSE accepts the 1'-COOH-S-2840 endpoints for consideration due to the very good visual fit and passed t-test. As 1'-COOH-S-2840 has been taken for consideration, the corresponding fit of the parent, inpyrfluxam, in the same modelling run has been used to determine parent endpoints in this soil.

#### Penn

The applicant has selected an SFO fit for the parent and metabolites. HSE finds that a DFOP-parent (with  $k_2$  fixed), and SFO-metabolites model produces a better statistical fit.

Metabolite endpoints were not considered robust for the Penn soil. This is due to the poorly defined formation and decline phases, and the low formation fraction for 1'-COOH-S-2840.

#### Woodside

The applicant has selected a HS model for the parent. HSE finds that a DFOP-parent (with  $k_2$  fixed), and SFO-metabolites model produces a better statistical fit. Unconstrained fits were found to optimise  $M_0$  to zero for the metabolite 3'-OH-S-2840, when it was present at an AR of 1.9% . As the unconstrained fit failed the t-test for metabolite degradation,  $M_0$  was fixed at 1.9% AR, and remodelled. This resulted in the metabolite degradation rate for 3'-OH-S-2840 passing the t-test. Therefore HSE proposes a DFOP-parent (with  $k_2$  fixed), and SFO-metabolites ( $M_0$



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fixed to the observed AR%) in this instance, as the fit when  $M_0$  is optimised is not acceptable.

A metabolite endpoint for 1'-COOH-S-2840 was taken forward for regulatory consideration for this fit, as a very good visual fit was achieved, and while the t-test is failed, it is by a small margin. The fit of 3'-OH-S-2840 was not considered robust, due to the observed systematic pattern of residuals in the fit. As 1'-COOH-S-2840 has been taken for consideration, the corresponding fit of the parent, inpyrfluxam, in the same modelling run has been used to determine parent endpoints in this soil.

**Table B.8.1.1.2.1-16 Applicant's modelling endpoints for parent and metabolites, associated with applicant's parent only modelling endpoint kinetic fit selection**

Soil	Model (parent-metabolites)	Compound	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	ff	VA	χ <sup>2</sup> err (%)	k (1/d)	Prob > t	Other parameters
Atwater	SFO-SFO	Inpyrfluxam	121	402	-	good	1.14	0.00572	< 0.05	M <sub>0</sub> : 94.1
		3'-OH-S-2840	>1,000	>1,000	0.433 1	good	3.93	2.58E-15	0.500	-
		1'-COOH-S-2840	34.2	114	0.517 1	good	11.6	2.03E-02	< 0.05	-
Newhaven	HS-SFO	Inpyrfluxam	85	710	-	acceptable	2.95	k <sub>1</sub> : 0.0252 k <sub>2</sub> : 2.58E-03	k <sub>1</sub> : < 0.05 k <sub>2</sub> : < 0.05	M <sub>0</sub> : 96.7 t <sub>b</sub> : 20.9
		3'-OH-S-2840	597	1983	0.216 3	good	8.62	1.16E-03	0.291	-
		1'-COOH-S-2840	188	624	0.542 6	good	6.38	3.69E-03	< 0.05	-
Penn	SFO-SFO	Inpyrfluxam	213	706	-	unacceptable	1.70	3.26E-03	< 0.05	M <sub>0</sub> :91.2
		3'-OH-S-2840	241	799	0.375 4	unacceptable	32.1	2.88E-03	0.331	-
		1'-COOH-S-2840	20.7	68.8	0.624 6	unacceptable	29.3	3.35E-02	0.041	-
Woodside		Inpyrfluxam	91.1	455	-	good	1.19	k <sub>1</sub> : 0.0108 k <sub>2</sub> : 0.00443	k <sub>1</sub> : < 0.05 k <sub>2</sub> : < 0.05	M <sub>0</sub> : 94.3 t <sub>b</sub> : 45.5

	HS-SFO	3'-OH-S- 2840	76.2	253	0.289 3	acceptable	12.4	9.10E-03	< 0.05	-
		1'-COOH-S- 2840	555	1845	0.630 4	good	3.48	1.25E-03	0.134	-

VA = Visual acceptability

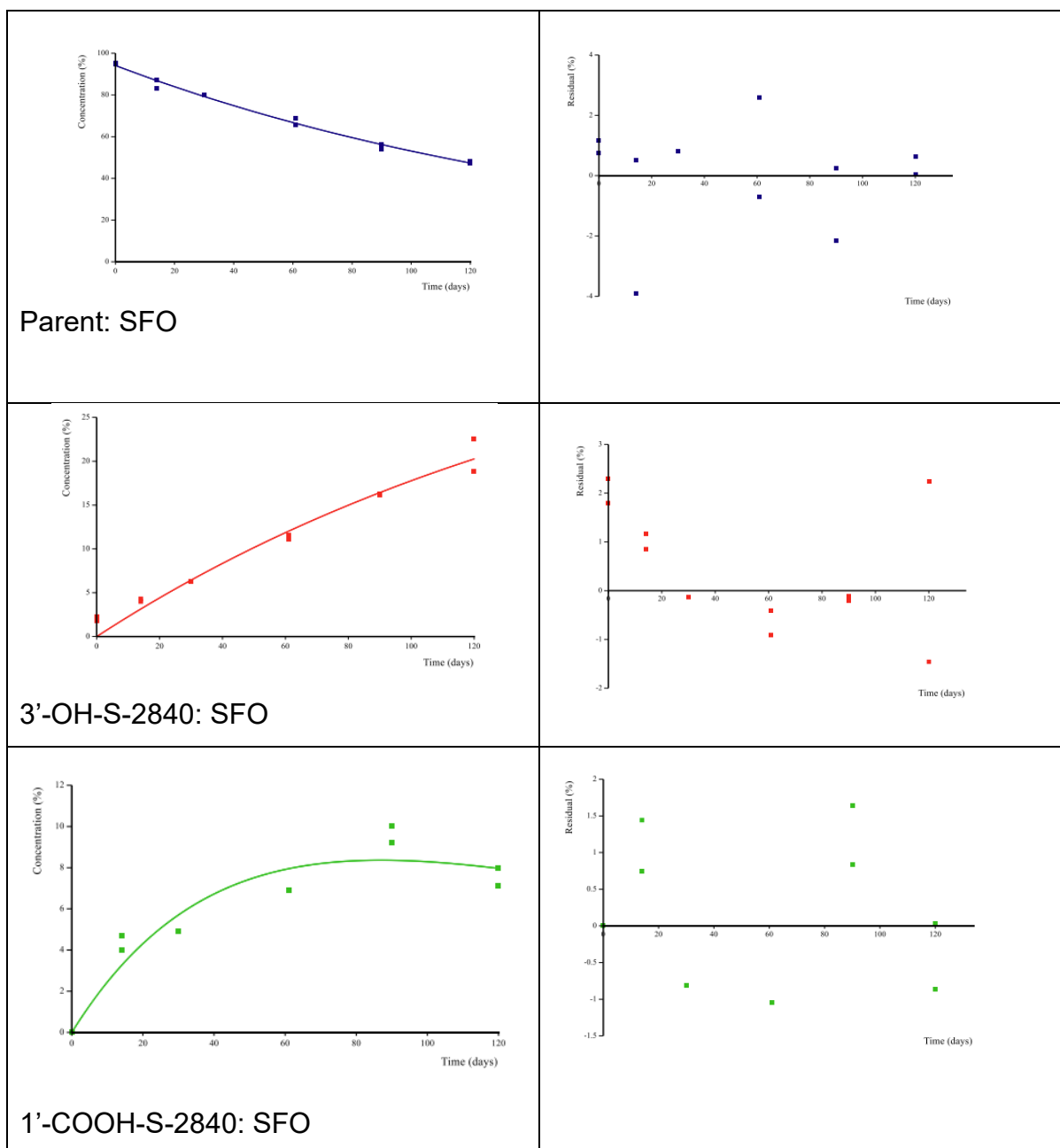
**Table B.8.1.1.2.1-17 HSE modelling endpoints for parent and metabolites, associated with HSE parent only modelling endpoint fit selection (fits taken for regulatory consideration shown in bold)**

Soil	Model (parent- metabolite s)	Compound	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	ff	VA	χ <sup>2</sup> err (%)	k (1/d)	Prob > t	Other parameters
Atwater	SFO-SFO	Inpyrfluxam	121	404	-	very good	1.14	5.71E-03	< 0.05	M <sub>0</sub> : 94.1
		3'-OH-S-2840	>1,000	>1,000	0.397 8	good	6.02	2.34E-14	0.500	M <sub>0</sub> : 1.31
		1'-COOH-S- 2840	37.2	124	0.519 3	good	11.6	2.03E-02	< 0.05	-
Newhaven	DFOP(k <sub>2</sub> fixed)-SFO	Inpyrfluxam	<b>69.7</b>	<b>&gt;1,000</b>	-	<b>very good</b>	<b>0.898</b>	<b>k<sub>1</sub>: 6.27E-02</b> <b>k<sub>2</sub>: 6.93E-04</b>	<b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: -</b>	<b>M<sub>0</sub>: 96.61</b> <b>g: 0.4816</b>
		3'-OH-S-2840	>1,000	>1,000	0.209 6	good	14.1	6.34E-04	0.409	M <sub>0</sub> : 0
		<b>1'-COOH-S- 2840</b>	<b>207</b>	<b>689</b>	<b>0.534</b> <b>8</b>	<b>very good</b>	<b>5.18</b>	<b>3.33E-04</b>	<b>&lt; 0.05</b>	-
Penn	DFOP(k <sub>2</sub> fixed)-SFO	Inpyrfluxam	243	>1,000	-	very good	1.06	k <sub>1</sub> :7.42E-03 k <sub>2</sub> : 6.93E-04	k <sub>1</sub> : < 0.05 k <sub>2</sub> : -	M <sub>0</sub> : 92.3 g: 0.5074
		3'-OH-S-2840	>1,000	>1,000	0.409 3	acceptable	10.2	0	0.5	M <sub>0</sub> : 0.9207
		1'-COOH-S- 2840	38.9	129	0.562 7	unacceptable	14	1.78E-02	< 0.05	-

Woodside	DFOP( $k_2$ fixed)-SFO	Inpyrfluxam	86.2	>1,000	-	very good	0.687	$k_1$ : 2.18E-02 $k_2$ : 6.93E-04	$k_1$ : < 0.05 $k_2$ : -	$M_0$ : 94.69 g: 0.5603
		3'-OH-S-2840	81.1	272	0.278 6	unacceptable	17.7	8.47E-03	0.05912	$M_0$ : 0
		1'-COOH-S-2840	787	>1,000	0.612	very good	2.66	8.81E-04	0.1681	-
<b>Woodside (3'-OH-S-2840 M0 fixed, 1.9% AR)</b>	<b>DFOP(<math>k_2</math> fixed)-SFO</b>	<b>Inpyrfluxam</b>	<b>86.1</b>	<b>&gt;1,000</b>	-	<b>Very good</b>	<b>0.697</b>	<b><math>k_1</math>: 2.19E-02 <math>k_2</math>: 6.93E-04</b>	<b><math>k_1</math>: &lt; 0.05 <math>k_2</math>: -</b>	<b><math>M_0</math>: 94.70 g: 0.559</b>
		3'-OH-S-2840	148	493	0.189 5	Unacceptable	7.51	8.67E-03	< 0.05	-
		<b>1'-COOH-S-2840</b>	<b>840</b>	<b>&gt;1,000</b>	<b>0.609 5</b>	<b>Very good</b>	<b>2.65</b>	<b>3.41E-03</b>	<b>0.187</b>	-

VA = Visual acceptability

Final fit selection in **bold**



**Figure B.8.1.1.2.1-11 Applicant visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Atwater soil under aerobic conditions (SFO-SFO)**

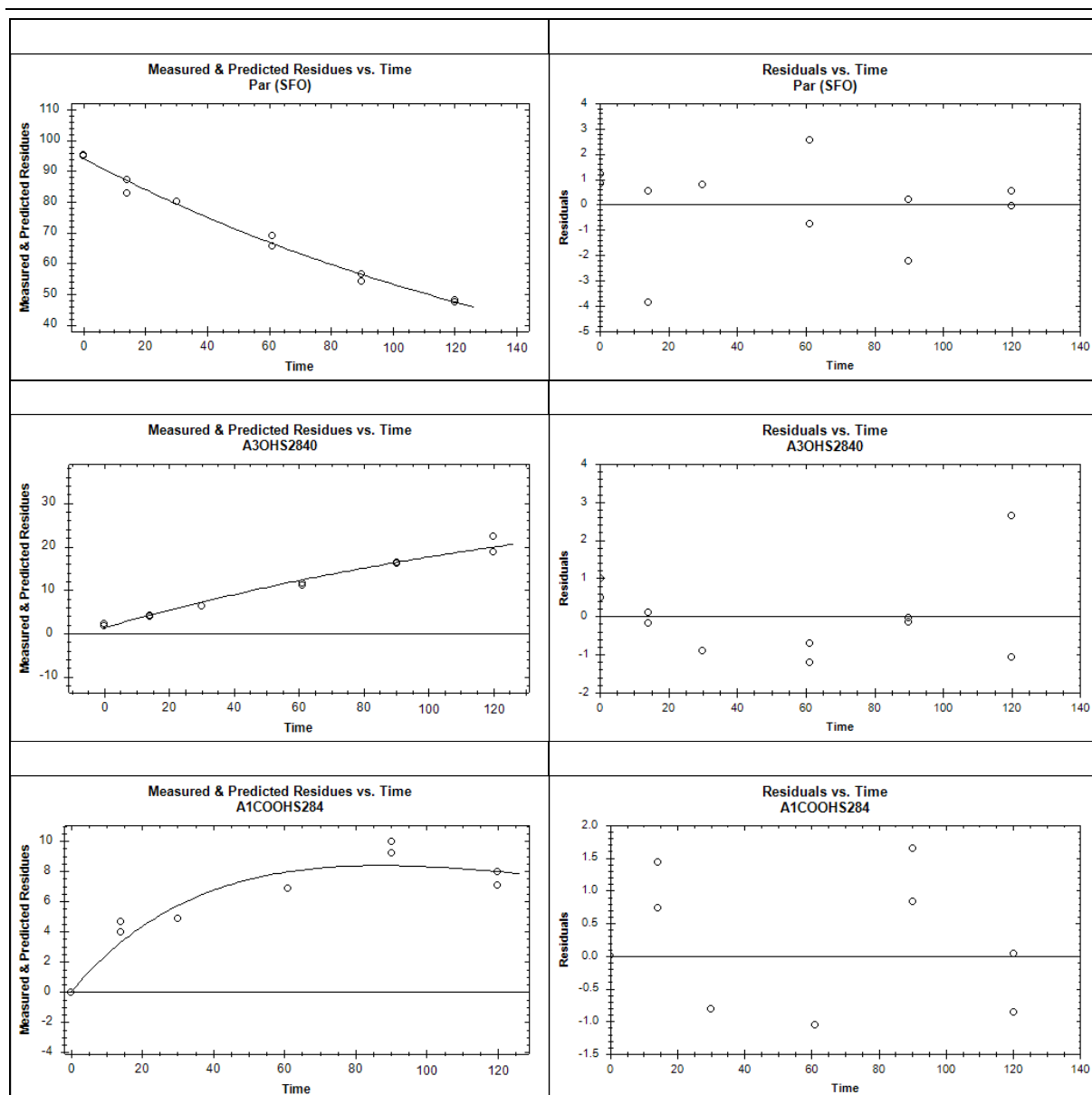
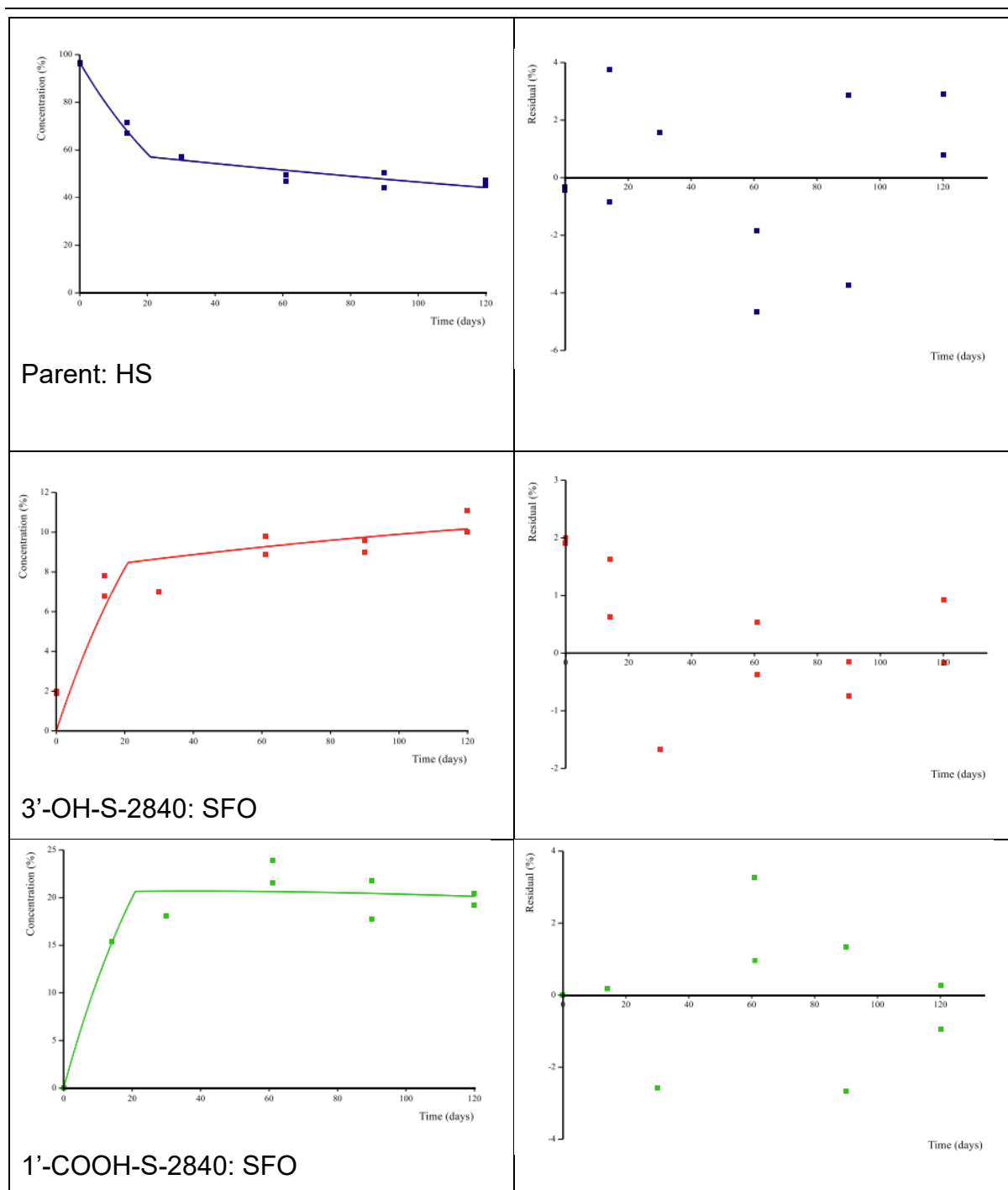
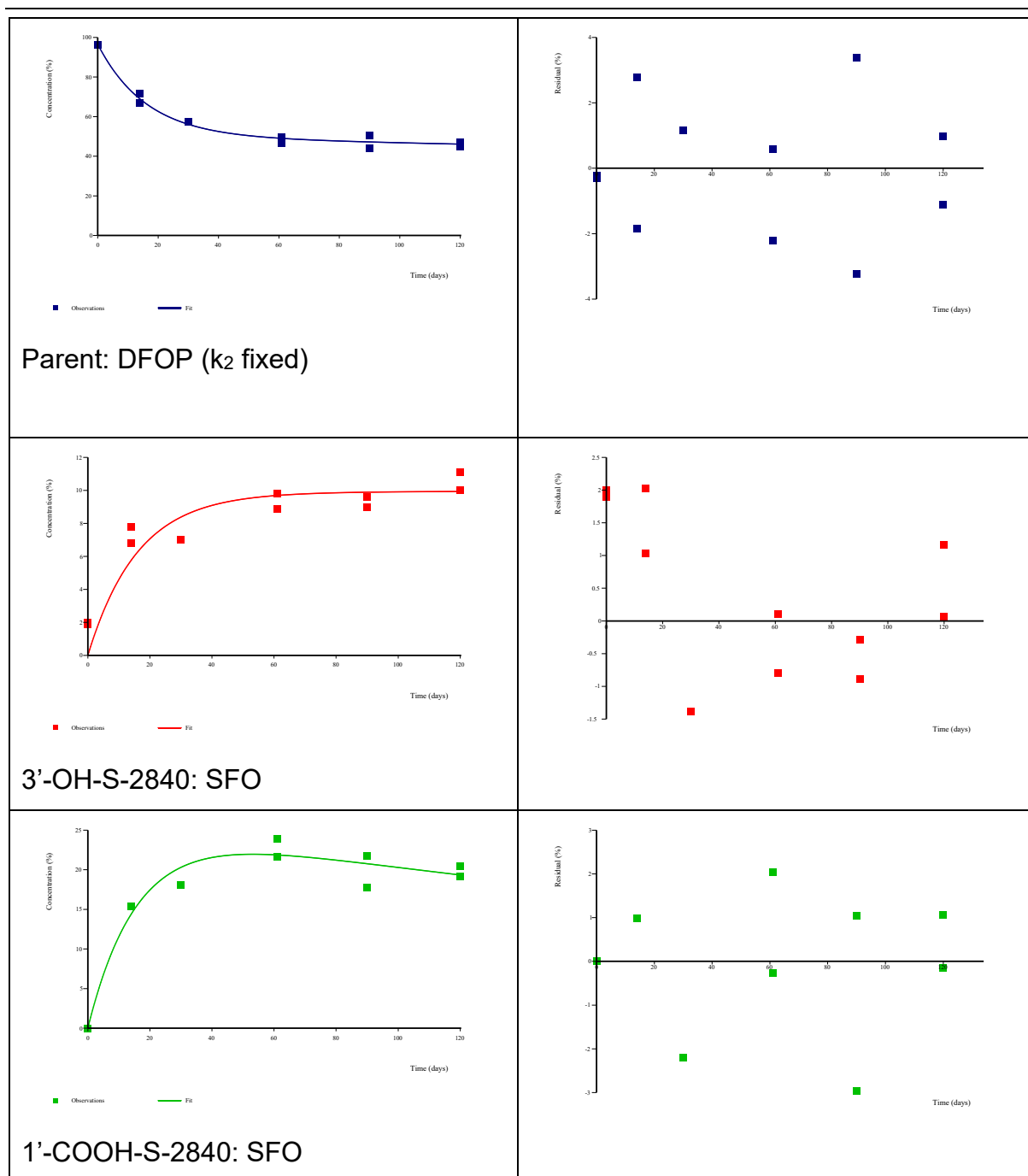


Figure B.8.1.1.2.1-12 HSE visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Atwater soil under aerobic conditions (SFO-SFO)

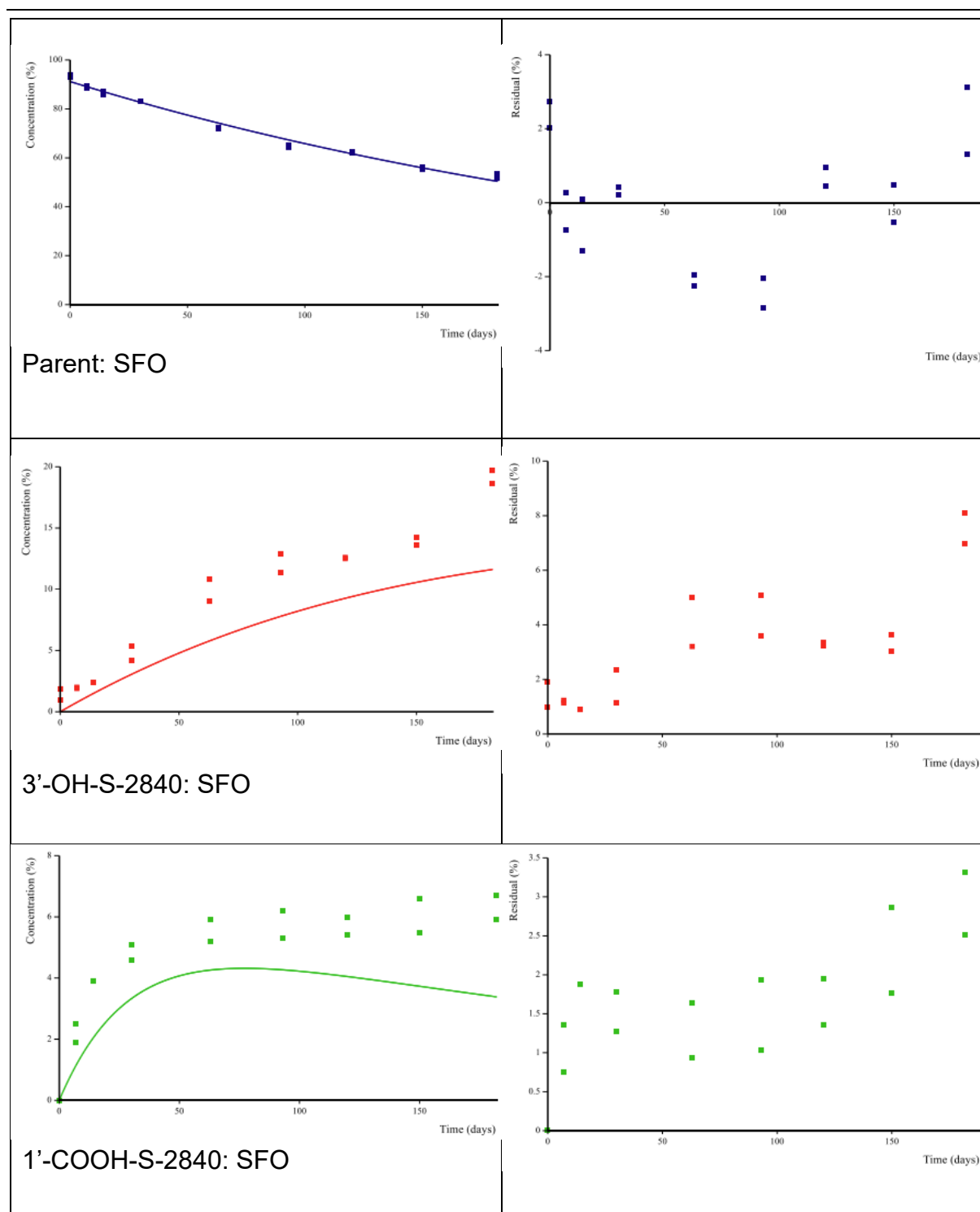


**Figure B.8.1.1.2.1-13 Applicant visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Newhaven soil under aerobic conditions (HS-SFO)**

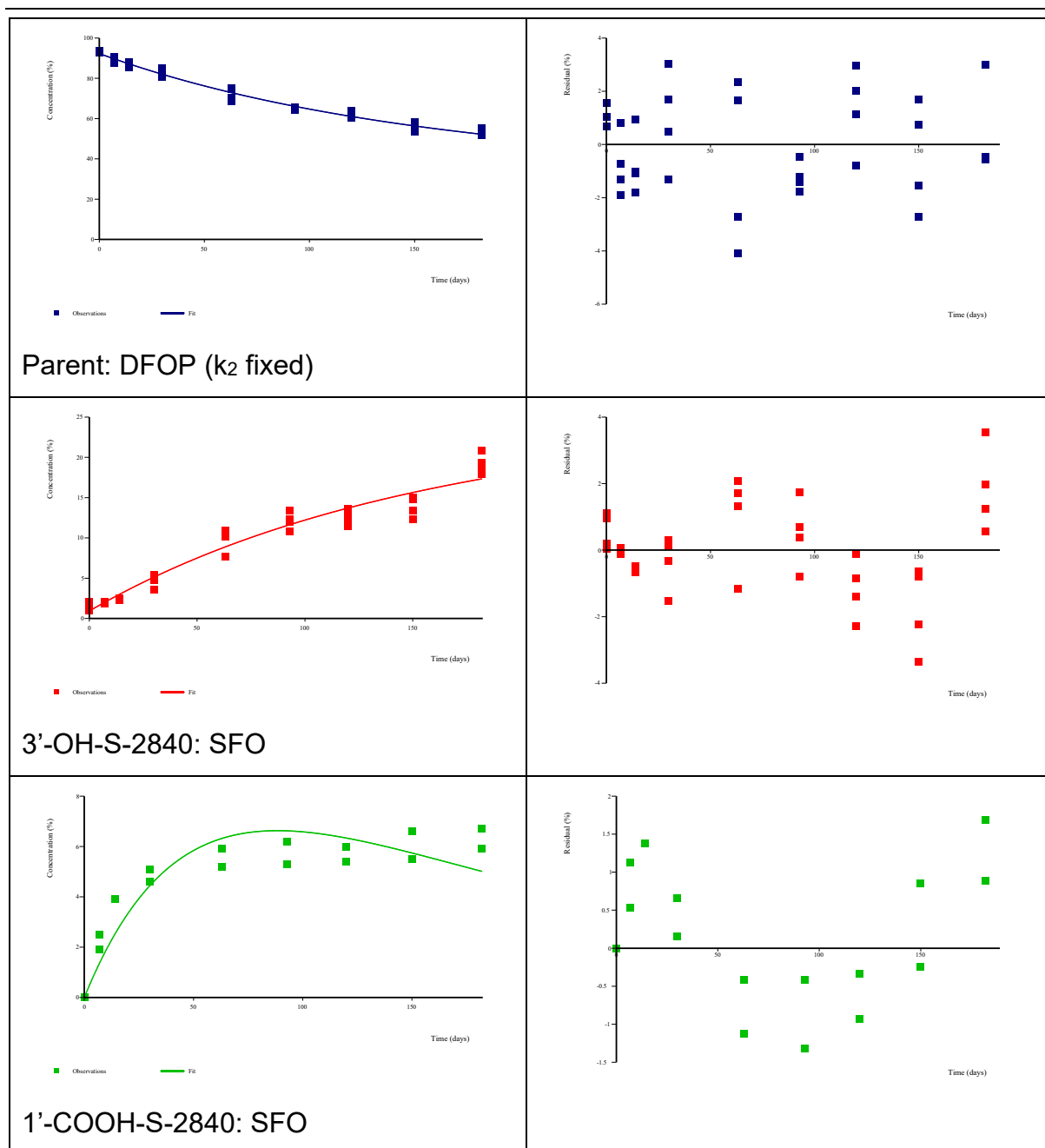




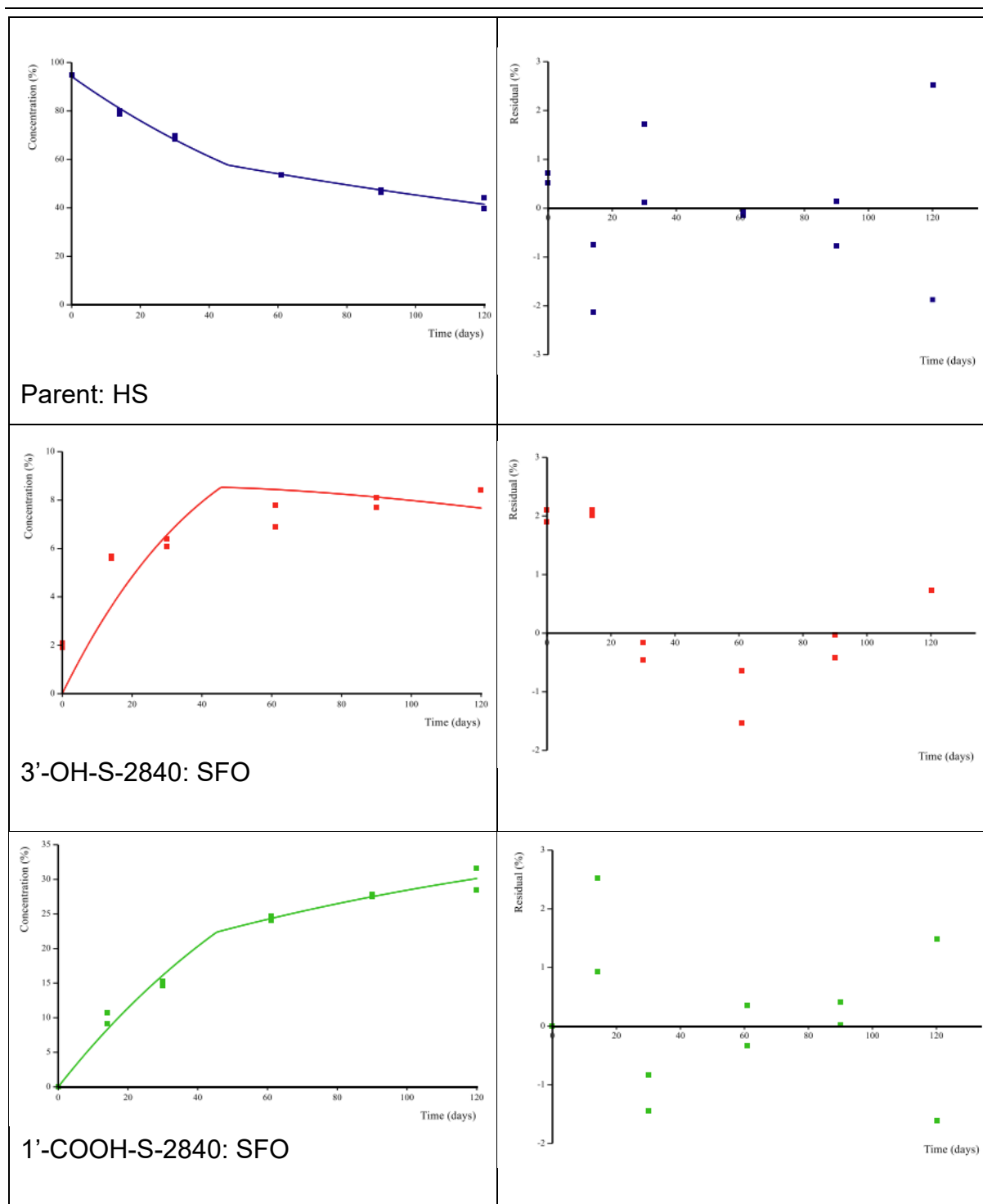
**Figure B.8.1.1.2.1-14 HSE visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Newhaven soil under aerobic conditions (DFOP( $k_2$  fixed)-SFO)**



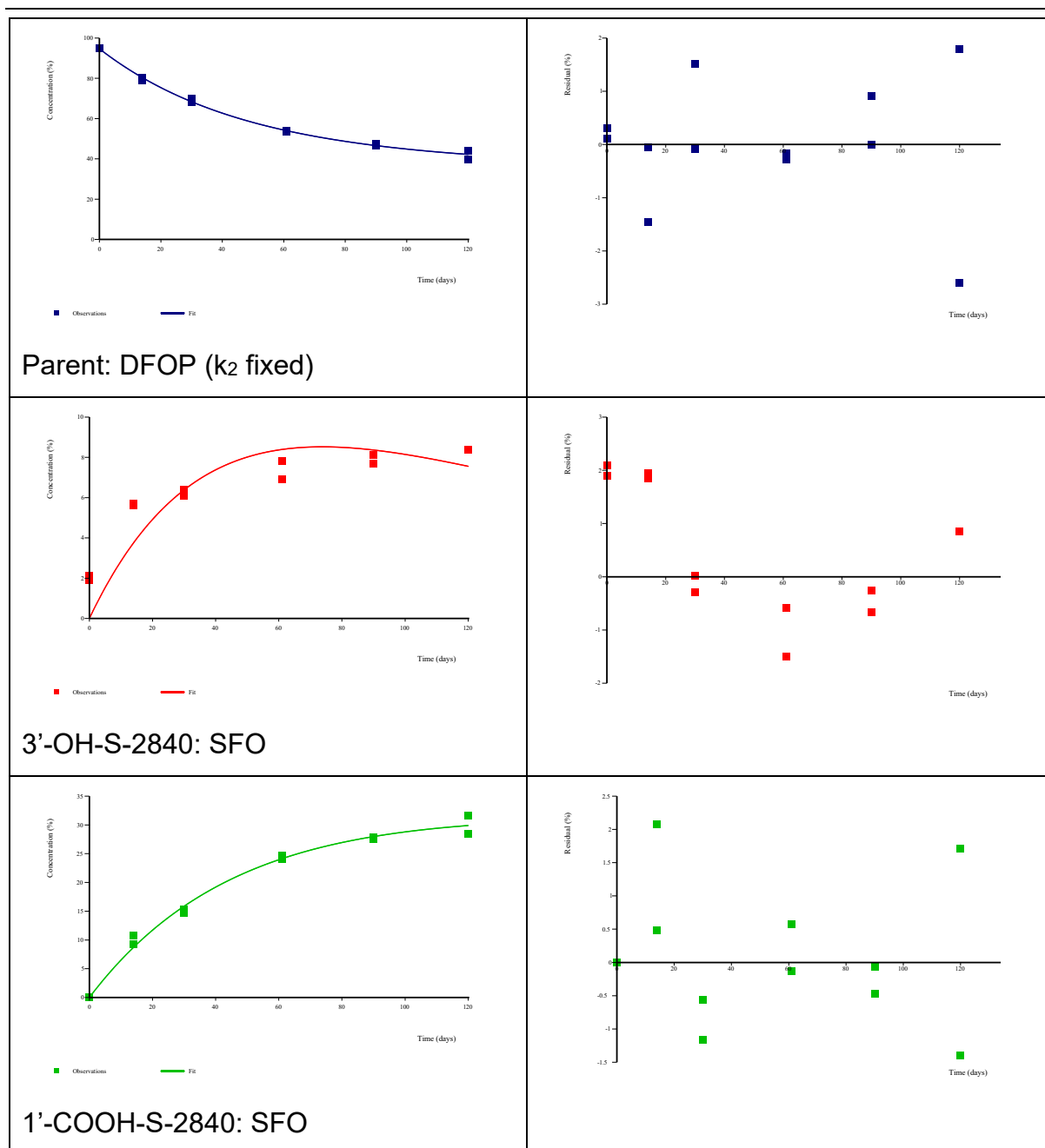
**Figure B.8.1.1.2.1-15 Applicant visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Penn soil under aerobic conditions (SFO-SFO)**



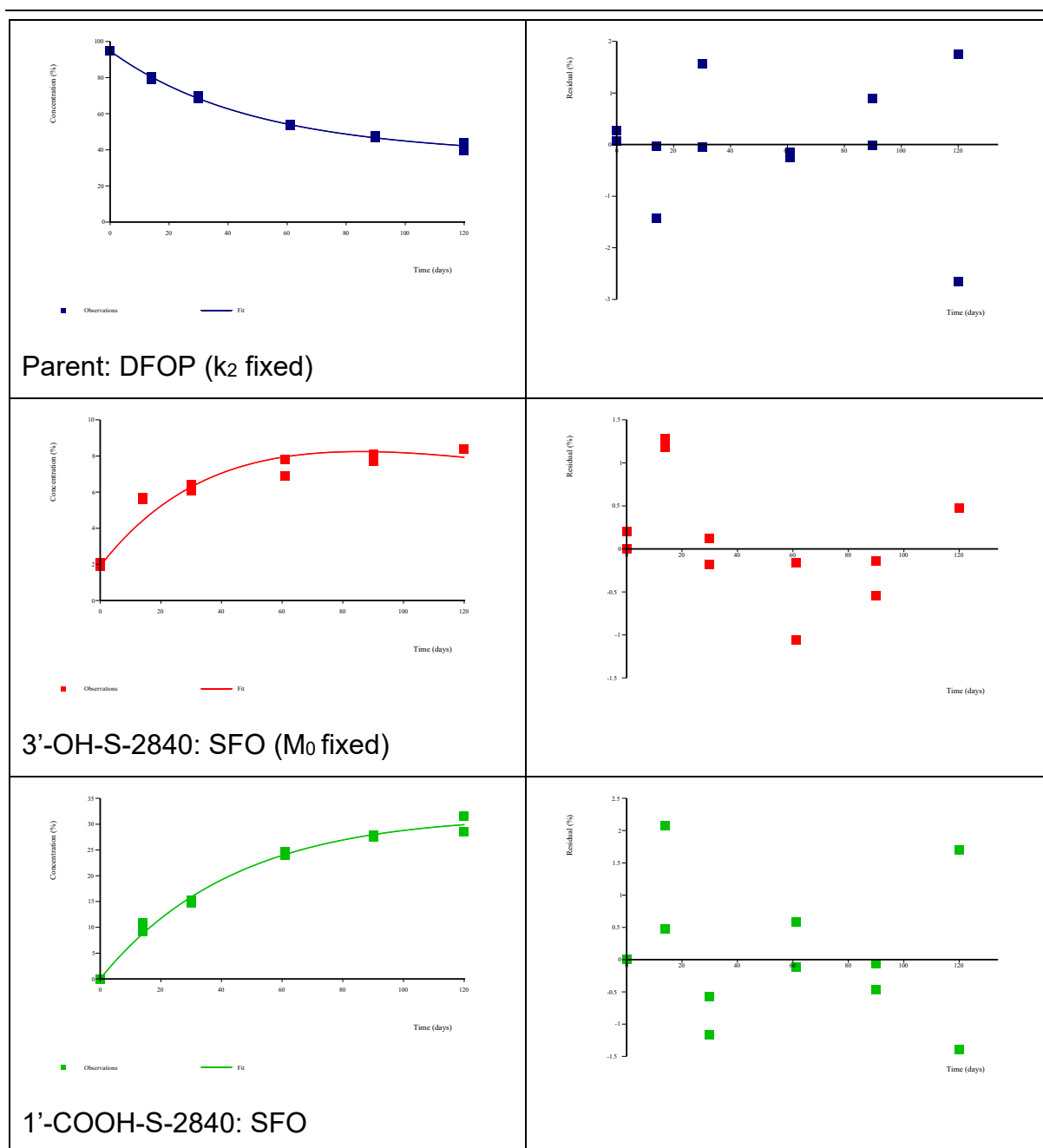
**Figure B.8.1.1.2.1-16 HSE visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Penn soil under aerobic conditions (DFOP( $k_2$  fixed)-SFO)**



**Figure B.8.1.1.2.1-17 Applicant visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Woodside soil under aerobic conditions (HS-SFO)**



**Figure B.8.1.1.2.1-18 HSE visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Woodside soil under aerobic conditions (DFOP( $k_2$  fixed)-SFO)**



**Figure B.8.1.1.2.1-19 HSE visual-fits and residual plots of the degradation of inpyrfluxam and metabolites in Woodside soil under aerobic conditions (DFOP( $k_2$  fixed)-SFO), 3'-OH-S-2840  $M_0$  fixed to 1.9% AR**

As endpoints were taken forward for metabolites in the Newhaven and Woodside soils, the endpoints for inpyrfluxam were also taken from the same modelling fits in these soils. Trigger and modelling endpoints for inpyrfluxam are summarised below in Table B.8.1.1.2.1-18 and Table B.8.1.1.2.1-19 respectively. Endpoints for metabolites taken forward for regulatory consideration are given in Table B.8.1.1.2.1-20.

**Table B.8.1.1.2.1-18 inpyrfluxam Laboratory studies, aerobic conditions, trigger / best fit endpoints**

<b>Soil</b>	<b>Classification</b>	<b>pH</b>	<b>DT<sub>50</sub> (d)</b>	<b>Model</b>
Atwater	Sandy loam	7.5	121	SFO
Newhaven	Silt loam	5.7	69.7	DFOP (k <sub>2</sub> fixed 1000d)
Penn	Loam	6.8	254	DFOP (k <sub>2</sub> fixed 1000d)
Woodside	Loam	7.5	86.1	DFOP (k <sub>2</sub> fixed 1000d, 3' OH S 2840 M <sub>0</sub> fixed to 1.9% AR)

**Table B.8.1.1.2.1-19 inpyrfluxam, Laboratory studies, aerobic conditions, parent modelling endpoints**

Soil name	Soil type (x)	pH (x)	t.°C	Moisture (unit)	$\chi^2$ (%)	Kinetic model	Estimated parameters (and t-test result)	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	SFO k / DFOP k <sub>1</sub> , d <sup>-1</sup> (equivalent days) 20°C pF2/10 kPa	SFO k / DFOP k <sub>2</sub> , d <sup>-1</sup> (equivalent days) 20°C pF2/10 kPa	DFOP g parameter	Pseudo-SFO DT <sub>50</sub> (d) (d <sup>-1</sup> ) 20°C pF2/10 kPa**	Notes / Reference	Model used to determine endpoints
Atwater	Sandy loam	7.5	20 ± 2	16.1	1.14	SFO	M <sub>0</sub> : 94.2	121	402	0.00573 (121)	0.00573 (121)	-	121	KCA 7.1.1.1_02	Parent-only model as no metabolite endpoints taken forward
Newhaven	Silt loam	5.7	20 ± 2	38.4	0.898	DFOP*	M <sub>0</sub> : 96.61 k <sub>1</sub> : (< 0.05)	69.7	>1,000	0.0627 (11.1)	0.00069 (1000)	0.482	1000	k <sub>2</sub> variable fixed / KCA 7.1.1.1_02	Parent and metabolite model as 1'-COOH-S-2840 endpoint taken forward
Penn	Loam	6.8	20 ± 2	24.1	1.02	DFOP*	M <sub>0</sub> : 92.62 k <sub>1</sub> : (< 0.05)	254	>1,000	0.008606 (81.2)	0.00069 (1000)	0.468	1000	k <sub>2</sub> variable fixed / KCA 7.1.1.1_01	Parent-only model as no metabolite endpoints taken forward
Woodside	Loam	7.5	20 ± 2	28.9	0.680	DFOP*	M <sub>0</sub> : 94.7 k <sub>1</sub> : (< 0.05)	86.1	>1,000	0.0219 (31.7)	0.00069 (1000)	0.559	1000	k <sub>2</sub> variable fixed, 3'-OH S-2840 M <sub>0</sub> fixed to 1.9% AR / KCA 7.1.1.1_02	Parent and metabolite model as 1'-COOH-S-2840 endpoint taken forward
Geomean (n = x)										(43.1)	(590)		590		
Arithmetic mean (n = x)												0.503			
Final endpoints for PEC modelling: Pseudo-SFO DT <sub>50</sub> = 590 and/or k <sub>1</sub> = 43.1, k <sub>2</sub> = 590, g = 0.503 ,															
pH-dependency: No															



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\*CAKE 3.7 allows rate input to five decimal places. DFOP  $k_2$  parameter fixed as  $0.00069 \text{ d}^{-1}$ , representative of 1005 d  $\text{DT}_{50}$ , as closest possible fixed  $k_2$  input to 0.000693 required by FOCUS guidance for representative 1000 d  $\text{DT}_{50}$ .

\*\*Soil moisture in all instances was above the pF2 soil moisture value. By FOCUS guidance no correction to pF2 was made.

**Table B.8.1.1.2.1-20 Metabolite endpoints retained for regulatory consideration from optimised fittings**

Soil	Metabolite endpoint (or reason endpoint not taken for regulatory consideration)	
	3'-OH-S-2840	1'-COOH-S-2840
	DT <sub>50</sub> (d)	DT <sub>50</sub> (d)
Atwater	(t-test failed)	(Poorly defined decline phase)
Newhaven	(t-test failed)	<b>207 (ff 0.535)</b>
Penn	(Poorly defined decline phase)	(Poorly defined decline phase)
Woodside	(Visually unacceptable fit)	<b>840 (ff 0.612)</b>

### Fixed Metabolite-Dosed Endpoints

HSE agrees with the applicants assessment that the absence of clear decline phases of the metabolites inhibits robust endpoints from being determined in all soils. HSE agrees with the applicant's decision to provide separate, metabolite-dosed studies, provided at KCA 7.1.2.1.2\_01 & KCA 7.1.2.1.2\_02. However HSE has performed additional kinetic modelling to test whether the behaviour in the metabolite dosed soils is also representative of the behaviour seen in the parent dosed trials.

HSE notes that while good visual fits could be determined for the freely optimised parent dosed systems, with low  $\chi^2$  values, the degradation rate of 3'-OH-S-2840 failed the t-test in the Atwater, Newhaven, and Penn soils and was therefore uncertain. The geomean best-fit metabolite DT<sub>50</sub> derived from the metabolite-dosed study was therefore used in the parent dosed system models considered here, by fixing the 3'-OH-S-2840 k value, when the freely optimised k value in the whole-system model failed the t-test. This was done to test the representativeness of the behaviour seen in the metabolite dosed systems.

A geomean best fit DT<sub>50</sub> of 314 d was determined for 3'-OH-S-2840 from the metabolite dosed study (B.8.1.1.2.2). This corresponds to a k value of  $2.21 \times 10^{-3}$  for 3'-OH-S-2840. For the HSE best-fit metabolite models, the t-test is failed for 3'-OH-S-2840 in the Atwater, Newhaven, and Penn soils. These k values were therefore fixed in the Atwater and Newhaven soils. Further fitting was not performed on the Penn soil, as the relatively low metabolite formation levels, and poorly defined decline phases did not support robust fitting.

While the Woodside soil failed the t-test for 1'-COOH-S-2840, this degradation rate was not fixed to that determined from the metabolite dosed study, as the DT<sub>50</sub>'s were vastly different (840 days from the parent-dosed study, 73.6 days from the

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metabolite dosed study) and would not lead to a better fit. Furthermore, the freely optimised fit of 1'-COOH-S-2840 fails the t-test by only a small margin, and so the criteria were relaxed and the fit accepted, based on the very good visual fit.

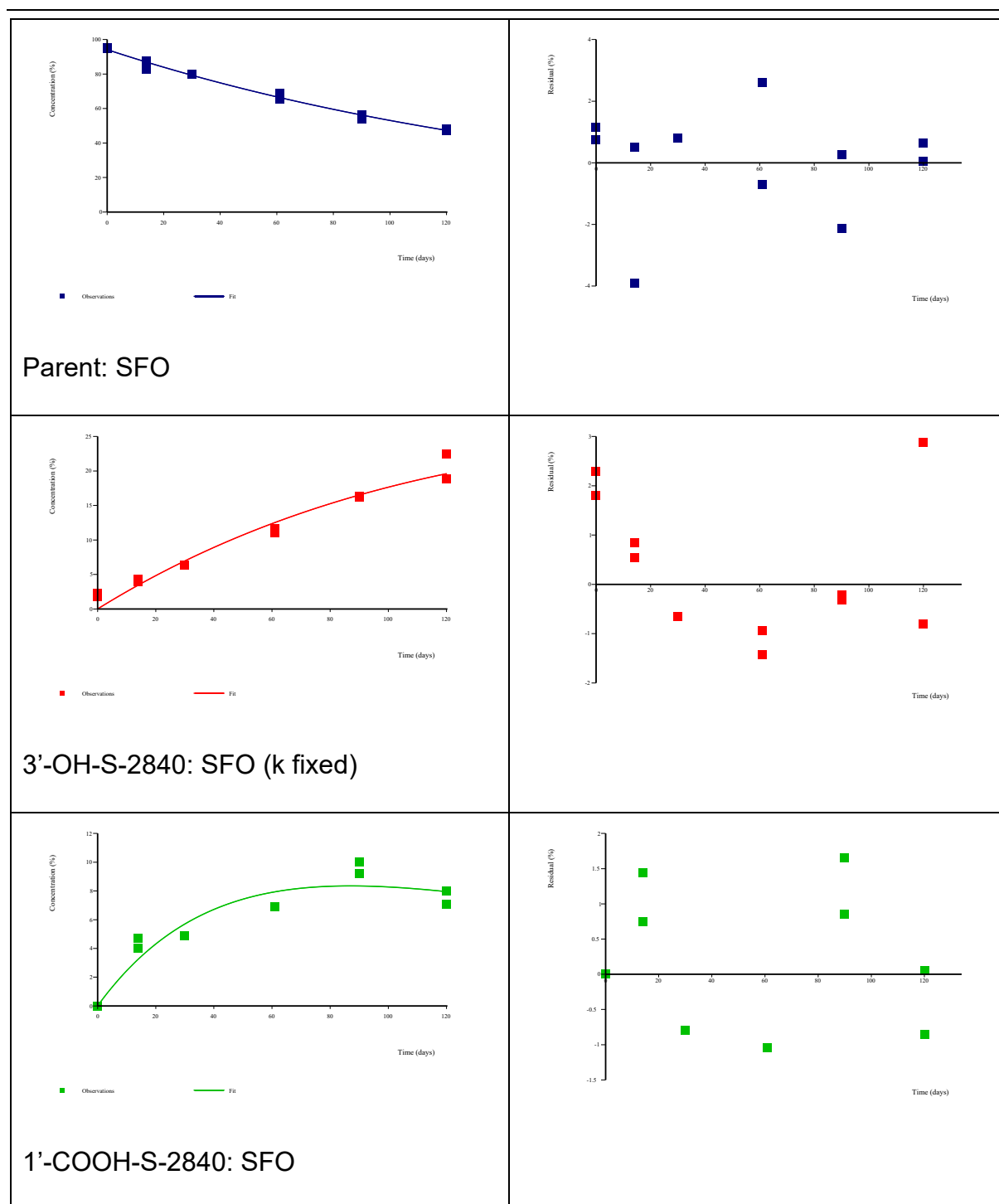
The goodness-of-fit of the whole-system Atwater and Newhaven models, with  $k$  values fixed was then assessed, in order to determine if the behaviour in the metabolite dosed trials is representative of the behaviour when metabolites are formed in situ from parent dosed trials.

When a DFOP model was used for the parent compartment, both  $k_1$  and  $k_2$  were allowed to be optimised by the model, with the aim of attaining the best fit for the whole system. This can result in  $k_2$  failing the t-test, however, this exercise was not

intended to test confidence in the degradation rates of the parent compound. This has been performed previously in the Parent-only Modelling and Parent and Metabolite Modelling sections.

**Table B.8.1.1.2.1-21 HSE statistics of degradation of inpyrfluxam in Atwater soil under aerobic conditions (parent and metabolite fits, 3' OH degradation rate fixed to metabolite-dosed value)**

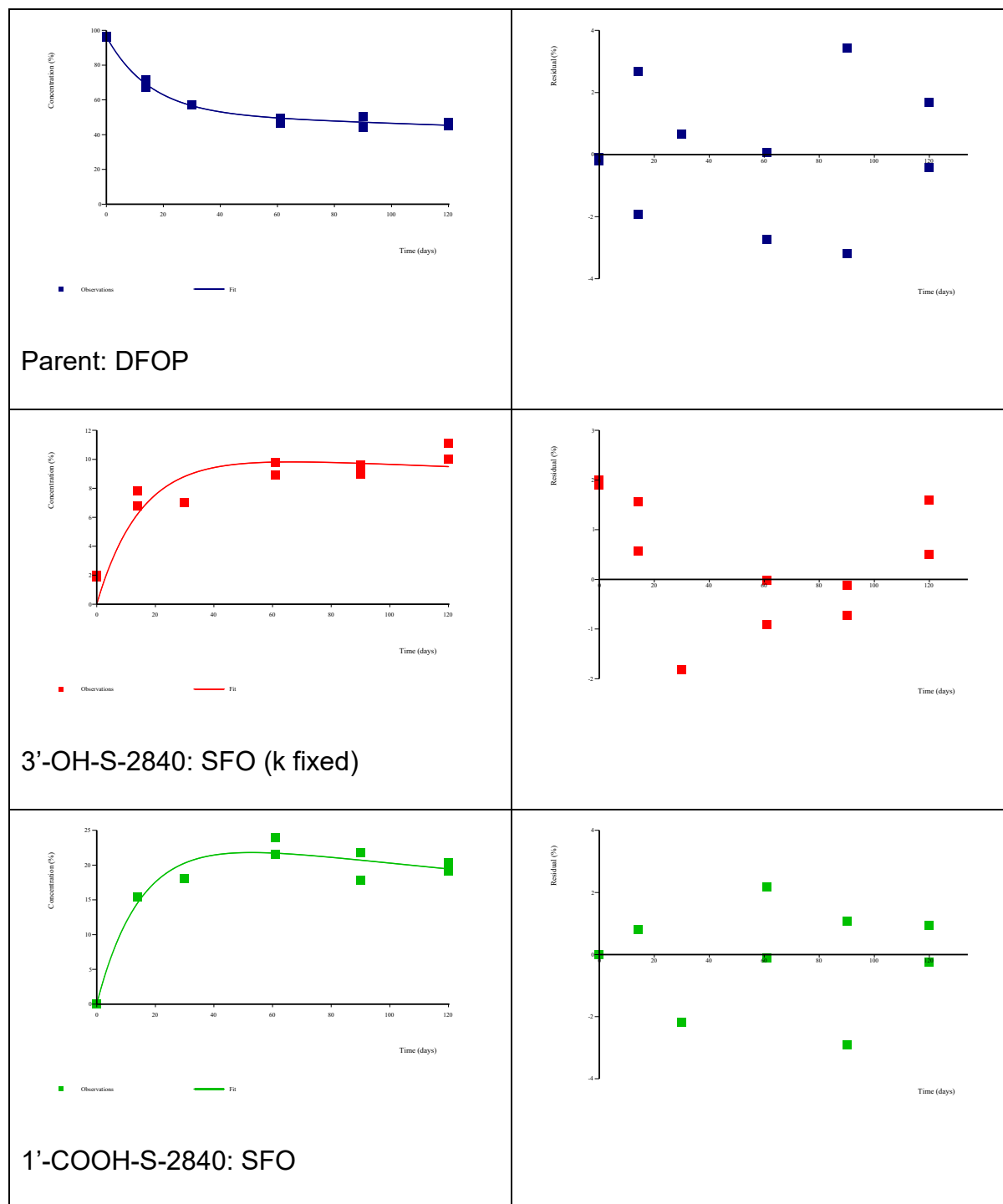
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
parent: SFO	very good	<b>1.14</b>	Mo: 94.2 k: 0.005728	K: <0.05	121	402
3'-OH-S-2840: SFO from parent	good	<b>8.87</b>	ffm: 0.4843	-	314	>1,000
1'-COOH-S-2840: SFO from parent	good	<b>11.6</b>	k: 0.02024 ffm: 0.5157	k: <0.05	34.3	114
<p>Data for all compartments could be fitted satisfactorily when the 3'-OH-S-2840 k value was fixed, giving acceptable <math>\chi^2</math> values and good visual fits. No endpoints were accepted for 1'-COOH-S-2840 due to the lack of a decline phase in this Atwater soil parent dosed study. However, the modelling has been kept here for information purposes only.</p> <p><b>Conclusion: Metabolite-dosed study k value accurately represents metabolism of 3'-OH-S-2840 in this Atwater soil parent dosed study.</b></p>						



**Figure B.8.1.1.2.1-20 HSE parent and metabolite fits in Atwater soil ( 3'-OH-S-2840 degradation rate fixed to metabolite-dosed values)**

**Table B.8.1.1.2.1-22 HSE statistics of degradation of inpyrfluxam in Newhaven soil under aerobic conditions (parent and metabolite fits, 3'-OH-S-2840 degradation rate fixed to metabolite-dosed value)**

Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
parent: DFOP	very good	<b>1.12</b>	M <sub>0</sub> : 96.5 k <sub>1</sub> : 0.06615 k <sub>2</sub> : 0.001207 g: 0.4563	k <sub>1</sub> : <0.05 k <sub>2</sub> : 0.0779	74.8	>1,000
3'-OH-S-2840: SFO from parent	good	<b>13.4</b>	ffm: 0.231	-	314	>1,000
1'-COOH-S-2840: SFO from parent	very good	<b>5.1</b>	k: 0.003708 ffm: 0.547	k: <0.05	187	621
Data for all compartments could be fitted satisfactorily when the 3'-OH-S-2840 k value was fixed, giving acceptable $\chi^2$ values and good visual fits						
<b>Conclusion: Metabolite-dosed study k value accurately represents metabolism of 3'-OH-S-2840 in this Newhaven soil parent dosed study</b>						



**Figure B.8.1.1.2.1-21 HSE parent and metabolite fits in Newhaven soil ( 3'-OH-S-2840 degradation rate fixed to metabolite-dosed values)**

The fits produced when the 3'-OH-S-2840 k value was fixed in Atwater and Newhaven soils were of a high quality. HSE considers this to add credibility to the

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DT<sub>50</sub> values determined from the metabolite-dosed studies, as being representative of in-situ formation and degradation.

For 1'-COOH-S-2840, reliable fits were determined in the Newhaven and Woodside soils, with the freely optimised DT<sub>50</sub>'s appearing to be longer than the geometric mean derived from the separate metabolite dosed studies. Although the data is variable across the four soils tested, there is some indication of longer persistence of this metabolite when formed from parent inpyrfluxam. HSE therefore proposes to retain the freely optimised fits for the Newhaven and Woodside soils, and add these to the regulatory database of DT<sub>50</sub>'s from metabolite dosed soils.

## Conclusion

HSE has determined robust DT<sub>50</sub> and DT<sub>90</sub> values for inpyrfluxam, for the purposes of triggering further studies and modelling environmental persistence. While visually acceptable models of parent and metabolite degradation were produced in some soils, endpoint values for metabolites could not be determined in all soils from this parent dosed study, due to a lack of metabolite decline phase within the study duration resulting in high uncertainty in determined metabolite half-lives.

HSE determined inpyrfluxam DT<sub>50</sub>'s in all soils to be over 60 days, in agreement with the applicant, therefore triggering field dissipation studies by EU regulations 283/2014 & 284/2013. These have been provided. HSE determined that inpyrfluxam degraded according to biphasic conditions in three of the four soils. Therefore HSE have determined separate fast and slow phase modelling endpoints of 43.9 & 590 days respectively. HSE determined an overall pseudo SFO DT<sub>50</sub> of 590 days, in comparison to the applicant's proposed 193 days. These values were calculated by taking the geometric mean, in the absence of clear evidence of pH dependence.

Kinetic fitting has been performed for parent to metabolite pathway fits. Many visually acceptable fits were produced, however metabolite k values often failed the t-test and metabolite levels were noted to be low and variable in some soils. In an attempt to improve fitting and to support the values obtained in metabolite dosed studies, metabolite k-values were therefore fixed to values taken from metabolite-dosed studies in soils that supported robust fitting, but had failed t-tests when freely optimised. The geometric mean k value of 3'-OH-S-2840 in metabolite-dosed studies was found to be representative of its degradation in the inpyrfluxam Atwater and Newhaven parent-dosed studies. This provides some confidence that the endpoints derived from the metabolite dosed soils are representative of behaviour when this metabolite is formed from parent inpyrfluxam. The use of the geometric mean DT<sub>50</sub> from the metabolite dosed studies for 3'-OH-S-2840 was considered reasonable by HSE.

For the 1'-COOH-S-2840 metabolite in the Penn and Atwater soils parent dosed soils, levels of formation were generally lower than in the other two soils, and with no



clear pattern described, robust kinetic fitting was not supported in these two soils. Reliable and/or visually acceptable fits were determined for 1'-COOH-S-2840 in the Newhaven and Woodside soils, with the freely optimised DT<sub>50</sub>'s appearing to be longer than the geometric mean derived from the separate metabolite dosed studies. Although data is variable across the tested soils, there is some indication of longer persistence of this metabolite when formed from parent inpyrfluxam. HSE therefore proposes to retain the freely optimised fits for the Newhaven and Woodside parent dosed soils, and add these to the regulatory database of DT<sub>50</sub>'s from the 1'-COOH-S-2840 metabolite dosed soils.

All HSE accepted results for metabolites, including those from metabolite and parent dosed studies are tabulated below in Table B.8.1.1.2.1-23.

**Table B.8.1.1.2.1-23 Summary of FOCUS Kinetic modelling to derive triggering/PECsoil endpoints for metabolites of inpyrfluxam, 1'-COOH-S-2840 and 3'-OH-S-2840 (laboratory studies only)**

Study type	Soil	Model	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	χ <sup>2</sup> err (%)	Visual fit
1'-COOH-S-2840						
Metabolite dosed	Speyer 5M soil	SFO	91.3	303	3.56	Good
	Newhaven soil	DFOP	24.5	623	3.39	Good
	Atwater soil	SFO	148	491	2.59	Good
Parent dosed	Newhaven soil	SFO	207	689	5.18	Very good
	Woodside soil	SFO	840	2790	2.65	Very good
3'-OH-S-2840						
Metabolite dosed	Speyer 5M soil	SFO	369	1226	2.5	Good
	Newhaven soil	SFO	303	1006	3.05	Good
	Atwater soil	SFO	276	917	0.863	Good

**B.8.1.1.2.2. Aerobic rate of degradation of metabolites**

<b>Data Point:</b>	KCA 7.1.2.1.1/03
<b>Report Author:</b>	██████ & ██████
<b>Report Year:</b>	2023
<b>Report Title:</b>	Recalculation of the laboratory aerobic degradation rate of the metabolites of S-2399 in soil according to FOCUS Kinetics Guidance
<b>Report No:</b>	20063620.UK0 - 9637
<b>Guideline(s) followed in study:</b>	FOCUS (2006), FOCUS (2014)
<b>Previous evaluation:</b>	New data, submitted for purpose of review
<b>GLP:</b>	Underlying aerobic study: Yes Kinetic Modelling: Not applicable

<b>Deviations</b>	<b>HSE assessment of deviations</b>
At day 0, the applicant has used the % AR for the parent compound. However, small amounts of unresolved background were recovered at day 0.	HSE has set the applied substance to the material balance at 0 DAT and has repeated the modelling.
<p style="text-align: center;"><b>HSE conclusions</b></p> <p style="text-align: center;">HSE accepts this study overall.</p>	

**INTRODUCTION**

Laboratory degradation studies were submitted and have been evaluated for the metabolites of inpyrfluxam 3'-OH-S-2840 and 1'-COOH-S-2840 and are presented in sections B.8.1.1.1.3 and B.8.1.1.1.4. A kinetic evaluation has been conducted to derive modelling and triggering endpoints according to the recommendations of the FOCUS workgroup on degradation kinetics (FOCUS 2006 and 2014). Both metabolites were applied to soil and modelled as the parent material in separate studies. The applicant derived endpoints using CAKE 3.7 and these have been validated by HSE using KinGUI.

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## DATA HANDLING

As the laboratory degradation studies were conducted at 20 °C and pF2, no normalisation was required.

For 1'-COOH-S-2840 the total % AR was used, as no enantiomeric shift was noted during the course of the study. For both substances, replicates at each time point have been plotted as separate data points. There are no samples close to the LOD or LOQ and therefore no adjustment was needed. At day 0, the applicant has used the % AR for the parent compound. In all soils and for both test items, small amounts of unresolved background were recovered at day 0. Consequently, HSE has set the applied substance to the material balance at 0 DAT, which is in accordance with FOCUS Kinetics Guidance and has repeated the modelling. The fits presented below are those derived by HSE and the impact of the different  $M_0$  value has been considered below to determine the impact on the  $DT_{50}$  values derived by the applicant.

The fit of the kinetic models SFO, FOMC, DFOP and HS were investigated sequentially according to the decision trees for modelling and triggering endpoints (Figures 7.1 and 7.2) in the FOCUS Kinetics Guidance document. The applicant assessed the suitability of the models based on the visual fit and the statistical fit according to the  $\chi^2$  error and a t-test to determine if parameters were significantly different to 0 (acceptability criteria  $P \leq 0.05$ ). The applicant rated the fits using the following scale:

- Not acceptable: the fitted curve does not represent the trend of the data points and residuals show strong deviations from random distribution.
- Acceptable: the fitted curve describes the trend of the data points, residuals may show some deviations from random distribution but it is not significant.
- Good: the fitted curve closely follows all the data points (limited scatter of data points); residuals are randomly distributed (no bias of residuals).
- Very good: no bias of residuals or scatter of data points.

HSE has also followed these definitions in its own, independent assessment. HSE has verified the fits selected by the applicant using the decision trees from Figures 7-1 and 7-2 of the FOCUS Kinetics Guidance document. Although the applicant has run SFO, FOMC and DFOP models, HSE did not consider it necessary to run more than SFO and FOMC in the majority of cases as SFO was usually the best fit model for triggering endpoints or a sufficiently good fit for modelling endpoints.

**KINETIC EVALUATION FOR 1'-COOH-S-2840****Table B.8.1.1.2.2-01 Input data for the kinetic evaluation: 3'-OH-S-2840**

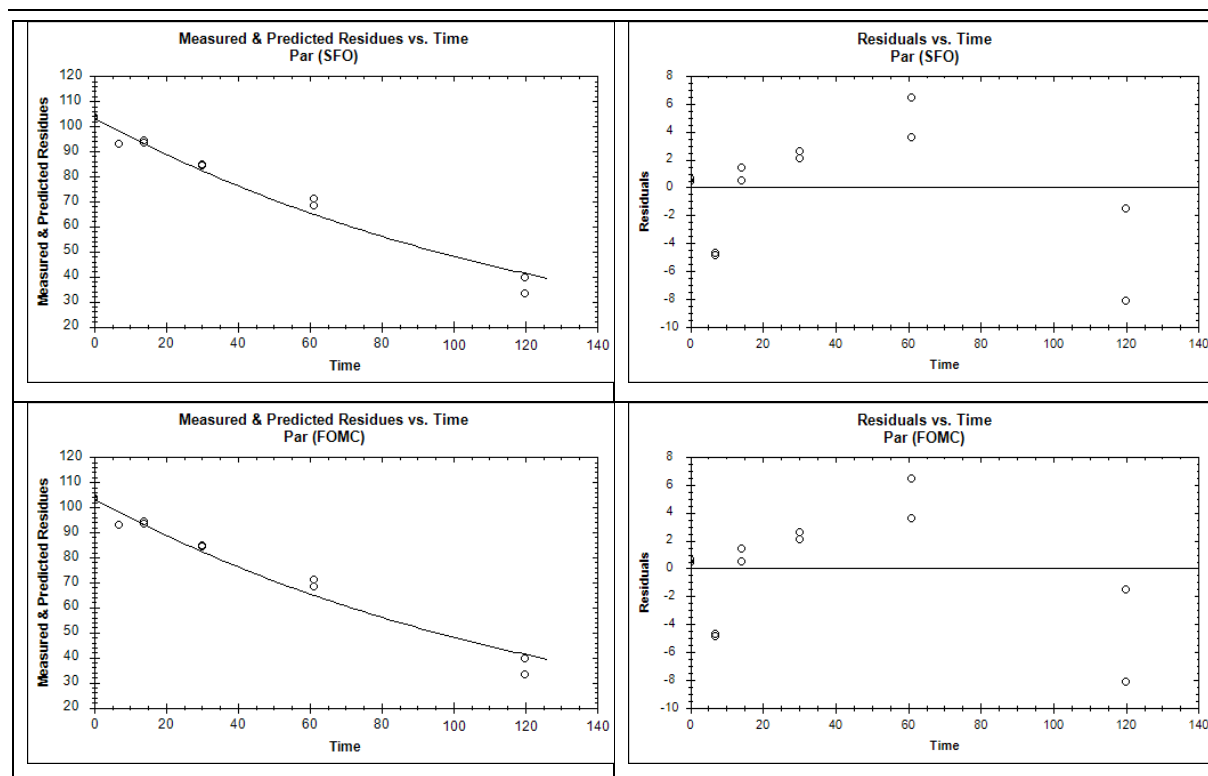
<b>Sampling interval (d)</b>	<b>Replicate</b>	<b>Speyer 5M</b>	<b>Newhaven</b>	<b>Atwater</b>
0	1	99.6	96.6	99.6
	2	97.7	101.0	97.7
7	1	96.6	92.7	95.0
	2	96.6	93.0	96.4
14	1	91.7	88.0	94.5
	2	93.6	91.0	94.5
30	1	92.9	89.4	89.0
	2	94.9	88.9	89.8
61	1	83.6	76.0	82.8
	2	78.7	77.6	81.9
120	1	78.3	78.8	75.7
	2	81.4	71.7	70.6

**Table B.8.1.1.2.2-02 Input data for the kinetic evaluation: 1'-COOH-S-2840**

<b>Sampling interval (d)</b>	<b>Replicate</b>	<b>Speyer 5M</b>	<b>Newhaven</b>	<b>Atwater</b>
0	1	104.0	103.6	103.8
	2	103.7	102.5	98.2
7	1	93.1	69.7	91.7
	2	93.2	68.4	94.2
14	1	93.3	61.8	92.7
	2	94.2	62.2	89.1
30	1	84.2	47.7	89.0
	2	84.7	45.3	85.0
61	1	71.2	45.8	85.0
	2	68.3	43.8	73.0
120	1	33.1	36.5	53.3
	2	39.7	38.5	53.8

**Speyer 5M soil****Figure B.8.1.1.2.2 -01 Kinetic fits for 1'COOH-S-2840 in the Speyer 5M soil (HSE fits)**

<b>Speyer 5M (KCA 7.1.2.1/02)</b>						
<b>Model</b>	<b>Visual Assessme nt</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>Good</b>	<b>3.564</b>	<b>M<sub>0</sub>: 103.3 k: 7.665 x 10<sup>-3</sup></b>	<b>k: &lt; 0.05</b>	<b>90.43</b>	<b>300.4</b>
FOMC	Good	3.927	M <sub>0</sub> : 103.3 alpha: 4155 beta: 54200	not applicable	90.43	300.5
<p>The visual fit of the SFO model to the data set is good, with the starting concentrations and the decline phase accurately represented. The plot of the residuals shows some systematic errors between 14 and 61 days, but the low <math>\chi^2</math> value of 3.56 % nevertheless demonstrates that the model is providing a good representation of the data set.</p> <p>The FOMC model gives a very similar visual fit, with a slightly higher <math>\chi^2</math> value of 3.93 % (likely due to the additional parameter and one less degree of freedom when fitting the same data to the FOMC model).</p> <p>The SFO model is considered to be sufficiently good to derive modelling endpoints, while the FOMC model does not offer any improvement over SFO. <b>Conclusion: SFO is selected for both trigger and modelling endpoints (DT<sub>50</sub> = 90.43 days, DT<sub>90</sub> = 300.4 days)</b></p> <p><b>Selected kinetics in bold</b></p>						



**Newhaven Soil****Figure B.8.1.1.2.2-02 Kinetic fits for 1'COOH-S-2840 in the Newhaven soil (HSE fits)**

<b>Newhaven (KCA 7.1.2.1/02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	Poor	16.55	M <sub>0</sub> : 81.8 k: $9.988 \times 10^{-3}$	k: <0.05	69.4	230.5
FOMC	Good	2.97	M <sub>0</sub> : 103.1 alpha: 0.236 beta: 1.562	not applicable	27.93	>1000
<b>DFOP</b>	<b>Good</b>	<b>3.456</b>	<b>M<sub>0</sub>: 102.6</b> <b>k<sub>1</sub>: <math>1.279 \times 10^{-1}</math></b> <b>k<sub>2</sub>: <math>2.654 \times 10^{-3}</math></b> <b>g: 0.496</b>	<b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b>	<b>23.06</b>	<b>609.5</b>
HS	Good	7.904	M <sub>0</sub> : 103.0 k <sub>1</sub> : $1.115 \times 10^{-1}$ k <sub>2</sub> : $5.643 \times 10^{-3}$ t <sub>b</sub> : 4.246	k <sub>1</sub> : <0.05 k <sub>2</sub> : <0.05	43.18	328.4
<p>The SFO model does not give a good fit. Starting concentrations are substantially under-represented and the model underestimates the rate of decline resulting in systematic errors in the plot of the residuals. The rate of decline is overestimated by the final time point. Additionally, the <math>\chi^2</math> value is fairly high at 16.55 %.</p> <p>The applicant investigated biphasic models to see if they improved the fit. The visual fit was much improved with the FOMC model, with good reproduction of the starting concentration, decline phase and final time point and consequently random errors in the plot of the residuals. The <math>\chi^2</math> value is also much lower than for SFO at 2.97 %.</p> <p>The DFOP visual fit is similar to the FOMC model. DFOP offers a marginally improved fit, but the plot of the residuals shows generally smaller errors for FOMC than for DFOP. The <math>\chi^2</math> value for DFOP is 3.46 %, which is slightly higher than for FOMC. The HS model also offers a good visual fit, but the plot of residuals and</p>						

the  $\chi^2$  value of 7.90 % indicate a worse fit than for FOMC or DFOP. The  $k_1$  and  $k_2$  parameters passed the t-test for both the HS and DFOP models.

The applicant's selection of the DFOP model for modelling endpoints is accepted by HSE. The SFO model has been selected for other soils in the data set and therefore DFOP is preferred to FOMC for modelling endpoints, as it makes combining SFO and non-SFO parameters easier. At study end, 38.5 % of the applied amount remains in soil. As the  $DT_{90}$  is not reached within the study it is not appropriate to use  $DT_{90}/3.32$  to derive a conservative modelling  $DT_{50}$ . A  $DT_{50}$  derived from the DFOP  $k_2$  parameter for DFOP is appropriate for this terminal metabolite. The applicant derived a  $DT_{50}$  value of 266 days from the  $k_2$  parameter; the equivalent HSE value is 261 days due to the differing treatment in  $M_0$  values.

For the triggering and persistence endpoint, the actual fitted  $DT_{90}$  and the  $DT_{50}$  with no back-calculation should be used. For the PECsoil endpoint, a pseudo SFO should be calculated by back calculating PECsoil using the tier 1 spreadsheet, or the kinetic parameters should be used if using ESCAPE. The applicant selected the FOMC model for triggering endpoints. The FOMC  $DT_{90}$  value is >1000 days, meaning it is not possible to derive an exact pseudo  $DT_{50}$  value by  $DT_{90}/3.32$ . It is also not recommended to use FOMC for very long term PECsoil accumulation calculations. Consequently, HSE has selected the DFOP model for triggering endpoints.

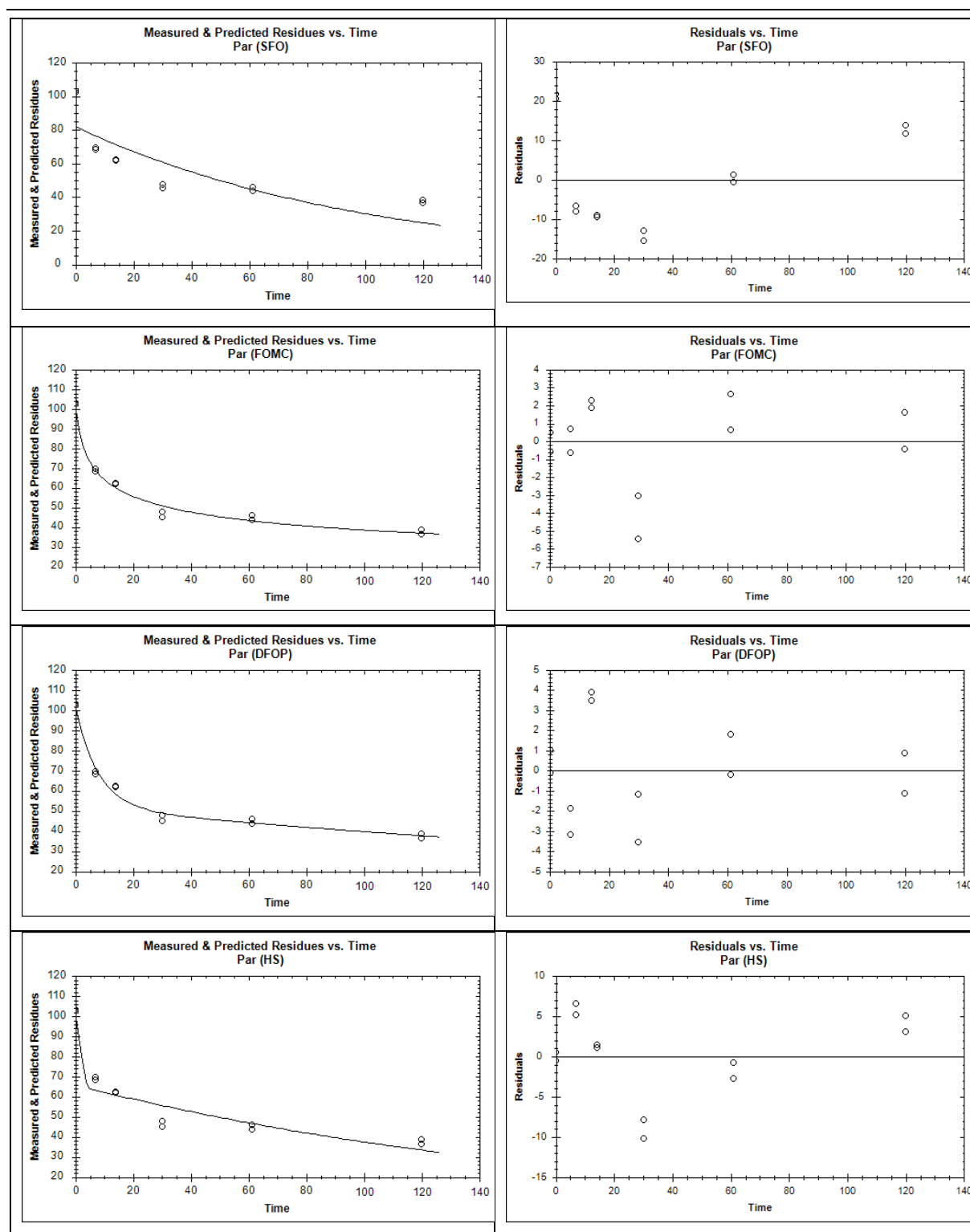
**Conclusion: DFOP is selected for both trigger and modelling endpoints**

**Trigger and persistence endpoints:  $DT_{50} = 23.06$  days,  $DT_{90} = 609.5$  days**

**Modelling endpoints:  $DT_{50} = \ln(2)/k_2 = 261.2$  d**

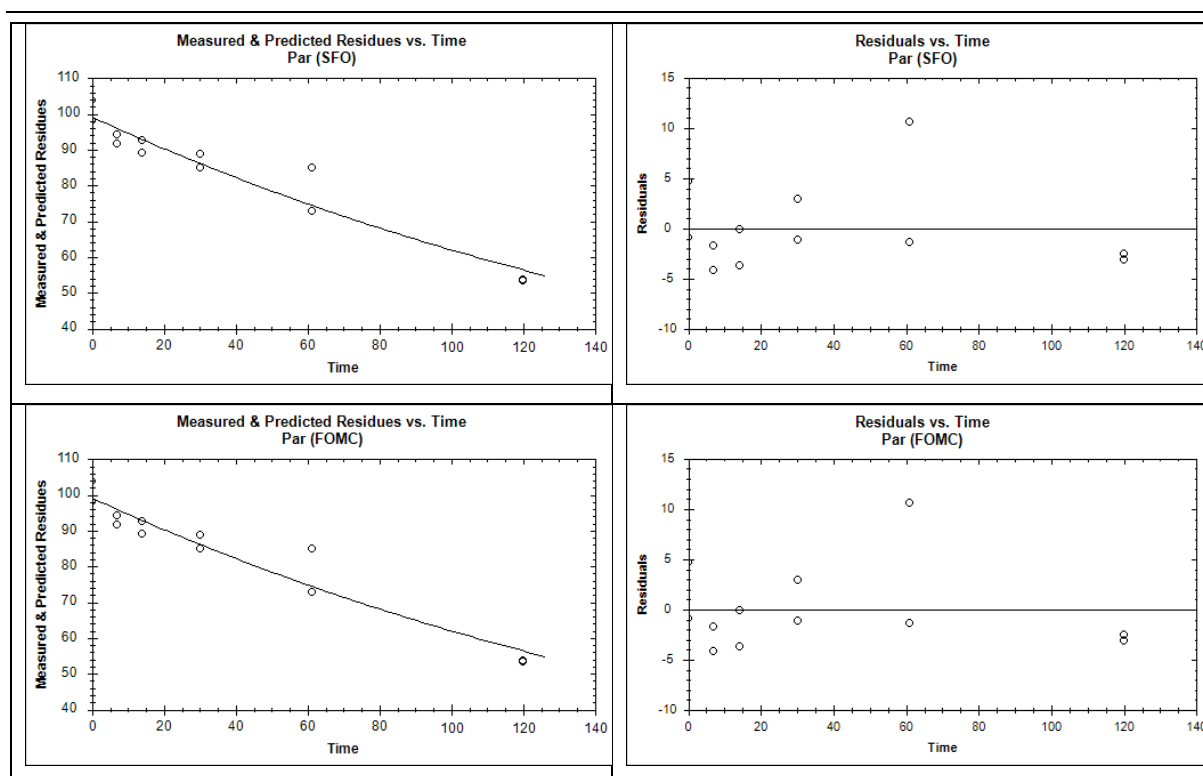
**Selected kinetics in bold**





**Atwater soil****Figure B.8.1.1.2.2-03 Kinetic fits for 1'COOH-S-2840 in the Atwater soil (HSE fits)**

<b>Atwater (KCA 7.1.2.1/02)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>Good</b>	<b>2.616</b>	<b>M<sub>0</sub>: 99.1 k: 4.703 x 10<sup>-3</sup></b>	<b>k: &lt;0.05</b>	<b>147.4</b>	<b>489.6</b>
FOMC	Good	2.883	M <sub>0</sub> : 99.1 alpha: 5149 beta: 1.095 x 10 <sup>6</sup>	not applicable	147.4	489.7
<p>The visual fit of the SFO model to the data set is good, with the starting concentrations and the decline phase well represented by the model, as is also shown in the plot of the residuals. The <math>\chi^2</math> value of 2.62 % is low.</p> <p>The FOMC model gives a very similar visual fit, with a slightly higher <math>\chi^2</math> value of 2.88 %.</p> <p>The SFO model is considered to be sufficiently good to derive modelling endpoints, while the FOMC model does not offer any improvement over SFO.</p> <p><b>Conclusion: SFO is selected for both trigger and modelling endpoints (DT<sub>50</sub> = 147.4 days, DT<sub>90</sub> = 489.6 days)</b></p> <p><b>Selected kinetics in bold</b></p>						



## KINETIC EVALUATION FOR 3'-OH-S-2840

### Speyer 5M Soil

Figure B.8.1.1.2.2-04 Kinetic fits for 3'-OH-S-2840 in the Speyer 5M soil (HSE fits)

Speyer 5M (KCA 7.1.2.1/01)						
Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	Good	2.439	M <sub>0</sub> : 97.1 k: 0.001898	k: <0.05	365.2	>1000
FOMC	Good	2.218	M <sub>0</sub> : 98.9 alpha: 0.1637 beta: 39.257	not applicable	>1000	>1000
The visual fit of the SFO model to the data set is good, with the starting concentrations only slightly underestimated and the decline phase accurately						

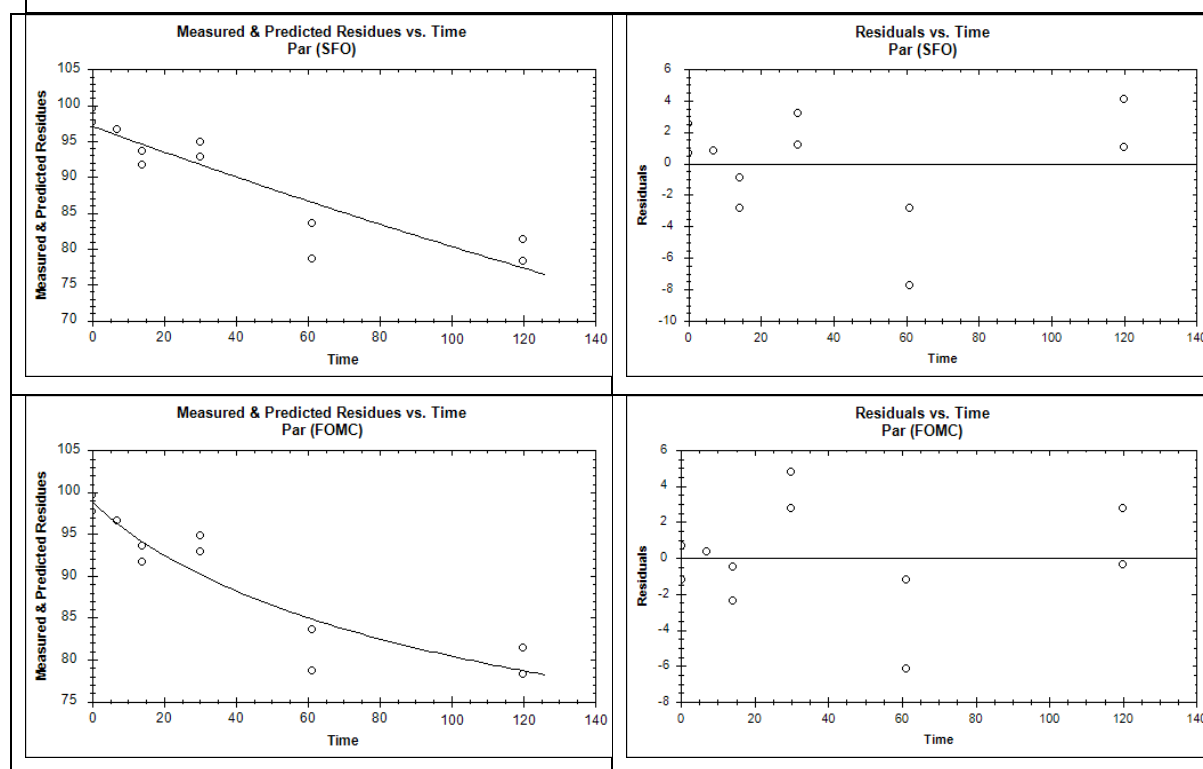
represented and this is also shown in the plot of the residuals. The  $\chi^2$  value of 2.44 % is low.

The FOMC model gives a very similar visual fit. The starting concentrations are slightly better represented than by the SFO model and the  $\chi^2$  value of 2.22 % is slightly lower.

The SFO model is considered to be sufficiently good to derive modelling endpoints, while the SFO and FOMC models are sufficiently similar that the FOMC model offers only marginal improvement over SFO. The SFO model is therefore selected for both modelling and triggering endpoints.

**Conclusion: SFO is selected for both trigger and modelling endpoints ( $DT_{50} = 365.2$  days,  $DT_{90} = >1000$  days)**

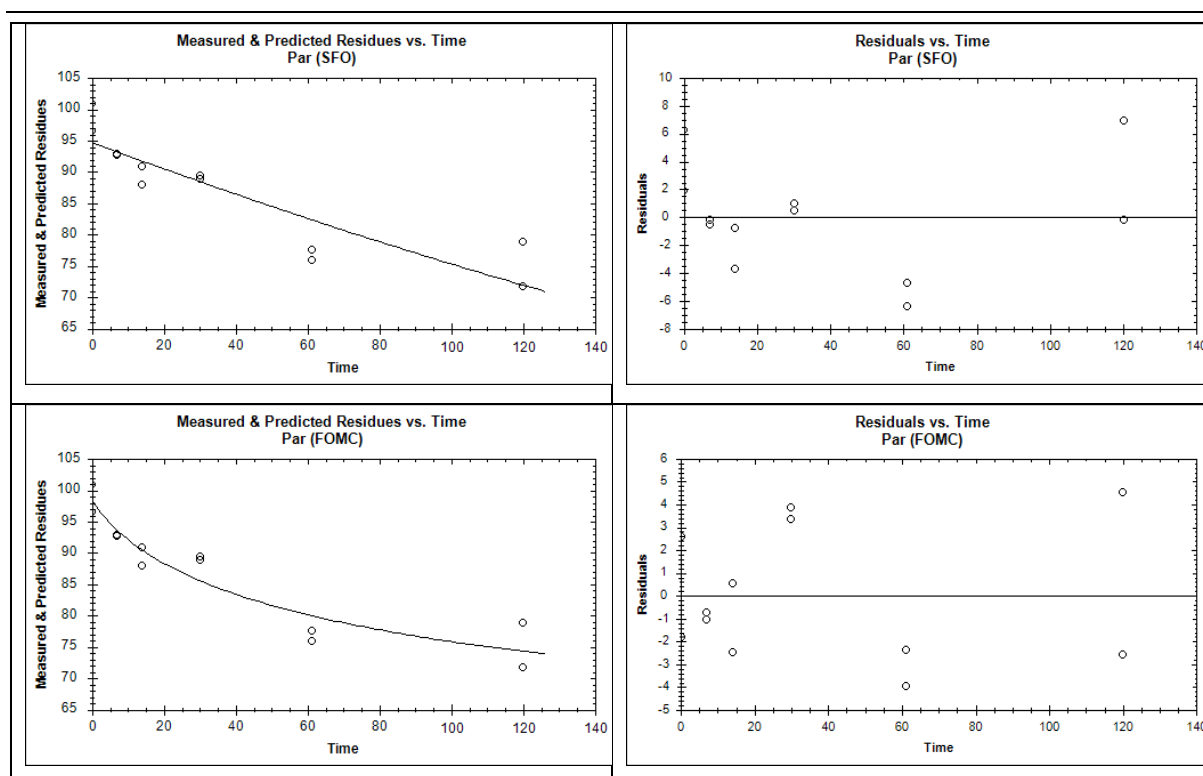
**Selected kinetics in bold**



## Newhaven Soil

**Figure B.8.1.1.2.2-05 Kinetic fits for 3'-OH-S-2840 in the Newhaven soil (HSE fits)**

<b>Newhaven (KCA 7.1.2.1/01)</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>Good</b>	<b>2.986</b>	<b>M<sub>0</sub>: 94.7 k: 2.297 x 10<sup>-3</sup></b>	<b>k: &lt;0.05</b>	<b>301.7</b>	<b>&gt;1000</b>
FOMC	Good	2.094	M <sub>0</sub> : 98.4 alpha: 0.1284 beta: 15.124	not applicable	>1000	>1000
<p>The visual fit of the SFO model to the data set is good, with the starting concentrations only slightly underestimated and the decline phase accurately represented and this is also shown in the plot of the residuals. The <math>\chi^2</math> value of 2.99 % is low.</p> <p>The FOMC model gives a very similar visual fit. The starting concentrations are slightly better represented than by the SFO model and the <math>\chi^2</math> value of 2.09 % is slightly lower.</p> <p>The SFO model is considered to be sufficiently good to derive modelling endpoints, while the SFO and FOMC models are sufficiently similar that the FOMC model offers only marginal improvement over SFO.</p> <p><b>Conclusion: SFO is selected for both trigger and modelling endpoints (DT<sub>50</sub> = 301.7 days, DT<sub>90</sub> = &gt;1000 days)</b></p> <p><b>Selected kinetics in bold</b></p>						



## Atwater

Figure B.8.1.1.2.2-06 Kinetic fits for 3'-OH-S-2840 in the Atwater soil (HSE fits)

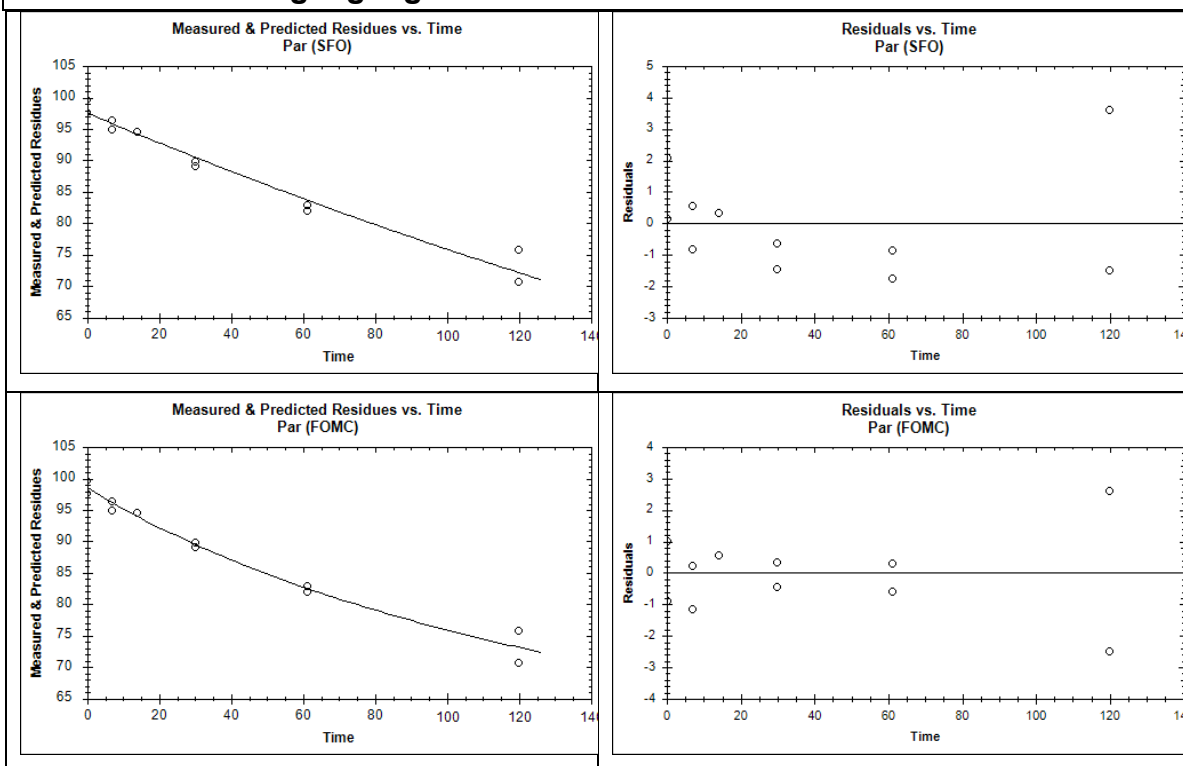
Atwater (KCA 7.1.2.1/01)						
Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	Good	0.8357	M <sub>0</sub> : 97.5 k: $2.519 \times 10^{-3}$	k: <0.05	275.2	914.2
FOMC	Good	0.2999	M <sub>0</sub> : 98.6 alpha: 0.4154 beta: 113.78	not applicable	489.8	>1000
The visual fit of the SFO model to the data set is good, with the starting concentrations and the decline phase accurately represented and this is also shown in the plot of the residuals. The $\chi^2$ value of 0.836 % is low.						

The FOMC model gives a very similar visual fit. The starting concentrations are slightly better represented than by the SFO model and the  $\chi^2$  value of 0.300 % is slightly lower.

The SFO model is considered to be sufficiently good to derive modelling endpoints, while the SFO and FOMC models are sufficiently similar that the FOMC model offers only marginal improvement over SFO.

**Conclusion: SFO is selected for both trigger and modelling endpoints ( $DT_{50} = 275.2$  days,  $DT_{90} = >1000$  days)**

**Selected modelling highlighted in bold**



## Comparison of HSE and applicant modelling

The applicant has submitted modelling according to FOCUS Kinetics (2006, 2014) to derive modelling and triggering endpoints, which has been validated by HSE. It was noted that the applicant has used the % AR recovered for the parent compound as M0 rather than the material balance at 0 DAT and therefore HSE has rerun the modelling to determine the impact of this discrepancy. HSE and applicant modelling are compared below.

**Table B.8.1.1.2.2-03 Summary and comparison of HSE and applicant modelling**

HSE					Applicant				
Mod el	DT <sub>50</sub>	DT <sub>90</sub>	χ <sup>2</sup>	Visu al fit	Mod el	DT <sub>50</sub>	DT <sub>90</sub>	χ <sup>2</sup>	Visual fit
1'COOH-S-2840, Speyer 5M soil									
SFO	90.4 3	300. 4	3.564	Good	SFO	91.3	303	3.56	Very good
FOM C	90.4 3	300. 5	3.927	Good	FOM C	91.3	306	3.95	Very good
DFO P	Not run				DFO P	91.3	303	4.48	Very good
1'COOH-S-2840, Newhaven soil									
SFO	69.4	230. 5	16.55	Poor	SFO	70.7	235	16.2	Acceptable
FOM C	27.9 3	>100 0	2.97	Good	FOM C	29.5	>10,00 0	2.98	Very good
DFO P	23.0 6	609. 5	3.456	Good	DFO P	24.5	623	3.39	Very good
HS	43.1 8	328. 4	7.904	Good	HS	37.9	403	5.82	Very good
1'COOH-S-2840, Atwater soil									
SFO	147. 4	489. 6	2.616	Good	SFO	148	491	2.59	Very good
FOM C	147. 4	489. 7	2.883	Good	FOM C	148	500	2.86	Very good
DFO P	Not run				DFO P	147	501	153	Very good
3'-OH-S-2840, Speyer 5M soil									
SFO	365. 2	>100 0	2.439	Good	SFO	369	1226	2.5	Very good
FOM C	>100 0	>100 0	2.218	Good	FOM C	>10,00 0	>10,00 0	2.3	Very good
DFO P	Not run				DFO P	>10,00 0	>10,00 0	2.51	Very good
3'-OH-S-2840, Newhaven soil									
SFO	301. 7	>100 0	2.986	Good	SFO	303	1006	3.05	Very good
FOM C	>100 0	>100 0	2.094	Good	FOM C	3324	>10,00 0	2.16	Very good
DFO P	Not run				DFO P	>10,00 0	>10,00 0	2.36	Very good
3'-OH-S-2840, Atwater soil									



<b>SFO</b>	<b>275.2</b>	<b>914.2</b>	<b>0.8357</b>	<b>Good</b>	<b>SFO</b>	<b>276</b>	<b>917</b>	<b>0.863</b>	<b>Very good</b>
FOM C	489.8	>1000	0.2999	Good	FOM C	486	>10,000	0.368	Very good
DFO P	Not run				DFO P	>10,000	>10,000	0.37	Very good

**Selected modelling highlighted in bold**

The change for  $M_0$  does not alter the conclusion as to which model is appropriate for any soil or for either metabolite. In all cases, the applicant's modelling results in marginally longer  $DT_{50}$  and  $DT_{90}$  values. Both metabolites form from parent and, as other metabolites, e.g. N-des-Me-DFPA, DFPA forming later in the degradation scheme are classed as minor (<10 % or <5 % at two consecutive timepoints) are terminal metabolites in exposure modelling schemes. The  $DT_{50}$  used for 3'-OH-S-2840 and 1'-COOH-S-2840 does not therefore impact on any following metabolites. As the difference between applicant and HSE kinetic modelling endpoints is marginal and applicant  $DT_{50}/DT_{90}$  values are slightly more conservative, applicant values are accepted.  $DT_{50}$  and  $DT_{90}$  values for both metabolites for use in the exposure assessment are summarised below.

**Table B.8.1.1.2.2-04 Summary of FOCUS Kinetic modelling to derive triggering/PECsoil endpoints for metabolites of inpyrfluxam 1'-COOH-S-2840 and 3'-OH-S-2840**

	<b>Model</b>	<b><math>DT_{50}</math></b>	<b><math>DT_{90}</math></b>	<b><math>\chi^2</math></b>	<b>Visual fit</b>
<b>1'-COOH-S-2840</b>					
Speyer 5M soil	SFO	91.3	303	3.56	Very good
Newhaven soil	DFOP*	24.5	623	3.39	Very good
Atwater soil	SFO	148	491	2.59	Very good
<b>3'-OH-S-2840</b>					
Speyer 5M soil	SFO	369	1226	2.5	Very good
Newhaven soil	SFO	303	1006	3.05	Very good
Atwater soil	SFO	276	917	0.863	Very good

\*For the PECsoil endpoint, a pseudo SFO should be calculated by back calculating PECsoil using the tier 1 spreadsheet, or the kinetic parameters should be used if using ESCAPE

**B.8.1.1.2.3. Anaerobic rate of degradation of the active substance**

<b>KCA number:</b>	KCA 7.1.2.1.3/01
<b>Report Title:</b>	S-2399: Anaerobic Soil Metabolism
<b>Study Author &amp; Year:</b>	██████ ██████ K, 2017
<b>Document number:</b>	VP-39081
<b>Guidelines:</b>	OECD Guideline 307 anaerobic transformation in soil
<b>Previous evaluations</b>	None, new data submitted for evaluation
<b>GLP:</b>	Yes

<b>KCA number:</b>	7.1.2.1.3/02
<b>Report Title:</b>	S-2399: Degradation under Anaerobic Conditions in Soil - Rate Studies
<b>Study Author &amp; Year:</b>	██████ ██████ K, 2017
<b>Document number:</b>	VP-39087
<b>Guidelines:</b>	OECD Guideline 307 anaerobic transformation in soil
<b>Previous evaluations</b>	None, new data submitted for evaluation
<b>GLP:</b>	Yes

<b>Deviations from OECD 307 guideline</b>	<b>HSE assessment of deviations</b>
Sampling site pesticide history not provided for Penn soil	This is a soil where pesticides would not normally be applied, and the degradation properties of the soil are not outliers from the other soils used (see B.8.1.1.1.2). Therefore HSE considers the soil microbes as unlikely to be adapted to metabolise inpyrfluxam, and accepts its use here.
Soil transport containers not defined	Minor omission. Loose polyethylene bags are recommended by OECD 307 to minimise changes in soil water

	content. As the applicant has adjusted soil water content prior to test initiation, HSE does not consider this omission to jeopardise study validity. Biomass measurements demonstrate that the soils are viable throughout the study, HSE therefore considers that the transport conditions are acceptable.
Storage temperature at the test centre not provided	Minor omission. Soil is recorded as having been refrigerated.
Use of 1.0 M NaOH volatile traps in aerobic phase. Deviation from 2.0 M NaOH given in OECD 307 guideline.	Minor deviation. Suitable mass balances suggest concentration used was suitable.
28 day aerobic onset period used rather than OECD 307 guideline 30 days	Minor deviation. Relatively small difference in duration from guideline recommendation.
Study exceeds OECD 307 guideline maximum duration of 120 days	Minor deviation. Anaerobic period lasts for 126 days, a minor exceedance. Furthermore, microbial biomass measurements demonstrate that the soil is still biologically active at study termination.
<b>HSE conclusion on deviations</b>	
None of the deviations from the guidelines are considered to void the study validity.	

## Introduction

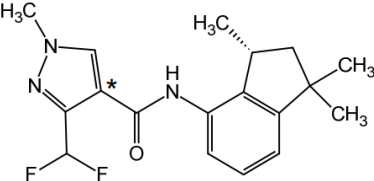
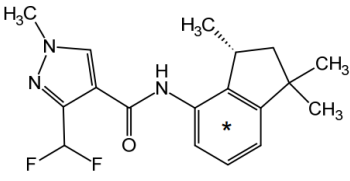
The degradation behaviour of inpyrfluxam in UK and US soils under anaerobic laboratory conditions in the dark was investigated according to OECD guidelines (OECD 307: Aerobic and anaerobic transformation in soil). The evaluation was conducted to derive kinetic parameters that are suitable to potentially trigger additional studies (trigger endpoints).

Rate studies were performed on a total of four soils. A US (Penn) soil in 7.1.2.1.3/01, and another US soil (Atwater) and two UK soils (Newhaven and Empingham) in 7.1.2.1.3/02. These two studies, which were conducted under GLP, have been compiled and summarised here.

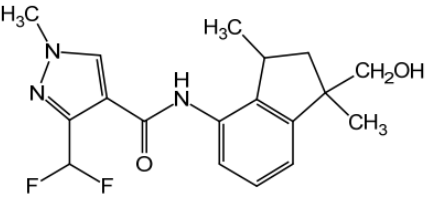
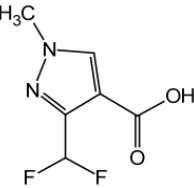
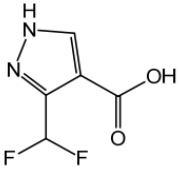
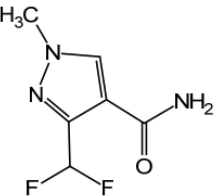
## Materials

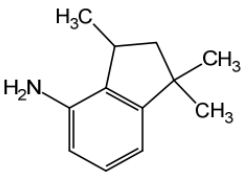
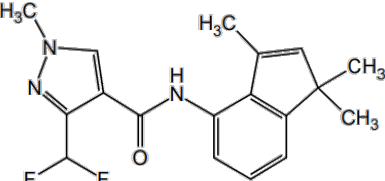
Detailed information on the radiolabelled test and reference materials used for the study are presented below in Table B.8.1.1.2.3-01.

**Table B.8.1.1.2.3-01 Test and reference materials**

	<b>7.1.2.13/01 (Penn studies)</b>	<b>7.1.2.1.3/02 (Newhaven, Empingham, Atwater studies)</b>
<b>1. Test Material</b>	<b>[pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PYR-label)</b>	
Lot/Batch:	CFQ41802	
Specific activity:	2.11 GBq/mmol	
Purity:	Radiochemical purity 95.2 % (in the dosing solution)	
CAS number:	Not assigned	
Structure:	 <p><u>* denotes <sup>14</sup>C label position</u></p>	
<b>2. Test Material</b>	<b>[phenyl-<sup>14</sup>C] inpyrfluxam (PHE-label)</b>	Not used in this study.
Lot/Batch number:	CFQ41803	-
Specific activity:	4.51 GBq/mmol	-
Purity:	Radiochemical purity 99.1 % (in the dosing solution)	-
CAS number:	Not assigned	-
Stability of compound:	Not stated	-
Structure:	 <p><u>* denotes <sup>14</sup>C label position</u></p>	-
<b>3. Reference Material</b>	<b>Inpyrfluxam (Pure 3'R isomer)</b> <b>S-2840 (Contains 2 enantiomers: 3'R &amp; 3'S)</b> <b>S-2940 (Pure 3'S isomer)</b>	
Chemical Name:	N-[(3R)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide	
Lot Numbers, (chemical purity %)	AS2375a (inpyrfluxam) (95.3 %) AS2375b (inpyrfluxam) (99.8 %) AS2375c (inpyrfluxam) (95.4%) AS2387a (S-2940) (99.8%)	

Structure:	
<b>4. Reference Material</b>	<b>N-des-Me-S-2840 (Contains 2 enantiomers: 3'R &amp; 3'S)</b>
Chemical Name:	N-[(3RS)-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
Lot Number (chemical purity %)	AS2380a (97.5 %)
Structure:	
<b>5. Reference Material</b>	<b>3'-OH-S-2840 (Contains 2 enantiomers: 3'R &amp; 3'S)</b>
Chemical Name:	N-[(3RS)-3-hydroxy-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide
Lot Number (chemical purity %)	AS2379a (99.7 %)
Structure:	
<b>6. Reference Material</b>	<b>1'-COOH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-COOH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)</b>
Chemical Name:	(1RS,3RS)-(1RS,3SR)-2,3-dihydro-1,3-dimethyl-4-[[1-methyl-3-(difluoromethyl)-1H-pyrazole-4-ylcarbonyl]amino]-1H-indene-1-carboxylic acid
Lot Numbers (chemical purity %)	AS2393a (1'-COOH-S-2840A) (100 %) AS2394a (1'-COOH-S-2840B) (99.6 %)
Structure:	
<b>7. Reference Material</b>	<b>1'-CH2OH-S-2840A (2 enantiomers: 1'R,3'S and 1'S,3'R) 1'-CH2OH-S-2840B (2 enantiomers: 1'R,3'R and 1'S,3'S)</b>
Chemical Name:	N-[(1RS,3RS)-(1RS,3SR)-2,3-dihydro-1-(hydroxymethyl)-1,3-dimethyl-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide

Lot Numbers (chemical purity %)	AS2395a (1'-CH <sub>2</sub> OH-S-2840A) (100 %) AS2396a (1'-CH <sub>2</sub> OH-S-2840B) (99.5 %)	
Structure:		
<b>8. Reference Material</b>	<b>DFPA</b>	
Chemical Name:	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid	
Lot Number (chemical purity %)	AS2378a (99.2 %)	
Structure:		
<b>9. Reference Material</b>	<b>N-des-Me-DFPA</b>	
Chemical Name:	3-difluoromethyl-1H-pyrazole-4-carboxylic acid	
Lot Number (chemical purity %)	AS2381a (97.8 %)	
Structure:		
<b>10. Reference Material</b>	<b>DFPA-CONH<sub>2</sub></b>	
Chemical Name:	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxamide	
Lot Number (chemical purity %)	AS2382a (99.2 %)	
Structure:		
<b>11. Reference Material</b>	<b>ATMI</b>	Not used in this study.
Chemical Name:	(3RS)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-amine	-
Lot Number (chemical purity %)	AS2383a (99.7 %)	-

Structure:		-
<b>12. Reference Material</b>	<b>3'-OH-S-2840-dehydrate</b>	
Chemical Name:	3-difluoromethyl-1-methyl-N-[1,1,3-trimethyl-1H-inden-4-yl]-1H-pyrazole-4-carboxamide	
Lot Number chemical purity (%)	AS2413a (98.2 %)	
Structure:		

## Soil collection

After collection from the sampling site using a shovel all soils were passed through a 2 mm sieve. On arrival the Newhaven and Empingham soils were stored in the dark at 5°C until application of the test item, which was within 3 months. HSE notes that the Atwater and Penn soils were stored at 'ambient temperature'. This significantly exceeds the OECD 307 recommended storage conditions of 4°C ± 2°C. However, despite the higher than recommended storage temperature, the microbial activity of these soils remained within the acceptable range, therefore HSE deems this to be a minor deviation as it had no significant impact on the study outcomes.

HSE notes that for the Penn soil, no details of treatments with chemicals, treatments with organic and inorganic fertilisers, additions of biological materials or other contamination regarding the soil collection site have been made available, as is required in OECD 307. HSE considers this a major deviation which potentially jeopardises the validity of the test. Inpyrfluxam is a new active substance and was not authorised for use in USA at the time of soil collection and is therefore unlikely to have been used commercially in the four years prior at the test site. However, there are a number of structurally related compounds in the pyrazole carboxamide class that could have been used. Therefore there is the potential that soil microbes at this site may have been exposed to structural analogues which may impact degradation rates. HSE has therefore requested this information from the applicant to proceed with the study validation. The applicant has since stated in a response to an RAI that "Due to the changing nature of the fields used for the study a pesticide history is unavailable." Whilst HSE would not normally accept this justification in isolation, it is noted that pesticide use histories were available from the other laboratory soil collection sites (see table B.8.1.1.2.3-02). The metabolite profile and parent degradation in this US soil was considered comparable to the behaviour seen at

other sites (where pesticide histories were available). Furthermore, the applicant has stated that it was sampled from a location which would not normally have pesticide applied. Overall HSE concluded that the results from this study could be considered valid in this case.

Soil collection data from KCA 7.1.2.1.3/01 & KCA 7.1.2.1.3/02 have been compiled and summarised below in Table B.8.1.1.2.3-02. Soil properties are given in Table B.8.1.1.2.3-03.

**Table B.8.1.1.2.3-02 Soil collection and storage properties**

<b>Soil</b>	<b>Newhaven</b>	<b>Empingham</b>	<b>Atwater</b>	<b>Penn</b>
Geographic locations	Derbyshire, UK: GPS [REDACTED]	Rutland, UK: GPS [REDACTED]	Atwater, CA, USA GPS [REDACTED]	Baptistown, N, USA. J: GPS [REDACTED]
Pesticide use history at the collection site	None (at least since 1991), rough grassland used for cattle grazing	None recorded (at least since 2002)	Not available, however pesticides would normally not be applied at the site.	Not available, however pesticides would normally not be applied at the site.
Sampling depth for soil	Top 5 - 20 cm	Top 12 - 20 cm	Top 0-7.6 cm	Top 0-7.6 cm
Date of collection	26/11/2015	26/11/2015	03/12/2015	24/08/2015
Storage conditions during transport	Kept at 5°C	Kept at 5°C	Kept at ambient temperature	Kept at ambient temperature
Storage conditions at test facility	Kept in refrigerator	Kept in refrigerator	Kept in refrigerator	Kept in refrigerator



storage length from collection to study initiation (days)	15	15	8	23
Soil moisture (%)	28.00	32.45	6.58	16.20
Soil moisture after adjustment to 50% MWHC* (%)	37.47	40.92	13.97	24.57
Maximum water holding capacity (%)	74.94	81.83	27.93	49.14

\*Maximum Water Holding Capacity

## Soil properties

**Table B.8.1.1.2.3-03 Soil properties**

	<b>Newhaven</b>	<b>Empingham</b>	<b>Atwater</b>	<b>Penn</b>
Soil classification (BBA classification)	Clayey Silt	Sandy Loam	Silty Sand	Silty Loam
Soil texture (USDA)	Sand 26%, Silt 65%, Clay 9%	Sand 46%, Silt 37%, Clay 17%	Sand 84%, Silt 13%, Clay 3%	Sand 28%, Silt 48%, Clay 24%
Organic matter (%)	6.1	11.3	1.2	2.3
Organic Carbon (%)	3.5	6.6	0.67	1.3*
Soil pH (1:1 soil:H <sub>2</sub> O)	5.5	7.3	6.0	6.1
Bulk density (disturbed) (g/cm <sup>3</sup> )	0.92	0.99	1.33	1.04

1/3 bar moisture (pF 2.5) (%)	24.5	28.1	5.7	26.0
1/10 bar moisture (pF 2) (%)	32.3	33.6	6.6	32.6
Cation Exchange Capacity (CEC; meq/100 g of dry soil)	12.6	31.4	5.1	8.8
Olsen phosphorus (ppm)	12	29	21	6
Salinity (electrical conductivity; mmhos/cm)	0.24	0.51	0.10	0.22
Sodium (ppm)	20	34	12	13
Calcium (ppm)	1133	5292	591	761
Magnesium (ppm)	49	136	96	155
Total nitrogen (%)	0.35	0.63	0.07	0.14

\*OC not provided by applicant, HSE notes however that  $OC = OM / 1.724$

## Incubation apparatus

### Aerobic test system

Duplicate soil samples (wet weight equivalent to 50 g dry weight, adjusted to ca. 50% water holding capacity) were placed inside the incubation chamber in open individual 100 mL glass beakers. One open Petri dish, containing 50 mL of 1.0 M NaOH solution was placed inside the chamber to capture evolved  $^{14}\text{CO}_2$ . Several open Petri dishes full of water were also placed inside the chamber to maintain high air humidity. Chamber air was continuously evacuated through the chamber air outlet, which was connected to a vacuum source, at a steady rate of approximately 10 mL per minute. The evacuated air passed through a tetraglyme trap to capture any organic  $^{14}\text{C}$  volatiles present in the chamber's air followed by two consecutive NaOH traps to capture any  $^{14}\text{CO}_2$  present in the chamber's air. The incubation apparatus was incubated in the dark at  $20 \pm 2^\circ\text{C}$ .

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**Anaerobic test system**

After incubating aerobically for 28 days, the incubation conditions were changed to establish and maintain anaerobic conditions. HSE notes that the OECD 307 guidelines recommend that an aerobic period of 30 days should be maintained before anaerobic conditions are initiated when the test substance DT50 is longer than 30 days (as is the case here). The applicant has used a 28 day onset period, and has not provided reasoning for this deviation. HSE views this as a minor deviation due to the relatively small difference in duration from the guideline 30 days and so is accepted by HSE.

Each individual soil sample was quantitatively transferred with 80, 85 and 70 mL of N<sub>2</sub> purged HPLC water into each of the biometer flasks, this gave waterlogged soils with a ca. 2 – 3 cm water layer. Dried ground alfalfa was added to each biometer at a rate of 0.1% (0.05 g) of the soil dry weight to speed the formation of anaerobic conditions and to ensure the survival of the microbial anaerobes. The <sup>14</sup>CO<sub>2</sub> side-arm trap was charged with 25 mL of 2 M NaOH solution (N<sub>2</sub> purged). The biometers were assembled and sparged with a stream of nitrogen for approximately 0.25 hours to facilitate the formation of anaerobic conditions in the test system. Following sparging, the flasks were sealed and incubated in the dark at 20 ± 2 °C. Anaerobic conditions were verified through pH, O<sub>2</sub>, and redox potential measurements. These are given below in Table B.8.1.1.2.3-04 to Table B.8.1.1.2.3-12. HSE agrees that the measurements taken suitably demonstrate that anaerobic conditions have been initiated and maintained.

**Table B.8.1.1.2.3-04 Dissolved oxygen, redox potential, and pH measurements - Newhaven**

<b>DAT (study initiation)</b>	<b>0</b>			<b>28</b>			<b>61</b>		
<b>Days after anaerobic onset</b>	<b>-</b>			<b>0</b>			<b>33</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	NA	NA	NA	NA	NA	NA	7.2	7.01	7.11
pH (soil phase)	NA	NA	NA	NA	NA	NA	6.7	6.74	6.72
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	0.52	0.50	0.51
Redox potential H <sub>2</sub> O (mV)	NA	NA	NA	NA	NA	NA	242	205	224
Redox potential Soil (mV)	NA	NA	NA	NA	NA	NA	183	200	192

NA = Not analysed

**Table B.8.1.1.2.3-05 Dissolved oxygen, redox potential, and pH measurements - Newhaven (cont.)**

<b>DAT (study initiation)</b>	<b>90</b>			<b>123</b>			<b>153</b>		
<b>Days after anaerobic onset</b>	<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	7.12	7.60	7.36	7.83	7.79	7.81	7.82	7.83	7.83
pH (soil phase)	7.16	7.30	7.23	7.53	7.53	7.53	7.69	7.48	7.59
Dissolved Oxygen (ppm)	0.03	0.04	0.04	0.09	0.05	0.07	0.09	0.07	0.08
Redox potential H <sub>2</sub> O (mV)	54	-56	-1	-109	-171	-140	-142	-129	-136
Redox potential Soil (mV)	-56	-82	-69	-173	-195	-184	-177	-173	-175

**Table B.8.1.1.2.3-06 Dissolved oxygen, redox potential, and pH measurements - Empingham**

<b>DAT (study initiation)</b>	<b>0</b>			<b>28</b>			<b>61</b>		
<b>Days after anaerobic onset</b>	<b>-</b>			<b>0</b>			<b>33</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	NA	NA	NA	NA	NA	NA	8.02	8.09	8.06
pH (soil phase)	NA	NA	NA	NA	NA	NA	7.74	7.81	7.78
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	0.12	0.28	0.20
Redox potential H <sub>2</sub> O (mV)	NA	NA	NA	NA	NA	NA	120	100	110
Redox potential Soil (mV)	NA	NA	NA	NA	NA	NA	60	70	65

NA = Not analysed

**Table B.8.1.1.2.3-07 Dissolved oxygen, redox potential, and pH measurements - Empingham (cont.)**

<b>DAT (study initiation)</b>	<b>90</b>			<b>123</b>			<b>153</b>		
<b>Days after anaerobic onset</b>	<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	7.60	7.80	7.70	7.92	7.75	7.84	8.00	7.8	7.90
pH (soil phase)	7.57	7.77	7.67	7.68	7.78	7.73	7.89	7.7	7.80
Dissolved Oxygen (ppm)	0.11	0.10	0.11	0.18	0.05	0.12	0.08	0.09	0.09
Redox potential H <sub>2</sub> O (mV)	73	115	94	-116	-145	-130	-101	-101	-101
Redox potential Soil (mV)	8	77	43	-180	-198	-189	-152	-144	-148

**Table B.8.1.1.2.3-08 Dissolved oxygen, redox potential, and pH measurements - Atwater**

<b>DAT (study initiation)</b>	<b>0</b>			<b>28</b>			<b>61</b>		
<b>Days after anaerobic onset</b>	<b>-</b>			<b>0</b>			<b>33</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	NA	NA	NA	NA	NA	NA	8.78	8.78	8.78
pH (soil phase)	NA	NA	NA	NA	NA	NA	8.81	8.77	8.79
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	0.04	0.04	0.04
Redox potential H <sub>2</sub> O (mV)	NA	NA	NA	NA	NA	NA	34	-21	7
Redox potential Soil (mV)	NA	NA	NA	NA	NA	NA	-160	-220	-190

NA = Not analysed



**Table B.8.1.1.2.3-09 Dissolved oxygen, redox potential, and pH measurements - Atwater (cont.)**

<b>DAT (study initiation)</b>	<b>90</b>			<b>123</b>			<b>153</b>		
<b>Days after anaerobic onset</b>	<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	8.60	8.69	8.65	7.92	7.75	7.84	8.00	7.8	7.90
pH (soil phase)	8.75	8.74	8.75	7.68	7.78	7.73	7.89	7.7	7.80
Dissolved Oxygen (ppm)	0.09	0.08	0.09	0.18	0.05	0.12	0.08	0.09	0.09
Redox potential H <sub>2</sub> O (mV)	-139	-135	-137	-116	-145	-130	-101	-101	-101
Redox potential Soil (mV)	-201	-188	-195	-180	-198	-189	-152	-144	-148

NA = Not analysed

**Table B.8.1.1.2.3-10 Dissolved oxygen, redox potential, and pH measurements – Penn, phenyl label**

<b>DAT (study initiation)</b>	<b>0</b>			<b>28</b>			<b>42</b>			<b>57</b>		
<b>Days after anaerobic onset</b>	<b>-</b>			<b>0<sup>1</sup></b>			<b>14</b>			<b>29</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	NA	NA	NA	NA	NA	NA	7.08	7.01	7.05	7.02	7.40	7.21
pH (soil phase)	NA	NA	NA	NA	NA	NA	6.70	6.67	6.69	7.00	7.11	7.06
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	1.79	0.61	1.20	0.53	0.62	0.58
Redox potential H <sub>2</sub> O (mV)	NA	NA	NA	NA	NA	NA	198	210	204	62	125	94
Redox potential Soil (mV)	NA	NA	NA	NA	NA	NA	205	178	192	50	90	70

<sup>1</sup>The changeover from aerobic to anaerobic was made at 28DAT.

NA = Not analysed

**Table B.8.1.1.2.3-11 Dissolved oxygen, redox potential, and pH measurements – Penn, phenyl label (cont.)**

<b>DAT (study initiation)</b>	<b>85</b>			<b>122</b>			<b>155</b>		
<b>Days after anaerobic onset</b>	<b>57</b>			<b>94</b>			<b>127</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
pH (water phase)	7.83	7.66	7.75	8.67	8.60	8.64	8.60	7.58	8.09
pH (soil phase)	7.59	7.49	7.54	8.20	8.08	8.14	8.32	7.53	7.93
Dissolved Oxygen (ppm)	0.31	0.37	0.34	0.18	0.38	0.28	0.34	0.25	0.30
Redox potential H <sub>2</sub> O (mV)	188	134	161	-160	-56	-108	-178	64	-57
Redox potential Soil (mV)	162	21	91	-188	-134	-161	-195	-35	-115

NA = Not analysed

**Table B.8.1.1.2.3-12 Dissolved oxygen, redox potential, and pH measurements – Penn, pyrazolyl**

<b>DAT (study initiation)</b>	<b>0</b>			<b>28</b>			<b>42</b>			<b>57</b>			<b>85</b>			<b>122</b>			<b>155</b>		
<b>Days after anaerobi c onset</b>	<b>-</b>			<b>0</b>			<b>14</b>			<b>29</b>			<b>57</b>			<b>94</b>			<b>127</b>		
<b>Sample No.</b>	<b>Re p 1</b>	<b>Re p 2</b>	<b>Av g.</b>	<b>Re p 1</b>	<b>Re p 2</b>	<b>Av g.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg .</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg .</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg .</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg .</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg .</b>
<b>pH (water phase)</b>	NA	NA	NA	NA	NA	NA	7.00	6.97	6.97	7.05	7.51	7.28	7.16	7.31	7.24	8.02	8.46	8.24	8.35	8.33	8.34
<b>pH (soil phase)</b>	NA	NA	NA	NA	NA	NA	6.51	6.60	6.55	6.82	6.97	6.90	7.15	7.50	7.33	7.62	8.07	7.85	7.92	7.98	7.95
<b>Dissolved Oxygen (ppm)</b>	NA	NA	NA	NA	NA	NA	0.27	0.50	0.37	0.57	0.53	0.55	0.27	0.28	0.28	0.33	0.37	0.35	0.04	0.23	0.14
<b>Redox potential H<sub>2</sub>O (mV)</b>	NA	NA	NA	NA	NA	NA	223	220	222	100	150	125	179	147	163	40	-142	-51	-157	-100	-129
<b>Redox potential Soil (mV)</b>	NA	NA	NA	NA	NA	NA	192	200	196	131	121	126	128	95	111	-17	-153	-85	-205	-191	-198

NA = Not analysed

## Treatments

The treatment groups of KCA 7.1.2.1.3/01 are summarised below in Table B.8.1.1.2.3-13.

**Table B.8.1.1.2.3-13 Treatment groups of KCA 7.1.2.1.3/01**

<b>Treatment group</b>	<b>Dosing substance</b>	<b>Soil used</b>	<b>Dosing rate (ppm) in solvent (μL)</b>	<b>Sample type</b>	<b>Purpose</b>
<b>A</b>	NA	Penn	NA	Untreated control	Measure microbial biomass
<b>B</b>	Acetonitrile	Penn	250 μL acetonitrile solvent only	Organic solvent controls	Determine effect of organic solvent on microbial biomass
<b>C</b>	Non-radiolabelled inpyrfluxam	Penn	0.662 ppm in 250μL acetonitrile solvent	Non-radiolabelled inpyrfluxam	Determine effect of inpyrfluxam and organic solvent on microbial biomass
<b>D</b>	[phenyl <sup>14</sup> C]S 2399	Penn	0.696 ppm in 250μL acetonitrile solvent	radiolabelled inpyrfluxam	Determine the test substance dissipation rate during the aerobic and anaerobic phases, and its metabolite formation
<b>E</b>	[pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	Penn	0.676 ppm in 250μL acetonitrile solvent	radiolabelled inpyrfluxam	Determine the test substance dissipation rate during the aerobic and anaerobic phases, and its metabolite formation

The treatment groups of KCA 7.1.2.1.3/02 are summarised below in Table B.8.1.1.2.3-14.

**Table B.8.1.1.2.3-14 Treatment groups of KCA 7.1.2.1.3/02**

<b>Treatment group</b>	<b>Dosing substance</b>	<b>Soil used</b>	<b>Dosing rate (ppm) in solvent (μL)</b>	<b>Sample type</b>	<b>Purpose</b>
<b>A</b>	NA	Newhaven, Empingham, Atwater	NA	Untreated control	Measure microbial biomass
<b>B</b>	Acetonitrile	Newhaven, Empingham, Atwater	250 μL acetonitrile solvent only	Organic solvent controls	Determine effect of organic solvent on microbial biomass
<b>C</b>	Non-radiolabelled inpyrfluxam	Newhaven, Empingham, Atwater	0.662 ppm in 250μL acetonitrile solvent	Non-radiolabelled inpyrfluxam	Determine effect of inpyrfluxam and organic solvent on microbial biomass
<b>D</b>	[pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	Newhaven	0.665 ppm in 250μL acetonitrile solvent	radiolabelled inpyrfluxam	Determine the test substance dissipation rate during the aerobic and anaerobic phases, and its metabolite formation
<b>E</b>	[pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	Empingham	0.665 ppm in 250μL acetonitrile solvent	radiolabelled inpyrfluxam	Determine the test substance dissipation rate during the aerobic and anaerobic phases, and its metabolite formation
<b>F</b>	[pyrazolyl-4- <sup>14</sup> C] inpyrfluxam	Atwater	0.665 ppm in 250μL acetonitrile solvent	radiolabelled inpyrfluxam	Determine the test substance dissipation rate during the aerobic and anaerobic phases, and its

					metabolite formation
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### Test substance application

After aerobic acclimation, the test substances were added to the aerobic test systems at the dosing rates specified in Table B.8.1.1.2.3-13 and Table B.8.1.1.2.3-14. The test substances were delivered in 250 µL of acetonitrile and applied drop wise to the soil surface with a syringe and the soil mixed with a metal spatula for 5 minutes. For the Penn soil study, the control samples (A, B and C) were placed in a common incubation chamber. The treatment samples (D and E) were placed in separate incubation chambers. For the Newhaven, Empingham and Atwater soils, the control samples (A, B and C) were placed in a common incubation chamber. The treatment samples (D, E and F) were placed in separate incubation chambers.

### Sampling, sample processing and analysis

Each sample consisted of one glass beaker (aerobic soil, tetraglyme and NaOH traps) or one biometer flask (anaerobic soil/water mixture, NaOH trap, and headspace gases). Two samples were removed for analysis according to the following schedule: Treatment D, E and F samples were analysed at 0, 28, 61, 90, 123 and 153 days after dosing (aerobic phase – 0 to 28 DAT; anaerobic phase – 28 to 153 DAT). The control samples of Treatment A were sampled at 0, 130, and 154-155 DAT. Treatments B and C were sampled at 154-155 DAT.

Aerobic samples were measured for  $^{14}\text{CO}_2$  production and then extracted with organic solvents. Anaerobic samples were measured for  $^{14}\text{CH}_4$  production, dissolved oxygen concentration, redox potential, and pH in that order at the time of analysis. Samples were analysed immediately after removal from the incubator and were not stored prior to any of the physical-chemical property or chemical analyses.

### Aerobic test system - NaOH and $^{14}\text{C}$ -volatiles (tetraglyme) traps

NaOH and tetraglyme traps were removed for analysis at 28 DAT. The volume of each NaOH and tetraglyme trap were measured and three aliquots of each trap were removed and radioactivity counted by LSC and normalised to the number of samples in the incubation chamber.

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**Aerobic test system - extraction of radiolabelled compounds in soil using organic solvents**

At each sampling interval duplicate soil samples from both labels were extracted and analysed. The aerobic phase samples were extracted with a neutral organic solution to yield extract #1 (acetone once and acetone:water 3:2 (v/v) twice) and extracted with a weakly acidic organic solution to yield extract #2 (acetone:water:HCl 60:40:1 (v/v/v) once). Neutral extract: the soil sample was quantitatively transferred out of the beaker into a separate pre-weighed centrifuge bottle. The beaker was rinsed with 150 mL of acetone, which was added to the centrifuge bottle. The centrifuge bottle was shaken for 20 minutes (on a reciprocal shaker) and sonicated for 5 minutes. The phases were separated by centrifugation for 15 - 20 minutes at 7,000 RPM (~10,000 RCF). The supernatant was decanted into a graduated cylinder through a glass funnel loosely packed with glass wool. The extraction was repeated two more times with 150 mL of acetone:water 3:2 (v/v) and the three supernatants combined in the graduated cylinder (neutral extract). The volume of each extract was measured and aliquots were removed from each extract for LSC.

The neutral sample extract was rotary evaporated to dryness at 25 - 35 °C. The remaining residues were dissolved in acetonitrile/water (1/1, v/v) and transferred into a small vial. An ultrasonic bath was used to help dislodge and solubilise the residues. The final volumes were measured and an aliquot was removed for LSC. The extracts were analysed by HPLC-RAM and/or thin layer chromatography (TLC)-autoradiography.

**Anaerobic test system - NaOH traps**

The volume of each NaOH solution in the side-arm trap was measured and three aliquots removed for LSC. Radioactivity present in each trap was determined and evolved  $^{14}\text{CO}_2$  was calculated as a percentage of applied radioactivity and ppm inpyrfluxam equivalents.

**Anaerobic test system - separation of water and soil phases**

The water from each biometer was carefully decanted into a pre-weighed 250 mL centrifuge bottle (pore water stays with the soil). The bottle was capped and centrifuged at 7,000 RPM (~10,000 RCF) for 10 min. The water phase was carefully decanted into a pre-weighed glass bottle. The bottle was weighed to determine the water volume and aliquots taken for LSC. Water and soil phases were analysed separately.

**Anaerobic test system - water analysis**

The water phase contained sufficient amounts of activity to analyse directly by HPLC-RAM and/or TLC-autoradiography.



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**Anaerobic test system - soil analysis**

The soil remaining in the biometer was transferred (with solvent) into the pre-weighed 250 mL centrifuge bottle that the water phase was decanted out of. The remaining soil was extracted with a neutral organic solution to yield extract #1 (acetone once and 3:2 acetone:water (v/v) twice) and then extracted once with an acidic organic solution to yield extract #2 (60:40:1 acetone:water:HCl (v/v/v)).

**Neutral extract:**

The soil sample in the centrifuge bottle was extracted with 150 mL of acetone. The centrifuge bottle was shaken for 20 minutes (reciprocal shaker) and sonicated for 5 minutes. The phases were separated by centrifugation (15 minutes at 7,000 RPM (~10,000 RCF)). The supernatant was decanted into a graduated cylinder through a glass funnel loosely packed with glass wool. The extraction was repeated two more times with 3:2 acetone: water (v/v) and centrifuged for 15 - 25 minutes. The three supernatants combined in the graduated cylinder (neutral extract). The volume of the combined neutral extracts were measured and aliquots were removed from the extracts for LSC. The neutral extracts were rotary evaporated at 25 - 35 °C to dryness. The remaining residues were dissolved in acetonitrile/water (1/1 or 65/35, v/v) and transferred into a small vial. An ultrasonic bath was used to help dislodge and solubilise the residues. The final volumes were measured and an aliquot was removed for LSC. The extracts were analysed by HPLC-RAM and/or TLC-autoradiography.

**Acidic extract:**

The remaining soil pellet was re-suspended in 150 mL of 60:40:1 acetone:water:HCl (v/v/v) and the sample was shaken for 20 minutes (reciprocal shaker) and sonicated for 5 minutes. The phases were separated by centrifugation (15 to 25 minutes at 7,000 RPM (~10,000 RCF)). The supernatant was decanted into a graduated cylinder through a glass funnel loosely packed with glass wool (acidic extract). The volume of each extract was measured and aliquots were removed from each extract for LSC. The acidic extracts were transferred into round bottom flasks and organic portion removed using rotary evaporation under vacuum and at  $\leq 35$  °C. The remaining aqueous portion was partitioned with 75 mL ethyl acetate three times. The volume of the aqueous and organic phases were measured in graduated cylinders and aliquots taken for LSC. The ethyl acetate was rotary evaporated to dryness at  $\leq 35$  °C. The remaining residues were dissolved in acetonitrile or acetonitrile/water and transferred into a small vial. The concentrated extract volumes were measured and their radioactivity determined by LSC. The extracts were analysed by HPLC-RAM and/or TLC-autoradiography. The concentration recoveries for soil the anaerobic (61 - 153 DAT) acidic extracts were acceptable:  $96.8\% \pm 8.3\%$ .

The post extraction soil (PES) was weighed and sub-samples were combusted using a sample oxidizer and its content of radioactivity was determined.

### **Limits of detection and quantification**

Limits of detection (LOD) and quantification (LOQ) for LSC were determined as 10 dpm and 42 dpm respectively.

The applicant has confirmed that the values are the same as the LOD and LOQs reported in study 7.1.1.2/02 (Anaerobic Route of Degradation). The LOD for LSC is reported as << 1% AR, and for LOQ; 0.02 % AR for acidic extracts, 0.06 % AR for neutral extracts and 0.01 % AR for water extracts (pyrazolyl label); 0.01 % AR for acidic extracts, 0.03 % AR for neutral extracts and 0.01 % AR for water extracts (phenyl label).

For HPLC-RAM, the applicant stated that LOD varied by matrix and analyst judgement was used. However, what was detectable was generally clearly distinguishable from background, in this case << 1 % AR. For LOQ, these were reported as 0.45 % AR for acidic extracts, 0.37 % AR for neutral extracts and 0.74 % AR for water extracts in the phenyl label. In the pyrazolyl label, these were 0.20 % AR for acidic extracts, 0.40 % AR for neutral extracts and 0.60 % AR for water extracts.

No LOD and LOQ values were reported for HPLC-UV and TLC as the applicant stated these were used for qualitative confirmation of the compound identities only.

HSE accepts that the limits of detection and quantification are in line with OECD 307, along with the applicant's justification that no limits are required for HPLC-UV and TLC.

## **Results and discussion**

### **Mass Balances**

The amount of inpyrfluxam ranged between 89.3 and 64.0 % AR at anaerobic stage onset, and 86.8 to 60.6 % AR at study termination. The primary route of metabolism in the Newhaven and the Empingham systems was oxidation to 1'-COOH-S2840B with a maximum of 17% and 15% AR respectively (Atwater produced 3% AR). The predominant metabolism route in the Atwater soil was oxidation to form 3'-OH-S-2840 at 5% AR. For the Penn soil, The predominant metabolism route was oxidation to form 3'-OH-S-2840 to a maximum of 9.5% AR and 1'-COOH-S2840B to 6.8% AR. Degradation of inpyrfluxam was found to primarily occur during the aerobic onset period for all soils. Radioactivity distribution by fraction, and total radioactivity recovered for all studies are given in Annex A, and lie within the 90-110 % range specified by OECD 307. Summary tables of mass balances are given below in Table

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B.8.1.1.2.3-15 and B.8.1.1.2.3-16, and radioactivity distributions by compound are given in Table B.8.1.1.2.3-17 to B.8.1.1.2.3-21.

**Table B.8.1.1.2.3-15 Sample mass balances for Newhaven, Empingham, and Atwater soils**

Time after application (days)																		
	0			28			61			90			123			153		
Time after switch to anaerobic conditions (days)																		
	-			0			33			62			95			125		
Sample Rep.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.
Newhaven	101.8	100.9	101.4	100.5	102.9	101.7	102.8	103.1	102.9	101.6	102.1	101.8	101.1	100.6	100.9	104.4	103.8	104.1
Empingham	104.4	102.8	103.6	103.4	101.6	102.5	103.5	103.5	103.5	102.8	104.4	103.6	104.0	102.5	103.3	103.8	104.6	104.2
Atwater	103.8	102.7	103.2	103.8	102.5	103.2	103.7	102.5	103.1	103.2	102.6	102.9	102.2	103.4	102.8	103.9	104.3	104.1

**Table B.8.1.1.2.3-16 Sample mass balances for Penn soil samples**

Time after application (days)												
	0			28			42			57		
Time after switch to anaerobic conditions (days)												
	-			0			14			14		
Sample Rep.	1	2	Avg.	1	2	Avg.	1	2	Avg.	3	4	Avg.
Penn, phenyl	100.2	101.5	100.8	100.6	104.3	102.5	100.2	102.7	101.4	99.3	100.8	100.1
Penn, pyrazolyl	98.1	101.3	99.7	99.0	99.8	99.4	102.7	101.5	102.1	99.3	103.1	101.2
Time after application (days)												
	85			122			155					
Time after switch to anaerobic conditions (days)												
	57			94			122					
Sample Rep.	1	2	Avg.	1	2	Avg.	1	2	Avg.			
Penn, phenyl	93.0	92.0	92.5	101.6	100.2	100.9	98.6	100.4	99.5			
Penn, pyrazolyl	99.0	99.3	99.2	98.5	100.6	99.6	99.9	97.3	98.6			

**Newhaven****Table B.8.1.1.2.3-17 Radioactivity distribution of the Newhaven soil (Treatment D) anaerobic soil study (neutral and acidic soil and water extracts combined) as percent of applied radioactivity.**

<b>Time after application (days)</b>	<b>0</b>			<b>28</b>			<b>61</b>			<b>90</b>			<b>123</b>			<b>153</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>-</b>			<b>0</b>			<b>33</b>			<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>1</b>	<b>2</b>	<b>Avg.</b>	<b>7</b>	<b>12</b>	<b>Avg.</b>	<b>15</b>	<b>18</b>	<b>Avg.</b>	<b>6</b>	<b>10</b>	<b>Avg.</b>	<b>5</b>	<b>8</b>	<b>Avg.</b>	<b>3</b>	<b>9</b>	<b>Avg.</b>
N-des-Me-DFPA	0	0	0	1.9	1.7	1.8	2.2	2.1	2.1	1.6	1.8	1.7	0.9	1.7	1.3	1.8	1.5	1.6
DFPA-CONH <sub>2</sub>	0.4	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFPA	0	0	0	0	0	0	0	0	0	0.2	0.4	0.3	1.2	0.9	1.0	0.9	1.4	1.1
1'-CH <sub>2</sub> OH-S-2840A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840A	0	0	0	4.1	4.6	4.3	4.9	4.7	4.8	5.1	5.4	5.2	6.0	5.7	5.9	5.6	6.3	6.0
1'-CH <sub>2</sub> OH-S-2840B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840B	0	0	0	13.0	14.9	14.0	16.8	17.8	17.3	17.0	17.0	17.0	16.0	16.3	16.1	17.9	16.8	17.4
3'-OH-S-2840 <sup>1</sup>	4.6	4.4	4.5	8.8	9.4	9.1	8.1	7.5	7.8	8.6	8.7	8.7	8.2	8.7	8.4	9.2	9.1	9.1
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inpyrfluxam	95.5	94.9	95.2	64.7	63.3	64.0	63.9	63.1	63.5	61	61.1	61.0	59.1	59.8	59.4	60.5	60.6	60.6
Unknown A	0.5	0.4	0.5	0	0	0	0.6	0.7	0.7	0.8	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.7

Other unknowns	0	0	0	1.0	1.6	1.3	0.6	1.6	1.1	2.1	1.6	1.8	3.1	1.3	2.2	1.0	1.5	1.3
<b>Total</b>	101.1	100.2	100.6	91.6	93.8	92.7	95.0	95.4	95.2	94.6	94.9	94.8	94.1	93.4	93.8	95.9	96.3	96.1

<sup>13</sup>'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840 dehydrate, which co-elutes with N-des-Me-S-2840 on HPLC and is identified on TLC. Total 3'-OH-S-2840 includes both forms in the acidic extract

**Empingham****Table B.8.1.1.2.3-18 Radioactivity distribution of the Empingham wood soil (Treatment E) anaerobic soil study (neutral and acidic soil and water extracts combined) as percent of applied radioactivity.**

<b>Time after application (days)</b>	<b>0</b>			<b>28</b>			<b>61</b>			<b>90</b>			<b>123</b>			<b>153</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>-</b>			<b>0<sup>1</sup></b>			<b>33</b>			<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>21</b>	<b>22</b>	<b>Avg.</b>	<b>27</b>	<b>35</b>	<b>Avg.</b>	<b>26</b>	<b>29</b>	<b>Avg.</b>	<b>31</b>	<b>33</b>	<b>Avg.</b>	<b>25</b>	<b>36</b>	<b>Avg.</b>	<b>28</b>	<b>32</b>	<b>Avg.</b>
N-des-Me-DFPA	0	0	0	1.3	1.4	1.3	0.6	1.5	1	0.5	1.1	0.8	1.2	1.0	1.1	0.9	1.1	1
DFPA-CONH <sub>2</sub>	0.7	0.6	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFPA	0	0	0	0.9	1.0	1.0	1.9	2.0	1.9	1.4	2.4	1.9	1.9	2.6	2.2	2.6	2.2	2.4
1'-CH <sub>2</sub> OH-S-2840A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840A	0	0	0	4.1	4.0	4.1	4.7	4.0	4.3	4.7	5.0	4.8	4.5	4.9	4.7	4.6	4.6	4.6
1'-CH <sub>2</sub> OH-S-2840B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840B	0	0	0	15.1	14.4	14.7	15.6	14.9	15.2	16.0	16.6	16.3	15.4	15	15.2	15.6	14.3	14.9
3'-OH-S-2840 <sup>1</sup>	4.5	4.5	4.5	6.1	6.7	6.4	6.1	5.9	6.0	5.9	5.6	5.7	5.6	5.5	5.6	6.0	5.3	5.7



N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inpyrfluxam	98.1	96.5	97.3	69.7	67.7	68.7	67.9	69.2	68.5	66.8	67.0	66.9	66.6	64.4	65.5	65.2	67.4	66.3
Unknown A	0.2	0.5	0.4	0	0	0	0.5	0.6	0.5	0.7	0.6	0.6	0.4	0.6	0.5	0.5	0.6	0.6
Other unknowns	0	0	0	1.0	1.0	1.0	1.4	0.8	1.1	1.0	0.6	0.8	1.1	1.3	1.2	0.8	0.8	0.8
<b>Total</b>	<b>103.6</b>	<b>102.1</b>	<b>102.9</b>	<b>98.1</b>	<b>96.1</b>	<b>97.1</b>	<b>98.7</b>	<b>98.8</b>	<b>98.7</b>	<b>97.0</b>	<b>98.9</b>	<b>97.9</b>	<b>96.6</b>	<b>95.2</b>	<b>95.9</b>	<b>96.2</b>	<b>96.4</b>	<b>96.3</b>

<sup>13</sup>C-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840 dehydrate, which co-elutes with N-des-Me-S-2840 on HPLC and is identified on TLC. Total 3'-OH-S-2840 includes both forms in the acidic extract

**Atwater****Table B.8.1.1.2.3-19 Radioactivity distribution of the Atwater soil (Treatment F) anaerobic soil study (neutral and acidic soil and water extracts combined) as percent of applied radioactivity.**

<b>Time after application (days)</b>	<b>0</b>			<b>28</b>			<b>61</b>			<b>90</b>			<b>123</b>			<b>153</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>-</b>			<b>0<sup>2</sup></b>			<b>33</b>			<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>41</b>	<b>42</b>	<b>Avg.</b>	<b>47</b>	<b>56</b>	<b>Avg.</b>	<b>46</b>	<b>52</b>	<b>Avg.</b>	<b>43</b>	<b>50</b>	<b>Avg.</b>	<b>45</b>	<b>54</b>	<b>Avg.</b>	<b>51</b>	<b>53</b>	<b>Avg.</b>
N-des-Me-DFPA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFPA-CONH <sub>2</sub>	0.3	0.6	0.4	0.6	0.6	0.6	0.8	1.3	1	0	0	0	0.7	0.5	0.6	0.9	0.5	0.7
DFPA	0	0	0	1.8	2.1	1.9	3	2.2	2.6	2.7	3	2.9	3.3	2.8	3	3	2.8	2.9
1'-CH <sub>2</sub> OH-S-2840A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840A	0	0	0	2.9	2.8	2.8	3.4	3.9	3.6	3.7	3.4	3.6	4.4	3.7	4.1	3.5	3.6	3.5
1'-CH <sub>2</sub> OH-S-2840B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840B	0	0	0	1.7	1.9	1.8	1.6	2.3	1.9	2.4	2.5	2.5	2.7	2.4	2.6	2.6	2.4	2.5
3'-OH-S-2840 <sup>1</sup>	4.6	4.1	4.3	5.5	5.1	5.3	5.7	5.7	5.7	5.3	5.7	5.5	4.9	5.6	5.2	5.2	5.6	5.4

N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Inpyrfluxam	98.0	97.3	97.7	89.9	88.6	89.3	87.1	85.3	86.2	87.6	86.5	87.1	84.0	86.1	85.1	86.4	87.1	86.8
Unknown A	0.7	0.6	0.6	0	0	0	0.8	0.4	0.6	0	0	0	0.5	0.7	0.6	0.6	0.7	0.6
Other unknowns	0.7	0.6	0.6	1.0	1.0	1.0	0.8	0.4	0.6	0.5	0.5	0.5	0.5	0.7	0.6	0.6	0.7	0.6
Total	104.3	103.1	103.7	103.3	102.0	102.0	103.1	101.5	102.3	102.3	101.8	102.0	101.0	102.5	101.8	102.9	103.5	103.2

<sup>13</sup>C-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840 dehydrate, which co-elutes with N-des-Me-S-2840 on HPLC and is identified on TLC. Total

3'-OH-S-2840 includes both forms in the acidic extract

**Penn (PHE)****Table B.8.1.1.2.3-20 Radioactivity distribution of the Penn soil phenyl label (Treatment D) anaerobic soil study (neutral and acidic soil and water extracts combined) as percent of applied radioactivity**

Fraction	Days After Treatment (DAT)												Days After Treatment (DAT)								
	0			28			42			57			85			122			155		
	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
ATMI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-CH <sub>2</sub> OH-S-2840 (total) <sup>1</sup>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840 (total) <sup>2</sup>	0	0	0	10.6	12.0	11.2	10.3	10.1	10.2	10.0	10.6	10.3	9.9	9.4	9.7	8.9	9.3	9.1	8.0	8.1	8.1
3'-OH-S-2840 <sup>3</sup>	2.6	3.9	3.2	9.1	9.8	9.4	9.0	8.3	8.6	8.1	8.5	8.3	7.5	6.8	7.1	6.8	7.4	7.1	8.0	6.9	7.4
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inpyrfluxam	96.9	96.9	96.9	74.7	76.6	75.6	75.0	78.4	76.7	73.9	74.4	74.1	68.2	68.8	68.5	76.8	75.1	75.9	74.6	78.7	76.7
Unknown A	0	0	0	0	0	0	0.2	0.3	0.3	0.4	0.4	0.4	0.5	0.3	0.4	0.3	0.5	0.4	0.4	0.2	0.3
Other unknowns	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Total</b>	<b>99.5</b>	<b>100.8</b>	<b>100.1</b>	<b>94.3</b>	<b>98.4</b>	<b>96.4</b>	<b>94.5</b>	<b>97.0</b>	<b>95.8</b>	<b>92.4</b>	<b>94.0</b>	<b>93.2</b>	<b>86.0</b>	<b>85.4</b>	<b>85.7</b>	<b>92.8</b>	<b>92.3</b>	<b>92.5</b>	<b>91.0</b>	<b>93.9</b>	<b>92.4</b>

<sup>1</sup> As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

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<sup>2</sup> As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

<sup>3</sup> 3'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840-dehydrate, which coelutes with N-des-Me-S-2840 on HPLC and is identified on TLC. Total 3'-OH-S-2840 includes both forms in the acidic extract.

**Penn (PYR)****Table B.8.1.1.2.3-21 Radioactivity distribution of the Penn soil pyrazolyl label (Treatment E) anaerobic soil study (neutral soil and acidic soil and water extracts combined) as percent of applied radioactivity**

Fraction	Days After Treatment (DAT)												Days After Treatment (DAT)								
	0			28			42			57			85			122			155		
	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
N-des-Me-DFPA	0	0	0	2.1	2.4	2.3	3	2.5	2.7	2.7	2.9	2.8	1.7	2	1.8	2.6	2.2	2.4	2.7	1.1	1.9
DFPA-CONH <sub>2</sub>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFPA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.3	1	1.1
1'-CH <sub>2</sub> OH-S-2840 (total) <sup>1</sup>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1'-COOH-S-2840 (total) <sup>2</sup>	0	0	0	5.7	6.3	6	6.9	7.9	7.4	7.6	8.7	8.1	6.8	5.1	5.9	7.6	7.5	7.6	7.4	7	7.3
3'-OH-S-2840 <sup>3</sup>	4.8	4.4	4.6	9.2	9.6	9.4	9.6	9.5	9.5	8.6	9.8	9.2	7.5	0.6	4.1	7.8	7.8	7.8	6.9	7.3	7.1
N-des-Me-S-2840	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inpyrfluxam	92.7	95.7	94.2	67.9	67.6	67.8	74.4	74.3	74.4	72.9	72.3	72.6	75	74	74.5	72.1	72.9	72.5	70.8	69.1	70
Unknown A	0	0.6	0.3	1.1	0.8	0.9	0.9	1.3	1.1	1.2	0.9	1.1	0.5	0	0.3	0.9	1.4	1.2	0.8	1.1	1
Other unknowns	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0.4

<b>Total</b>	<b>97.5</b>	<b>100.7</b>	<b>99.1</b>	<b>86.1</b>	<b>86.7</b>	<b>86.4</b>	<b>95.2</b>	<b>95.5</b>	<b>95.3</b>	<b>92.9</b>	<b>94.5</b>	<b>93.7</b>	<b>91.5</b>	<b>81.7</b>	<b>86.6</b>	<b>91.1</b>	<b>91.8</b>	<b>91.4</b>	<b>89.9</b>	<b>87.3</b>	<b>88.6</b>
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<sup>1</sup>As the sum of both isomers = 1'-CH<sub>2</sub>OH-S-2840 A + 1'-CH<sub>2</sub>OH-S-2840 B

<sup>2</sup>As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

<sup>3</sup>3'-OH-S-2840 can transform in the acidic extract to 3'-OH-S-2840-dehydrate, which coelutes with N-des-Me-S-2840 on HPLC and is identified on TLC. Total 3'-OH-S-2840 includes both forms in the acidic extract.

## Quantification of released CO<sub>2</sub> and other volatile compounds

The maximum percent of volatiles released from any sample was 0.5 %. The percent of applied radioactivity in isolated fractions is given in full in Annex B.

## HSE Kinetic Analysis

HSE notes that the applicant provided a kinetic analysis. HSE does not consider the kinetic analysis provided by the applicant as acceptable. The applicant has not modelled the raw data, and has instead performed a natural-log transformation followed by an  $r^2$  test.

For the aerobic phase, where samples are provided at two time points, near-perfect  $r^2$  values are derived. This is not indicative of a good model, but of a lack of data. This is because two data points will always sit on the straight line which  $r^2$  measures goodness-of-fit against. The applicant is not required to model the aerobic phase data as this is an anaerobic study, and this assessment should be removed.

The applicant has also only assumed that the inpyrfluxam degradation follows SFO kinetics, and not demonstrated it to be so by modelling. This is prerequisite to applying the ln-transformation required for an  $r^2$  fit.

The applicant has not provided a visual assessment, chi-squared error test, or t-test for the untransformed anaerobic data. Plots of the data and models will be required alongside the stated metrics in line with FOCUS guidelines. HSE has therefore conducted an independent kinetic analysis using the untransformed data.

HSE performed a kinetic analysis of the anaerobic phase data provided by the applicant using modelling software CAKE 3.7. The initiation of anaerobic conditions was taken as time zero. Pyrazolyl and Phenyl labels of inpyrfluxam in the Penn was soil were considered as true replicates and fitted together. The results are summarised in Table B.8.1.1.2.3-22, and plots of the fitted data and the residuals are given in Figure B.8.1.1.2.3-01 to Figure B.8.1.1.2.3-08.

**Table B.8.1.1.2.3-22 HSE inpyrfluxam best-fit endpoints kinetic analysis summary**

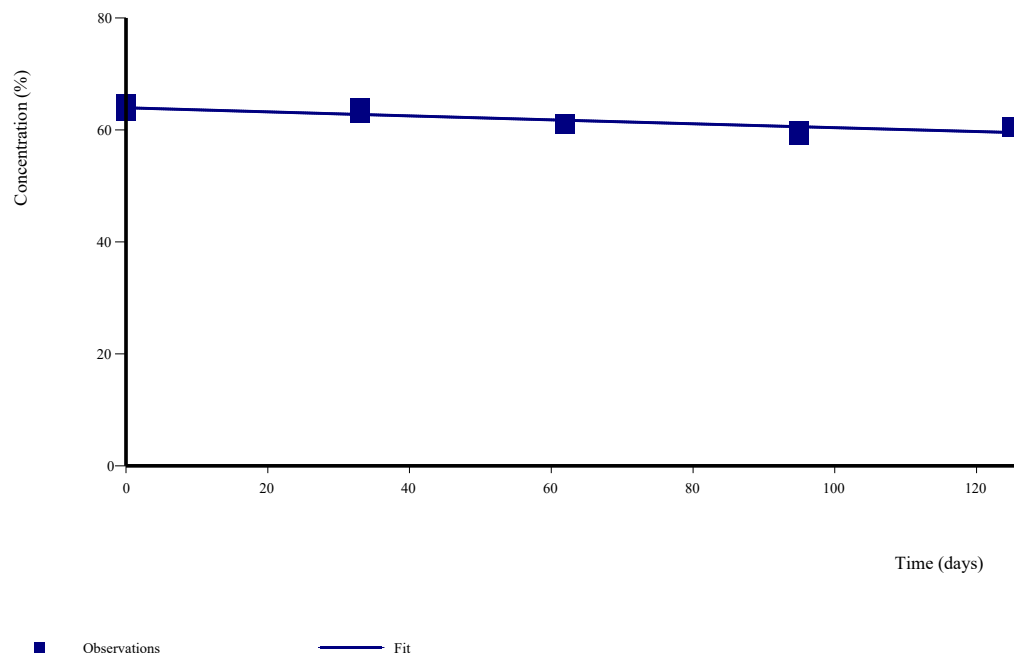
Soil	Best fit Kinetic Model	Visual assessment	$\chi^2$ error (%)	Estimated parameters	Prob > t	DT <sub>50</sub>	DT <sub>90</sub>
Newhaven	SFO	Good	1.05	M <sub>0</sub> : 64.0	< 0.05	1210	4030



				k: 0.00057			
Empingham	SFO	Very good	0.69	M <sub>0</sub> : 68.8 k: 0.00038	< 0.05	1850	6140
Atwater	SFO	Very good	0.97	M <sub>0</sub> : 68.8 k: 0.00023	< 0.05	2970	9880
Penn	SFO	Good	1.51	M <sub>0</sub> : 73.7 k: 5.11E-014	0.5	>10,000	>10,000

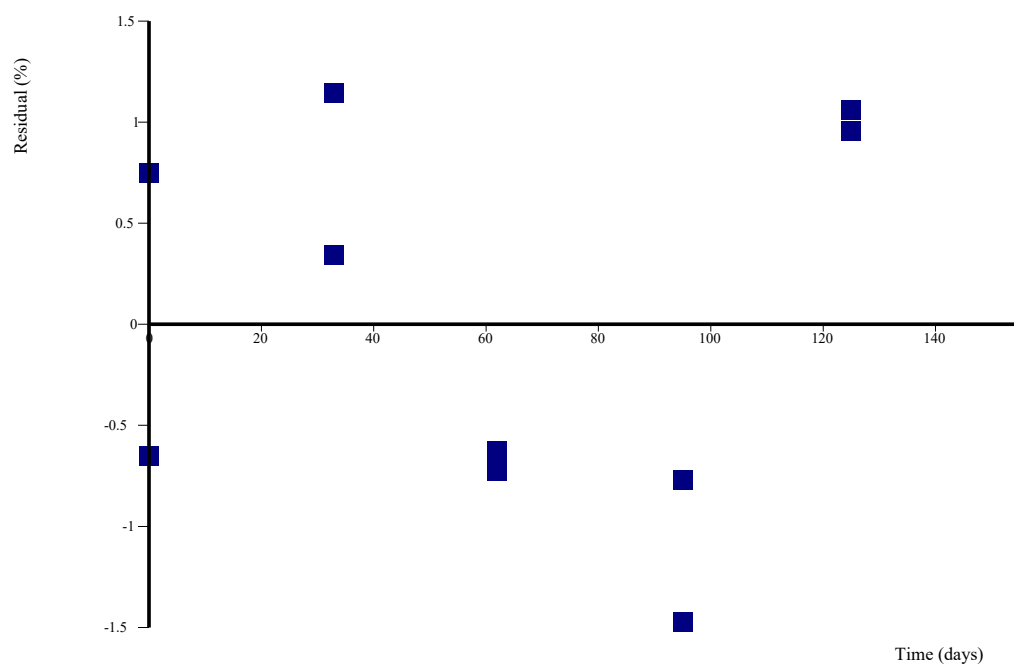
**Table B.8.1.1.2.3-23 HSE kinetic fit statistics for inpyrfluxam in the Newhaven soil**

<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	Good	1.05	M <sub>0</sub> : 64.0 k: 0.00057	k: <0.05	1210	4030
<p><b>SFO:</b> Good visual fit, residuals appear randomly distributed, <math>\chi^2</math> error level 1.05% and t-test passed</p> <p><b>Conclusion: select SFO as best-fit for trigger endpoints (DT<sub>50</sub> = &gt; 1,000 days, DT<sub>90</sub> = &gt; 1,000 days)</b></p>						



**Figure B.8.1.1.2.3-01 HSE kinetic fit of the Newhaven soil**

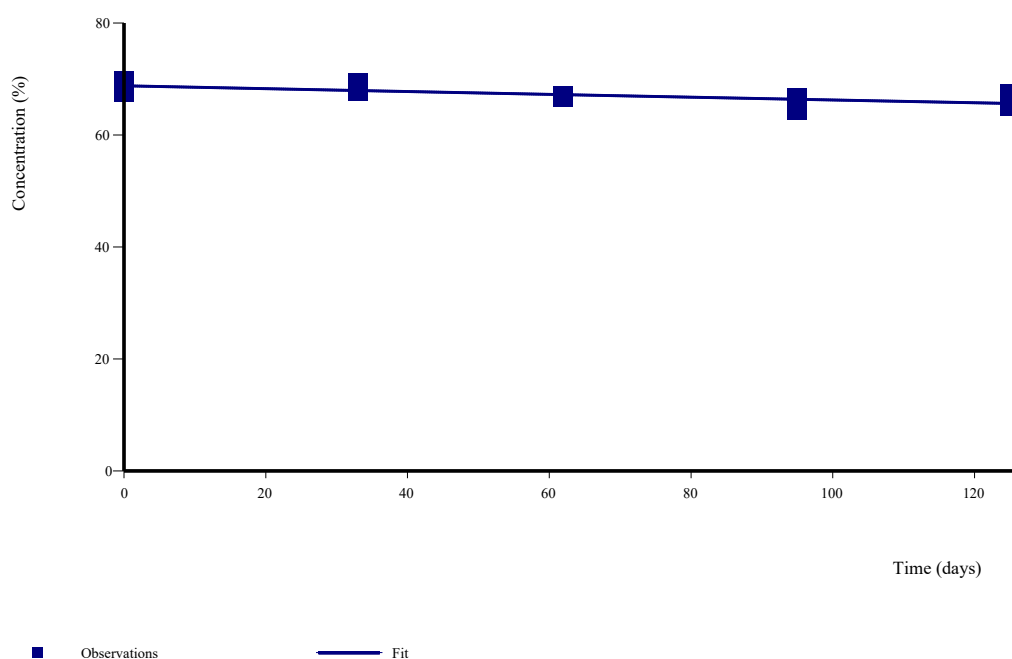
**Residuals:**

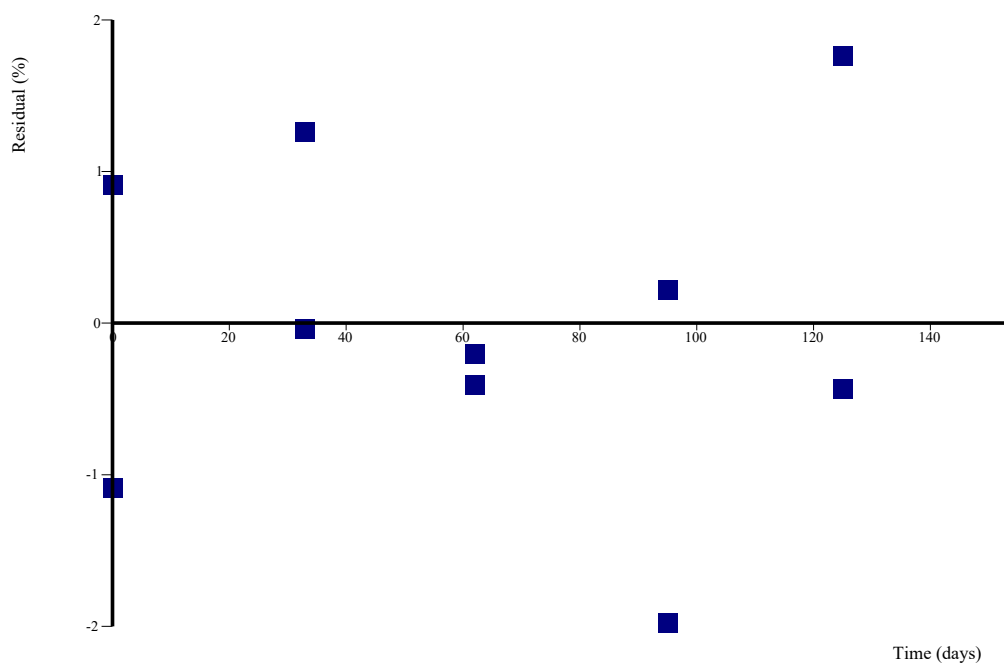


**Figure B.8.1.1.2.3-02 Residuals of the HSE kinetic fit of the Newhaven soil**

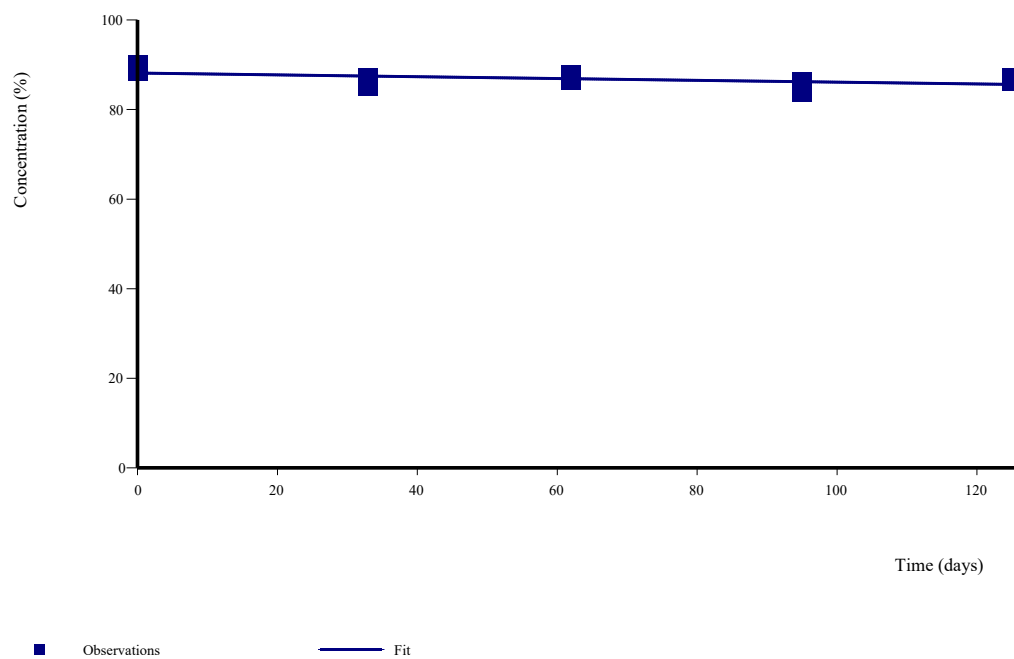
**Table B.8.1.1.2.3-24 HSE kinetic fit statistics for S 2399 in the Empingham soil**

Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	Very good	0.69	M <sub>0</sub> : 68.8 k: 0.00038	k: < 0.05	1850	6140
SFO: Very good visual fit, residuals appear randomly distributed, $\chi^2$ error level 0.69 % and t-test passed.						
<b>Conclusion: select SFO as best-fit for trigger endpoints (DT<sub>50</sub> = &gt; 1,000 days, DT<sub>90</sub> = &gt; 1,000 days)</b>						

**Figure B.8.1.1.2.3-03 HSE kinetic fit of the Empingham soil**

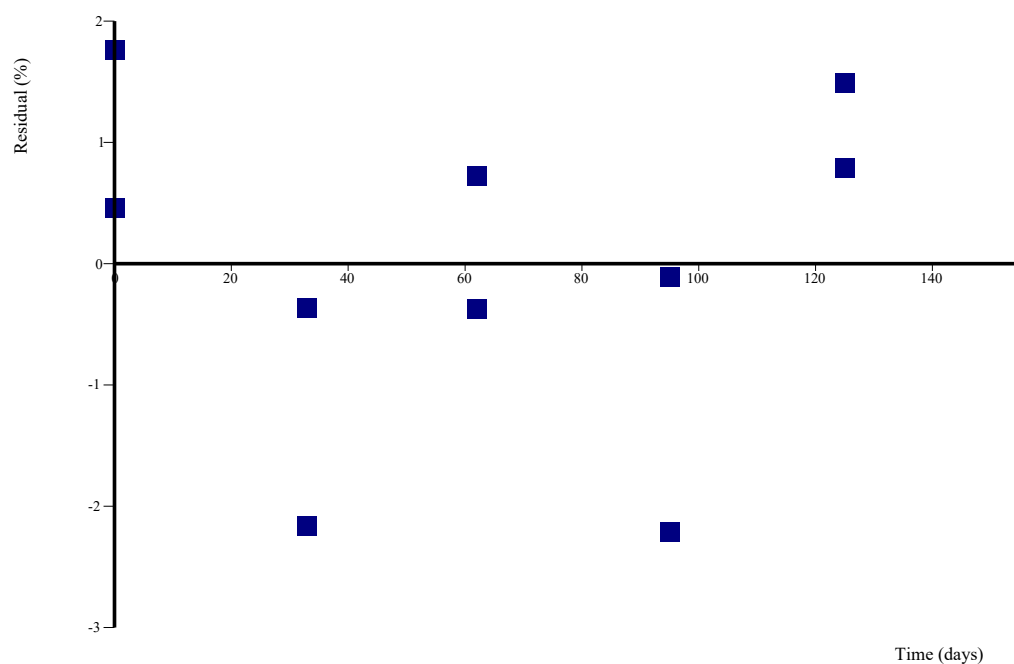
**Residuals:****Figure B.8.1.1.2.3-04 Residuals of the HSE kinetic fit of the Empingham soil****Table B.8.1.1.2.3-25 HSE kinetic fit statistics for inpyrfluxam in the Atwater soil**

Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	Very good	0.97	M <sub>0</sub> : 68.8 k: 0.00023	k: < 0.05	2970	9880
SFO: Very good visual fit, residuals appear randomly distributed, $\chi^2$ error level of 0.97 %, and t-test passed.						
<b>Conclusion: select SFO as best-fit for trigger endpoints (DT<sub>50</sub> = &gt; 1,000 days, DT<sub>90</sub> = &gt; 1,000 days)</b>						



**Figure B.8.1.1.2.3-05 HSE kinetic fit of the Atwater soil**

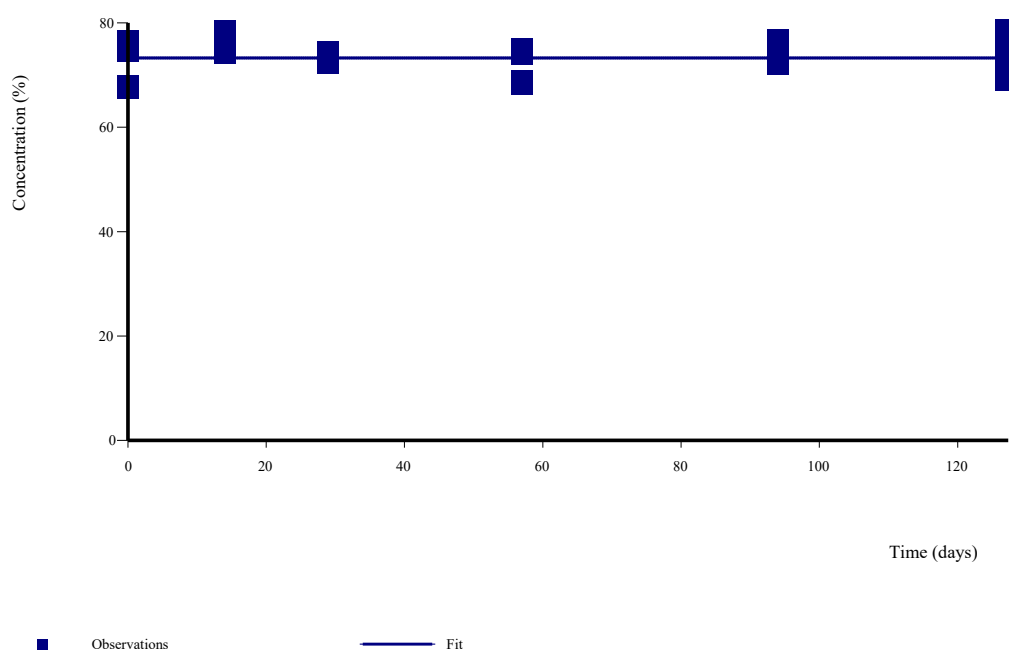
**Residuals:**



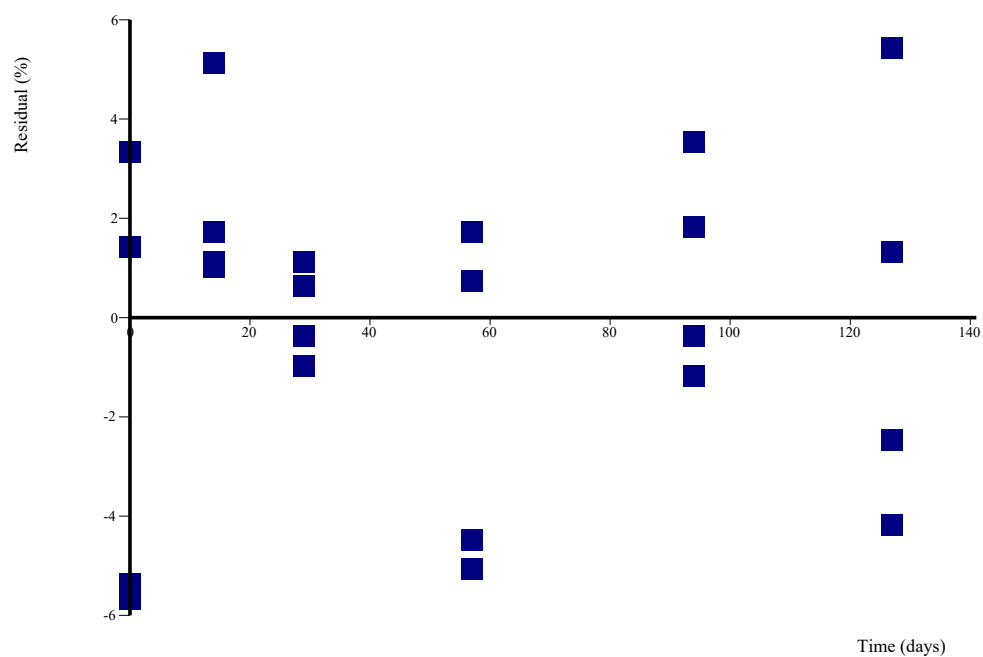
**Figure B.8.1.1.2.3-06 *Residuals of the HSE kinetic fit of the Atwater soil***

**Table B.8.1.1.2.3-26 HSE kinetic statistics for inpyrfluxam in the Penn soil**

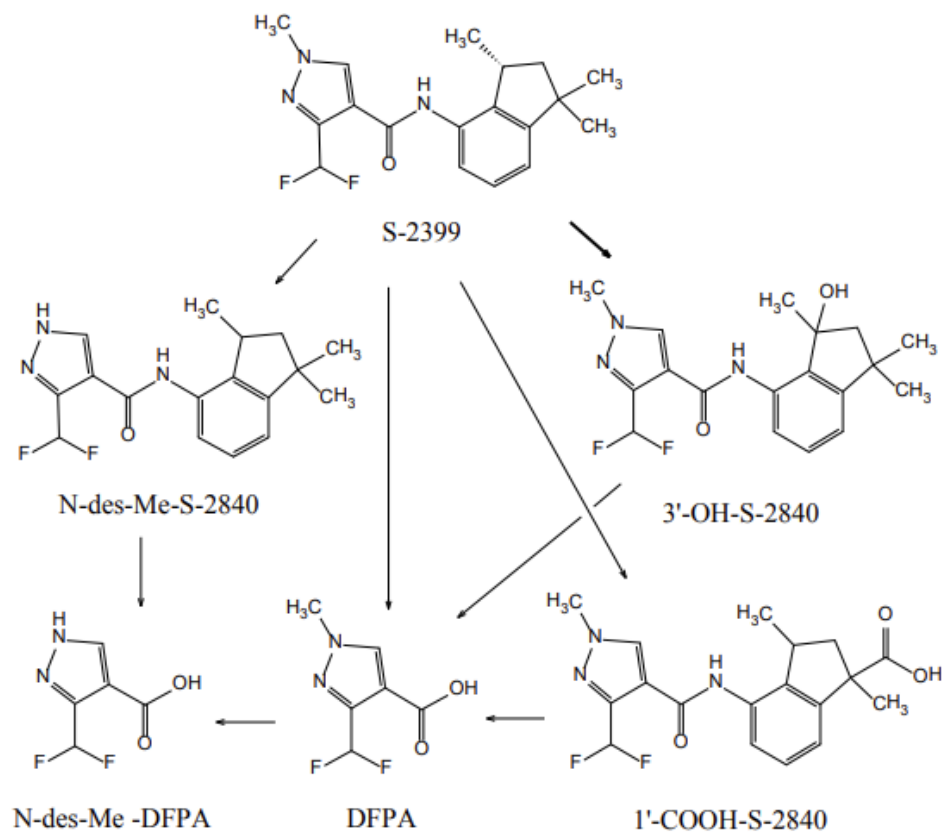
Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
SFO	Good	1.51	M <sub>0</sub> : 73.7 k: 5.11E-014	k: 0.5	> 10,000	> 10,000
<p>SFO: Good visual fit, residuals appear randomly distributed, <math>\chi^2</math> error level of 1.51 %, however t-test is failed. Degradation rate is not statistically distinguishable from zero. This is however expected as no degradation appears to take place.</p> <p><b>Conclusion: select SFO as best-fit for trigger endpoints (DT<sub>50</sub> = &gt; 1,000 days, DT<sub>90</sub> = &gt; 1,000 days)</b></p>						

**Figure B.8.1.1.2.3-07 HSE kinetic fit of the Penn soil**

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**Residuals:**

**Figure B.8.1.1.2.3-08 Residuals of the HSE kinetic fit of the Penn soil**



**Figure B.8.1.1.2.3-09** Proposed anaerobic soil degradation pathway of inpyrfluxam (metabolites were primarily formed during the aerobic phase).



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## Conclusions

HSE agrees with the applicant's assessment that the primary route of metabolism in the Newhaven and the Empingham systems was oxidation to 1'-COOH-S2840B to 17% and 15% AR (Atwater produced 3% of the AR). The predominant metabolism route in the Atwater and Penn soils was oxidation to form 3'-OH-S-2840 <10% AR. The Newhaven and the Empingham soils produced 3'-OH-S-2840 as 9% and 6% of the AR, respectively. Minor amounts of 1'-COOH-S-2840A, N-des-Me-DFPA, DFPA and DFPA-CONH<sub>2</sub> were observed (<6 % AR). HSE also agrees with the applicant that degradation products are primarily formed during the aerobic phase.

HSE does not consider the applicants use of  $r^2$  kinetics as sufficient or applicable for kinetic analysis by FOCUS guidance. HSE has therefore conducted a kinetic analysis in line with FOCUS guidance, and derived endpoints from this modelling which showed very slow or negligible anaerobic degradation of inpyrfluxam as DT<sub>50</sub> values for all soils were >1000 days.

## Annex A

Table B.8.1.1.2.3-27 Percent of applied radioactivity in isolated fractions (Newhaven)

Percent of applied radioactivity in isolated fractions (Newhaven) <sup>1</sup>																		
Time after application (days)	0			28			61			90			123			153		
Time after switch to anaerobic conditions (days)	-			0 <sup>2</sup>			33			62			95			125		
Sample No.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.
Water Phase	NA	NA	NA	NA	NA	NA	11.8	12.1	11.9	12.7	14.6	13.6	13.6	13.2	13.4	14.0	14.4	14.2
<b>Soil extracts</b>																		
1st soil ext. (neutral)	101.1	100	101	88.4	90.2	89.3	81.0	80.9	81.0	78.4	77.4	77.9	75.9	76.9	76.4	78.4	77.8	78.1
2nd soil ext. (acidic)	0.5	0.5	0.5	5.5	5.7	5.6	4.7	4.8	4.8	5.0	4.8	4.9	5.8	5.1	5.4	5.6	5.6	5.6
Total soil ext.	101.5	101	101	93.9	95.9	94.9	85.7	85.8	85.8	83.4	82.2	82.8	81.8	82.0	81.9	84.1	83.5	83.8
Soil-bound (PES)	0.3	0.2	0.3	6.4	6.8	6.6	5.0	5.0	5.0	5.2	4.9	5.1	5.4	5.1	5.2	5.8	5.5	5.6
<b>Volatiles</b>																		
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.4	0.4
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0
Biometer rinse	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0

Sample balance	101.8	100.9	101.4	100.5	102.9	101.7	102.8	103.1	102.9	101.6	102.1	101.8	101.1	100.6	100.9	104.4	103.8	104.1
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<sup>1</sup>Duplicate samples were analysed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following

fractions: First soil ext. + second soil ext. + Soil-bound (PES) + Water phase + <sup>14</sup>CO<sub>2</sub> + <sup>14</sup>CH<sub>4</sub>

<sup>2</sup>The changeover from aerobic to anaerobic was made at 28DAT.

NA = Not analysed

Table B.8.1.1.2.3-28 Percent of applied radioactivity in isolated fractions (Empingham)

Percent of applied radioactivity in isolated fractions (Empingham) <sup>1</sup>																		
Time after application (days)	0			28			61			90			123			153		
Time after switch to anaerobic conditions (days)	-			0 <sup>2</sup>			33			62			95			125		
Sample No.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.	Rep 1	Rep 2	Avg.
Water Phase	NA	NA	NA	NA	NA	NA	12.3	12.7	12.5	12.5	12.9	12.7	11.8	12.2	12.0	11.5	11.0	11.3
<b>Soil extracts</b>																		
1st soil ext. (neutral)	103.6	102.1	102.9	95.4	93.2	94.3	84.6	83.9	84.2	81.9	83.4	82.6	82.9	80.9	81.9	82.6	83.4	83.0
2nd soil ext. (acidic)	0.4	0.4	0.4	2.9	3.1	3.0	2.0	2.2	2.1	2.6	3.0	2.8	2.0	2.2	2.1	2.1	2.1	2.1
Total soil ext.	104	102.5	103.3	98.3	96.3	97.3	86.5	86.2	86.3	84.6	86.3	85.5	84.9	83.2	84	84.7	85.5	85.1
Soil-bound (PES)	0.4	0.3	0.3	5.1	5.2	5.2	4.7	4.6	4.6	5.6	5.1	5.4	7.2	7.0	7.1	7.5	8.0	7.7
<b>Volatiles</b>																		
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0
Biometer/	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0

beaker rinse																		
Sample balance	104.4	102.8	103.6	103.4	101.6	102.5	103.5	103.5	103.5	102.8	104.4	103.6	104.0	102.5	103.3	103.8	104.6	104.2

<sup>1</sup>Duplicate samples were analysed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following

fractions: First soil ext. + second soil ext. + Soil-bound (PES) + Water phase + <sup>14</sup>CO<sub>2</sub> + <sup>14</sup>CH<sub>4</sub>

<sup>2</sup>The changeover from aerobic to anaerobic was made at 28DAT.

NA = Not analysed

**Table B.8.1.1.2.3-29 Percent of applied radioactivity in isolated fractions (Atwater)**

<b>Percent of applied radioactivity in isolated fractions (Atwater)<sup>1</sup></b>																		
<b>Time after application (days)</b>	<b>0</b>			<b>28</b>			<b>61</b>			<b>90</b>			<b>123</b>			<b>153</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>-</b>			<b>0<sup>2</sup></b>			<b>33</b>			<b>62</b>			<b>95</b>			<b>125</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
Water Phase	NA	NA	NA	NA	NA	NA	26.6	26.3	26.4	26.8	26.8	26.8	26.6	27	26.8	27.6	27.6	27.6
<b>Soil extracts</b>																		
1st soil ext. (neutral)	103.6	102.5	103.1	100.9	99.6	100.2	74.6	73.7	74.2	73.6	73.1	73.3	72.7	73.6	73.1	73.4	73.8	73.6
2nd soil ext. (acidic)	0.1	0.1	0.1	1.7	1.7	1.7	1.3	1.2	1.3	1.6	1.5	1.5	1.3	1.4	1.4	1.4	1.5	1.5
Total soil ext.	103.7	102.6	103.2	102.5	101.3	101.9	75.9	75.0	75.4	75.1	74.6	74.9	74.1	75	74.5	74.9	75.4	75.1
Soil-bound (PES)	0.1	0	0.1	1.2	1.2	1.2	1.2	1.1	1.1	1.2	1.1	1.2	1.3	1.3	1.3	1.2	1.2	1.2
<b>Volatiles</b>																		
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0

Biometer rinse	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0.1	0	0
Sample balance	103.8	102.7	103.2	103.8	102.5	103.2	103.7	102.5	103.1	103.2	102.6	102.9	102.2	103.4	102.8	103.9	104.3	104.1

<sup>1</sup>Duplicate samples were analysed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following

fractions: First soil ext. + second soil ext. + Soil-bound (PES) + Water phase + <sup>14</sup>CO<sub>2</sub> + <sup>14</sup>CH<sub>4</sub>

<sup>2</sup>The changeover from aerobic to anaerobic was made at 28DAT

NA = Not analysed

**Table B.8.1.1.2.3-30 Percent of applied radioactivity in isolated fractions (Penn PHE)**

<b>Distribution of [phenyl-<sup>14</sup>C] inpyrfluxam label as % of applied radioactivity in isolated fractions in Penn soil<sup>1</sup></b>												
<b>Time after application (days)</b>	<b>0</b>			<b>28</b>			<b>42</b>			<b>57</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>-</b>			<b>0<sup>2</sup></b>			<b>14</b>			<b>29</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
Water Phase	NA	NA	NA	NA	NA	NA	10.3	9.7	10.0	10.7	9.7	10.2
<b>Soil extracts</b>												
1st soil ext. (neutral)	99.5	100.8	100.1	87.0	91.2	89.1	74.6	78.1	76.3	69.6	72.6	71.1
2nd soil ext. (acidic)	0.6	0.6	0.6	7.8	8.3	8.1	10.1	9.8	10	12.9	12.3	12.6
Total soil ext.	100.1	101.3	100.7	94.7	99.5	97.1	84.7	88.0	86.3	82.5	84.9	83.7
Soil-bound (PES)	0.1	0.1	0.1	5.6	4.6	5.1	4.9	4.6	4.8	5.8	5.9	5.8
<b>Volatiles</b>												
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Biometer/ beaker rinse	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Sample balance	100.2	101.5	100.8	100.6	104.3	102.5	100.2	102.7	101.4	99.3	100.8	100.1
<b>Continued</b>												



<b>Time after application (days)</b>	<b>85</b>			<b>122</b>			<b>155</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>57</b>			<b>94</b>			<b>127</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
Water Phase	11.2	11.0	11.1	11.3	11.8	11.5	12.0	11.2	11.6
<b>Soil extracts</b>									
1st soil ext. (neutral)	60.8	59.6	60.2	63	64.6	63.8	59.9	66.6	63.3
2nd soil ext. (acidic)	14.9	15.3	15.1	19.7	16.6	18.2	19.3	15.6	17.4
Total soil ext.	75.8	74.9	75.4	82.8	81.2	82.0	79.2	82.2	80.7
Soil-bound (PES)	5.6	5.7	5.6	7.2	6.8	7.0	7.0	6.5	6.7
<b>Volatiles</b>									
<sup>14</sup> CO <sub>2</sub>	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
<sup>14</sup> CH <sub>4</sub>	0	0	0	0	0	0	0	0	0
Biometer/ beaker rinse	0	0	0	0	0	0	0	0	0
Sample balance	93.0	92.0	92.5	101.6	100.2	100.9	98.6	100.4	99.5

<sup>1</sup>Duplicate samples were analysed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following fractions: First soil ext. (neutral) + second soil ext. (acidic) + Soil-bound (PES) + Water phase + <sup>14</sup>CO<sub>2</sub> + <sup>14</sup>CH<sub>4</sub> + Biometer rinse.

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$^{22}\text{BaCl}_2$  precipitation of NaOH trapped activity performed to confirm  $^{14}\text{CO}_2$  as  $\text{Na}_2^{14}\text{CO}_3$ . 28 DAT only. The changeover from aerobic to anaerobic was made on 28DAT.

NA = Not analysed

**Table B.8.1.1.2.3-31 Percent of applied radioactivity in isolated fractions (Penn PYR)**

<b>Distribution of [pyrazolyl-<sup>14</sup>C] inpyrfluxam Label as % of Applied Radioactivity in Isolated Fractions of Penn soil<sup>1</sup></b>												
<b>Time after application (days)</b>	<b>0</b>			<b>28</b>			<b>42</b>			<b>57</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>-</b>			<b>0<sup>2</sup></b>			<b>14</b>			<b>29</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
Water Phase	NA	NA	NA	NA	NA	NA	10.9	10.7	10.8	11	9.7	10.3
<b>Soil extracts</b>												
1st soil ext. (neutral)	97.5	100.7	99.1	86.1	86.7	86.4	76.1	76.1	76.1	69.6	72.6	71.1
2nd soil ext. (acidic)	0.6	0.6	0.6	8.4	8.3	8.3	10.8	10.1	10.5	12.9	12.3	12.6
Total soil ext.	98.1	101.3	99.7	94.5	95.0	94.8	86.9	86.2	86.5	82.5	84.9	83.7
Soil-bound (PES)	0.1	0.1	0.1	4.4	4.7	4.5	4.9	4.5	4.7	5.5	5.6	5.5
<b>Volatiles</b>												
<sup>14</sup> CO <sub>2</sub>	NA	NA	NA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<sup>14</sup> CH <sub>4</sub>	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Biometer/beaker rinse	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Sample balance	98.1	101.3	99.7	99.0	99.8	99.4	102.7	101.5	102.1	99.3	103.1	101.2
<b>Continued</b>												

<b>Time after application (days)</b>	<b>85</b>			<b>122</b>			<b>155</b>		
<b>Time after switch to anaerobic conditions (days)</b>	<b>57</b>			<b>94</b>			<b>127</b>		
<b>Sample No.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg.</b>
Water Phase	9.3	13.1	11.2	13	13.2	12.8	12.5	12.1	12.3
<b>Soil extracts</b>									
1st soil ext. (neutral)	69.2	65.0	67.1	66.6	64.7	65.7	62.1	59.2	60.7
2nd soil ext. (acidic)	15.2	15.8	15.5	14.0	16.5	15.2	18.8	19.4	19.1
Total soil ext.	84.4	80.8	82.6	80.6	81.2	80.9	80.9	78.7	79.8
Soil-bound (PES)	5.2	5.3	5.3	5.3	6.1	5.7	6.4	6.3	6.4
<b>Volatiles</b>									
<sup>14</sup> CO <sub>2</sub>	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<sup>14</sup> CH <sub>4</sub>	0	0	0	0	0	0	0	0	0
Biometer/beaker rinse	0	0	0	0	0	0	0	0	0
Sample balance	99.0	99.3	99.2	98.5	100.6	99.6	99.9	97.3	98.6

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<sup>1</sup>Duplicate samples were analysed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following fractions: First soil ext. (neutral) + second soil ext. (acidic) + Soil-bound (PES) + Water phase + <sup>14</sup>CO<sub>2</sub> + <sup>14</sup>CH<sub>4</sub> + Biometer rinse.

<sup>2</sup>BaCl<sub>2</sub> precipitation of NaOH trapped activity performed to confirm <sup>14</sup>CO<sub>2</sub> as Na<sub>2</sub><sup>14</sup>CO<sub>3</sub>. 28 DAT only. The changeover from aerobic to anaerobic was made on 28DAT.

NA = Not analysed

### B.8.1.2. Field studies

The results of the laboratory aerobic soil studies for inpyrfluxam, 1' COOH-S-2840 and 3'-OH-S-2840 triggered the conduct of field dissipation studies to investigate dissipation under more realistic field conditions. The applicant has provided field dissipation studies from four field sites in Europe and five field sites in the USA and Canada.

#### B.8.1.2.1. Soil dissipation studies

##### B.8.1.2.1.1 US field dissipation studies

According to European guidance retained by Great Britain, the results of field dissipation studies conducted at non-European sites can be accepted for use in European assessments provided that the conditions during the study are representative of European conditions. The applicant performed an assessment to determine comparability of conditions at the sites to European conditions. The assessment was performed using the OECD ENASGIPS tool which uses an 'ecoregion' approach to compare soil and climatic conditions in the North American sites to European situations. Since this analysis will determine the relevance on non-European field studies to GB conditions this has been presented before the individual field study evaluations.

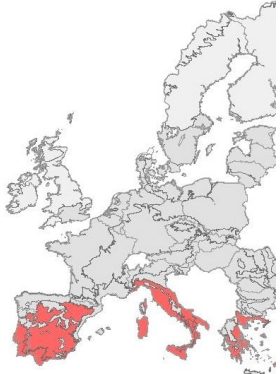

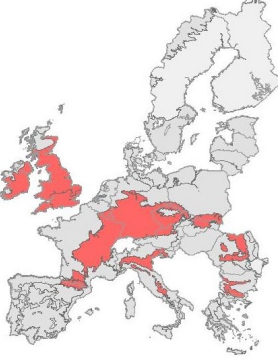


<b>Data Point:</b>	KCA 7.1.2.2.1/06
<b>Report Author:</b>	██████████ and ██████████
<b>Report Year:</b>	2018a
<b>Report Title:</b>	Normalisation of S-2399 DT <sub>50</sub> data from terrestrial field dissipation studies in North America.
<b>Study number</b>	Exponent International Ltd., UK. Report No. 1403863.UK0-2381 Sumitomo Chemical Co., Ltd. Report No: TPR-0083
<b>Guideline(s) followed in study:</b>	“EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT <sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil”, EFSA European Food Safety Authority (2014), EFSA Journal 2014;12(5):3662. Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. FOCUS (2014) version 1.1 (18 December 2014). NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies, Regulatory Directive DIR2006-01, March 2006.

	OECD (2016), Guidance document for conducting Pesticide Terrestrial Field Dissipation studies. ENV/JM/MONO(2016)6 – 29 Feb 2016.  OECD 307. Guideline for the testing of chemicals. Aerobic and anaerobic transformation in soil. Adopted 24 April 2002.
<b>GLP?</b>	Non-GLP, unpublished

The applicant has provided five terrestrial field dissipation studies conducted in North America. The trial sites were located in California, North Dakota, Ontario, Washington and Mississippi. The Mississippi trial site was excluded from this comparison as the applicant expressed concern on the scientific validity of the study due to flooding at the site and resulting variability in parent levels detected. HSE would agree with the exclusion of this site from the data set to be assessed. To support the use of the remaining four trials the applicant provided an 'ecoregion crosswalk' assessment and kinetic evaluations for the derivation of 'trigger' and 'modelling' endpoints.

The applicant used the OECD ENASGIPS tool to determine ecoregions in Europe that are similar to the ecoregions containing the terrestrial field dissipation sites. The coordinates of each site were then used to establish a root ecoregion code, noting that some sites were on the border of two ecoregions in which case both were assessed. Using a default threshold of 80% the applicants determined matching ecoregions using the Holistic Ecoregion Similarity Model and parameters of temperature, precipitation, percent organic carbon, soil pH and soil texture. The results of the ENASGIPS ecoregion similarity assessment for the four North American field sites are shown in Figure B.8.1.2.1.1-01.

**Figure B.8.1.2.1.1-01 The locations in Europe with an overall similarity >80% with respect to the relevant North American experimental sites**

North American Field site	Relevant EU Ecoregion(s)	
California		
Ontario* (NA0407, left) (NA0414, right)  (*Site located on the border of 2 different root ecoregions)		
Washington		
North Dakota* (NA0810)  (*Site located on the border of 2 different root ecoregions)		



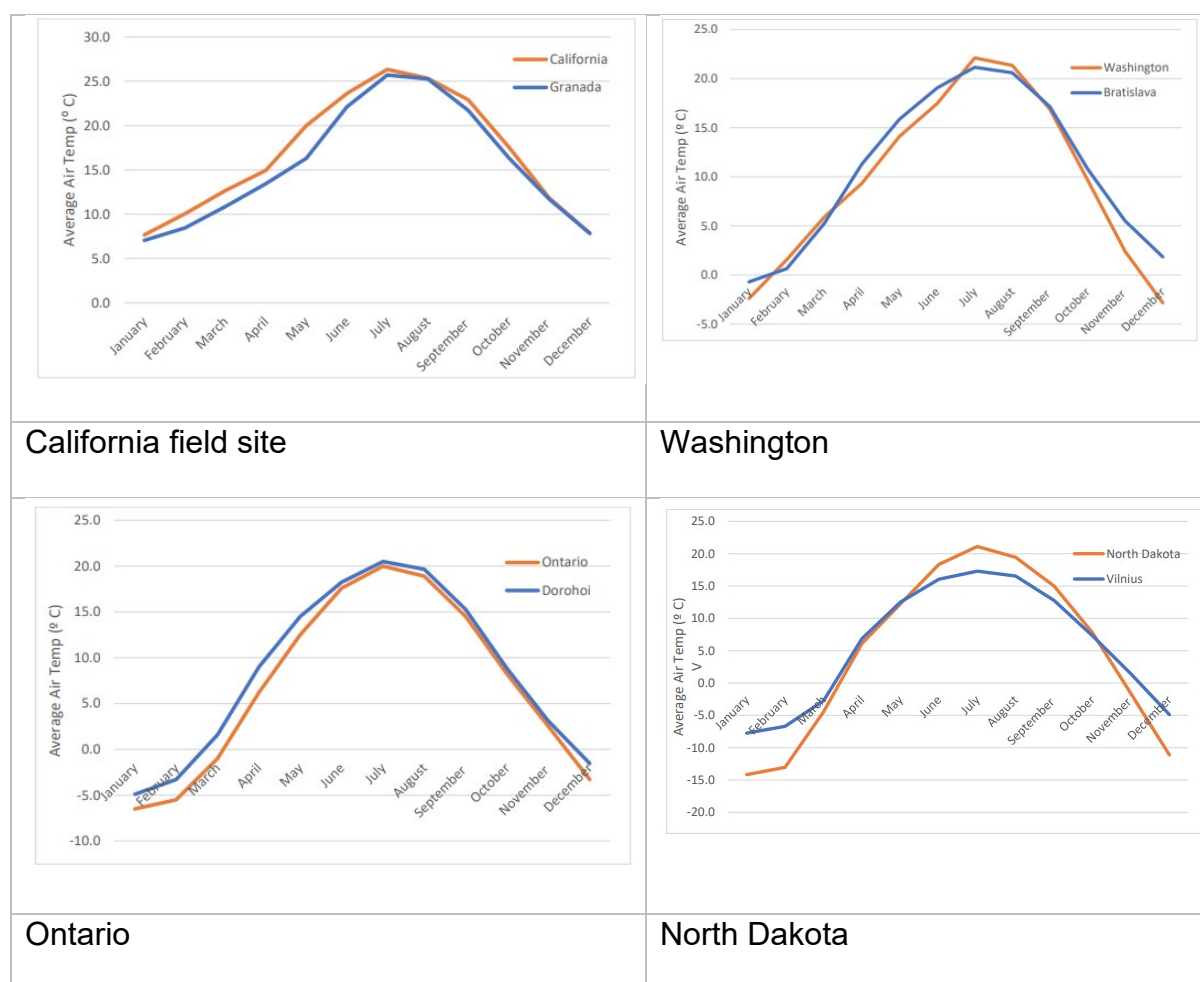
HSE have concerns over the use of this method as the sole determinant of whether a field dissipation study site has comparable conditions to those in Europe. It is considered that information on the comparability of the site-specific soil and weather conditions during the actual course of the study in relation to European conditions is more appropriate evidence than an ENASGIPS assessment, which relies on historic climate data. This is based on EFSA (2014) guidance (EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT<sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil) which is retained for use in GB. The applicant was requested to provide a further comparison of the climate (temperature and precipitation/irrigation) and soil conditions at the US field sites during the study with EU conditions, where cereals are likely to be grown. It was suggested that at least monthly average air temperature and precipitation at the sites should be considered along with the representativeness of the soil to European conditions. The mineral properties of the soil (proportions of sand, silt and clay) together with organic carbon and pH need to be considered, alongside a confirmation that comparable soils can be found in the EU. The main aim of this interpretation is to avoid accepting studies from sites where individual properties might be within the ranges of those seen in Europe as a whole but where the combination is not seen in any European region and therefore unrepresentative of European locations. In response to this the applicant provided further comparative assessment.

<b>Data Point:</b>	KCA 7.2.2.1/11
<b>Report Author:</b>	██████ and ██████
<b>Report Year:</b>	2024
<b>Report Title:</b>	The relevance of North American field dissipation studies on inpyrfluxam to EU conditions
<b>Study number</b>	2006362.UK0-9770
<b>Guideline(s) followed in study:</b>	n/a
<b>GLP?</b>	No

### Consideration of climatic conditions

To address climatic relevance of the North American field sites the applicant considered European locations in the indicated matching ecoregions and using the published UK Meteorological Office Tables of temperature relative humidity

precipitation and sunshine for the world part III which provides long term monthly average temperature and rainfall in European locations for multiple years (generally in the period 1931 - 1960; actual duration depends on the particular site). The applicant considered the sites of Granada (Spain), Bratislava (Slovakia) and Dorohoi (Romania) to be comparable to that of California, Washington and Ontario respectively. They noted that a clear match for North Dakota could not be found and proposed that Vilnius (Lithuania) showed some correspondence. No comparison of rainfall was presented as the applicant stated that field dissipation site plots were additionally irrigated and it was considered that irrigation for agricultural need would also occur at some of the selected EU locations. Graphical representation of the 30 year average air temperature comparisons presented by the applicant are shown in Figure B.8.1.2.1.1-02.

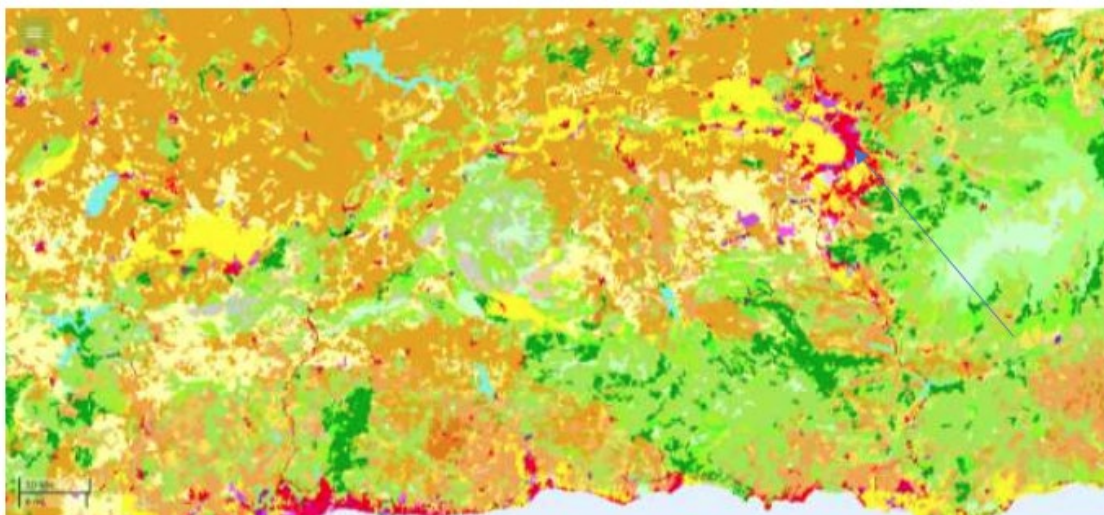


**Figure B.8.1.2.1.1-02 Long term average air temperatures for the US field sites compared to locations in the EU**

The applicant considered that with the exception of the North Dakota site the average air temperatures of the North American field sites were shown to be comparable to particular locations in the EU.

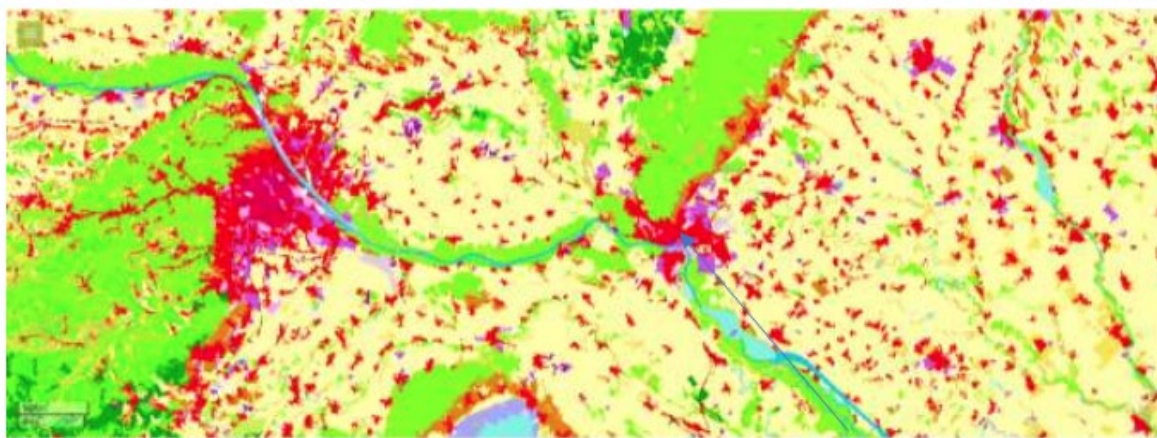
As a further check of the agricultural relevance of selected EU locations, the applicant used the Corine database (2018) to determine the nature of the land use in each region. Figures 8.1.2.1.1-03 (a-d) show the detail of this assessment for the vicinity of Granada, Bratislava, Dorohoi and Vilnius respectively and the applicants conclusions on this.

**Figure B.8.1.2.1.1-03 The agricultural relevance of the selected EU areas identified**



Arrow shows location of Granada

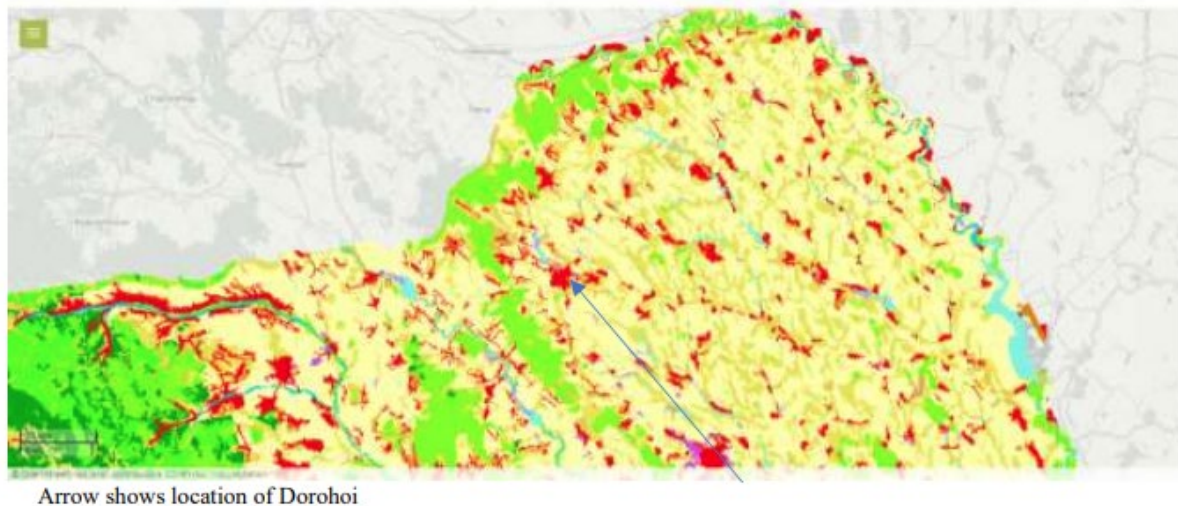
a) The area around Granada shows a significant proportion of permanently irrigated land (strong yellow), non-irrigated arable land (pale yellow) and olive groves (brown).



Arrow shows location of Bratislava

b) The area around Bratislava is predominantly non-irrigated arable land (pale yellow)





c) The area around Dorohoi is predominantly non-irrigated arable land (pale yellow)



c) The area around Dorohoi is predominantly non-irrigated arable land (pale yellow)

The applicant has presented long term (10-30 years) historical average monthly temperature and rainfall data taken from collection weather sites at 1 – 37 kilometres from the four dissipation sites. They have noted that irrigation was additionally applied at all sites during the trials, which was in line with relevant guidance. In the case of the Ontario and North Dakota sites this was to ensure the long term average rainfall amount was met (107% and 137% respectively). In the case of Washington and California it was assumed to be in order to mimic normal agricultural practice. Therefore comparisons of rainfall between the North American field sites and the EU locations has not been presented as all field sites were additionally irrigated and the applicant consider it to be probable that irrigation for agricultural need would also occur at some of the selected EU locations. HSE consider this further in the site specific assessments.

## Consideration of soil characteristics

In line with standard reporting the applicant has provided details of the soil characteristics present at the North American terrestrial field dissipation trials sites. The applicant proposes that these soils meet the soil types found in Europe and are similar to the 'Hamburg' scenario used in FOCUS modelling. HSE notes that this is the case for the soil properties at the Ontario site. In addition the applicant notes that some sites have sandy soils with a lower organic matter content which they consider represent more vulnerable scenarios. HSE do not consider this latter point relevant for the purpose of determining relevance of the conditions to EU scenarios.

**Table B.8.1.2.1.1-01 Soil characteristics in the 0-30 cm depth at the US field sites**

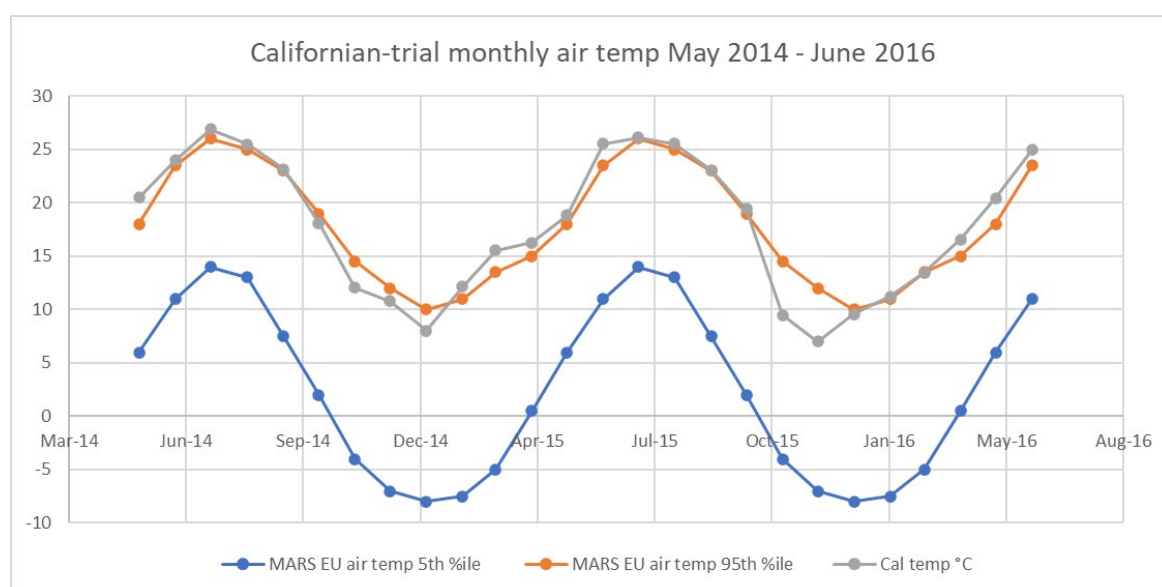
	California	Washington	Ontario	N.Dakota
USDA Textural Classification	Loamy sand	Loamy sand	Sandy loam	Sandy loam
% sand	82	79	67	71
% silt	11	18	27	14
% clay	7	3	6	15
pH (1:1 soil: water)	7.5	8.0	5.4	7.2
Percent Organic Matter (Walkley Black)	0.45	0.77	2.3	1.9
Cation exchange capacity (meq/100g)	6.1	10.4	6.7	14.7
Bulk Density (disturbed, g/cm <sup>3</sup> )	1.33	1.42	1.21	1.07
Moisture at 1/3 bar (%)	8.4	15.5	22.8	20.3
Moisture at 1/10 bar (%) – pF 2	12.0	19.9	Not stated	31.3

Based upon their presentation the applicant consider all four sites are likely representative of EU conditions. However, they note that North Dakota may be an exception due to more extreme air temperatures.

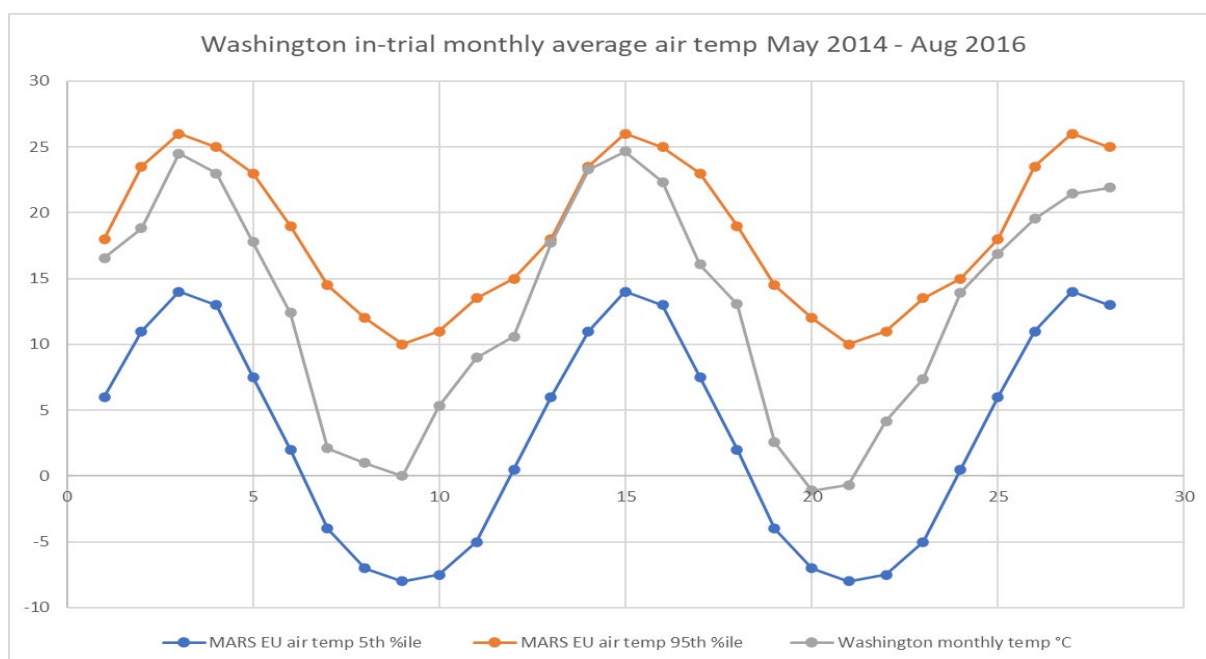
## HSE consideration of the US field sites

The applicant presented comparison of the long term average air temperature values for the area of the trial and compared these with sites in Europe that are linked to arable growing areas. However, HSE consider that a more appropriate assessment is to consider the actual conditions occurring at the US trial site during the trial and relate those to the average EU conditions. HSE has made a further consideration of the climate characteristics measured at each site location (up to 4 km away) and comparison with weather data from the MARS database. The MARS weather data sets are based upon 25 km x 25 km grid squares. In addition the CORINE landcover database was used to determine arable areas from the agricultural land area. By use of these datasets, statistical data for monthly average temperature and precipitation for the European arable area can be determined. The precipitation data were generated from 1986 – 2015 and the CORINE data set was from 1981 – 2010. HSE has considered the 5<sup>th</sup> and 95<sup>th</sup> percentile values of the data and used these to compare to conditions in the trials. For each trial site the average monthly rainfall and temperature values taken during the conduct of the trial have been used where available. Graphical comparisons of the temperature (Figure B.8.1.2.1.1-04) and rainfall/irrigation (Figure B.8.1.2.1.1-05) data from each site are detailed below.

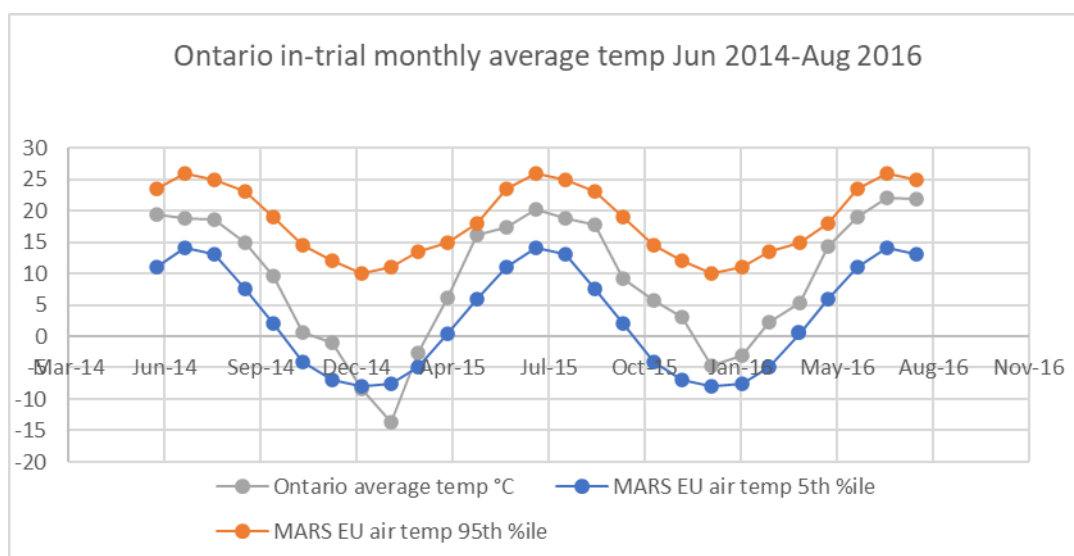
### a) California trial site



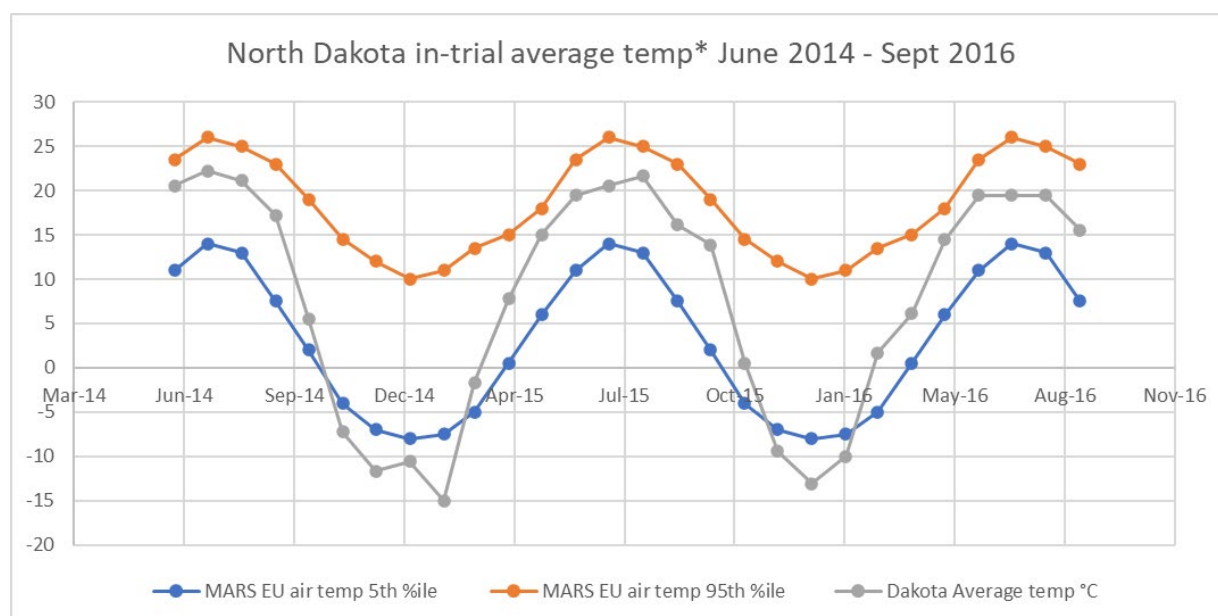
## b) Washington trial site



## c) Ontario trial site



## d) North Dakota trial site\*



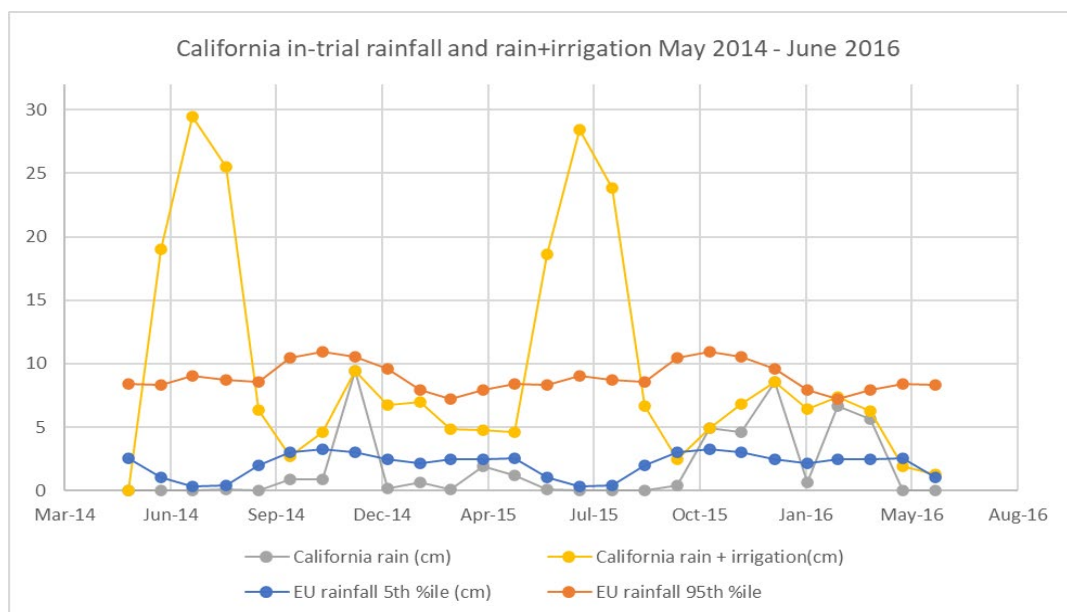
\* Calculated as the mean of the low and high temperatures reported

**Figure B.8.1.2.1.1-04: Comparison of average monthly temperature at US field dissipation study sites with 30 year average MARS data for arable land in Europe**

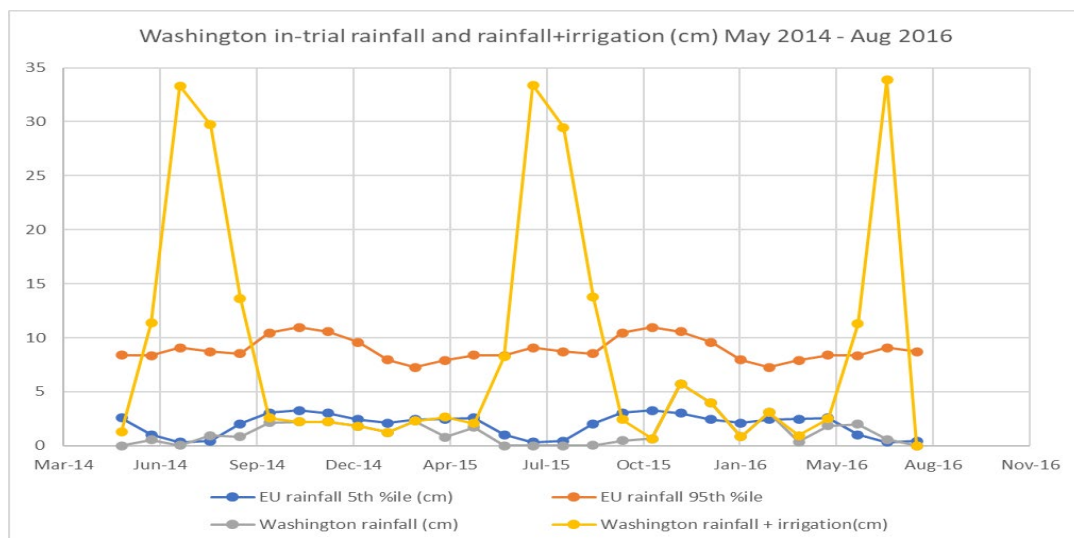
In considering site temperatures with those of arable areas across Europe it can be considered that the sites of Washington and Ontario had good correlation with average values within the 5<sup>th</sup> and 95<sup>th</sup> percentile values of European sites. The north Dakota site had good correlation for the spring and summer months but was below the 5<sup>th</sup> percentile for the winter months of November – January and therefore had a large amplitude in temperatures during the course of the trial. The Californian site average air temperatures were at or above the 95<sup>th</sup> percentile of European conditions throughout the trial, with temperatures above the 95<sup>th</sup> percentile in 11 of the months tested during the 26 month trial. The climate during this trial is not therefore considered to be European like with regard to temperature.



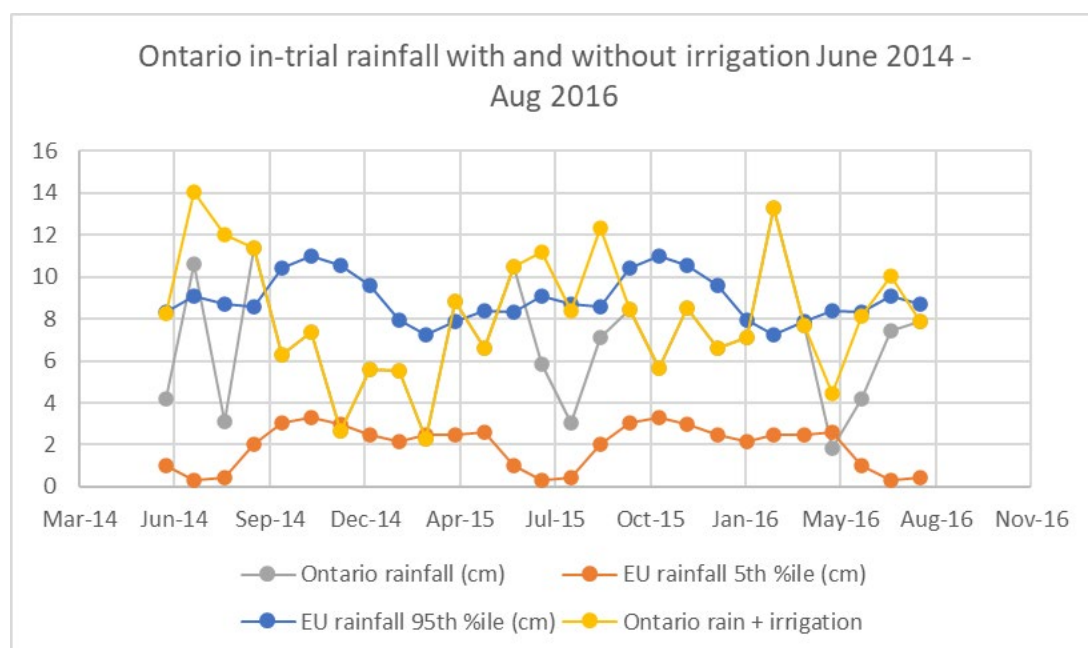
## a) California trial site



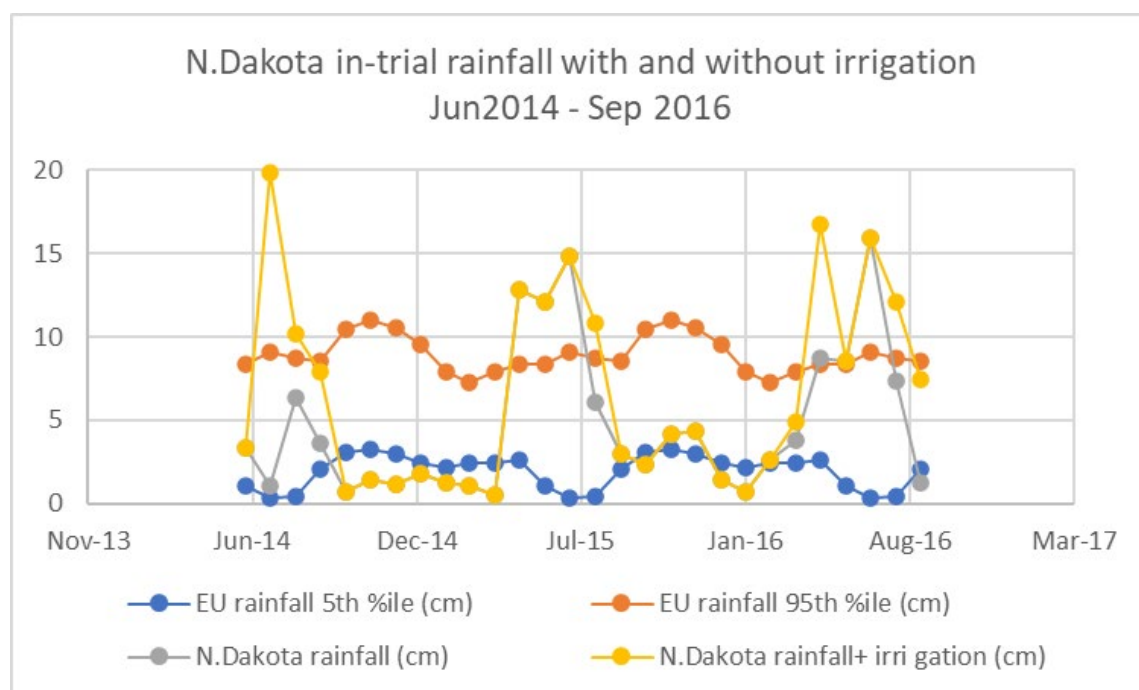
## b) Washington



## c) Ontario



## d) North Dakota



**Figure B.8.1.2.1.1-05: Comparison of average monthly temperature at US field dissipation study sites with 30 year average MARS data for arable land in Europe**

In the California and Washington sites precipitation was lower than would be expected in European arable situations for at least 75% of the study period, although this was compensated to an extent by the application of irrigation. However, the

application of irrigation at both sites gave total precipitation and irrigation far in excess of precipitation at European arable sites in 6 of the 26 months in California and 9 out of the 28 months in Washington. It is recognised that irrigation could also be applied to European field sites in order to support crop development, however the irrigation applied at the California and Washington sites is significantly above the 95% precipitation values for the European sites making these conditions unlikely to be representative of EU conditions. The precipitation in Ontario was within the 5<sup>th</sup> and 95th percentile of European conditions for 20 months of the 27 month trial. The precipitation in North Dakota was outside the 5th and 95th percentile of European conditions for 17 months of the 28 month trial and therefore is considered to have extreme amplitude in terms of precipitation for the majority of the trial period.

## **Summary**

### **California**

Whilst the ecoregion assessment indicated a good agreement of the root ecoregion (ENASGIPS California Central Valley grasslands) with eight Southern European ecoregions, the average temperature data from the actual study period indicate that the site was warmer than the majority of European arable situations for 11 months of the 26 month comparison. Precipitation at the site was also lower than would be expected in European arable situations in over half the months of the study period, although this was compensated to an extent by the application of irrigation. However, the application of irrigation gave total precipitation and irrigation far in excess of precipitation at European arable sites in 6 of the 26 months. HSE consideration of the soil properties based upon the Harmonised World Soil Database (HWSD) and using a search criteria of Coarse texture and OC of 0.2 – 0.5% found very few matches, these being mainly limited to a few grid squares in the Iberian peninsula. Including a search term for pH of 7.0 – 8.0 eliminated nearly all matching grid squares with the exception of what appear to be two grid squares, one being in the Iberian peninsula and the other in Greece. Thus it appears that the soil will be likely to have an extremely limited representation in Europe. Taking account of the information on temperature and precipitation during the study period compared to long-term average weather data in European arable areas, the HSE does not accept that the site may be used for the purposes of normalisation of degradation rates.

### **Washington**

The ecoregion assessment indicated a good agreement of the root ecoregion (ENASGIPS Snake-Columbia shrub steppe) with seven Southern European ecoregions. The average temperature data from the actual study period indicated temperatures in a similar range compared to long-term monthly European climate data. Precipitation at the site was also lower than would be expected in European arable situations in over 75% of the months of the study period. This was compensated by the application of irrigation. However, the application of irrigation

gave total precipitation and irrigation far in excess of precipitation at European arable sites in 9 of the 28 months in the trial. HSE consideration of the soil properties based upon the Harmonised World Soil Database (HWSD) and using a search criteria of Coarse texture and OC of 0.2 – 0.5% found very few matches, these being mainly limited to a few grid squares in the Iberian peninsula. Including a search term for pH of 7.0 – 8.0 eliminated nearly all matching grid squares with the exception of what appear to be two grid squares, one being in the Iberian peninsula and the other in Greece. Thus it appears that the soil will be likely to have an extremely limited representation in Europe. Taking account of the information on precipitation during the study period compared to long-term average weather data in European arable areas, the HSE does not accept that the site may be used for the purposes of normalisation of degradation rates.

## **Ontario**

The ecoregion assessment indicated a good agreement of the root ecoregions (ENASGIPS Eastern Great Lakes lowland forest and the Southern Great Lakes forest) with 14 similar ecoregions in Europe. These ecoregions are mostly found in Central Europe, the United Kingdom and selected mountainous regions such as the Pyrenees, Carpathian Mountains, and Apennine Mountains.

The monthly climate data during the trial indicated precipitation and temperatures in a similar range compared to long-term monthly European climate data. There also appears to be a reasonable comparison of soil texture, pH and organic carbon content to the soil used in the EU field trials. The HSE considers that the site can be used for the purposes of normalisation of degradation rates for inpyrfluxam.

## **North Dakota**

The ecoregion assessment indicated a good agreement of the root ecoregions (ENASGIPS Northern mixed grasslands) with 1 similar ecoregion in eastern Europe. The monthly climate data from the trial indicated the precipitation in North Dakota was within the 5th and 95th percentile of European conditions for 11 months of the 28 month trial and lower than would be expected in European arable situations in 10 of the months of the study period. This was compensated by the application of irrigation. However, the application of irrigation gave total precipitation and irrigation far in excess of precipitation at European arable sites in 10 of the 28 months in the trial. The average temperature data from the actual study period indicated temperatures in a similar range compared to long-term monthly European climate data for 21 of the 28 month study. However this was also present with extreme low temperatures being recorded at the trial site during the winter months of Nov – Feb which resulted in very large amplitude in the temperatures recorded during the trial period. The characteristics of the soil from the North Dakota site were considered to have a reasonable comparison of soil texture, pH and organic carbon content to the soils used in the EU field trials. On balance for the North Dakota it is considered that

the extremes of weather at this site and impact on soil temperatures are unlikely to be experienced in EU arable areas and this site is therefore not considered comparable to EU conditions.

Given that the field dissipation studies from California, Washington, North Dakota and Mississippi are not to be used in the environmental exposure and risk assessment, the applicant executive summaries of the studies have been presented only for information and not for risk assessment purposes.

<b>Data Point:</b>	KCA 7.1.2.2.1/01
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2017a
<b>Report Title:</b>	S-2399: Terrestrial Field Soil Dissipation of S-2399 on Bare Ground in Mississippi
<b>Study number</b>	Study No: V-14-38553 Sumitomo Chemical Co. Ltd. Report No: TPR-0031
<b>Guideline(s) followed in study:</b>	EPA Guideline Number OPPTS 835.6100, Terrestrial Field Dissipation  PMRA Environmental Chemistry and Fate – Terrestrial DACO No.: 8.3.2
<b>GLP?</b>	Yes

**THE STUDY IS NOT CONSIDERED TO BE REPRESENTATIVE OF EUROPEAN CONDITIONS AND IS NOT USED FOR RISK ASSESSMENT PURPOSES. THE STUDY SUMMARY IS PRESENTED FOR INFORMATION ONLY**

### Executive Summary

The dissipation and mobility of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B were assessed following two applications of inpyrfluxam 2.84 SC (30.5% w/v inpyrfluxam) to bare (uncropped) soil in Mississippi at a nominal rate of 115 g a.s./ha and 100 g a.s./ha on the 10<sup>th</sup> July 2014 and 24<sup>th</sup> July 2014, respectively (14 day interval).

Inpyrfluxam readily dissipated in soil under field conditions with a calculated intermediate order rate (IORE) half-life of 42.3 days. However, this value is highly uncertain due to the high variability in the data and several outliers which may be due to significant flood events which occurred during the study. 3'-OH-S-2840

reached a maximum of 0.0186 mg/kg (9.8% of the maximum individual residue of inpyrfluxam detected after the second application (0.190 mg/kg). No residues of 3'-OH-S-2840 were found in the deeper layers of soil at any time point. Residues of 1'-COOH-S-2840A and 1'-COOH-S-2840B were not detected throughout the study. Although on isolated occasions residues of inpyrfluxam were found in deeper soil layers (up to 90 cm), it was concluded that the leaching potential of inpyrfluxam and its degradates is very low in the loam soil at the site even when there had been excessive rainfall during the study period.

<b>Data Point:</b>	KCA 7.1.2.2.1/02
<b>Report Author:</b>	
<b>Report Year:</b>	2017b
<b>Report Title:</b>	S-2399: Terrestrial Field Soil Dissipation of S-2399 on Bare Ground in California
<b>Study number</b>	Study No: V-14-38586; Sumitomo Chemical Co. Ltd. Report No: TPR-0032
<b>Guideline(s) followed in study:</b>	EPA Guideline Number OPPTS 835.6100, Terrestrial Field Dissipation  PMRA Environmental Chemistry and Fate – Terrestrial DACO No.: 8.3.2
<b>GLP?</b>	Yes

**THE STUDY IS NOT CONSIDERED TO BE REPRESENTATIVE OF EUROPEAN CONDITIONS AND IS NOT USED FOR RISK ASSESSMENT PURPOSES. THE STUDY SUMMARY IS PRESENTED FOR INFORMATION ONLY**

### Executive Summary

The study investigated the dissipation of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B in the field following two applications of inpyrfluxam 2.84 SC (30.5% w/v inpyrfluxam) to bare (uncropped) ground in California at a nominal rate of 115 g a.s./ha and 100 g a.s./ha on the 29<sup>th</sup> May 2014 and 12<sup>th</sup> June 2014, respectively (14 day interval).

Under field conditions, inpyrfluxam readily dissipated in soil and was calculated to have a double first-order in parallel (DFOP) half-life of 6.55 days. The metabolite 3'-OH-S-2840 was detected above the limit of detection (LOD; 0.005 mg/kg) but less than the limit of quantification (LOQ; 0.01 mg/kg) in 10 samples over the study. The

maximum individual residue of 3'-OH-S-2840 observed (0.00818 mg/kg) was equivalent to 19% of the maximum individual residue of inpyrfluxam detected (0.0430 mg/kg) after the second application.

Residues of 1'-COOH-S-2840A and 1'-COOH-S-2840B were not detected throughout the study and residues of inpyrfluxam and 3'-OH-S-2840 were found only in the top 15 cm of the soil. The lack of residues below the top layer of soil demonstrates that the leaching potential of inpyrfluxam and its degradates is very low in the fine sandy loam soil at the field site.

<b>Data Point:</b>	KCA 7.1.2.2.1/03
<b>Report Author:</b>	██████
<b>Report Year:</b>	2017a
<b>Report Title:</b>	S-2399: Terrestrial Field Soil Dissipation of S-2399 2.84 SC on Bare Ground in North Dakota
<b>Study number</b>	Study No: V-14-38603; Sumitomo Chemical Co. Ltd. Report No: TPR-0034
<b>Guideline(s) followed in study:</b>	EPA Guideline Number OPPTS 835.6100, Terrestrial Field Dissipation  PMRA Environmental Chemistry and Fate – Terrestrial DACO No.: 8.3.2
<b>GLP?</b>	Yes

**THE STUDY IS NOT CONSIDERED TO BE REPRESENTATIVE OF EUROPEAN CONDITIONS AND IS NOT USED FOR RISK ASSESSMENT PURPOSES. THE STUDY SUMMARY IS PRESENTED FOR INFORMATION ONLY**

### Executive Summary

The dissipation and mobility of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B were assessed following two applications of inpyrfluxam 2.84 SC (30.5% w/v inpyrfluxam) to bare (uncropped) soil in North Dakota at a nominal rate of 115 g a.s./ha and 100 g a.s./ha on the 25<sup>th</sup> June 2014 and 09<sup>th</sup> July 2014, respectively (14 day interval).

inpyrfluxam and 3'-OH-S-2840 residues were found only in the top 15 cm and no residues were detected in deeper layers at any timepoint. The degradates 1'-COOH-S-2840A and 1'-COOH-S-2840B were not detected throughout the study. As no residues were detected below the top layer of soil during the study it was concluded that the leaching potential of inpyrfluxam and 3'-OH-S-2840 is very low in the sandy loam soil at the test site.

inpyrfluxam readily dissipated in soil under field conditions with a calculated intermediate order rate (IORE) half-life of 24 days. 3'-OH-S-2840 was detected during the study at levels above the LOQ (0.01 mg/kg) in four samples. The maximum individual residue of 3'-OH-S-2840 observed was 0.0117 mg/kg which was equivalent to 12% of the maximum individual residue of inpyrfluxam detected after the second application (0.0942 mg/kg).

<b>Data Point:</b>	KCA 7.1.2.2.1/04
<b>Report Author:</b>	
<b>Report Year:</b>	2017c
<b>Report Title:</b>	S-2399: Terrestrial Field Soil Dissipation of S-2399 on Bare Ground in Washington.
<b>Study number</b>	Study No: V-14-38546; Sumitomo Chemical Co. Ltd. Report No: TPR-0053
<b>Guideline(s) followed in study:</b>	EPA Guideline Number OPPTS 835.6100, Terrestrial Field Dissipation PMRA Environmental Chemistry and Fate – Terrestrial DACO No.: 8.3.2
<b>GLP?</b>	Yes

**THE STUDY IS NOT CONSIDERED TO BE REPRESENTATIVE OF EUROPEAN CONDITIONS AND IS NOT USED FOR RISK ASSESSMENT PURPOSES. THE STUDY SUMMARY IS PRESENTED FOR INFORMATION ONLY**

### Executive Summary

The field study assessed the dissipation of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B following two applications of inpyrfluxam 2.84 SC (30.5% w/v inpyrfluxam) to bare (uncropped) soil in Ephrata,



Washington at a nominal rate of 115 g a.s./ha and 100 g a.s./ha on the 20<sup>th</sup> May 2014 and 3<sup>rd</sup> June 2014, respectively.

Inpyrfluxam and 3'-OH-S-2840 residues were found only in the top 15 cm and no residues were detected in deeper layers at any timepoint. The degradates 1'-COOH-S-2840A and 1'-COOH-S-2840B were not detected throughout the study. As no residues were detected below the top layer of soil during the study, this demonstrated that the leaching potential of inpyrfluxam and 3'-OH-S-2840 is very low in the loamy fine sand soil at the test site.

Inpyrfluxam readily dissipated in soil under field conditions with a calculated intermediate order rate (IORE) half-life of 29.3 days. 3'-OH-S-2840 was detected during the study at levels above the LOQ (0.01 mg/kg) in one sample (0.0104 mg/kg at 630 days after the second application). This maximum individual residue of 3'-OH-S-2840 equated to 16% of the maximum individual residue of inpyrfluxam detected after the second application (0.0659 mg/kg).

<b>Data Point:</b>	KCA 7.1.2.2.1/05
<b>Report Author:</b>	
<b>Report Year:</b>	2017b
<b>Report Title:</b>	S-2399: Terrestrial Field Soil Dissipation of S-2399 on Bare Ground in Ontario, Canada.
<b>Study number</b>	Study No: V-14-38593; Sumitomo Chemical Co. Ltd. Report No: TPR-0033
<b>Guideline(s) followed in study:</b>	EPA Guideline Number OPPTS 835.6100, Terrestrial Field Dissipation  PMRA Environmental Chemistry and Fate – Terrestrial DACO No.: 8.3.2
<b>GLP?</b>	Yes

## Introduction

The decline of inpyrfluxam was studied following treatment of bare ground with the formulation 'inpyrfluxam 2.84 SC', containing 30.5% w/v of the active substance inpyrfluxam. Two applications of the formulation at a nominal rate of 115 g a.s./ha and 100 g a.s./ha were made on the 5<sup>th</sup> June 2014 and 20<sup>th</sup> June 2014, respectively

(14 day interval). Soil cores from the site were analysed for inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B.

## MATERIALS AND METHODS

### Test item

<b>Formulation name</b>	<b>S-2399 2.84 SC</b>
<b>Description</b>	White emulsion; stored between 13.3 and 21.3°C before application
<b>Formulation type</b>	SC
<b>Active ingredient</b>	Inpyrfluxam
<b>Chemical name</b>	3-(Difluoromethyl)-1- methyl-N-[(3'R)- 1',1',3'-trimethyl-2',3'- dihydro-1'H-inden-4'- yl]-1H-pyrazole-4- carboxamide
<b>Nominal content</b>	30.5% w/v inpyrfluxam

The soil characteristics at the site are detailed in Table B.8.1.2.1.1-02 and the site usage and management history for the previous 3 years are presented in Table B.8.1.2.1.1-03. The treatment area was divided into three plots (plot A, B and C), each of which was further divided into 36 equal subplots (of 5 x 1.5 m) and this area was a distance of 51.2 m downslope from the one control plot which was divided into 20 equal subplots (each 5 x 1.5 m). The site was maintained as bare soil during the study by the application of paraquat, glyphosate and atrazine for weed control.

**Table B.8.1.2.1.1-02 Test Site Soil Properties**

<b>Branchton, Ontario Test Plot</b>			
	<b>Depth (cm)</b>		
	<b>0-30</b>	<b>30-60</b>	<b>60-90</b>
<b>USDA Textural Classification</b>	Sandy loam	Sandy loam	Sandy loam
<b>% sand</b>	67	65	61
<b>% silt</b>	27	29	35
<b>% clay</b>	6	6	4
<b>pH (1:1 soil: water)</b>	5.4	5.2	5.9
<b>Percent Organic Matter (Walkley Black)</b>	2.3	1.5	0.36
<b>Cation exchange capacity (meq/100g)</b>	6.7	6.3	4.2
<b>Bulk Density (disturbed, g/cm<sup>3</sup>)</b>	1.21	1.19	1.31
<b>Moisture at 1/3 bar (%)</b>	22.8	23.9	23.0
<b>Moisture at 1/10 bar (%) – pF 2</b>	Not stated	Not stated	Not stated

**Table B.8.1.2.1.1-03 Site Usage and Management History for the previous three years**

<b>Use</b>	<b>Year</b>	<b>Treated area</b>
Crops grown	Previous year	Edible beans – cranberry beans; Fallow
	2 years previous	Soybeans
	3 years previous	Soybeans
Pesticides used	Previous year	Pethoximide; S-metolachlor; Glyphos
	2 years previous	RoundUp WeatherMax
	3 years previous	RoundUp WeatherMax
Cultivation methods (e.g. tillage)	Previous years	Not provided

Following application of the 30.5% w/v SC formulation of inpyrfluxam via a tractor mounted sprayer to bare (uncropped) soil at a nominal rate of 115 g a.s./ha and 100 g a.s./ha on the 5<sup>th</sup> June 2014 and 20<sup>th</sup> June 2014, respectively. Verification pads were included to confirm rates which were verified using an LC/MS-MS analytical method RM-50V. The validation of this method is reported in Volume 3CA – B5 Methods of Analysis (Section B.5.1.2.1). Supplementary irrigation events (25 in total) were applied via a sprinkler system when required. Daily weather data were recorded at Branchton, Ontario, (approx. 2.7 km from the site). A datalogger installed at the test site monitored soil volumetric water content, soil temperature and rainfall.

### Sampling

Soil cores from each plot and the control were taken on day -1, 0 (after first application) and 13, and following the second application on day 0 (after second application), 1, 7, 14, 21, 31, 59, 89, 119, 367, 419, 489, 705, 740, and 790 post application. At each sampling event, five soil cores per subplot were taken (sampling depth 0-15cm and 15-90 cm). For each core, the 0-15 cm segment was collected followed by a 15-90 cm core. Samples were frozen at the site and stored at the laboratory (maximum storage length 672 days). Samples were then cut into 15 cm segments (15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm and 75-90 cm) and corresponding depth segments from each plot were hand mixed for analysis.

### Analytical method

The analytical method Valent Residue Method RM-50S was used to determine residues of inpyrfluxam, 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B in soil. The validation of this method is reported in Section B.5.1.2.1. Soil extractions involved two acetone/water extractions followed by a third extraction using acetone and 0.5 M HCl. All extractions were combined and partitioned with dichloromethane. The dichloromethane fraction was evaporated to dryness and re-dissolved in

methanol/water. Samples were analysed using HPLC with tandem LC-MS/MS (with turbo-ion spray ionisation in positive and negative ion modes. The LOD and LOQ for each compound were 0.005 mg/kg and 0.01 mg/kg, respectively.

## RESULTS

The procedural recoveries for inpyrfluxam and its metabolites were between 70% and 120% as shown in Table B.8.1.2.1.1-04.

**Table B.8.1.2.1.1-04 Procedural recoveries from soils spiked with 0.01 or 0.1 mg/kg analyte**

	<b>Summary of method performance: percent recovery<sup>a</sup></b>							
	<b>Inpyrfluxam</b>		<b>3'-OH-S-2840</b>		<b>1'-COOH-S-2840A</b>		<b>1'-COOH-S-2840B</b>	
<b>Spike conc. (mg/kg)</b>	0.0100	0.100	0.0100	0.100	0.0100	0.100	0.0100	0.100
<b>Mean ± SD (%)</b>	96.5 ± 8.8	92.3 ± 6.5	95.0 ± 7.9	93.5 ± 7.0	90.6 ± 7.1	89.2 ± 8.2	86.3 ± 10.4	87.7 ± 9.5
<b>Range (%)</b>	71.6 - 110	76.7 - 109	82.8 - 107	84.6 - 113	75.0 - 104	75.2 - 113	70.4 - 106	66.9 - 103
<b>RSD (%)</b>	9.1	7.0	8.3	7.5	7.8	9.2	12.1	10.8
<b>Observations (n)</b>	18	18	18	18	18	18	18	18

<sup>a</sup> One set of concurrent recovery samples was re-partitioned and re-analysed due to percent recoveries outside the acceptance criteria (70-120%); only results of the re-analysis were included in the calculations.

The results of all soil analyses are presented in Table B.8.1.2.1.1-05. The mean zero time concentration of inpyrfluxam in the top 0-15 cm soil segment after the first application was 87.4% of the amount applied. Average residues declined to < LOQ (0.01 mg/kg) by 367 days after the second application, noting that the LOQ for the analytical method is at 15% of the measured applied substance, which reduces confidence in analytical measurements around the DT<sub>90</sub>. No residues were detected in deeper soil layers at any timepoint.

The application to bare soil was made at two separate times with a 14 day interval, which makes the assessment of degradation of the active substance and subsequent metabolites difficult. It is further noted that the experimental set up did not take necessary measures to minimise the impact of soil surface processes. In the absence of any incorporation of the test substance into the soil, irrigation, or application of soil onto the ground after application the guidance (EFSA, 2014) recommends that only data points after cumulative rainfall and/or irrigation of 10 mm has occurred are to be used to conclude on the degradation rate of the decline curve. The daily weather data indicate that between the first and second application 9.4 mm of rainfall occurred and three days after the second application 24.2 mm rainfall was recorded (this coincided with 1.6 cm of irrigation being applied). Therefore any data points recorded before three days after the second application

cannot be used to determine the degradation rate. Based upon the study set up the first data point that can be considered to determine degradation endpoints was recorded seven days after the second application which was the 27<sup>th</sup> June. In addition it is noted that the guidance (EFSA, 2014) indicates that a study should not usually be used for calculating the degradation rate of any primary metabolite that is formed before 10 mm of rainfall has occurred. Based upon the data presented in Table B.8.1.2.1.1-05 this study is not appropriate for determination of degradation of the primary metabolite 3'-OH-S-2840 as this was formed prior to the second application being made and the required amount of rainfall/irrigation being applied. In addition the metabolites 1'-COOH-S-2840A and 1'-COOH-S-2840B were not detected and are therefore not included in the kinetic assessment.

**Table B.8.1.2.1.1-05 Concentration of inpyrfluxam and its metabolites in soil at 0-15 cm depth from the Branchton, Ontario field site (mg/kg)**

Sampling Event	DALA <sup>a</sup>	Residues in Branchton, Ontario soil expressed as mg/kg					
		Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
1	-1	A	0-15	N.D. <sup>d</sup>	N.D.	N.D.	N.D.
		B	0-15	N.D.	N.D.	N.D.	N.D.
		C	0-15	N.D.	N.D.	N.D.	N.D.
2	0 <sup>b</sup>	A	0-15	0.0497	N.D.	N.D.	N.D.
		B	0-15	0.0324	N.D.	N.D.	N.D.
		C	0-15	0.0748	N.D.	N.D.	N.D.
3	13	A	0-15	0.0234	N.D.	N.D.	N.D.
		B	0-15	0.0252	0.00710	N.D.	N.D.
		C	0-15	0.0227	0.00557	N.D.	N.D.
4	0 <sup>c</sup>	A	0-15	0.0541	N.D.	N.D.	N.D.
		B	0-15	0.0659	0.00683	N.D.	N.D.
		C	0-15	0.0730	0.00637	N.D.	N.D.
5	1	A	0-15	0.0335	N.D.	N.D.	N.D.
		B	0-15	0.0795	0.00881	N.D.	N.D.
		C	0-15	0.0431	N.D.	N.D.	N.D.
6	7	A	0-15	0.0243	N.D.	N.D.	N.D.
		B	0-15	0.0257	N.D.	N.D.	N.D.
		C	0-15	0.0696	0.0113	N.D.	N.D.
7	14	A	0-15	0.0233	0.00583	N.D.	N.D.
		B	0-15	0.0336	0.00673	N.D.	N.D.
		C	0-15	0.0188	N.D.	N.D.	N.D.
8	21	A	0-15	0.0307	0.00621	N.D.	N.D.
		B	0-15	0.0166	N.D.	N.D.	N.D.

Sampling Event	DALA <sup>a</sup>	Residues in Branchton, Ontario soil expressed as mg/kg					
		Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
		C	0-15	0.0276	0.00648	N.D.	N.D.
9	31	A	0-15	0.0312	N.D.	N.D.	N.D.
		B	0-15	0.0153	N.D.	N.D.	N.D.
		C	0-15	0.0277	0.00739	N.D.	N.D.
10	59	A	0-15	0.0152	0.00697	N.D.	N.D.
		B	0-15	0.0106	N.D.	N.D.	N.D.
		C	0-15	0.0290	0.00996	N.D.	N.D.
11	89	A	0-15	0.0120	N.D.	N.D.	N.D.
		B	0-15	0.0244	0.00859	N.D.	N.D.
		C	0-15	0.0326	0.0135	N.D.	N.D.
12	119	A	0-15	0.0136	0.00675	N.D.	N.D.
		B	0-15	0.0138	0.00944	N.D.	N.D.
		C	0-15	0.0266	0.0132	N.D.	N.D.
13	367	A	0-15	0.0108	0.00731	N.D.	N.D.
		B	0-15	0.0115	0.00707	N.D.	N.D.
		C	0-15	0.00571	N.D.	N.D.	N.D.
14	419	A	0-15	N.D.	N.D.	N.D.	N.D.
		B	0-15	0.0134	0.00915	N.D.	N.D.
		C	0-15	0.00803	0.00628	N.D.	N.D.
15	489	A	0-15	0.00779	0.00577	N.D.	N.D.
		B	0-15	0.00645	N.D.	N.D.	N.D.
		C	0-15	0.00813	0.00915	N.D.	N.D.

<sup>a</sup> Days after last application;

<sup>b</sup> First application; cores sampled after the application when the spray had dried – typically collection started between 1 and 3 hours

<sup>c</sup> Second application; cores sampled after the application when the spray had dried – typically collection started between 1 and 3 hours

<sup>d</sup> Not detected (<0.005 mg/kg)

<sup>e</sup> No residues were detected below 15 cm.

## CONCLUSION

This study can be used to support the degradation assessment of inpyrfluxam in soil. The conditions of application are such that data points prior to three days post second application must be excluded from the assessment. Sufficient degradation was met within the study with average residues at < LOQ (0.01 mg/kg) by 367 days after the second application. No residues were detected in deeper soil layers at any timepoint.

The normalisation of inpyrfluxam data from the terrestrial field dissipation study conducted in Ontario is detailed in [REDACTED] and [REDACTED] (2018a). As detailed

above the data from seven days post the second application can be used for degradation analysis of inpyrfluxam. Daily soil temperature and moisture values were determined throughout the duration of the study with moisture values determined as volumetric values (v/v). For normalisation of day length the reference soil water holding capacity for the test site should be measured at pF2 or 10 KPa. At the Ontario study site a soil water holding capacity of 22.5% was measured at pF2.5. Therefore the applicant proposed the use of the default value of 27% as detailed in the FOCUS (2014) guidance for the sandy loam soil present at this trial site. HSE consider this is an acceptable approach based upon the pF2.5 measurement presented and in the absence of a measured value at pF2. The levels of inpyrfluxam determined throughout the trial as adjusted for normalised day length are presented in Table B.8.1.2.1.1-06

**Table B.8.1.2.1.1-06 Residues of inpyrfluxam in soil at 0-15 cm depth from the Branchton, Ontario field site (mg/kg)**

<b>Days after second treatment<sup>a</sup></b>	<b>Days after zero time point for degradation analysis</b>	<b>Normalised days</b>	<b>Plot</b>	<b>Depth (cm)</b>	<b>Inpyrfluxam (mg/kg)</b>
7	0	0	A	0-15	0.0243
			B	0-15	0.0257
			C	0-15	0.0696
14	7	9.3	A	0-15	0.0233
			B	0-15	0.0336
			C	0-15	0.0188
21	14	17.2	A	0-15	0.0307
			B	0-15	0.0166
			C	0-15	0.0276
31	24	27.6	A	0-15	0.0312
			B	0-15	0.0153
			C	0-15	0.0277
59	52	55.6	A	0-15	0.0152
			B	0-15	0.0106
			C	0-15	0.0290
89	82	83.6	A	0-15	0.0120
			B	0-15	0.0244
			C	0-15	0.0326
119	112	101.3	A	0-15	0.0136
			B	0-15	0.0138
			C	0-15	0.0266
367	360	161.6	A	0-15	0.0108

Days after second treatment <sup>a</sup>	Days after zero time point for degradation analysis	Normalised days	Plot	Depth (cm)	Inpyrfluxam (mg/kg)
			B	0-15	0.0115
			C	0-15	0.00571
419	412	211.6	A	0-15	N.D.
			B	0-15	0.0134
			C	0-15	0.00803
489	482	266.7	A	0-15	0.00779
			B	0-15	0.00645
			C	0-15	0.00813

<sup>a</sup>the sampling date of 7 days after the second treatment is determined as day zero for the purposes of assessing compound degradation

Based upon the above data the result of the kinetic fitting for inpyrfluxam degradation at the Ontario site was proposed by the applicant as DT<sub>50</sub> of 104 days (at reference conditions of 20°C and pF2). HSE validation of the fitting using day seven sampling date post the second application to soil also indicated a DT<sub>50</sub> of 104 days with SFO kinetics. The outputs are detailed in Table B.8.1.2.1.1-07 and Figure B.8.1.2.1.1-08

The applicant rated the fits using the following scale:

- Not acceptable: the fitted curve does not represent the trend of the data points and residuals show strong deviations from random distribution.
- Acceptable: the fitted curve describes the trend of the data points, residuals may show some deviations from random distribution but it is not significant.
- Good: the fitted curve closely follows all the data points (limited scatter of data points); residuals are randomly distributed (no bias of residuals).
- Very good: no bias of residuals or scatter of data points.

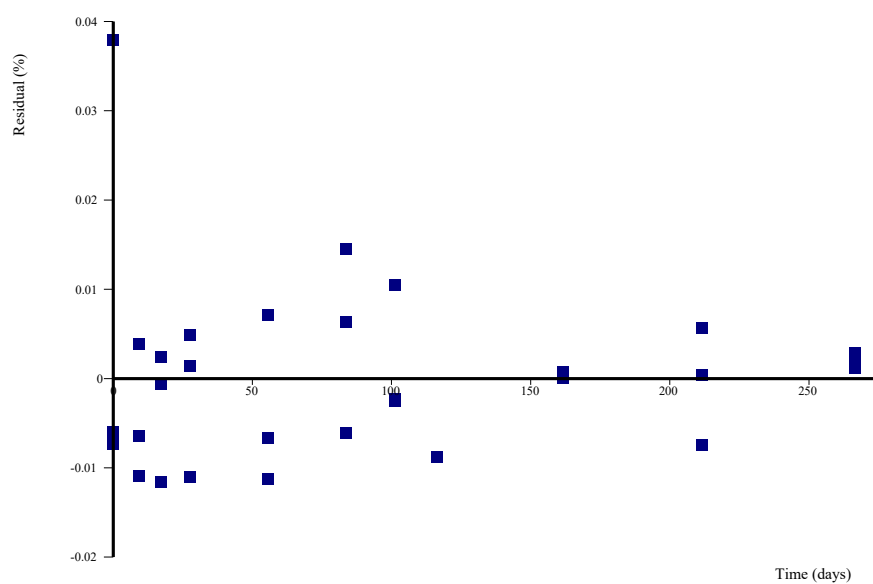
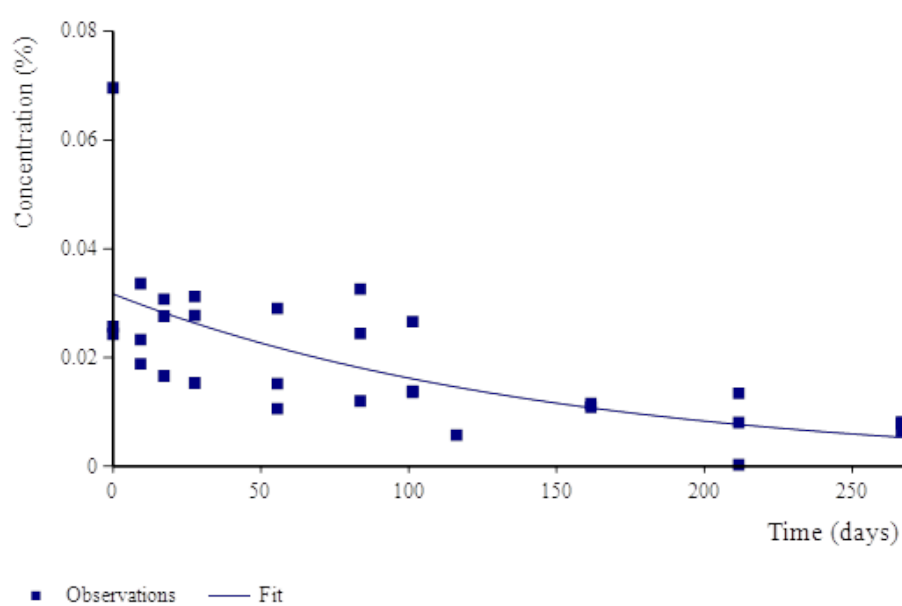
HSE has also followed these definitions in its own, independent assessment.

**Table B.8.1.2.1.1-07 Results of the Kinetic determination for inpyrfluxam from the Ontario field site data when normalised to 20°C and pF2.**

Parameter	Compound inpyrfluxam
Model	SFO



Visual fit	acceptable
DT <sub>50</sub> (d)	104
DT <sub>90</sub> (d)	344
$\chi^2$ error (%)	19.5
k (days <sup>-1</sup> )	0.00669
P value	7.37E-04



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**Figure B.8.1.2.1.1-06 Observations and fitted model with residual plot**

It is noted that the initial value for plot C is a significantly higher value than the other two replicates. However the residual plot indicates no systematic deviation for the SFO model and all other points fit well. The  $\chi^2$  error exceeds 15 but the p value is <0.05 and the visual fit is acceptable. Therefore it is considered the SFO fit is acceptable and the normalised degradation endpoints proposed for inpyrfluxam are accepted for modelling purposes.

**Consideration of non-normalised degradation for inpyrfluxam**

The applicant has presented kinetic fitting of the non-normalised data for the purposes of determining trigger and persistence endpoints for inpyrfluxam from the data at the Ontario site. They have used the FOCUS Kinetics Guidance 2014 and fits generated with CAKE 3.7 software and IRLS fitting. Determination of metabolite dissipation was not made due to low levels determined. HSE agrees with this approach.

HSE has validated this assessment using the data from zero DALA as detailed in Table 8.1.2.1.1-07 below. HSE considered SFO, FOMC and DFOP and HS fitting for the Ontario site data. The outputs are detailed in Table 8.1.2.1.1-08 below with the visual fits and residual plots detailed in Figure 8.1.2.1.1-07.

**Table 8.1.2.1.1-08 Residues of inpyrfluxam in soil at 0-15 cm depth from the Branchton, Ontario field site (mg/kg)**

Days after second treatment	Plot	Depth (cm)	Inpyrfluxam (mg/kg)
0	A	0-15	0.0541
	B	0-15	0.0659
	C	0-15	0.0730
1	A	0-15	0.0335
	B	0-15	0.0795
	C	0-15	0.0431
7	A	0-15	0.0243
	B	0-15	0.0257
	C	0-15	0.0696
14	A	0-15	0.0233
	B	0-15	0.0336
	C	0-15	0.0188
21	A	0-15	0.0307
	B	0-15	0.0166
	C	0-15	0.0276
31	A	0-15	0.0312
	B	0-15	0.0153
	C	0-15	0.0277
59	A	0-15	0.0152

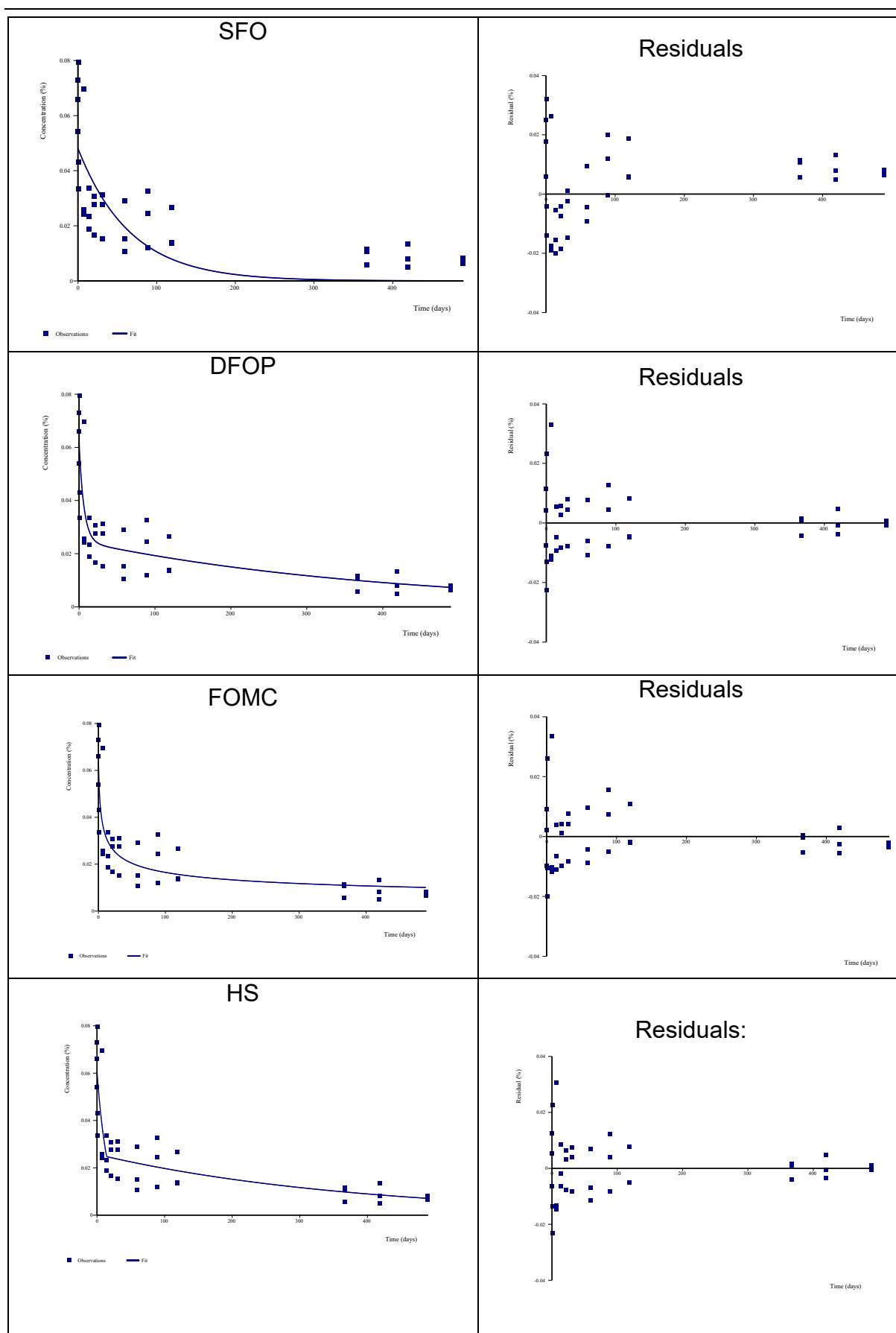
Days after second treatment	Plot	Depth (cm)	Inpyrfluxam (mg/kg)
89	B	0-15	0.0106
	C	0-15	0.0290
	A	0-15	0.0120
119	B	0-15	0.0244
	C	0-15	0.0326
	A	0-15	0.0136
367	B	0-15	0.0138
	C	0-15	0.0266
	A	0-15	0.0108
419	B	0-15	0.0115
	C	0-15	0.00571
	A	0-15	0.005
489	B	0-15	0.0134
	C	0-15	0.00803
	A	0-15	0.00779
	B	0-15	0.00645
	C	0-15	0.00813
	A	0-15	0.00779

**Table B.8.1.2.1.1-09 Kinetic fittings for inpyrfluxam using non-normalised data from the Ontario site for the determination of triggering endpoints**

Parameter	Ontario Site			
Model	SFO	DFOP	FOMC	HS
$\chi^2$ error (%)	28.6	7.81	9.05	7.59
k (days <sup>-1</sup> )*	0.0151 (6.14x10 <sup>-4</sup> )		0.06377 alpha: 0.313 beta: 1.34	
k <sub>1</sub> *(days <sup>-1</sup> )	-	0.156 (0.03)	-	0.0627 (3.2E-04)
k <sub>2</sub> *(days <sup>-1</sup> )	-	0.00248 (0.024)	-	0.00263 (0.0161)
G	-	0.598	-	-
t <sub>b</sub>	-	-	-	14.26
Statistical fit	Poor	Good	Poor	Good
Visual fit	Poor	Good	Acceptable	Good
DT <sub>50</sub> (d)**	45.8	<b>10.9</b> (279)	11	11.1 (264)
DT <sub>90</sub> (d)	152	560	2120	550

\*P value from the t-test is given in brackets.

\*\* values in brackets represent slow phase.



### Figure 8.1.2.1.1-07 Visual-fits and residual plots of degradation of inpyrfluxam at the Ontario field site

The SFO fitting is visually unacceptable and the fitting for the FOMC is also poor with the indicated DT<sub>90</sub> predicted significantly beyond the study duration. DFOP and HS fits were comparable, and give the statistical fits with the lowest  $\chi^2$  errors and supporting visual fits with residuals evenly placed. HSE accepts the overall DT<sub>50</sub> of 10.9 days and DT<sub>90</sub> of 560 d determined by DFOP fitting as proposed by the applicant as the best fit for the determination of persistence and triggering endpoints at the Ontario site.

#### B.8.1.2.1.2 EU field dissipation studies

<b>Data Point:</b>	KCA 7.1.2.2.1./07
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2018
<b>Report Title:</b>	Soil dissipation study after application of S-2399 at four different locations in Europe – 2016/2018
<b>Study number</b>	267-2016
<b>Guideline(s) followed in study:</b>	<p>Regulation (EC) N°1107/2009 of 21 October 2009 (Repealing the Council Directive 91/414/EEC) concerning the placing of plant protection products on the market</p> <p>Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market</p> <p>“EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT<sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil”, EFSA European Food Safety Authority (2014), EFSA Journal 2014;12(5):3662.</p> <p>NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies, Regulatory Directive DIR2006-01, March 2006.</p> <p>US EPA (2008), OPPTS 835.6100: Terrestrial field dissipation</p>

	Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 - SANCO/3029/99 rev.4, 11 July 2000
<b>GLP?</b>	Yes

<b>Data Point:</b>	KCA 7.1.2.2.1/07
<b>Report Author:</b>	██████████ and ██████████
<b>Report Year:</b>	2018
<b>Report Title:</b>	Normalisation of data from four field studies in Europe and determination of normalised field DT <sub>50</sub> values for S-2399 and metabolites
<b>Study number</b>	1403863.UK0-7449  TPR 0079
<b>Guideline(s) followed in study:</b>	Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2014)
<b>GLP?</b>	No - modelling
<p style="text-align: center;"><b>HSE conclusion</b></p> <p>The approach taken with regard to deriving kinetic endpoints had not followed the FOCUS guidance (2014) therefore the study is not acceptable to derive modelling endpoints for use in the exposure assessment.</p>	

<b>Data Point:</b>	KCA 7.1.2.2.1/12
<b>Report Author:</b>	██████████ and ██████████
<b>Report Year:</b>	2023

<b>Report Title:</b>	Recalculation of the field dissipation/degradation rate of S-2399 (inpyrfluxam) in soil according to FOCUS Kinetics Guidance.
<b>Study number</b>	20063620.UK0 – 8865
<b>Guideline(s) followed in study:</b>	Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. FOCUS. Sanco/10058/2005 version: 1.1, Date: 18 December 2014
<b>GLP?</b>	No - modelling
<p style="text-align: center;"><b>HSE conclusion</b></p> <p>The study is acceptable to derive modelling and trigger endpoints for use in the exposure assessment.</p>	

## Introduction

The decline of inpyrfluxam was studied at 4 sites in Northern and Southern Europe following a single application of the formulated product to bare soil. The sites were located in Germany, Czech Republic, Italy and Spain and were chosen as typical of commercial agricultural fields in these countries and of the sites where the active substance will be used. Soil cores were analysed for residues of inpyrfluxam and its metabolites over a period of 2 years.

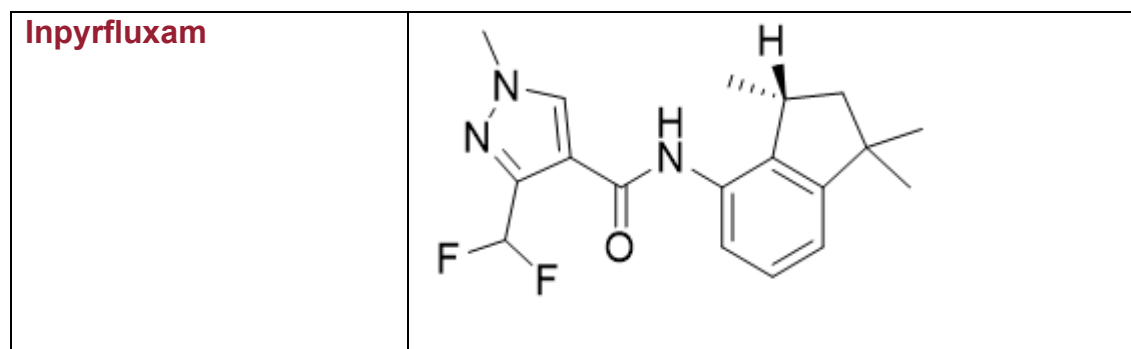
## MATERIALS AND METHODS

### Test item

<b>Formulation name</b>	<b>S-2399 40 SC</b>
Main use	Fungicide
Formulation type	SC
Active ingredient	Inpyrfluxam

Chemical name	<i>N</i> -[(3 <i>R</i> )-2,3-dihydro-1,1,3-trimethyl-1 <i>H</i> -inden-4-yl]-1-methyl-3-(difluoromethyl)-1 <i>H</i> -pyrazole-4-carboxamide  3-(difluoromethyl)- <i>N</i> -[( <i>R</i> )-2,3-dihydro-1,1,3-trimethyl-1 <i>H</i> -inden-4-yl]-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide (IUPAC)
CAS number	1352994-67-2
Nominal concentration	40 % w/v, 400 g/L
Nominal density	1.1g/mL as mentioned in the SDS
<b>Batch number</b>	<b>AJ11-10L601</b>
Sumitomo Code Recipe	AJ11-10
Expiry date	June 16, 2018
Actual concentration ( <b>total isomer content</b> )	38.61 % +/- 0.32 w/w or 386.1 +/- 3.2 g/kg or 422.3 +/- 3.5 g/L
Actual concentration ( <b>inpyrfluxam content (calculation)</b> )	37.31 % +/- 0.31 w/w or 373.1 +/- 3.1 g/kg or 408.1 +/- 3.4 g/L
Actual density	1.0938 g/mL
Certificate of analyses	SUMITOMO / FO 24239 / Ch.6547 / 2016 B dated 15/07/2016

### Test substance





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**Test system**

The location of the sites is shown in Figure B.8.1.2.1.2-01 below.



**Figure B.8.1.2.1.2-01 Geographic location of the trial sites (see Table B.8.1.2.1.2-01 for key to trial site codes)**

The selected sites were not liable to erosion or flooding, were not stony or overshadowed by trees and had no appreciable slope. The sites had not been cultivated for crops in several years and had not been treated with any of the SDHI family of products (boscalid, fluxapyroxad, bixafen, penthiopyrad, penflufen, sedaxane, isopyrazam and benzovindiflupyr) in the years 2013-2016 or soil disinfectants within the previous 3 years.

Soils were characterised to a depth of 30 cm; this is considerably less than the 1.5 m depth recommended by the OECD Guideline, but as residues remained almost exclusively in the top 30 cm, this is considered acceptable in this case.

**Table B.8.1.2.1.2-01 Chemical and physical characterisation of the soils used in the study**

<b>Country (Region)</b>	<b>Germany (North Rhine-Westphalia)</b>	<b>Czech Republic (Stredni Morava)</b>	<b>Italy (Emilia Romagna)</b>	<b>Spain (Ourense)</b>
Corresponding field trial number Of the present study	267-2016 GE01	267-2016 CZ02	267-2016 IT03	267-2016 SP04
GPS coordinates				
Altitude (m)	17	180	8	624
Sample Reference (*)	BIO F3	ATC F6	AGR F7	TRI F5
pH (water)	6.5	7.9	8.1	5.0
pH (0.01M CaCl <sub>2</sub> )	6.0	7.4	7.5	4.4
Organic Matter %	3.29	3.87	1.39	4.42
Organic Carbon %	1.91	2.24	0.81	2.57
Cation Exchange Capacity meq/100g	17.4	27.6	9.2	16.3
Water Holding Capacity at pF2 %	37.7	44.1	21.7	44.7
Sand %	50	12	63	43
Silt %	24	40	21	37
Clay %	26	48	16	20
Textural Class USDA Classification	Sandy Clay Loam	Clay/Silty Clay	Sandy Loam	Loam
Microbial biomass (mg C/kg)**				
Prior to application (7 to 0 days before)	957	846	250	282
365 days (± 7 days) after application	386	700	89	245

730 days ( $\pm$ 14 days) after application	497	472	198	263
% Biomass carbon as % TOC**				
Prior to application (7 to 0 days before)	3.06	3.69	5.10	1.01
365 days ( $\pm$ 7 days) after application	2.80	2.87	1.60	0.97
730 days ( $\pm$ 14 days) after application	1.71	2.06	3.30	1.10

All results on a dry soil basis

(\*) Sample Reference of the study CEMS-7522

(\*\*) On a dry soil basis

**Table B.8.1.2.1.2-02 Previous use of the trial sites**

	<b>Crop (2015)</b>	<b>Field status at application (2016)</b>
Germany	Grass (since 2013)	Bare soil
Czech Republic	Alfalfa (since 2013)*	Bare soil
Italy	Alfalfa (since 2012)	Bare soil
Spain	Potato (2015)*	Bare soil

\*Also see Table B.8.1.2.1.2-03 below

No fertilizers were applied at any site and no irrigation was used. Previous pesticide use was supplied for all 4 trial sites.

- At Trial site 1 (Germany), no plant protection products were applied between 2013 and the start of the trial. From June 2016 through to May 2018 glyphosate containing products were applied on 10 occasions, presumably to clear the site prior to application and to keep the plots clear of weeds during the trial. 'Summax' a product containing flumioxazin was also applied on one occasion in March 2017; this is also a herbicide.
- At Trial site 3 (Italy), no plant protection products were applied between 2012 and 2015. Glyphosate containing products were applied on 12 occasions between June 2016 and July 2018.

- The pesticide histories of plots 2 (Czech Republic) and 4 (Spain) were more complicated. The histories of these plots are summarised below.

**Table B.8.1.2.1.2-03 Site use and plant protection products applied at sites 2 and 4 (Czech Republic and Spain)**

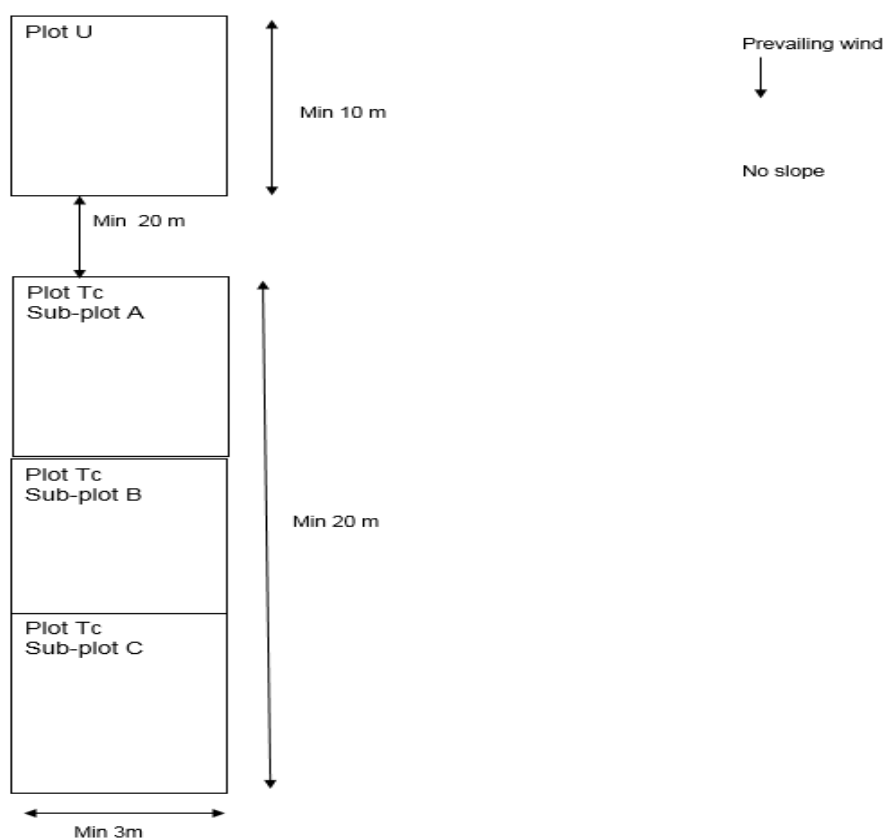
Year	Crop	Active Substance
Trial site 2 – Czech Republic		
2010	Winter wheat	Aminopyralid, florasulam, pyroxsulam 50 g/kg, chlormequat-chloride, quinoxifen, prochloraz, propiconazole, prothioconazole, tebuconazole
2011	Sugar beet	Desmedipham, phenmedipham,  Ethofumesate, propaquizafop, clopyralid, metamitron, desmedipham, phenmedipham, triflusal, trifluralin-methyl, desmedipham, phenmedipham, chloridazon, propaquizafop, flusilazole, carbendazim, flutriafol, thiophanate methyl, chlorpyrifos, cypermethrin
2012	Spring barley	Trinexapac-ethyl, 2,4-D, aminopyralid, florasulam, fenpropidin, propiconazole, pinoxaden, cypermethrin, cyproconazole, propiconazole, pinoxaden, cypermethrin, cyproconazole
2013	Alfalfa	None
2014	Alfalfa	Thiacloprid
2015	Alfalfa	Thiacloprid, diquat
2016	Bare soil	Glyphosate
2017	Bare soil	Glyphosate
2018	Bare soil	Glyphosate
Trial site 4 - Spain		
2011	Potato	Chlorpyrifos, linuron, cymoxanil - folpet - fosetyl aluminium, mancozeb + dimethomorph 695 wp, cymoxanil + mancozeb, dibromide
2012	Wheat	Pyrethrin

2013	Potato	Chlorpyrifos, linuron, cymoxanil - folpet - fosetyl aluminium, mancozeb + dimethomorph, cymoxanil + mancozeb, dibromide
2014	Wheat	Pyrethrin
2015	Potato	Chlorpyrifos, linuron, cymoxanil - folpet - fosetyl aluminium, mancozeb + dimethomorph, cymoxanil, dibromide
2016	Not applicable	Glyphosate
2017	Not applicable	Glyphosate
2018	Not applicable	Glyphosate-ammonium, glyphosate

Prior to application, at  $365 \pm 7$  days and at  $730 \pm 7$  days after application samples were tested for moisture and dry matter, microbial biomass (as mg/kg and as % of the total organic carbon of fresh soil), for organic matter and organic carbon content and for response to substrate glucose addition (determination of microbial biomass carbon by substrate induced respiration). For the soil microbial biomass, soil (4.0 kg) was sampled from a depth of 0-30 cm from 10 different areas on each occasion using an auger or a spade or shovel. Soil specimens were collected into polythene bags and kept at ambient temperature but not frozen and shipped to the analytical laboratory within 48-72 h.

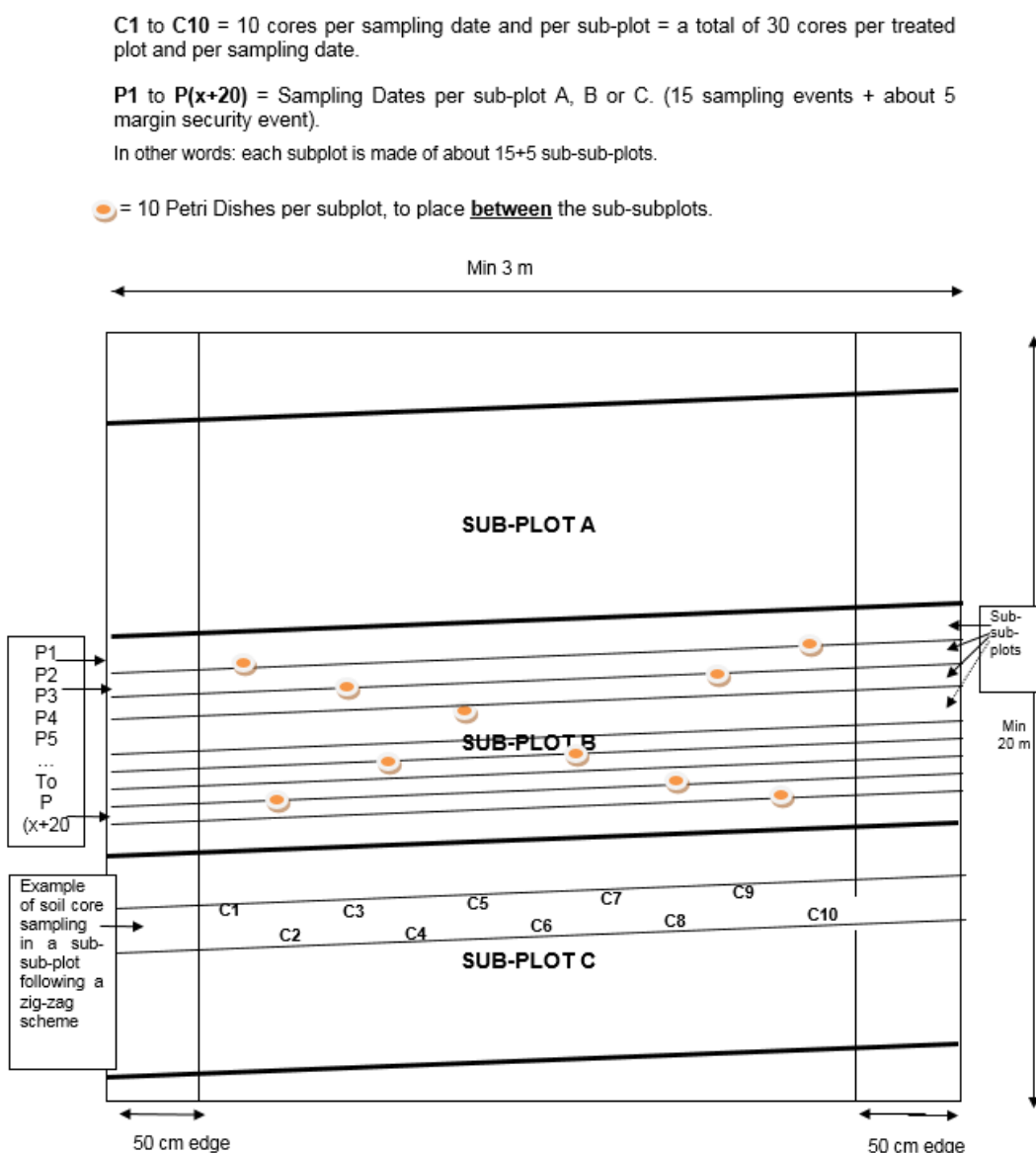
The sites were mechanically cleared of all vegetation and subsequently kept free of weeds via herbicide application at recommended commercial rates. Each trial site was divided into one main untreated plot (U) and one treated plot (Tc); the treated plot was divided into 3 sub-plots (TcA, TcB and TcC). Each of the sub-plots was then divided by the number of scheduled sampling events (sub-sub-plots). Ten soil cores were collected at each sampling date. The untreated plot was separated from the treated plots by a buffer zone  $\geq 20$  m to prevent contamination from application of the test item. The design of the study sites is shown below:

X



**Figure B.8.1.2.1.2-02 Geographic location of the trial sites (see Table B.8.1.2.1.2-01 for key to trial site codes)**

Plots were 3 x 20 m, divided into 3 sub-plots; this compares with the recommended typical plot size from the OECD Guideline of 4 x 10 m. The plot size is therefore considered to be acceptable.



### Application of test item

#### Figure B.8.1.2.1.2-03 Setup of a treated plot

Applications were made between 20 May and 02 June 2016. Prior to application, the surface of each plot was raked or hoed to produce a fine level tilth at about 5-10 cm depth. In spring 2016, the test item was uniformly applied to bare soil on the treated plots using a hand-carried boom sprayer to closely simulate a broadcast commercial-type treatment. Each boom had 6 flat fan nozzles with spacing of 50 cm. The sprayer equipment was calibrated before use using the volume/time method to deliver the desired spray volume per hectare. The target rate was 200 g a.s./ha (0.5 L formulated product/ha) in a spray volume of 200 L water/ha. The contents of the spray tank was sampled (10 mL) both just before application and just after application to ensure homogeneity; samples were immediately frozen in dry ice and shipped at  $\leq -18$  °C. The application rate was also checked by means of 10 petri

dishes per sub-plot containing sieved soil ( $20 \pm 2$  g) placed on the soil surface. Petri dishes were also placed between the sub-sub-plots to ensure that further soil samplings were not interfered with. Petri dishes were opened just before application and then closed and collected immediately following application and placed in polyethylene bags before being packed with gel packs or dry ice and transferred to freezer storage within 3 h. Petri dishes were subsequently shipped frozen ( $\leq -18$  °C).

Environmental conditions at the time of application, including air temperature, wind speed and direction, relative humidity, percentage cloud cover, soil temperature at 10 cm depth, rainfall within 24 h of application and soil surface moisture were recorded. Applications were conducted with a windspeed of  $<3$  m/s. There was no rainfall within 24 h of application at the Czech Republic, Italy and Spain sites. Rainfall of 0.8 mm occurred at the Germany site approximately 1 h after application and after incorporation of the test item into the soil.

After application, the test item was incorporated into the soil to a depth of 7 cm. It is stated that the test item was incorporated 'just after application'. HSE consider that this incorporation was sufficient to meet the EFSA DegT<sub>50</sub> guidance recommendation to minimise the impact of surface processes.

### *Soil cores*

Cores 5 cm in diameter were taken from both treated and control plots using metal soil corers with acetate tubes. Cores were taken just prior to application of the test substance. Cores were taken from 0-30 cm depth from prior to test substance application until 14 days, 0-50 cm depth for time points from 30-60 days and from either 0-90 cm or 0-100 cm from 90 days until 730 days. Ten cores were taken from the untreated plot just before test substance application, on day 365 and on day 730, while ten cores were taken from plots Tca, TcB and TcC at all timepoints; thus 30 cores were taken from each treated plot per sampling date.

Zero contamination soil sampling equipment with acetate tubes was used for collecting soil cores. Cores were not taken from areas where sampling had already occurred and holes in the treated plot were filled with soil collected from uncontaminated areas to prevent wash off of treated soil to lower depths. The following quality control measures were taken:

- Locating untreated plots at least 20 m from treated plots.
- Collecting specimens from the inner part of plots.
- Untreated specimens were collected first and stored separately from treated cores.
- Cleaning sampling equipment carefully after use.
- Using gel packs or dry ice to transport specimens.
- Wearing disposable gloves.
- Transporting and storing untreated and treated samples separately.



Cores were frozen within 12 h of collection and shipped frozen ( $\leq -18^{\circ}\text{C}$ ) to the analytical laboratory. Gel packs were used for transportation when the journey time exceeded 2 h. Specimens were stored at  $\leq -18^{\circ}\text{C}$  in the dark after their arrival at the laboratory.

#### *Preparation and analysis of specimens*

Samples were processed in order of expected lowest concentration to minimise contamination, so control specimens were processed first, followed by specimens from the later timepoints, proceeding to the earliest time points. Deeper horizons were also processed first. The total weight of the 10 cores from each sub-plot was recorded. Frozen soil cores were sliced into 10 cm layers whilst still frozen and each layer homogenised by milling and sieving (2 mm) with dry ice. Samples were stored deep frozen ( $\leq -18^{\circ}\text{C}$ ) and time outside of the freezer minimised to <60 minutes.

Soil from petri dishes were transferred to suitable containers, weighed and the diameter of petri dishes measured. Soil was homogenised by shaking ( $\geq 30$  min; 300 rpm).

Spray solutions were thawed and homogenised by vortexing (1 minute). An aliquot was diluted with methanol/water (80/20 (v/v)).

#### *Stock, fortification and standard solutions*

Stock solutions were prepared by dissolving an analyte (approximately 1000  $\mu\text{g/mL}$  after correction for purity) in methanol (10 mL). Separate stock solutions were prepared for parent and each metabolite. Stock solutions were further diluted for use in the procedural recovery process and as intermediate standard solutions for subsequent use as solvent calibration solutions and preparation of matrix-matched calibration solutions. Matrix-matched calibration solutions were prepared using final sample extracts of control (untreated) samples of soil which were spiked with solvent standard solutions. Stock and fortification solutions were stored refrigerated at 1-10  $^{\circ}\text{C}$  in brown glass vials under dark conditions. The weights of the analytes and solution volumes used are shown in Table B.8.1.2.1.2-04 below.

**Table B.8.1.2.1.2-04 Preparation of stock solutions**

Purity of reference item (%)	Weighed amount of reference item (mg)	Amount of analyte after correction for purity (mg)	Final volume (mL)	Equivalent concentration ( $\mu\text{g/mL}$ )
Inpyrfluxam				
99.9	10.3	10.29	10	1029
99.9	10.2	10.19	10	1019
99.9	10.8	10.79	10	1079

99.9	10.7	10.69	10	1069
99.9	10.5	10.49	10	1049
99.9	10.4	10.39	10	1039
99.9	10.8	10.79	10	1079
99.9	10.5	10.49	10	1049
99.9	10.5	10.49	10	1049
3'-OH-S-2840				
99.5	10.1	10.05	10	1005
99.5	10.4	10.35	10	1035
99.5	10.5	10.45	10	1045
99.5	10.7	10.65	10	1065
99.5	11.0	10.95	10	1095
99.5	10.6	10.55	10	1055
99.5	10.8	10.75	10	1075
99.5	10.4	10.35	10	1035
1'-COOH-S-2840A				
99.8	5.4	5.39	10	539
99.8	10.3	10.28	10	1028
99.8	10.2	10.18	10	1018
99.8	10.6	10.58	10	1058
99.8	10.1	10.08	10	1008
99.8	10.4	10.38	10	1038
99.8	10.3	10.28	10	1028
99.8	10.3	10.25	10	1028
1'-COOH-S-2840B				
99.5	10.9	10.85	10	1085
99.5	11.0	10.95	10	1095
99.5	10.1	10.05	10	1005
99.5	10.2	10.15	10	1015
99.5	10.8	10.75	10	1075
99.5	10.7	10.65	10	1065
99.5	10.6	10.55	10	1055
99.5	10.3	10.25	10	1025
99.5	10.5	10.45	10	1045
99.5	10.1	10.05	10	1005

Standards were stored at -18 °C.

#### *Analytical method*

Soil samples were prepared for biomass determination and sub samples taken for dry matter and organic matter. Specimens for organic matter were air dried (<32 °C)

and sieved (2 mm) and a sub-sample sieved again (0.5 mm). The dry matter and moisture content of specimens submitted for soil biomass analysis were determined using the CEMAS method and the water content determined using the gravimetric method by loss on drying at 105 °C. Soil microbial biomass was determined using substrate-induced respiration.

Soil samples (5.0 g) were extracted (x2) with acetone/water (12.5 mL; 4/1, v/v) mixture and agitated on a horizontal flatbed shaker (10 min) and centrifuged (4000 rpm; 5 min), followed by a further extraction with acetone/ 0.5 M HCl (12.5 mL; 4/1, v/v) and the samples agitated again on the flatbed shaker (10 min). Care was taken not to agitate the samples for longer than 10 minutes due to the acid instability of 3'-OH-S-2840. Aqueous 5 M NaOH (1 mL) was immediately added and the sample shaken by hand. Extracts were filtered through celite and the combined supernatants adjusted to 50 mL with acetone/water (80/20, v/v) as required. An aliquot (10 mL) of the acetone/water HCl phase was shaken on a horizontal flatbed shaker (10 mins) whilst being extracted with ethyl acetate (32 mL) and solid NaCl added and the samples shaken (2 mins). Na<sub>2</sub>SO<sub>4</sub> was added and the samples agitated again (2 min). An aliquot of the resulting supernatant (20 mL) was evaporated to dryness on a rotary evaporator (40 °C). The dried residues were reconstituted with LCMS grade (MeOH (0.125 mL) followed by HPLC grade water (0.375 mL), using an ultrasonic bath to aid dissolution.

Samples were analysed by LC-MS/MS resulting in LOQ values of 0.002 mg/kg for inpyrfluxam and 3'-OH-S-2840 and for COOH-S-2840A and 1'-COOH-S-2840B in soil with a LOQ of 0.001 mg/kg (all LOQ values are on a wet soil basis). A second method was also used in the study for which the LOQ values were the same. The LOQ value for inpyrfluxam represents 4.55 %, which is <5 % of the nominal applied amount and therefore acceptable (200 g a.s./ha is 0.044 mg/kg over a depth of 30 cm, assuming a bulk density of 1.52 g/cm<sup>3</sup>). The LOQ values for 3'-OH-S-2840 and for COOH-S-2840 represent 2.2 and 2.1 % of the nominal active substance concentration on a molar basis. Limit of detection (LOD) was set for 0.0004 mg/kg for inpyrfluxam and 3'-OH-S-2840 and 0.001 mg/kg with a limit of detection (LOD) of 0.0002 mg/kg for 1'-COOH-S-2840A and 1'-COOH-S-2840B (based on wet soil basis) for both methods. The second method was needed as the column was exchanged for another in order to obtain more reproducible peak shapes and recoveries. The methods were validated (see KCA 4.1.2/03).

With the exception of the petri dish samples, results were not corrected for recoveries. For petri dish samples, both corrected and uncorrected results were presented by the applicant.

Soil samples were calculated and reported in terms of dry matter and samples in petri dishes in terms of wet matter.

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### *Storage stability*

Soil cores, petri dishes and spray solution samples were stored at ( $\leq -18$  °C for a maximum of 679, 187 and 181 days respectively from sampling until extraction. The storage stability of inpyrfluxam and metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B under frozen conditions in soil over a period of 24 months was investigated in a separate study (KCA 7.1.2.2.1/10). Storage stability was demonstrated under these conditions, covering the duration and conditions of storage for samples in the current study.

In validation study S16-05522 (TPA-0043 (KCA 4.1.2/03)) it was shown that analytes in the extracts were not stable for 8 days when stored refrigerated (1-10 °C) in the dark. Based on the high variation of stabilities between analytes and different soils, it was recommended that samples be analysed within 24 h if possible. The maximum duration of storage of final sample extracts was 1 day at 1-10 °C. It is therefore considered that stability has been adequately demonstrated over the conditions and duration of storage used for the samples.

A deviation was observed in that a maximum temperature of -7 °C was recorded for c.8 h for the soil specimens due to an electric failure in the laboratory. This was addressed via a storage stability study in which soil specimens were stored at -7 °C for 7 days. The stability of the analytes was demonstrated during this period. It is therefore accepted that the deviation did not affect the outcome of the study.

## **RESULTS AND DISCUSSION**

### *Deviations*

The applicant has listed several deviations from the study plan. These include 3 occasions where the temperature during shipment of specimens was higher than -18 °C, reaching as high as -12 °C for up to 76 hours. The applicant reported that the samples remained hard frozen and concluded there was no impact on the study. HSE agrees with this conclusion. In addition, the applicant reported that the temperature of a laboratory freezer containing some samples was at -7 °C for a period of 28 h, which is above the intended temperature of -18 °C. The applicant arranged for a storage stability study to demonstrate stability of the samples at -7 °C for a period of 7 days. As the storage stability study demonstrated stability of residues under these conditions, the applicant concluded that the deviation had no adverse impact on the study. HSE agrees with this conclusion.

At the Italy site, another trial was also being conducted with inpyrfluxam. The applicant reports that the distance between the current trial and the neighbouring trial was 11.5 m, which is less than the 20 m stated in the study plan. The applicant concluded that a buffer of >10 m was sufficient and that there was therefore no impact on the study plan. The Rautmann spraydrift value for a distance of 10 m for a

single application to field crops is 0.27 %, which implies that a small amount of contamination from the adjacent trial is possible. The applicant has been asked to justify why the distance between the two trials is sufficient and gave the following response:

*'Given the drift percentage of 0.27 %, unless the adjacent trial were performed at significantly elevated application rates any deposition from that adjacent trial would have a negligible impact on any decline data being substantially lower than the variability observed in the field study and there is no evidence within the study that additional inputs from an adjacent trial occurred. In several locations in the study report it is stated that the selected sites were required to have, among other required criteria, not been treated with SDHI family products (boscalid, fluxapyroxad, bixafen, penthiopyrad, penflufen, sedaxane, isopyrazam and benzovindiflupyr ...) in 2013, 2014, 2015 and 2016. To demonstrate this, all treated and untreated control plots at all sites were sampled just prior to application on the same day as the application. No residues of parent or its metabolites were detected in the untreated specimens analysed as part of the study; hence, any unintended inputs from an adjacent trial prior to application were negligible and had no impact on the results of the study. Since the first application of the neighboring trial 268-2016 IT03 occurred just before the start of the 267-2016 IT03 trial in this study any deposition from the neighbouring trial would have been detected in the samples taken just prior to application. Furthermore, following the reported deviation a screen was later used for the application on the trial 268-2016 IT03 and additional TQA auditor was on site at the first application in each trial. An additional TQA audit was performed at the second application in trial 268-2016 IT03 with the SD as well (allowing to say that additional precautions were taken to have no cross-contamination).'*

*Control samples collected from untreated control plots at 365 and 730 days after application (DAA) also demonstrated all residues for parent and metabolites to be <LOD. Again, if there were any residues input from an adjacent trial, they were so low to have not been detected and therefore had no impact on the study results.*

*This is further supported by the kinetic evaluation performed for all four sites in the study of [REDACTED] and [REDACTED] (2018b; Report No: 1403863.UK0-7449) which demonstrated a good statistical fit to the data with a chi-squared error value of 14.9 for SFO kinetics. Although a large scatter of data was observed in the visual fit, this is common to several field trials and the visual fit demonstrated that the scatter of data was also random around the SFO decline curve.'*

It is agreed that the control plot samples have been well characterised and that there is no evidence of contamination from treated plots and the distance between the treated and control plots has had no effect on the outcome of the study.

The applicant reports that in the fortification samples, single recoveries were not in the range of 70 - 110 % but 70 - 120 % each, though mean recoveries at each fortification level were in the range of 70 - 110 %. HSE has examined the recovery data and notes that only occasional replicates are outside of the 70-110 % range. As only a very small number of replicates are >110 % and always <120 %, it is agreed that this deviation has not affected the outcome of the study.

#### *Application rate*

The target application rate was 200 g a.s./ha, equivalent to 0.5 L product/ha (400 g a.s./L). This is higher than the proposed application rate of 90 g a.s./ha but is accepted as HSE considers it unlikely that the degradation rate was influenced by concentration over this range, and noting that a higher rate makes it easier to analyse for parent and metabolites. The applicant reports that the actual application rate varied between 0.475 and 0.503 L product/ha. The application rate was verified by analysing the spray solutions and also by means of petri dishes containing soil placed on the treated plots. The results from both of these analyses are presented below.

**Table B.8.1.2.1.2-05 Analytical results for the concentration of inpyrfluxam in the spray solutions**

	<b>Timing</b>	<b>Inpyrfluxam (g/L)</b>	<b>Inpyrfluxam (% of target)</b>
Germany	Before application	0.759	76
	After application	0.706	71
Czech Republic	Before application	0.568	57
	After application	0.745	75
Italy	Before application	0.692	69
	After application	0.659	66
Spain	Before application	0.659	66
	After application	0.526	53

**Table B.8.1.2.1.2-06 Analytical results of the concentration of inpyrfluxam in the petri dishes**

	<b>Plot</b>	<b>Residue (mg/kg based on wet soil)</b>	<b>Residue (g/ha based on wet soil)</b>	<b>% of 200 g/ha target</b>
Germany	TcA	6.32	184	92
	TcB	5.89	171	86
	TcC	6.18	171	86
Czech Republic	TcA	4.75	149	75
	TcB	6.62	208	104
	TcC	6.27	197	99
Italy	TcA	9.58	199	100

Spain	TcB	7.60	157	79
	TcC	9.86	203	102
	TcA	8.93	266	133
	TcB	8.10	241	121
	TcC	7.13	214	107

Data corrected for daily recoveries on level 5 mg/kg or 10 mg/kg

The results from the spray solution analysis indicate that between 53 and 76 % of the target amount was present in the spray solution. This would amount to an application rate of 106 to 152 g a.s./ha. The results for the petri dish specimens indicate that between 75 and 133 % of the target amount was deposited on the soil surface. Overall the results are rather variable, but the petri dishes indicate a higher application rate closer to the target was sprayed.

### Soil cores

No residues were detected in the untreated specimens.

**Table B.8.1.2.1.2-07 Mean residues of inpyrfluxam and metabolites (mg/kg dry soil) for the Germany trial site**

Soil depth /timing	0 DAA	3 DAA	7 DAA	20 DAA	28 DAA	61 DAA	91 DAA
Inpyrfluxam (LOQ 0.002 mg/kg)							
0-10 cm	0.154	0.114	0.0936	0.132	0.0987	0.0755	0.0607
10-20 cm	0.00103*	0.000751*	< LOD	0.00287	0.00206	0.000942*	0.000647*
20-30 cm	0.000643*	< LOD	< LOD	0.000988*	< LOD	0.00926	< LOD
Sum	0.156	0.115	0.0936	0.136	0.101	0.0857	0.0613
	<b>179 DAA</b>	<b>270 DAA</b>	<b>359 DAA</b>	<b>448 DAA</b>	<b>543 DAA</b>	<b>629 DAA</b>	<b>728 DAA</b>
0-10 cm	0.0353	0.0395	0.0244	< LOD	0.00941	0.0127	0.00983
10-20 cm	0.000449*	< LOD	0.000566*	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.0357	0.0395	0.0250	-	0.00941	0.0127	0.00983
3'-OH-S-2840 (LOQ = 0.002 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>20 DAA</b>	<b>28 DAA</b>	<b>61 DAA</b>	<b>91 DAA</b>
0-10 cm	0.000589*	0.00167*	0.00182*	0.00638	0.00558	0.00790	0.00818
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	0.000857*	< LOD
Sum	0.000589*	0.00167*	0.00182*	0.00638	0.00558	0.00876	0.00818
	<b>179 DAA</b>	<b>270 DAA</b>	<b>359 DAA</b>	<b>448 DAA</b>	<b>543 DAA</b>	<b>629 DAA</b>	<b>728 DAA</b>
0-10 cm	0.00969	0.0106	0.00759	< LOD	0.00461	0.00633	0.00561
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00969	0.0106	0.00759	-	0.00461	0.00633	0.00561
1'-COOH-S-2840A (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>20 DAA</b>	<b>28 DAA</b>	<b>61 DAA</b>	<b>91 DAA</b>
0-10 cm	< LOD	< LOD	0.000816*	0.00259	0.0025	0.00125	0.000868*
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	-	-	0.000816*	0.00259	0.00250	0.00125	0.000868*
	<b>179 DAA</b>	<b>270 DAA</b>	<b>359 DAA</b>	<b>448 DAA</b>	<b>543 DAA</b>	<b>629 DAA</b>	<b>728 DAA</b>
0-10 cm	0.000955*	0.000417*	0.000549*	< LOD	< LOD	< LOD	0.000298*
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.000955*	0.000417*	0.000549*	-	-	-	0.000298*
1'-COOH-S-2840B (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>20 DAA</b>	<b>28 DAA</b>	<b>61 DAA</b>	<b>91 DAA</b>
0-10 cm	< LOD	< LOD	0.00146	0.00505	0.00424	0.00315	0.00194
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	0.000433*	< LOD
Sum	-	-	0.00146	0.00505	0.00424	0.00358	0.00194
	<b>179 DAA</b>	<b>270 DAA</b>	<b>359 DAA</b>	<b>448 DAA</b>	<b>543 DAA</b>	<b>629 DAA</b>	<b>728 DAA</b>
0-10 cm	0.00233	0.000987	0.0011	< LOD	0.000543*	0.000406*	0.000491*
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00233	0.000987	0.00110	-	0.000543*	0.000406*	0.000491*

\* values &lt; LOQ



Mean values were calculated with detected residues, where values < LOD were set to zero, while for values < LOQ, the detected residues was used for calculations.

**Table B.8.1.2.1.2-08 Mean residues of inpyrfluxam and metabolites (mg/kg dry soil) for the Czech Republic trial site**

Soil depth/timing	0 DAA	3 DAA	7 DAA	15 DAA	28 DAA	61 DAA	92 DAA
Inpyrfluxam (LOQ 0.002 mg/kg)							
0-10 cm	0.0967	0.149	0.113	0.125	0.0834	0.0810	0.0814
10-20 cm	0.00209	0.00110*	0.00124*	< LOD	0.00057 5*	< LOD	0.00101*
20-30 cm	0.00328	< LOD	< LOD	< LOD	0.00367	< LOD	< LOD
Sum	0.102	0.150	0.114	0.125	0.0876	0.0810	0.0824
	<b>182 DAA</b>	<b>265 DAA</b>	<b>360 DAA</b>	<b>455 DAA</b>	<b>540 DAA</b>	<b>629 DAA</b>	<b>733 DAA</b>
0-10 cm	0.0716	0.0842	0.0594	0.0441	0.0343	0.0323	0.0109
10-20 cm	0.00135*	0.00197*	0.00080 1*	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.0730	0.0862	0.0602	0.0441	0.0343	0.0323	0.0109
3'-OH-S-2840 (LOQ = 0.002 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>15 DAA</b>	<b>28 DAA</b>	<b>61 DAA</b>	<b>92 DAA</b>
0-10 cm	0.00097 7*	0.00247	0.00268	0.0023	0.00236	0.00511	0.0075
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00097 7*	0.00247	0.00268	0.0023 0	0.00236	0.00511	0.00750
	<b>182 DAA</b>	<b>265 DAA</b>	<b>360 DAA</b>	<b>455 DAA</b>	<b>540 DAA</b>	<b>629 DAA</b>	<b>733 DAA</b>
0-10 cm	0.00925	0.0122	0.0099	0.0089 5	0.00873	0.00867	0.00325
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00925	0.0122	0.00990	0.0089 5	0.00873	0.00867	0.00325
1'-COOH-S-2840A (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>15 DAA</b>	<b>28 DAA</b>	<b>61 DAA</b>	<b>92 DAA</b>
0-10 cm	< LOD	< LOD	0.00087 3*	0.0013	0.00281	0.00377	0.00377
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

Sum	-	-	0.00087 3*	0.0013 0	0.00281	0.00377	0.00377
	<b>182 DAA</b>	<b>265 DAA</b>	<b>360 DAA</b>	<b>455 DAA</b>	<b>540 DAA</b>	<b>629 DAA</b>	<b>733 DAA</b>
0-10 cm	0.00192	0.00237	0.00198	0.0022 5	0.00111	0.00051*	0.00033*
10-20 cm	0.00074 8*	0.00148	0.00041 6*	< LOD	0.00038 9*	0.00021 3*	< LOD
20-30 cm	< LOD	0.00044 6*	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00267	0.00430	0.00240	0.0022 5	0.00150	0.00072 3*	0.00033*
1'-COOH-S-2840B (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>15 DAA</b>	<b>28 DAA</b>	<b>61 DAA</b>	<b>92 DAA</b>
0-10 cm	< LOD	< LOD	0.00092 5	0.0012 3	0.00278	0.00388	0.00377
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	-	-	0.00092 5*	0.0012 3	0.00278	0.00388	0.00377
	<b>182 DAA</b>	<b>265 DAA</b>	<b>360 DAA</b>	<b>455 DAA</b>	<b>540 DAA</b>	<b>629 DAA</b>	<b>733 DAA</b>
0-10 cm	0.00222	0.00269	0.00205	0.0028 2	0.00137	0.00063 6*	0.00040 0*
10-20 cm	0.00068 4*	0.00149	0.00041 4'	< LOD	0.00050 3*	0.00037 6*	< LOD
20-30 cm	< LOD	0.00041 1*	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00290	0.00459	0.00246	0.0028 2	0.00187	0.00101	0.00040 0*

\* values < LOQ

Mean values were calculated with detected residues, where values < LOD were set to zero, while for values < LOQ, the detected residues was used for calculations.

**Table B.8.1.2.1.2-09 Mean residues of inpyrfluxam and metabolites (mg/kg dry soil) for the Italy trial site**

Soil depth/timing	0 DAA	3 DAA	6 DAA	14 DAA	28 DAA	60 DAA	89 DAA
Inpyrfluxam (LOQ 0.002 mg/kg)							
0-10 cm	0.0542	0.0375	0.0326	0.0507	0.0439	0.044	0.0334
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	0.00137 *	0.00125 *	< LOD	< LOD

Sum	0.0542	0.0375	0.0326	0.0521	0.0452	0.0440	0.0334
	<b>180 DAA</b>	<b>272 DAA</b>	<b>358 DAA</b>	<b>455 DAA</b>	<b>550 DAA</b>	<b>637 DAA</b>	<b>728 DAA</b>
0-10 cm	0.0284	0.0322	0.0238	0.0159	0.0154	0.0204	0.00877
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.0284	0.0322	0.0238	0.0159	0.0154	0.0204	0.00877
3'-OH-S-2840 (LOQ = 0.002 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>6 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>60 DAA</b>	<b>89 DAA</b>
0-10 cm	0.00088 6*	0.00055 3*	0.00096 8*	0.00167 *	0.00238	0.00252	0.0025
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00088 6	0.00055 3	0.00096 8	0.00167	0.00238	0.00252	0.00250
	<b>180 DAA</b>	<b>272 DAA</b>	<b>358 DAA</b>	<b>455 DAA</b>	<b>550 DAA</b>	<b>637 DAA</b>	<b>728 DAA</b>
0-10 cm	0.00351	0.0044	0.00401	0.00262	0.004	0.00489	0.00289
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00351	0.00440	0.00401	0.00262	0.00400	0.00489	0.00289
1'-COOH-S-2840A (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>6 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>60 DAA</b>	<b>89 DAA</b>
0-10 cm	< LOD	< LOD	< LOD	< LOD	0.00041 3*	0.00061 4*	0.00053 5*
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	-	-	-	-	0.00041 3	0.00061 4	0.00053 5
	<b>180 DAA</b>	<b>272 DAA</b>	<b>358 DAA</b>	<b>455 DAA</b>	<b>550 DAA</b>	<b>637 DAA</b>	<b>728 DAA</b>
0-10 cm	0.00064 9*	0.00020 4*	0.00026 6*	0.00023 7*	< LOD	< LOD	< LOD
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00064 9	0.00020 4	0.00026 6	0.00023 7	-	-	-
1'-COOH-S-2840B (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>6 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>60 DAA</b>	<b>89 DAA</b>
0-10 cm	< LOD	< LOD	< LOD	0.00025 5*	0.00061 0*	0.00079 2*	0.00069 3*
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	-	-	-	0.00025 5*	0.00061 0*	0.00079 2*	0.00069 3*

	<b>180 DAA</b>	<b>272 DAA</b>	<b>358 DAA</b>	<b>455 DAA</b>	<b>550 DAA</b>	<b>637 DAA</b>	<b>728 DAA</b>
0-10 cm	0.00081 9*	0.00034 2*	0.00034 4*	0.00036 1*	< LOD	< LOD	< LOD
10-20 cm	0.00021 3*	0.00029 5*	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	0.00020 5*	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00103	0.00084 2*	0.00034 4*	0.00036 1*	-	-	-

\* values < LOQ

Mean values were calculated with detected residues, where values < LOD were set to zero, while for values < LOQ, the detected residues was used for calculations.

**Table B.8.1.2.1.2-10 Mean residues of inpyrfluxam and metabolites (mg/kg dry soil) for the Spain trial site**

<b>Soil depth/timi ng</b>	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>62 DAA</b>	<b>89 DAA</b>
Inpyrfluxam (LOQ 0.002 mg/kg)							
0-10 cm	0.168	0.166	0.133	0.124	0.106	0.0796	0.0665
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	0.00094 4*	0.00064 1*
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.168	0.166	0.133	0.124	0.106	0.0805	0.0671
	<b>176 DAA</b>	<b>266 DAA</b>	<b>361 DAA</b>	<b>454 DAA</b>	<b>538 DAA</b>	<b>629 DAA</b>	<b>740 DAA</b>
0-10 cm	0.0526	0.0477	0.0474	0.028	0.0254	0.0206	0.0174
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.0526	0.0477	0.0474	0.0280	0.0254	0.0206	0.0174
3'-OH-S-2840 (LOQ = 0.002 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>62 DAA</b>	<b>89 DAA</b>
0-10 cm	0.0018 1*	0.00321	0.00254	0.00492	0.00736	0.0103	0.00913
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.0018 1*	0.00321	0.00254	0.00492	0.00736	0.0103	0.00913
	<b>176 DAA</b>	<b>266 DAA</b>	<b>361 DAA</b>	<b>454 DAA</b>	<b>538 DAA</b>	<b>629 DAA</b>	<b>740 DAA</b>
0-10 cm	0.0114	0.0132	0.00639	0.0088	0.00871	0.0071	0.00627
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.0114	0.0132	0.00639	0.00880	0.00871	0.00710	0.00627

1'-COOH-S-2840A (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>62 DAA</b>	<b>89 DAA</b>
0-10 cm	< LOD	0.00113	0.00246	0.00362	0.00421	0.00523	0.00484
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	-	0.00113	0.00246	0.00362	0.00421	0.00523	0.00484
	<b>176 DAA</b>	<b>266 DAA</b>	<b>361 DAA</b>	<b>454 DAA</b>	<b>538 DAA</b>	<b>629 DAA</b>	<b>740 DAA</b>
0-10 cm	0.00566	0.00571	0.00592	0.00591	0.0044	0.00434	0.00353
10-20 cm	< LOD	0.000367*	0.000427*	0.00026*	< LOD	< LOD	0.000707
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	0.00566	0.00608	0.00635	0.00617	0.00440	0.00434	0.00424
1'-COOH-S-2840B (LOQ = 0.001 mg/kg)							
	<b>0 DAA</b>	<b>3 DAA</b>	<b>7 DAA</b>	<b>14 DAA</b>	<b>28 DAA</b>	<b>62 DAA</b>	<b>89 DAA</b>
0-10 cm	< LOD	0.00143	0.00292	0.0048	0.00606	0.00877	0.00854
10-20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sum	-	-	0.00292	0.00480	0.00606	0.00877	0.00854
	<b>176 DAA</b>	<b>266 DAA</b>	<b>361 DAA</b>	<b>454 DAA</b>	<b>538 DAA</b>	<b>629 DAA</b>	<b>740 DAA</b>
0-10 cm	0.0105	0.0107	0.0104	0.0119	0.00904	0.00877	0.00765
10-20 cm	< LOD	0.000631*	0.00065*	0.000426*	0.000201*	0.000446*	0.0014
20-30 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.000257*
Sum	0.0105	0.0113	0.0111	0.0123	0.00924	0.00922	0.00931

\* values < LOQ

Mean values were calculated with detected residues, where values < LOD were set to zero, while for values < LOQ, the detected residues was used for calculations.

The OECD and EPA Guidelines state that the study duration should be sufficient to determine the DT<sub>90</sub> of the active substance and any metabolites. A simple calculation has been conducted, comparing the initial parent residues in the soil column with the total parent residues at the final time point. For the Czech Republic and Spain sites, approximately 90 % decline had occurred during the study, with 10.7 and 10.4 % of the residues at 0 DAA remaining at study end. For the Italy site, 16.2 % of residues remained. At the Germany site however, 63 % of the initial residues remained at study end, indicating that a longer study duration may have been appropriate at this site to achieve 90 % decline.

Translocation of inpyrfluxam residues down the soil column occurred only to a limited extent, and was more evident at the Germany and Czech Republic sites.

Metabolites 1'-COOH-S-2840A and B seemed to be the most mobile with residues

more frequently found >10 cm at the Czech Republic and Spain sites, while inpyrfluxam was more mobile at the Germany and Czech Republic sites. Between days 0 and 60 or 62 DAA, soil cores were taken to a depth of 30 cm. Residues >LOD at 20-30 cm depth were observed at the Germany, Czech Republic and Italy sites. Movement of residues downwards through the soil column was more limited overall at the Spain and Italy sites. OECD Guidance states that soil should be sampled to a depth where values <LOD are observed; this indicates that samples to >30 cm should have been taken at the start of the study as this is where the highest residues were found at lower levels. It is noted however that residues in the lowest layers at the earliest time points were low and were generally <LOQ; the exceptions were at the Czech Republic site at 0 DAA (TcA sub-plot) and at the Italy site at 14 TAA (TcC sub-plot) when residues >LOQ were reported. From 60 DAA soil was sampled to 90 or 100 cm depth and no parent residues were detected at >LOD at 20-30 cm depth at any time point after 60 DAA. HSE considers that this demonstrates that, while it would have been preferable to sample to depths lower than 30 cm at earlier time points, overall the sampling was acceptable.

For parent, residues gradually declined over time. At day 0, the sum of residues in the top 30 cm of soil ranged between 0.0542 and 0.168 mg/kg. Residues were highest in the Spain site and lowest in the Italy site. Residues had declined to 0.00627 mg/kg to <LOD by study end. Residues were generally concentrated in the top 10 cm of soil but even on 0 DAA residues >LOD were detected down to 30 cm depth.

Metabolites were generally detected at low levels and almost exclusively in the 0-10 cm soil level at all trial sites.

- At the Germany site, residues of 3'-OH-S-2840 over 30 cm depth reached around 0.008 to 0.010 mg/kg from 61 to 270 DAA before declining thereafter. At the Czech Republic site residues of the metabolite remained around 0.002 mg/kg from 3 DAA to 28 DAA and remained close to 0.01 mg/kg from 182 DAA to 629 DAA, before declining at study end. Residues in the Italy site remained much lower, reaching 0.002 to 0.004 mg/kg from 28 to 637 DAA and then declining. At the Spain site residues rose to a maximum of 0.0132 at 266 DAA before declining to 0.006 mg/kg by study end.
- Residues of metabolite 1'-COOH-S-2840A over 0-30 cm depth increase over time peaking in the middle of the study and declining towards study end. At the Germany site, residues increased to 0.003 mg/kg at 20-28 DAA before declining to low levels <LOQ by 91 DAA. In the Czech Republic site, residues increased to 0.004 mg/kg at 360 DAA before declining to <LOQ at study end. At the Italy site residues increased from <LOD but remained <0.001 mg/kg throughout the study. At the Spain site, residues increased from <LOD at the start of the study to around 0.006 mg/kg between 266 and 454 DAA before declining to 0.004 mg/kg by study end.

- Metabolite 1'-COOH-S-2840B increased from <LOD at study start, increased in concentration in the middle of the study and declined again to <LOQ by study end. At the Germany site, residues reached 0.005 mg/kg on 20 DAA. At the Czech Republic site, the peak occurred later on 265 DAA at 0.005 mg/kg, after which residues declined to <LOQ by study end. At the Italy site, residues remained <LOQ throughout the study. Residues at the Spain study increased from <LOD to 0.012 mg/kg by 538 DAA, but declined to <0.01 mg/kg by study end.

#### *Comparison of behaviour of 1'-COOH-S-2840 isomers in field studies*

Changes in enantiomeric excess were considered in section B.8.1.1.1.4 in accordance with the principles outlined in the EFSA Guidance document, 'Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers' (2019) and the 'GB Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers'. It was identified that changes in enantiomeric excess did not occur in two of the three soils tested. In the final soil (Newhaven), a change in enantiomeric excess of 11.8 % was observed but this was transient and enantiomeric excess was <10 % at study end. The guidance document considers changes >10 % to be potentially significant with respect to the environmental risk assessment.

The GB guidance, 'Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers' recommends that the DFOP model would provide a fully mechanistic description of preferential transformation of a pair of stereoisomers, as the model consists of separate first order rate constants to describe preferential transformation rates. As degradation of 1'-COOH-S-2840 was best described by DFOP kinetics, in contrast to the SFO kinetics used for the other two soils, further consideration was given to the transient change in enantiomeric excess in the Newhaven soil. Best fit kinetics were determined for each isomer separately. The behaviour of both isomers was biphasic, showing that the biphasic behaviour is due to factors other than behaviour of the individual isomers. The *k* values for the fast and slow phases were also similar for both isomers, which would not be the case if they were degrading at different rates. The overall degradation behaviour of 1'-COOH-S-2840 (A and B combined) has also been considered in section B.8.1.3. It was determined that overall, this metabolite follows SFO kinetics which would not be the case if the stereoisomers showed different degradation behaviour in soil.

It is not therefore considered that the changes in the enantiomeric excess for 1'-COOH-S-2840A and 1'-COOH-S-2840B are indicative of isomeric conversion. A single exposure assessment for the sum of the isomers was therefore considered appropriate.

The changes in enantiomeric excess observed in the field studies have also been considered in line with the Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. Metabolite 1'-COOH-S-2840 was not observed at the Ontario site at any time point and the metabolite was not assessed at the Italy site as levels were only >LOQ at three time points, so that it was considered unlikely that an acceptable fit could be obtained. The enantiomeric excess has therefore been considered for the other three field sites considered relevant to GB conditions: Germany, Czech Republic and Spain. The changes in enantiomeric excess were monitored from the peak formation of the total (A+B) metabolite.

**Table B.8.1.1.4-11 Changes in enantiomeric excess**

	<b>1'-COOH-S-2840A</b>	<b>1'-COOH-S-2840B</b>	<b>Enantiomeric excess</b>	<b>Change in enantiomeric excess</b>
<b>Germany (peak total metabolite 0.07640 at 20 DAA)</b>				
0 DAA	0	0	0	-
3 DAA	0	0	0	-
7 DAA	35.9	64.1	28.3	-
20 DAA	33.9	66.1	32.2	-
28 DAA	37.1	62.9	25.8	-6.4
61 DAA	25.9	74.1	48.2	16.0
91 DAA	30.9	69.1	38.2	6.0
179 DAA	29.1	70.9	41.9	9.7
270 DAA	29.7	70.3	40.6	8.4
359 DAA	33.3	66.7	33.4	1.2
448 DAA	0.0	0.0	0.0	-
543 DAA	0.0	100.0	100.0	-
629 DAA	0.0	100.0	100.0	-
728 DAA	37.8	62.2	24.5	-7.7
<b>Czech Republic (peak total metabolite 0.0889 at 265 DAA)</b>				
0 DAA	0	0	0	-
3 DAA	0	0	0	-
7 DAA	48.6	51.4	2.9	-
15 DAA	51.4	48.6	-2.8	-
28 DAA	50.3	49.7	-0.5	-
61 DAA	49.3	50.7	1.4	-
92 DAA	50.0	50.0	0.0	-
182 DAA	47.9	52.1	4.1	-
265 DAA	48.4	51.6	3.3	-



360 DAA	49.4	50.6	1.2	-2.0
455 DAA	44.4	55.6	11.2	8.0
540 DAA	44.5	55.5	11.0	7.7
629 DAA	41.7	58.3	16.6	13.3
733 DAA	45.2	54.8	9.6	6.3
<b>Spain (peak total metabolite 0.01847 mg/kg at 454 DAA)</b>				
0 DAA	0	0	0	-
3 DAA	100	0	-	-
7 DAA	45.7	54.3	8.6	-
14 DAA	43.0	57.0	14.0	-
28 DAA	41.0	59.0	18.0	-
62 DAA	37.4	62.6	25.3	-
89 DAA	36.2	63.8	27.7	-
176 DAA	35.0	65.0	30.0	-
266 DAA	35.0	65.0	30.0	-
361 DAA	36.4	63.6	27.2	-
454 DAA	33.4	66.6	33.2	-
538 DAA	32.3	67.7	35.5	2.3
629 DAA	32.0	68.0	36.0	2.8
740 DAA	31.3	68.7	37.4	4.2

Changes in the enantiomeric excess were compared against the first time point in each study where both the A and B isomers were observed. There were some transient changes in enantiomeric excess >10 % observed at the Germany and Czech Republic, but enantiomeric excess changes were <10 % by study end. As these changes were transient, they are not considered to be significant.

The Spain site differs from the other sites at which metabolite 1'-COOH-S-2840 was observed in that very little degradation of the metabolite was observed over the course of the study (see Figure B.8.1.2.1.2-17). At the other sites, 0 to 10 % remains at study end, while at the Spain site, approximately 73 % of the peak concentration of the total metabolite remains. Both the EFSA guidance document and the GB guidance document, 'Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers' state that the trigger of 10 % enantiomeric excess should be considered at the end of the study if at least 50 % of the bulk substance (sum of stereoisomers) has been degraded. This criterion has not been met and therefore it is not appropriate to consider the changes in stereoisomeric excess for the Spain site. The EFSA Guidance also states that the trigger can also be considered by extrapolation of the transformation pattern to consider what the situation would be should degradation continue. Values have therefore been calculated from the peak formation. The enantiomeric excess by

study end is <10 % and there are no indications that data from this site would indicate changes in enantiomeric excess >10 %.

There are thus no indications of changes in enantiomeric excess >10 % in the field study data. The field study data therefore corroborates the data from the laboratory degradation study and it is considered acceptable to calculate PEC values for total 1'-COOH-S-2840 in the exposure assessment.

#### *Weather data*

The applicant provided data for each trial site comparing the weather which occurred during the trials to the historical averages. These are summarised below on a monthly basis. Daily weather records for each site were provided in an appendix to the study report.

**Table B.8.1.2.1.2-12 Temperature and rainfall data for the Germany trial**

<b>Month</b>	<b>Average minimum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average minimum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Average maximum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average maximum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Rainfall Current year <sup>(1)</sup> (mm)</b>	<b>Rainfall Historical <sup>(2)</sup> (mm)</b>
<b>May 16 (*)</b>	12.3	7.6	19.2	18.9	57.6	53.7
<b>June 16</b>	14.4	10.3	21.8	21.5	207.8	69.1
<b>July 16</b>	14.3	13.1	24	24.0	44.2	91.2
<b>August 16</b>	13	12.3	23.6	23.8	56.8	95.6
<b>Sept. 16</b>	12	9.8	23.6	19.9	12.4	69.2
<b>Oct 16</b>	7	7.2	13.2	15.5	51.0	68.6
<b>Nov 16</b>	2.8	4.3	8.2	10.5	70.4	56.8
<b>Dec 16</b>	1.9	3.0	7.1	8.1	23.2	94.1
<b>(*) data starting from 18/05/16 for May 2016</b>						
<b>Jan 17</b>	-1.5	1.1	3.6	6.1	43.6	83.4
<b>Feb 17</b>	2.7	-0.2	8.1	6.3	64.6	52.9
<b>March 17</b>	4.5	1.6	13.3	10.9	63.0	38.8
<b>April 17</b>	3.1	4.1	13.1	14.9	16.6	53.3
<b>May 17</b>	10.9	8.6	21.4	18.7	43.8	62.1
<b>June 17</b>	13.7	11.0	23.8	21.4	32.4	92.7
<b>July 17</b>	14.1	13.7	23.2	24.5	89.4	78.8
<b>August 17</b>	12.8	12.4	22.8	24.0	37.6	81.8
<b>Sept 17</b>	10.3	10.0	18.5	20.4	99.4	63.6

<b>Oct 17</b>	10.2	7.3	16.3	14.9	42.4	66.4
<b>Nov 17</b>	4.2	4.2	9.4	9.9	62.0	69.6
<b>Dec 17</b>	2.8	2.8	6.4	8.0	83.2	72.6
<b>Jan 18</b>	3.6	0.5	7.7	5.5	80.7	72.8
<b>Feb 18</b>	-2.2	0.9	3.5	7.1	14.2	61.6
<b>March 18</b>	0.8	1.8	8.8	10.9	44.2	47.3
<b>April 18</b>	7.4	3.9	18.0	15.0	41.0	37.9
<b>May 18</b>	11.0	9.0	23.0	18.8	34.6	62.1
<b>Total rainfall (deviation to Norm):</b>					<b>1358.5 (- 20%)</b>	<b>1696.0</b>
<b>Source of data (air temperature and precipitation):</b> <b>Weather station in the trial field (0.0 km to trial site)</b> <b>DWD German Meteorological Service + Biochem Agrar Weather station (Kleve)</b> <b>(Historical data from 2011 until May 2016) (18 km to trial site)</b>						

Table B.8.1.2.1.2-13 Temperature and rainfall data for the Czech Republic trial

<b>Month</b>	<b>Average minimum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average minimum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Average maximum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average maximum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Rainfall Current year <sup>(1)</sup> (mm)</b>	<b>Rainfall Historical <sup>(2)</sup> (mm)</b>
<b>May 16</b>	9	8.3	21.2	20.0	52.6	69.0
<b>June 16</b>	12.4	11.7	26.3	23.3	83.8	86.0
<b>July 16</b>	14	12.8	27.7	25.1	172.4	82.0
<b>August 16</b>	12	12.5	25.4	24.6	52.6	78.0
<b>Sept. 16</b>	10.8	9.4	24.8	20.6	42.8	50.0
<b>Oct 16</b>	5	4.9	12.7	14.7	85.0	40.0
<b>Nov 16</b>	1.5	1.3	7.7	7.4	50.4	46.0
<b>Dec 16</b>	-3.7	-2.4	2.4	2.7	12.0	38.0
<b>Jan 17</b>	-9.4	-4.9	-1.5	0.9	8.2	31.0
<b>Feb 17</b>	-1.5	-3.2	5.3	3.6	33.6	30.0
<b>March 17</b>	1.7	0.0	13.4	8.9	25.0	32.0
<b>April 17</b>	3.7	4.0	13.9	15.0	72.4	43.0
<b>May 17</b>	8.6	8.3	21.8	20.0	36.0	69.0
<b>June 17</b>	12.3	11.7	27.3	23.3	45.6	86.0
<b>July 17</b>	13.7	12.8	27.7	25.1	79.6	82.0
<b>August 17</b>	14.2	12.5	29.1	24.6	55.0	78.0
<b>Sept 17</b>	10.2	9.4	19	20.6	115.2	50.0

<b>Oct 17</b>	6.2	4.9	15.4	14.7	124.0	40.0
<b>Nov 17</b>	1.6	1.3	8	7.4	45.8	46.0
<b>Dec 17</b>	-1.9	-2.4	4.4	2.7	49.6	38.0
<b>Jan 18</b>	-0.2	-4.9	5.2	0.9	43.6	31.0
<b>Feb 18</b>	-4.5	-3.2	0.6	3.6	16.6	30.0
<b>March 18</b>	-2.6	0.0	7.1	8.9	19.0	32.0
<b>April 18</b>	7.6	4.0	20.9	15.0	33.4	43.0
<b>May 18</b>	11.4	8.3	24.8	20.0	65.8	69.0
<b>Total rainfall (deviation to Norm):</b>					<b>1420.0 (+ 8%)</b>	<b>1319.0</b>
<b>Source of data (air temperature and precipitation):</b> <b>ATC-Agro Trial Center (Uhersky Ostroh) (trial current years data) 0.2 km to trial site</b> <b>National weather service (Uhersky Ostroh) (Historical weather data) 1.0 km to trial site</b>						

Table B.8.1.2.1.2-14 Temperature and rainfall data for the Italy trial

<b>Month</b>	<b>Average minimum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average minimum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Average maximum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average maximum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Rainfall Current year <sup>(1)</sup> (mm)</b>	<b>Rainfall Historical <sup>(2)</sup> (mm)</b>
<b>May 16</b>	11.5	10.9	23.2	24.2	93.8	65.4
<b>June 16</b>	15.2	15.1	27.9	29.2	82.6	47.2
<b>July 16</b>	17.9	17.6	32.1	31.9	36.2	38.3
<b>August 16</b>	15.7	17.4	30.3	32.3	53.4	30.0
<b>Sept. 16</b>	14.2	14.3	28	26.9	50.0	52.2
<b>Oct 16</b>	8.7	10.2	18	20.1	94.6	75.4
<b>Nov 16</b>	5	5.9	12.4	13.8	83.2	64.1
<b>Dec 16</b>	-0.5	0.4	7.2	8.3	39.4	24.9
<b>Jan 17</b>	-4.1	-0.6	5.9	7.9	4.0	44.1
<b>Feb 17</b>	1.8	0.2	10.9	9.1	68.8	110.6
<b>March 17</b>	4	3.4	19.1	15.6	12.2	70.0
<b>April 17</b>	7.1	7.5	21.2	19.8	28.8	65.1
<b>May 17</b>	11.5	11.1	24.6	23.7	98.4	77.3
<b>June 17</b>	16.4	15.1	31.3	29.0	34.0	53.8
<b>July 17</b>	17.5	17.9	32.4	32.3	18.8	34.9
<b>August 17</b>	17.9	17.0	33.2	31.6	24.0	40.7
<b>Sept 17</b>	12.7	13.9	24.3	26.6	98.6	56.4

<b>Oct 17</b>	8.9	10.6	21.6	19.6	3.6	85.0
<b>Nov 17</b>	3.7	6.2	12.5	13.8	139.2	74.4
<b>Dec 17</b>	-1.1	0.5	8.2	8.0	24.0	28.6
<b>Jan 18</b>	0.7	-0.8	9.5	7.8	10.4	42.9
<b>Feb 18</b>	-0.2	1.6	6.7	10.1	172.8	116.1
<b>March 18</b>	2.6	3.6	11.7	15.5	77.4	72.2
<b>April 18</b>	8.9	7.6	21.9	20.3	14.0	54.1
<b>May 18</b>	13.6	11.3	24.9	23.8	98.0	82.4
<b>Total rainfall (deviation to Norm):</b>					<b>1460.2 (- 3%)</b>	<b>1506.1</b>
<b>Source of data (air temperature and precipitation):</b>						
<b>Arpa, Emilia Romagna region (Mezzolara) 6.0 km to trial site</b>						

**Table B.8.1.2.1.2-15 Temperature and rainfall data for the Spain trial**

<b>Month</b>	<b>Average minimum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average minimum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Average maximum air temperature Current year <sup>(1)</sup> (°C)</b>	<b>Average maximum air temperature Historical <sup>(2)</sup> (°C)</b>	<b>Rainfall Current year <sup>(1)</sup> (mm)</b>	<b>Rainfall Historical <sup>(2)</sup> (mm)</b>
<b>June 16</b>	9.9	10.6	27.9	26.4	0	30.0
<b>July 16</b>	10.4	13.3	28.9	29.1	0	16.9
<b>August 16</b>	9.5	14.0	29.6	28.5	0	22.8
<b>Sept. 16</b>	7.7	12.5	23.7	27.9	38.6	36.1
<b>Oct 16</b>	6.3	9.6	19.8	21.5	22.2	75.7
<b>Nov 16</b>	3.3	5.9	12.7	14.7	90.2	78.9
<b>Dec 16</b>	1.3	3.4	11.9	12.8	26.8	79.8
<b>Jan 17</b>	2.8	5.2	13.9	13.8	120.4	123.1
<b>Feb 17</b>	6.7	4.5	16.7	10.9	208.8	84.9
<b>March 17</b>	7.4	6.2	18.5	14.9	119	71.8
<b>April 17</b>	2.3	8.1	21.8	16.9	0.6	67.6
<b>May 17</b>	7.5	10.1	21.8	20.6	80.0	54.1
<b>June 17</b>	11.4	13.8	26.4	23.7	33.2	34.4
<b>July 17</b>	19	17.3	28.3	26.4	10.2	16.4
<b>August 17</b>	18.7	17.2	28.3	25.2	14.6	26.6
<b>Sept 17</b>	14.6	14.6	23.5	24.6	0.4	44.5
<b>Oct 17</b>	13.2	10.9	24.2	18.9	26.4	225.0
<b>Nov 17</b>	7.1	7.3	14	13.7	64.2	97.6
<b>Dec 17</b>	5.4	4.7	10.6	11.4	110.6	85.8
<b>Jan 18</b>	1.2	4.9	10.4	14.2	76.6	147.1
<b>Feb 18</b>	-0.9	5.2	9.4	12.6	47.0	126.7
<b>March 18</b>	2	6.3	10.1	16.0	240.0	95.6

<b>April 18</b>	4.5	7.3	15.9	19.3	71.2	44.6
<b>May 18</b>	5.6	9.8	19.5	21.9	57.6	57.1
<b>Total rainfall (deviation to Norm):</b>					<b>1458.6</b> <b>(- 16%)</b>	<b>1743.1</b>
<b>Source of data (air temperature and precipitation:</b> <b>Kilma 31 (current trial period) 0.5 km to trial site</b> <a href="http://www.aemet.es">www.aemet.es</a> (Orense) (historical weather data) 40 km to trial site						

The applicant has supplied weather data for each trial site. The 'EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT<sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil' (2014) provides advice regarding the acceptability of rainfall data which has not been collected at the trial site in field studies. This states that rainfall data from weather stations at a distance of <1 km is preferred and that weather stations at no more than 20 km distance from the experimental field are recommended. In addition, it should be made clear that there are no climatological barriers, e.g. mountains or hills, between the rainfall station and the experimental field. Temperature data is not mentioned in the guidance, but temperature is generally considered to exhibit less spatial variability over the distances from trial sites to weather stations typically seen and therefore is of less concern, however temperature data collected from >20 km should also be treated with caution.

Weather data recorded during the trial period were available within 0.5 km of the trial site for the Germany, Czech Republic and Spain sites. For the Italy site all data, recorded during the trial and historically, were recorded at a single weather station located at 6 km from the field site. Historical weather data for the Germany, Czech Republic and Spain sites were recorded from weather stations at 18, 40 and 0.5 km away from the respective trials sites. Therefore the historical data from the Spain site was recorded outside the recommended 20 km distance which has been considered when making judgements as to the representativeness of the trial weather to longer term trends.

The rainfall data show that the rainfall recorded during the trials was within 20 % of the historical rainfall recorded for each site and is therefore similar to long term trends. For the temperature data:

- For the Germany site, comparison of the average minimum air temperature suggests that 2016 was a little cooler than average, but maximum data for 2016 and minimum and maximum air temperature data for 2017 suggest that air temperatures were similar to longer term trends.
- For the Czech Republic site, both the average minimum temperatures and average maximum temperatures recorded during the trial are slightly higher than the historical average values.
- For the Italy trial, the minimum average temperatures are similar to historical trends, while the average maximum air temperatures are generally slightly higher than the historical average maximums.

- At the Spain site, the average minimum temperatures during the study were generally slightly lower than the historical average minimums for much of the trial, but were higher from July 2017 onwards. The average maximum temperatures during the year of the trial were similar or slightly lower during 2016 but were higher from February 2017 onwards.

The weather conditions at the trial sites do not show significant differences from the historical data recorded. Therefore the location of the Spain site historical data collection, which was outside the recommended maximum of 20 km, can be accepted by HSE.

With regards to the weather data within the trial it is noted that the Italy site recorded data from a 6 km distance. However as there are recorded soil moisture and temperature values on site for the duration of the trial, this weather data has not been used to determine normalisation values and hence can be accepted as representative of weather conditions during the trials period. The only weather data that is to be used for the normalisation of the data for consideration of degradation is the Czech Republic site as there were no measured soil moisture and temperature values recorded on site for this trial. The in trial weather data for the Czech Republic site was recorded within 0.2 km of the trials site and hence this meets requirements.

## CONCLUSIONS

The applicant has submitted field studies conducted at 4 sites in Europe. inpyrfluxam was sprayed on 3 sub-plots at each sites at a target rate of 0.5 L product/ha (200 g a.s./ha) and soil cores taken from each sub-plot over a period of approximately 2 years. After application, residues were incorporated to a depth of 7 cm. It is not stated that the plots were rolled after incorporation as recommended in the OECD Guideline, but the incorporation of the test item into the soil is considered to be adequate. An untreated control plot was also included at each location.

Data show that inpyrfluxam declined at the treated plots during the course of the study. This was accompanied by low levels of formation of metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and B at all sites. Residues mostly remained in the top 10 cm of soil with detections down to levels of 30 cm.

Overall, HSE is satisfied that the study is suitable to derive information on the route and rate of decline of inpyrfluxam under field conditions for use in the exposure assessment.

### Time step normalisation and kinetic fitting of the field data

<b>Data Point:</b>	KCA 7.1.2.2.1/08
<b>Report Author:</b>	██████████ and ██████████
<b>Report Year:</b>	2018

<b>Report Title:</b>	Normalisation of data from four field studies in Europe and determination of normalised field DT <sub>50</sub> values for inpyrfluxam and metabolites.
<b>Study number</b>	Exponent International Ltd., UK. Report No. 1403863.UK0-7449 Sumitomo Chemical Co., Ltd. Report No: TPR-0079
<b>Guideline(s) followed in study:</b>	“EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT <sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil”, EFSA European Food Safety Authority (2014), EFSA Journal 2014;12(5):3662.  Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. FOCUS (2014) version 1.1 (18 December 2014).
<b>GLP?</b>	Non-GLP, unpublished

The applicant has submitted a report detailing normalisation procedures for the field study data from the sites in Germany, Czech Republic, Italy and Spain using PERSIST. The timestep normalisation of the field conditions was based on the availability of measured daily soil temperature and moisture content at the Germany, Italy and Spain sites and calculated daily soil temperature and moisture content (from air temperature and rainfall) at the Czech site. The following equation was used:

$$D_{\text{norm}} = (Q_{10}^{(T_{\text{act}} - T_{\text{ref}})/10}) * (\theta_{\text{act}}/\theta_{\text{ref}})^{0.7}$$

Where:

$Q_{10} = 2.58$ ,

$T_{\text{act}}$  = the measured/calculated soil temperature at 10 cm depth

$T_{\text{ref}}$  = the reference temperature of 20°C

$\theta_{\text{act}}$  = the measured/calculated volumetric/gravimetric moisture content at 10 cm depth

$\theta_{\text{ref}}$  = the reference volumetric/gravimetric moisture for the soil type at pF2

If  $T_{\text{act}}$  was <0°C then no degradation was considered to occur (i.e.  $D_{\text{norm}} = 0$ ) and where  $\theta_{\text{act}}$  was greater than  $\theta_{\text{ref}}$  then no correction for moisture was undertaken (i.e.  $(\theta_{\text{act}}/\theta_{\text{ref}})^{0.7} = 1$ )

A warm-up period was included to stabilise the program. Assumptions of soil bulk density of 1.5 g/cm<sup>3</sup> and soil depth of 10 cm were included.

For normalisation of day length the reference soil water holding capacity for the test site should be measured at pF2 or 10 KPa. The applicant has noted that the



measured soil moisture data for the sites was available as gravimetric values (w/w) whereas the soil moisture content determined during the studies was determined as a volumetric values (v/v). In order to determine the most appropriate input for the normalisation approach they have therefore also referred to the FOCUS (2014) default volumetric and gravimetric values for the soil types at each site ( see Table B.8.1.2.1.2-16).

**Table B.8.1.2.1.2-16 Reference soil and moisture content for the soil at each site**

<b>Trial</b>	<b>location</b>	<b>Soil type</b>	<b>Measured WHC at pF2 (w/w) %</b>	<b>Default* soil moisture at pF2 (w/w)%</b>	<b>Default* soil moisture at pF2 (v/v)%</b>	<b>Selected WHC at pF2 (v/v)%</b>
<b>GE01</b>	Germany	Sandy clay loam	37.7	22	31	37.7
<b>CZ02</b>	Czech Republic	Clay/Silty clay	44.1	40	46	44.1
<b>IT03</b>	Italy	Sandy loam	21.7	19	27	27
<b>SP04</b>	Spain	Loam	44.7	25	34	44.7

\*FOCUS (2014)

Based upon this comparison the applicant has selected to use the measured gravimetric values as reference volumetric values for the Germany, Czech Republic and Spanish sites. For the Italian site the measured gravimetric value for pF2 was considerably lower than the moisture content observed in the field conditions and hence the applicant proposed use of the default volumetric soil moisture content for this soil type at the Italian site. HSE notes that it is preferable to use measured values where possible and notes that the applicants approach in using the measured gravimetric values in the normalisation is accepted. The deviation from this in the Italian trial can also be accepted as conservative based upon the fact that the measured gravimetric value for pF2 is lower than the moisture contents observed under field conditions at the Italian site, with only 30 days having a measured moisture value below 21.7%. Therefore the approach taken is conservative for the active substance degradation.

Using the above values the applicant has determined normalised daylength for each site and the proposed normalised time points to be used in the kinetic assessment of the field data. HSE has validated the applicants values and determined slight differences in the adjusted time points to be used. However, with the exception of the Czech Republic data the HSE determined daylength values were generally lower and hence less conservative than those determined by the applicant, therefore HSE accepts the applicant values for the adjusted timepoints set for the Germany, Italy

and Spanish sites. For the Czech Republic site the HSE determined daylength values were used to determine the impact on normalised degradation rates.

**Table B.8.1.2.1.2-17 Applicant summary of time adjusted days for the different sites with comparison to HSE validation**

Germany			Czech Republic			Italy			Spain		
DAT	Norm DAT	HSE Norm DAT	DAT	Norm DAT	HSE Norm DAT	DAT	Norm DAT	HSE Norm DAT	DAT	Norm DAT	HSE Norm DAT
0	0.0	0	0	0.0	0	0	0.0	0.0	0	0.0	0
3	1.9	1.7	3	2.3	2.3	3	3.1	3.1	3	1.2	1.0
7	4.5	3.8	7	7.0	7	6	6.2	6.2	7	2.6	2.3
20	17.6	16.3	15	13.8	13.8	14	16.5	16.5	14	5.0	4.3
28	26.0	24.1	28	25.7	25.7	28	34.2	34.2	28	11.3	9.7
61	58.8	55.4	61	65.6	65.6	60	106.5	100.5	62	32.8	28.1
91	88.0	82.5	92	98.2	98.2	89	169.4	155.8	89	47.3	40.4
179	147.2	138.2	182	146.6	147.6	180	273.3	256.1	176	100.9	86.3
270	163.1	156.4	265	149.4	157.6	272	297.3	281.6	266	128.6	110
359	195.0	188.3	366	186.8	196.8	358	344.1	328.1	361	168.8	144.4
448	305.8	264.1	455	286.7	296.7	455	551.2	535.1	454	235.1	201.1
543	332.0	321.6	540	334.7	345.7	550	642.2	626.1	538	264.7	226.4
629	351.9	341.5	629	342.0	358.8	637	662.6	646.5	629	288.1	246.5
728	391.4	376	733	386.3	403.5	728	717.5	698.9	740	339.1	292.4

The applicant states that they have followed FOCUS Kinetics (2014) Guidance in the treatment of values around or close to the LOD and LOQ. Soil cores collected at each site were analysed in 10 cm segments. The majority of residues were concentrated in the top 0-10 cm of soil, with infrequent detects at lower levels. For the purpose of calculating DT<sub>50</sub> values residues >LOQ were summed to give a total residue level in the soil at each timepoint. Values close to the LOQ and LOD, including those at lower depths, were treated in accordance with FOCUS Kinetics Guidance. The applicant states that:

- For the parent, values <LOD directly after a detectable amount were set to 0.5LOD, samples between the LOD and LOQ were set to 0.5 (LOD+LOQ) and all subsequent samples <LOD were set to 0 unless there were later samples >LOQ.
- For metabolites, time 0 samples <LOD were set to 0.
- For metabolites, samples just before and after a detectable amount were set to 0.5 LOD. Samples between LOD and LOQ were set to the measured value or 0.5 (LOQ+LOD).
- Where sporadic samples were observed at 20-30 cm depth, samples before and after were taken to be 0 if <LOD. For first detects, if residues were only

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observed at one of the 3 subplots then the previous time point was recorded as 0 and not 0.5 LOD.

HSE did not always agree with the applicant's treatment of values around the LOD and LOQ values:

- For first detects where residues were only detected at one of the 3 sub-plots, the applicant recorded the previous time point as 0 and not 0.5 LOD. HSE would consider using 0.5 LOD as all sub-plots are considered individually rather than in conjunction with the others.

In addition it is recognised that the current FOCUS kinetics (2014) guidance does not necessarily provide comprehensive guidance on data handling in field dissipation studies and rules are open to a degree of interpretation. HSE proposed changes in approaches towards the following:

- For samples at deeper depths, the applicant has taken the samples at the time points before and after and in the next soil layer down to be 0. HSE would consider using 0.5 LOD for parent and metabolites. Examples would be:
  - At each time point with positive detection in a subsoil layer, the layer above with <LOD has been set to 0.5 LOD for that time point.
  - If there is movement down the soil profile, in the first layer below the lowest with detectable residues, time points where there is a detection >LOD in the layer immediately above are set to 0.5 LOD.

It is considered that the applicant has applied data handling principles with consideration of the existing FOCUS guidance. In light of the residue levels determined the additions of 0.5 LOD to the existing residues as proposed by HSE could have minimal impact on the outcome of the kinetic fitting. HSE has therefore recalculated some of the values shown for the DE site to consider the impact of the change in data handling and also the CZ site to consider variation in normalised day length values.

As confirmed above the LOQ value for analysis of inpyrfluxam and 3'-OH-S-2840 is 0.002 mg/kg and the LOD for these compounds was 0.0004 mg/kg. For metabolites 1'-COOH-S-2840A and B the LOQ is 0.002 mg/kg and the LOD was 0.0002 mg/kg. The residues for metabolites were corrected for molecular weight differences to convert them into parent equivalents. The molecular weights are:

- Parent: 333.38 g/mol
- 3'-OH-S-2840: 349.38 g/mol
- 1'-COOH-S-2840: 363.36 g/mol

Based on the behaviour and analysis of the individual isomer pairs (A and B) for metabolite 1'-COOH-S-2840 it is considered reasonable to treat this as a single substance for the purpose of kinetic fitting.

The applicant conducted kinetic fitting to determine the decline of the parent compound inpyrfluxam. They used the CAKE 3.2 software and considered the suitability of fit based upon the minimum % error required to pass the  $\chi^2$  test at a probability of 0.05 and a t-test (acceptability  $p \leq 0.05$ ) and initially plotted the parent only data using SFO and DFOP kinetics to determine the best fit for this compound. This approach enabled consideration of the impact of the subsequent metabolite fitting on the parent substance. Using the outcome from this initial parent only fitting they then fitted the data for the parent compound inpyrfluxam and its metabolites considering parallel degradation to the metabolites and the metabolites subsequently degrading to the sink compartment. This also included the estimation of the formation fractions from the parent compound. It was noted that at the Italian site the levels of the metabolite 1'-COOH-S-2840 were considered too low to enable any robust fits to be determined. HSE agrees with the approach taken in the kinetic fitting.

The applicant rated the fits using the following scale:

- Not acceptable: the fitted curve does not represent the trend of the data points and residuals show strong deviations from random distribution.
- Acceptable: the fitted curve describes the trend of the data points, residuals may show some deviations from random distribution but it is not significant.
- Good: the fitted curve closely follows all the data points (limited scatter of data points); residuals are randomly distributed (no bias of residuals).
- Very good: no bias of residuals or scatter of data points.

HSE has also followed these definitions in its own, independent assessment.

The field values for the German site presented by the applicant for use in the kinetic assessment are detailed in Table B.8.1.2.1.2-18 below. Residue are expressed as the sum of residues corrected as parent equivalents.

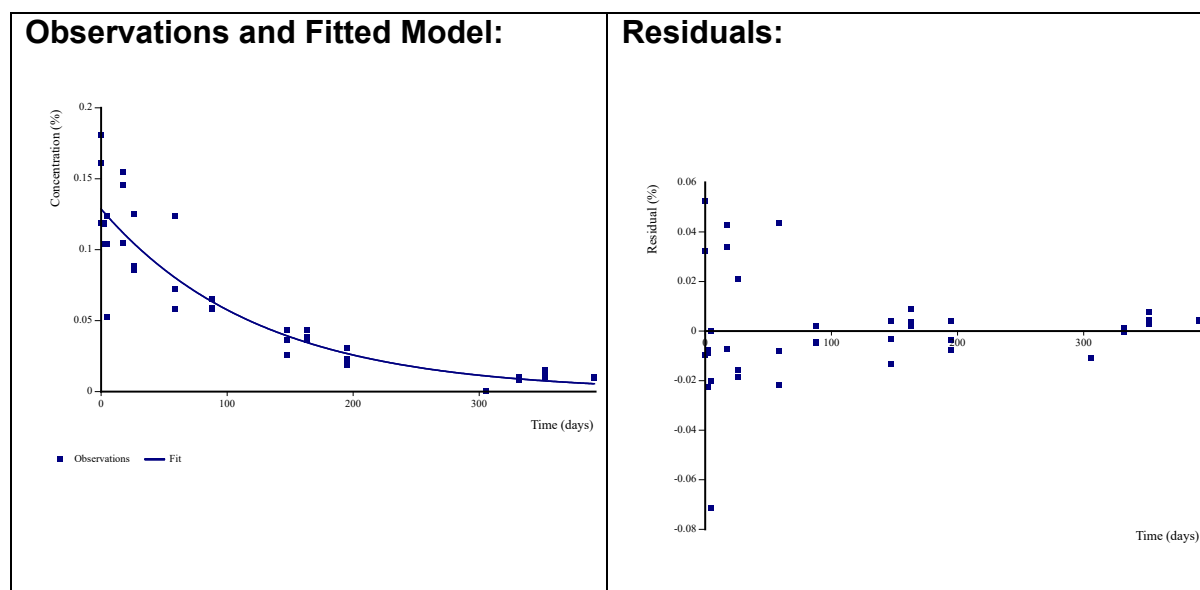
**Table B.8.1.2.1.2-18 Residues of inpyrfluxam and its metabolites in soil (mg/kg) – German site as presented by the applicant**

Sampling Event (days)	Plot	Soil layer (cm)		
		Inpyrfluxam	3'-OH-2840	1'-COOH-S-2840
0	A	0.119	0	0
	B	0.181	0	0

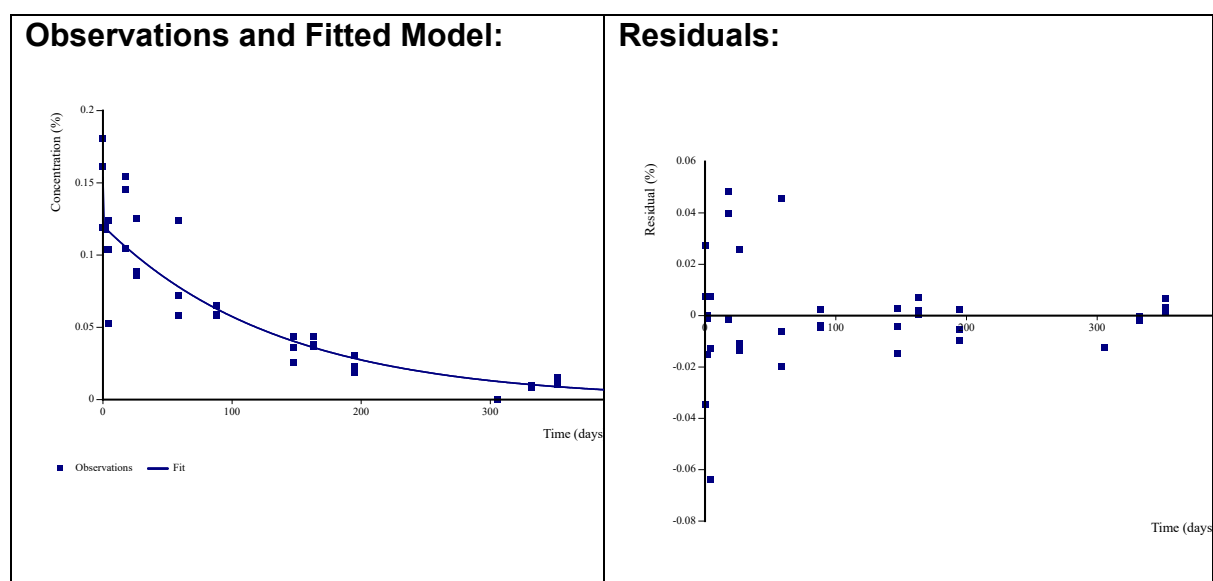
	C	0.161	0	0
3	A	0.118	0	0
	B	0.104	0	0
	C	0.119	0	0
7	A	0.053	0.00115	0.00110
	B	0.1042	0.00115	0.00206
	C	0.1242	0.00115	0.00219
20	A	0.10438	0.00534	0,00428
	B	0.15447	0.00607	0.00773
	C	0.14575	0.00684	0.00903
28	A	0.0859	0.00490	0.00534
	B	0.0888	0.00508	0.00544
	C	0.1254	0.00601	0.00778
61	A	0.0586	0.00583	0.00233
	B	0.07198	0.00685	0.00303
	C	0.0591	0.01238	0.00804
91	A	0.0591	0.00858	0.00236
	B	0.0582	0.00806	0.00203
	C	0.0652	0.00677	0.00261
179	A	0.026	0.00691	0.00215
	B	0.0364	0.00929	0.00248
	C	0.0435	0.01155	0.00344
270	A	0.0367	0.01097	0.00110
	B	0.0383	0.00992	0,00110
	C	0.0434	0.00954	0.00110
359	A	0.0308	0.00936	0.00183
	B	0.0191	0.00491	0.00110
	C	0.0232	0.00746	0.00172
448	A	0.0002	0.00019	0.00018
	B	0.0002	0.00019	0.00018
	C	0.0002	0.00019	0.00018
543	A	0.0097	0.00435	0
	B	0.0101	0.00495	0
	C	0.00843	0.00389	0
629	A	0.0105	0.00449	0
	B	0.0122	0.00595	0
	C	0.0155	0.00766	0
728	A	0.00968	0.00470	0
	B	0.0100	0.00535	0
	C	0.00978	0.00599	0

The applicant has presented the following kinetic fits using the CAKE 3.2 software and the data presented from the German trial site.

### SFO fitting



### DFOP fitting



**Figure B.8.1.2.1.2-04 Germany field data kinetic fitting inpyrfluxam alone**

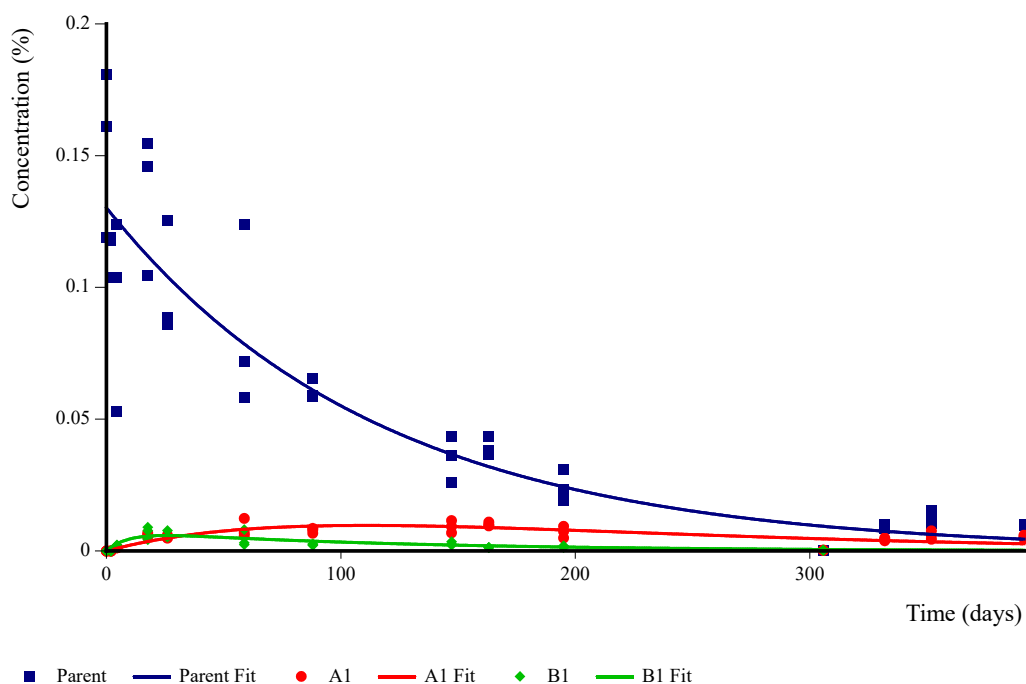
The  $\chi^2$  error value was comparable from both fittings, there is little difference between SFO and DFOP in the visual fit, and DT<sub>90</sub> values are comparable. Therefore in line with the FOCUS guidance and the desire to accept SFO for FOCUS modelling when the fit is good enough it was concluded that the SFO fitting for the parent compound is acceptable. HSE agree with this fitting for the parent compound.

**Table B.8.1.2.1.2-19 Results of the kinetic determinations for inpyrfluxam (alone)**

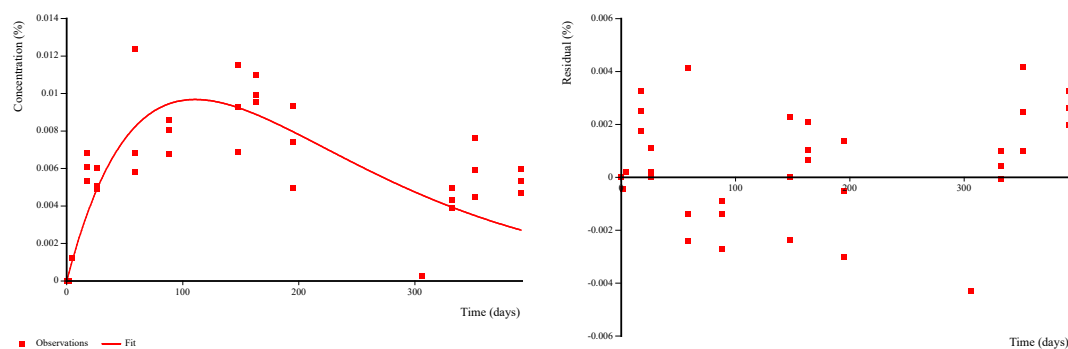
Parameter	German site
<b>Model</b>	<b>SFO</b>
$\chi^2$ error (%)	17.6
k (days <sup>-1</sup> )*	0.00805 (4.3x10 <sup>-10</sup> )
Statistical fit	Good
Visual fit	Good
DT <sub>50</sub> (d)	86.1
DT <sub>90</sub> (d)	286
<b>Model</b>	<b>DFOP</b>
$\chi^2$ error (%)	15.4
k1*(days <sup>-1</sup> )	3.91 (0.43)
k2*(days <sup>-1</sup> )	0.00740 (1.4x10 <sup>-10</sup> )
G	0.217
Statistical fit	Unacceptable
Visual fit	Good
DT <sub>50</sub> (d) (overall)**	60.6 (93.7)
DT <sub>90</sub> (d) (overall)	278

\*P value from the t-test is given in brackets.

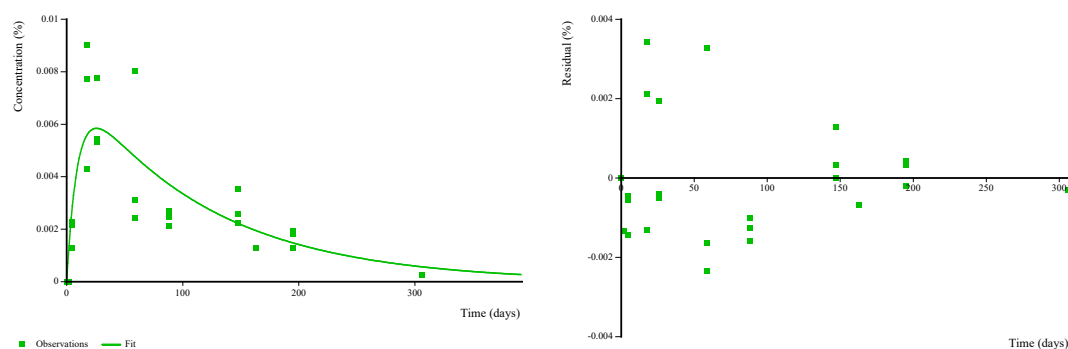
\*\* values in brackets represent slow phase.



3'-OH-S-2840



## 1'-COOH-S-2840



**Figure B.8.1.2.1.2-05 Germany field data SFO-SFO – inpyrfluxam with metabolites, applicant assessment.**

**Table B.8.1.2.1.2-20 Applicant assessment of kinetic fit outputs for the German site**

Soil	Inpyrfluxam	3'OH-S-2840	1'COOH-S-2840
Kinetic fit	SFO	SFO	SFO
Overall $\chi^2$ error%	29.1		
$\chi^2$ error%	17.8	27.4	25.9
Visual fit	Good	Acceptable	Acceptable
Statistical fit	Good	Acceptable	Acceptable
DT <sub>50</sub> /DT <sub>90</sub> inpyrfluxam	79.5 / 264	73.8 / 245	6.46 / 21.5
Formation fraction	-	0.209	0.68

The applicant has stated that they consider that the visual fit for the metabolites show a clear formation and decline and whilst there is some scatter effect due to the variability in the data points they consider that there is no bias of residuals and therefore they consider that the visual fits are acceptable. Regarding the statistical fits for the metabolites they note that the  $\chi^2$  error for both compounds is >15% and note that this is acceptable for field data and is due to the variability of the data values. Therefore they consider the statistical fit acceptable.



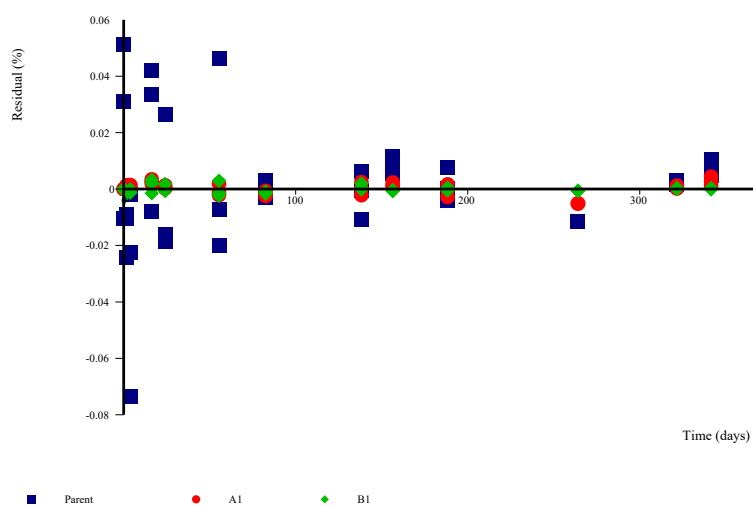
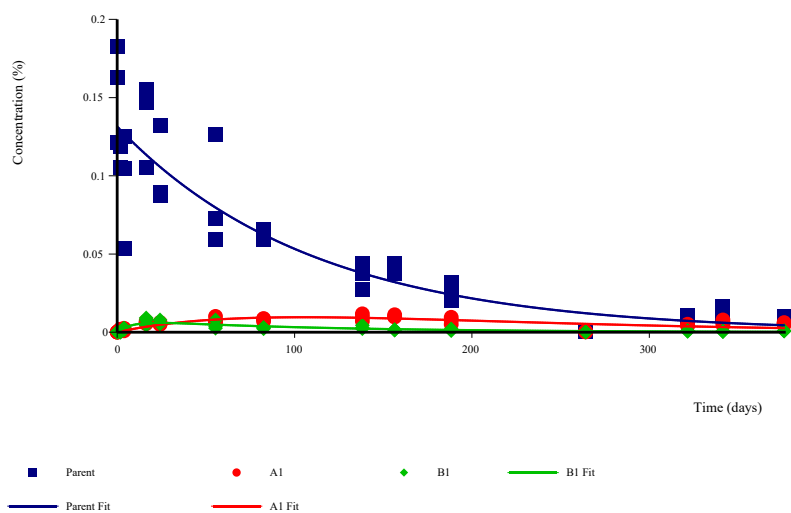
HSE has handled the presented data values from the German trial site using latest kinetic approaches to bracket residues in lower layers by time and depth and used measured values when reported. In all cases the sum of residues were corrected as parent equivalents. The residue values for use in the kinetic assessment are detailed in Table B.8.1.2.1.2-21. It is noted for the parent compound at 61 days the soil was sampled to 50cm depth and residues determined at the 30-40 cm horizon have been added to the overall residue for that timepoint. It is considered that this deeper sampling may have affected the residue determination for the 3'-OH-S-2840 and 1'-COOH-S-2840 metabolites at this time point as there were small amounts of residue detected at the 20-30cm horizon. However, as there was no evidence of leaching to lower layers at previous and subsequent timepoints this appeared consistent with sampling error and contamination of the 20-30 cm layer from the surface sampling. The residue levels at these time points were added to the surface residue and included for kinetic analysis but not bracketed with 0.5 LOD residues. In contrast at time point 270 d for the metabolite 1'-COOH-S-2840 (B) some evidence of movement into the 10-20 cm horizon was noted and this value has therefore been bracketed.

**Table B.8.1.2.1.2-21 Residues of inpyrfluxam and its metabolites in soil (mg/kg) – German site as presented by HSE**

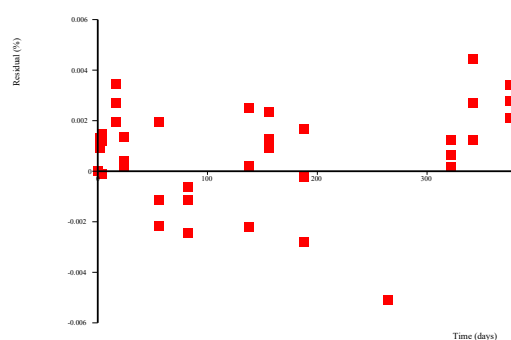
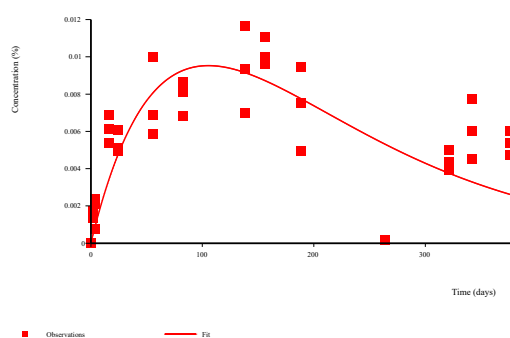
Sampling Event (days)	Normalised days	Plot	Compound		
			S2399	3'-OH-S-2840	1'-COOH-S-2840
0	0	A	0.12085	0	0
	0	B	0.18268	0	0
	0	C	0.16248	0	0
3	1.7	A	0.11878	0.00174	0
	1.7	B	0.10495	0.001731	0
	1.7	C	0.12013	0.001346	0
7	3.8	A	0.05320	0.000788	0.001367
	3.8	B	0.10440	0.002115	0.00233
	3.8	C	0.12512	0.002356	0.002569
20	16.3	A	0.10541	0.005385	0.004275
	16.3	B	0.15533	0.006115	0.007734
	16.3	C	0.14685	0.006894	0.009028
28	24.1	A	0.08701	0.004933	0.005339
	24.1	B	0.08936	0.005115	0.00544
	24.1	C	0.13222	0.006058	0.00778
61	55.4	A	0.05946	0.005875	0.00245
	55.4	B	0.07249	0.006904	0.003468
	55.4	C	0.12599	0.01	0.007771
91	82.5	A	0.06001	0.008644	0.002679
	82.5	B	0.05943	0.008125	0.002128
	82.5	C	0.06540	0.006827	0.002936

179	138.2	A	0.02701	0.006962	0.002165
	138.2	B	0.03713	0.009365	0.002752
	138.2	C	0.04390	0.011635	0.004275
270	156.4	A	0.03742	0.011058	0.00122
	156.4	B	0.03850	0.01	0.001394
	156.4	C	0.04360	0.009615	0.001581
359	188.3	A	0.03148	0.009433	0.001899
	188.3	B	0.01986	0.004952	0.000907
	188.3	C	0.02405	0.007519	0.001908
448	264.1	A	0.00040	0.000192	9.17E-05
	264.1	B	0.00040	0.000192	9.17E-05
	264.1	C	0.00040	0.000192	9.17E-05
543	321.6	A	0.00990	0.004385	0.000845
	321.6	B	0.01030	0.00499	0.000721
	321.6	C	0.00863	0.003923	0.000428
629	341.5	A	0.01070	0.004529	0.000265
	341.5	B	0.01240	0.006	0.000384
	341.5	C	0.01658	0.007721	0.000731
728	376	A	0.00988	0.00474	0.00071
	376	B	0.01020	0.005394	0.000759
	376	C	0.00998	0.006038	0.000702

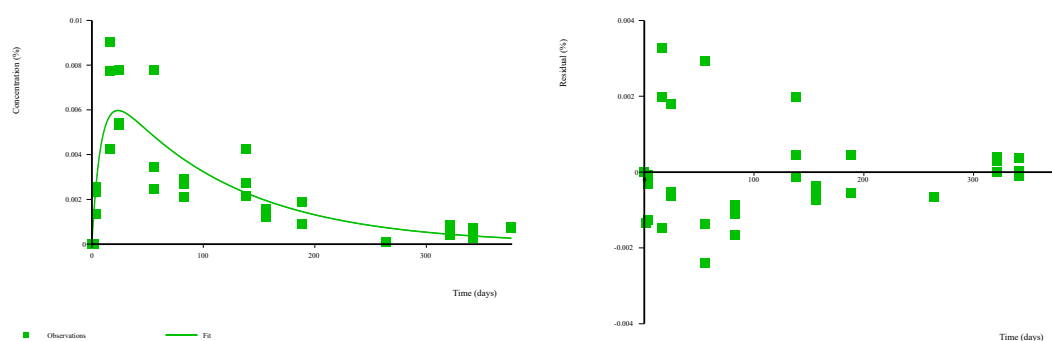
Using the data presented from the German trial site HSE has considered the kinetic fit using the CAKE 3.7 software with IRLS fitting and obtained the following fits for SFO-SFO kinetics.



## 3'-OH-S-2840



## 1'-COOH-S-2840

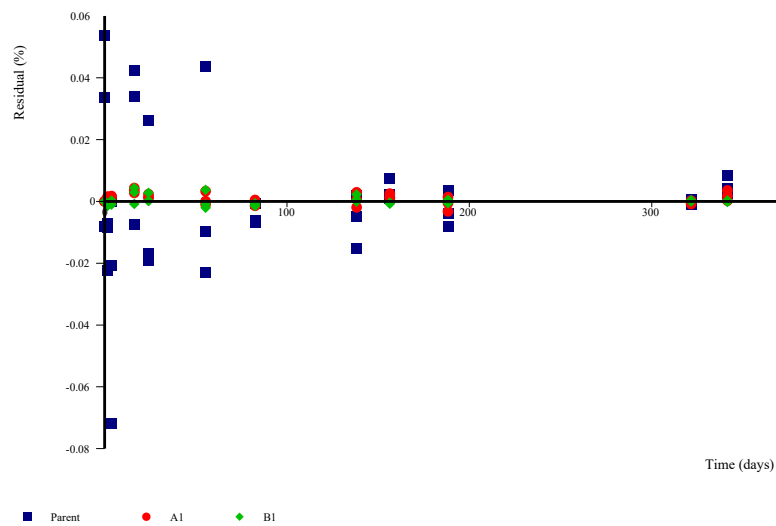
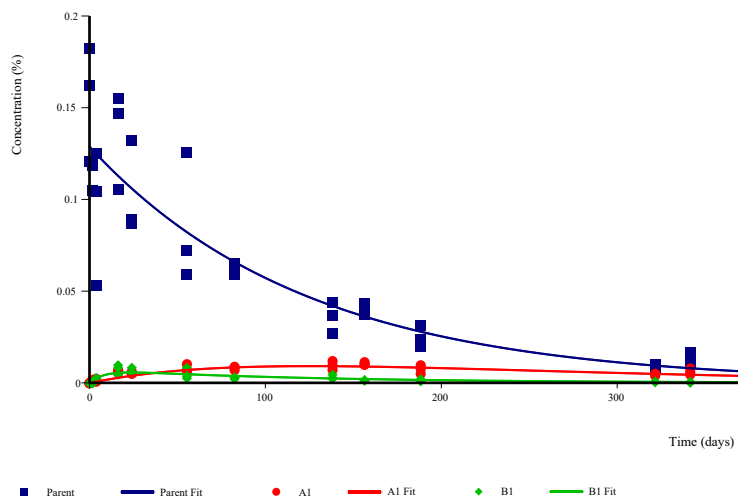


**Figure B.8.1.2.1.2-06 Kinetic fit from German trial site data SFO-SFO – inpyrfluxam with metabolites 3'-OH-S-2840 (A1) and 1'-COOH-S-2840 (B1).HSE assessment.**

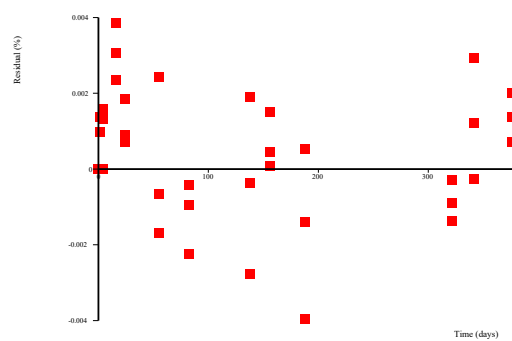
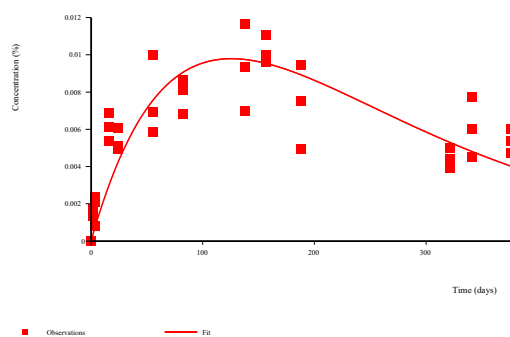
**Table B.8.1.2.1.2-22 HSE assessment of kinetic fit outputs for the German site**

Compound	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840
Kinetic fit	SFO	SFO	SFO
Overall X <sup>2</sup> error%	30.6		
X <sup>2</sup> error (%)	18	30.1	25.3
Visual fit	Good	Unacceptable	Unacceptable
k value	0.009	0.0099	0.119
P value	8.4E-16	7.7E-07	3.43E-04
Statistical fit	Good	Acceptable	Acceptable
DT <sub>50</sub> inpyrfluxam	76.7	69.7	5.83
DT <sub>90</sub>	255	232	19.4
ff	-	0.21	0.74

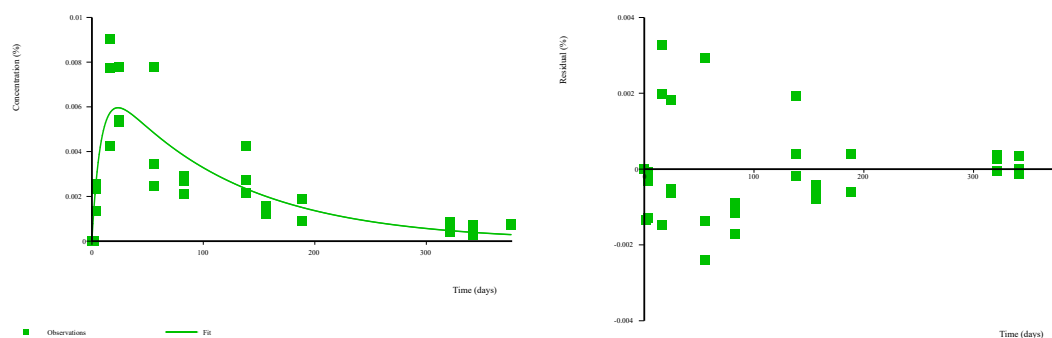
It is noted from the assessment that the HSE approach to handling of the data from the field site has not had a significant impact on the kinetic outcomes. As stated above this could be due to the levels at 0.5 LOD and 0.5 LOQ being insignificant as compared to the overall residue levels being assessed. However, unlike the applicant conclusion the HSE consider that the visual assessment of the data would indicate an unacceptable fit for the metabolite compounds. For 3'-OH-S-2840 the rate of decline at the later time points is over estimated and for 1'-COOH-S-2840 the maximum formation is under represented. It is noted that the residues sampled in field at 448 days (normalised to 264.1 days) from all plots were at <LOD, which appears inconsistent with the neighbouring timepoints sampled. This may indicate that the data at this timepoint is an outlier that should be removed. The kinetic fit was repeated with the 448 day timepoint removed to see if this improved the fitting of the later decline.



## Germany – 3'-OH-S-2840



## Germany – 1'-COOH-S-2840

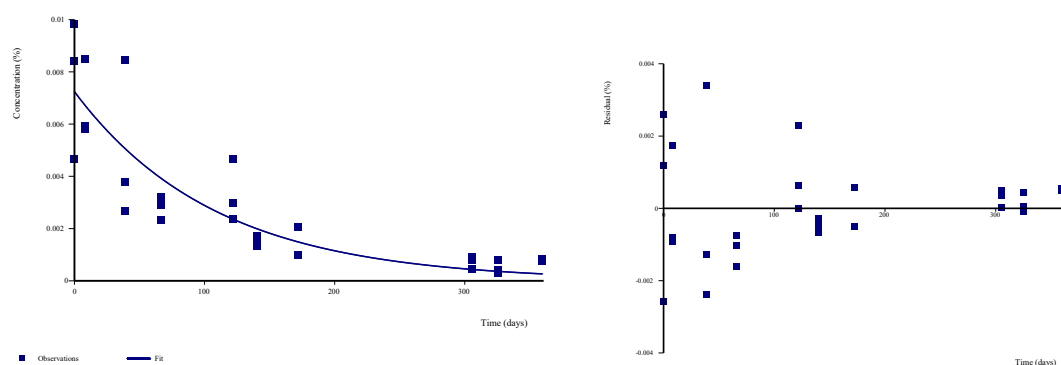


**Figure B.8.1.2.1.2-07 Kinetic fit from German trial site data SFO-SFO – inpyrfluxam with metabolites 3'-OH-S-2840 (A1) and 1'-COOH-S-2840 (B1) with the 448 day sample data removed. HSE assessment**

**Table B.8.1.2.1.2-23 HSE assessment of kinetic fit outputs for the German site with the in-field 448 day sample removed**

Compound	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840
Kinetic fit	SFO	SFO	SFO
Overall X <sup>2</sup> error%	28.2		
X <sup>2</sup> error (%)	16.7	18.2	23.7
Visual fit	Good	Acceptable	Unacceptable
k value	0.0088	0.007178	0.12
P value	3.6E-14	4.03E-08	5.2E-04
Statistical fit	Good	Unacceptable	Acceptable
DT <sub>50</sub>	78.8	96.6	5.76
DT <sub>90</sub>	262	321	19.1
ff	-	0.18	0.77

The visual fit for metabolite 3'-OH-S-2840 is improved by the removal of the 448 day timepoint and although the initial formation is slightly under represented the top down decline is visually acceptable in the opinion of HSE. The endpoints are affected in that they are of a longer duration than proposed by the applicant for this metabolite. The fit for the 1'-COOH-S-2840 is unaffected by the removal of the 448 d in field timepoint and the visual fit remains unacceptable in the opinion of HSE in that the initial formation is under represented. It is noted that the variability in the initial residues for the parent substance inpyrfluxam may have resulted in an under representation of initial parent values which would have an impact on the metabolite formation. However the fitting for the initial formation of 1'-COOH-S-2840 is not considered acceptable. To consider further the acceptability of the fitting for the decline phase a top down kinetic fit was conducted for the metabolite 1'-COOH-S-2840 using the residue values determined at the infield day 20 sampling point as 0 DAT.



**Figure B.8.1.2.1.2-08 Kinetic fit from German trial site data SFO fitting of the metabolite 1'-COOH-S-2840 decline. Using a top down fitting and the 448 day sample data removed. HSE assessment.**

**Table B.8.1.2.1.2-24 Kinetic fit output summary for top down assessment of 1'COOH-S-2840 residues decline at German field site**

<b>Compound</b>	<b>1'COOH-S-2840</b>
<b>Kinetic fit</b>	<b>SFO top down fit</b>
X <sup>2</sup> error (%)	14.4
Visual fit	Acceptable
k value	0.009199
P value	7.83E-7
Statistical fit	Acceptable
DT <sub>50</sub>	<b>75.4</b>
DT <sub>90</sub>	<b>250</b>

The top down kinetic fitting of the metabolite 1'COOH-S-2840 values indicate an acceptable visual and statistical fit with a lower  $\chi^2$  value and improved statistical fitting of the data. It is noted that this indicates a significant increase in the proposed degradation endpoint for this metabolite compound from the German trial site, but this is a conservative approach as it does not account for continued formation from parent when modelling via a top down approach. However the DT<sub>50</sub> value via this method is noted to be within the range determined in the other field sites. The values determined from the top down fitting are proposed for use in the setting of endpoints for the degradation of 1'COOH-S-2840 (noting also that as a terminal metabolite, the use of such a conservative approach is acceptable as it will not impact the formation of additional metabolites further down the degradation pathway). Although the sequential fitting did not provide an acceptable visual fit that matched the peak occurrence observed, the indicated formation fraction from the sequential fitting can be accepted by HSE. This is because taking into account the acceptable sequential

modelling for the 3'OH-S-2840 metabolite, which resulted in a formation fraction of 0.18, the fitted formation fraction for the 1'COOH-S-2840 of 0.77 is unlikely to underestimate the true formation fraction. Since both metabolites are formed in parallel from parent inpyrfluxam, and the fact that formation fractions from a single substance should sum to a maximum of 1, the absolute maximum formation fraction for 1'COOH-S-2840 can only be  $1 - 0.18 = 0.82$ . Hence the fitted value of 0.77 was accepted as sufficiently conservative in this case, particularly when coupled to the conservative top down dissipation rate for 1'COOH-S-2840.

The following endpoints as determined by HSE are agreed for the German field data:

**Table B.8.1.2.1.2-25 Endpoints for German field data determined by HSE**

Compound	Inpyrfluxam	3'OH-S-2840	1'COOH-S-2840
Kinetic fit	SFO	SFO	SFO
DT <sub>50</sub>	78.8	96.6	75.4
DT <sub>90</sub>	262	321	250
Ff (molar basis)	-	0.18	0.77

#### Kinetic assessment Czech Republic field site

The following data are presented by the applicant for the Czech Republic field site.

The field values for the Czech Republic site presented by the applicant for use in the kinetic assessment are detailed in Table B.8.1.2.1.2-26 below. Residue are expressed as the sum of residues corrected as parent equivalents.

**Table B.8.1.2.1.2-26 Residues of inpyrfluxam and its metabolites in soil (mg/kg) – Czech Republic site**

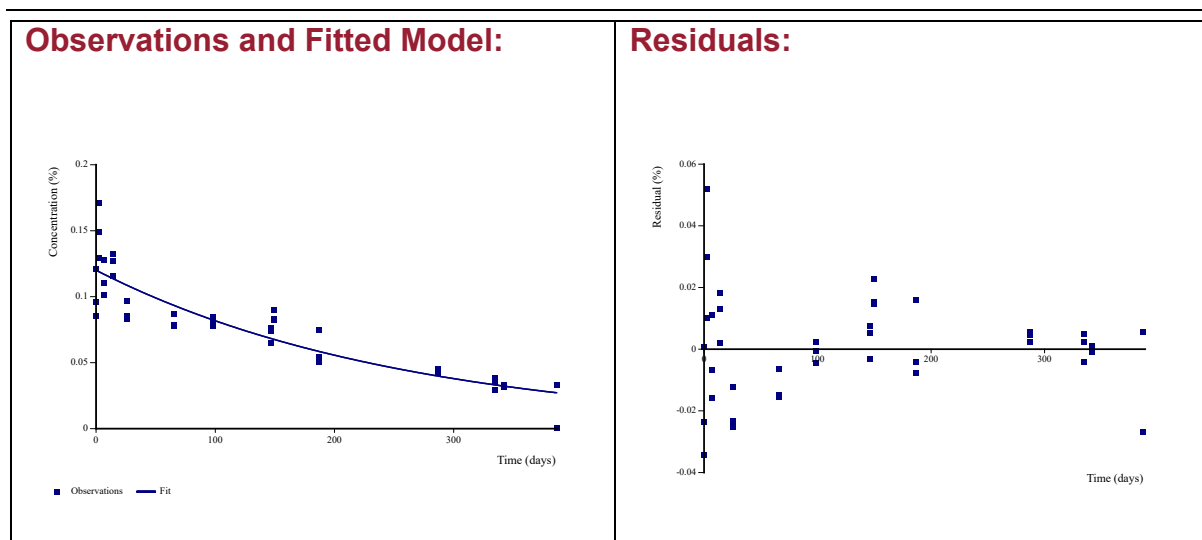
Sampling Event (days)	Plot	Compound		
		Inpyrfluxam	3'-OH-2840	1'-COOH-S-2840
0	A	0.12072	0	0
	B	0.0963	0	0
	C	0.0857	0	0
3	A	0.170	0.00241	0
	B	0.149	0.00115	0
	C	0.129	0.00257	0
7	A	0.101	0.00270	0
	B	0.128	0.00250	0
	C	0.11	0.00247	0



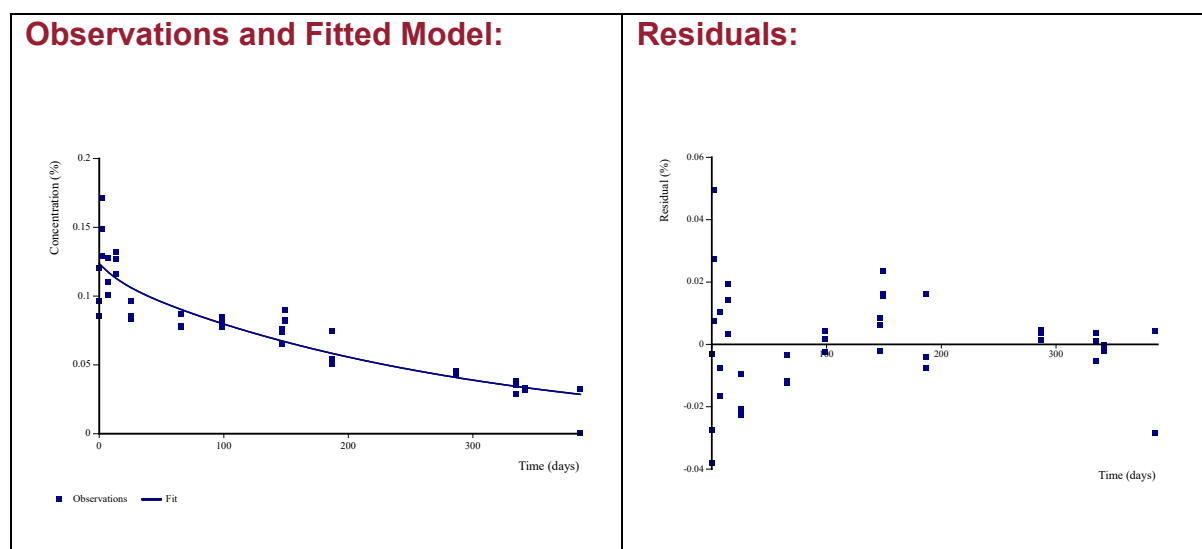
15	A	0.132	0.00115	0.00280
	B	0.127	0.00115	0.00172
	C	0.116	0.00115	0.00110
28	A	0.0855	0.00115	0.00522
	B	0.0834	0.00256	0.00588
	C	0.0965	0.00252	0.00428
61	A	0.0869	0.00509	0.00642
	B	0.0778	0.00437	0.00679
	C	0.0785	0.00518	0.00785
92	A	0.0778	0.00695	0.00736
	B	0.0818	0.00718	0.00670
	C	0.0845	0.00735	0.00669
182	A	0.076	0.00946	0.00365
	B	0.0652	0.00830	0.00396
	C	0.0739	0.00872	0.00676
265	A	0.0823	0.01231	0.00537
	B	0.083	0.01155	0.00834
	C	0.09023	0.01107	0.00774
366	A	0.0507	0.00834	0.00324
	B	0.0544	0.00926	0.00354
	C	0.0744	0.01078	0.00429
455	A	0.0423	0.00803	0.00433
	B	0.0444	0.00869	0.00502
	C	0.0455	0.00890	0.00461
540	A	0.0291	0.00725	0.00175
	B	0.0382	0.00906	0.00176
	C	0.0356	0.00866	0.00192
629	A	0.0323	0.00813	0.00110
	B	0.0332	0.00906	0.00110
	C	0.0314	0.00763	0.00110
733	A	0.0002	0.00019	0
	B	0.0002	0.00019	0
	C	0.0328	0.00930	0

The applicant has presented the following kinetic fits using the CAKE 3.2 software and the data presented from the Czech Republic trial site.

SFO fitting



## DFOP fitting



**Figure B.8.1.2.1.2-09: Czech Republic field data kinetic fitting– inpyrfluxam alone**

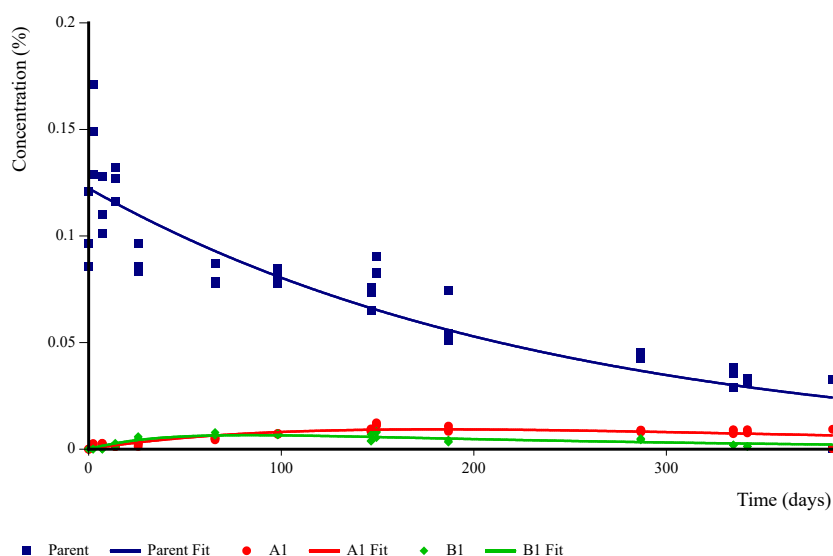
The  $\chi^2$  error value was comparable from both fittings. For the DFOP fitting the applicant noted that the t-test was non-significant for  $k_1$  and therefore the DFOP fit was considered statistically unacceptable. However HSE consider that there is little difference between SFO and DFOP in the visual fit, and  $DT_{90}$  values are comparable. Therefore in line with the FOCUS guidance and the desire to accept SFO for FOCUS modelling when the fit is good enough It was concluded that the SFO fitting for the parent compound is acceptable. HSE agree with this approach.

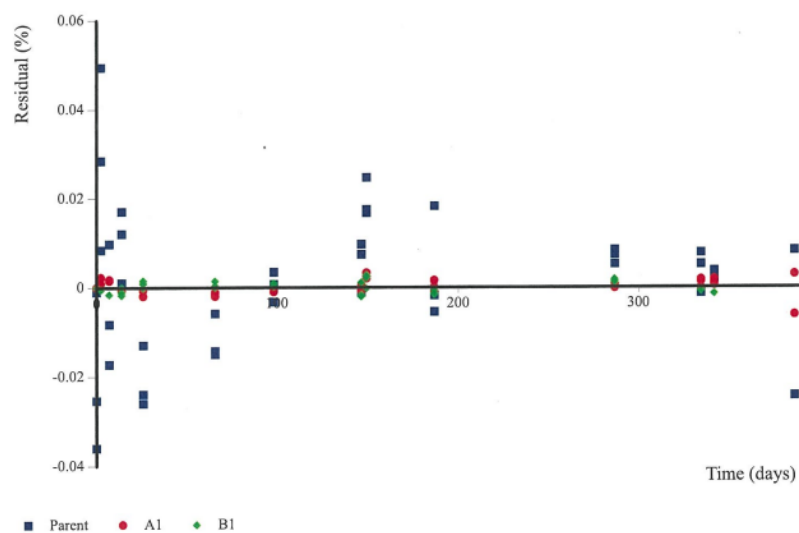
**Table B.8.1.2.1.2-27: Results of the kinetic determinations for inpyrfluxam (alone) applicant assessment.**

Parameter	Czech site
<b>Model</b>	<b>SFO</b>
$\chi^2$ error (%)	14.4
$k$ (days <sup>-1</sup> )*	0.00384 (7.8x10 <sup>-13</sup> )
Statistical fit	Good
Visual fit	Good
DT <sub>50</sub> (d)	181
DT <sub>90</sub> (d)	599
<b>Model</b>	<b>DFOP</b>
$\chi^2$ error (%)	15.3
$k_1^*$ (days <sup>-1</sup> )	0.0595 (0.376)
$k_2^*$ (days <sup>-1</sup> )	0.00358 (2.9x10 <sup>-6</sup> )
G	0.07813
Statistical fit	Unacceptable
Visual fit	Good
DT <sub>50</sub> (d) (overall)**	171 (194)
DT <sub>90</sub> (d) (overall)	620

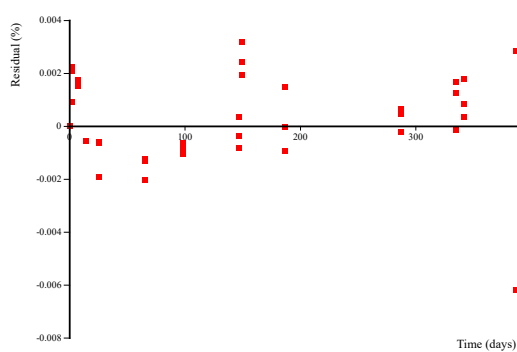
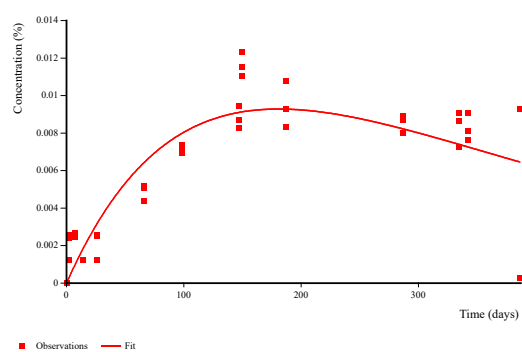
\*P value from the t-test is given in brackets.

\*\* values in brackets represent slow phase.

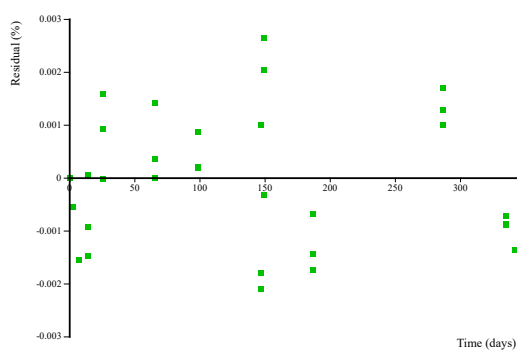
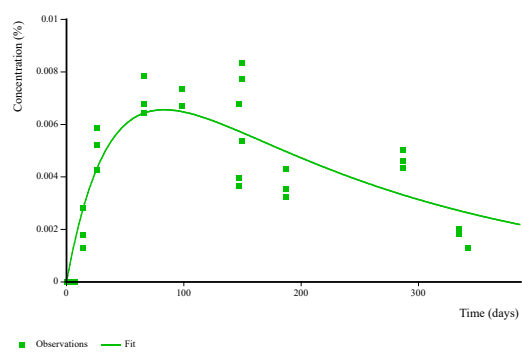




### 3'-OH-S-2840



### 1'-COOH-S-2840



**Figure B.8.1.2.1.2-10: Czech Republic field data SFO-SFO – inpyrfluxam with metabolites, applicant assessment**

**Table B.8.1.2.1.2-28 Applicant assessment of kinetic fit outputs for the Czech Republic site**

<b>Substance</b>	<b>Inpyrfluxam</b>	<b>3'OH-S-2840</b>	<b>1'COOH-S-2840</b>
kinetic fit	SFO	SFO	SFO
Overall $\chi^2$ error%	24.5	-	-
$\chi^2$ error%	14.6	20.2	24.1
Visual fit	Good	Acceptable	Acceptable
Statistical fit	Good	Acceptable	Acceptable
DT <sub>50</sub>	168	96.5	25.2
DT <sub>90</sub>	557	320	83.6
Formation fraction	-	0.276	0.502

The applicant has noted that they consider that the visual fit for the metabolites show a clear formation and decline and whilst there is some scatter effect due to the variability in the data points they consider that there is no bias of residuals and therefore they consider that the visual fit is acceptable. Regarding the statistical fits for the metabolite they note that the  $\chi^2$  error for both compounds is >15% and note that this is acceptable for field data and is due to the variability of the data values. Therefore they consider the statistical fit acceptable.

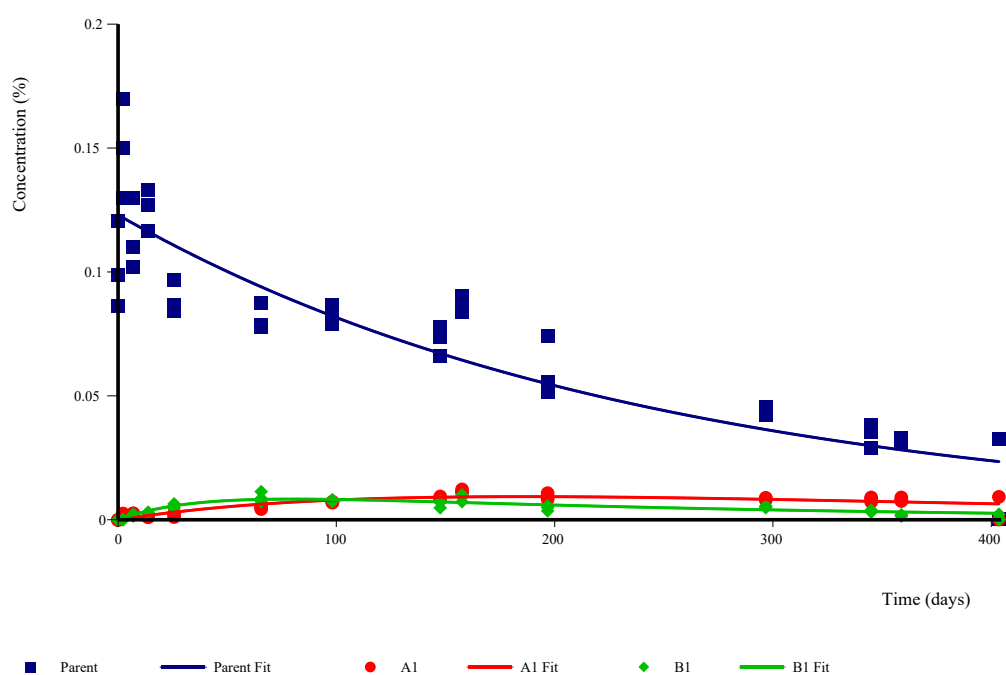
HSE have taken the presented data from the Czech Republic trial site using latest kinetic approaches to bracket residues in lower layers by time and depth the residue values for use in the kinetic assessment are detailed in in Table B.8.1.2.1-29. HSE has also used the normalised data points as determined in the HSE timestep normalisation for this site as part of the independent validation at this site.

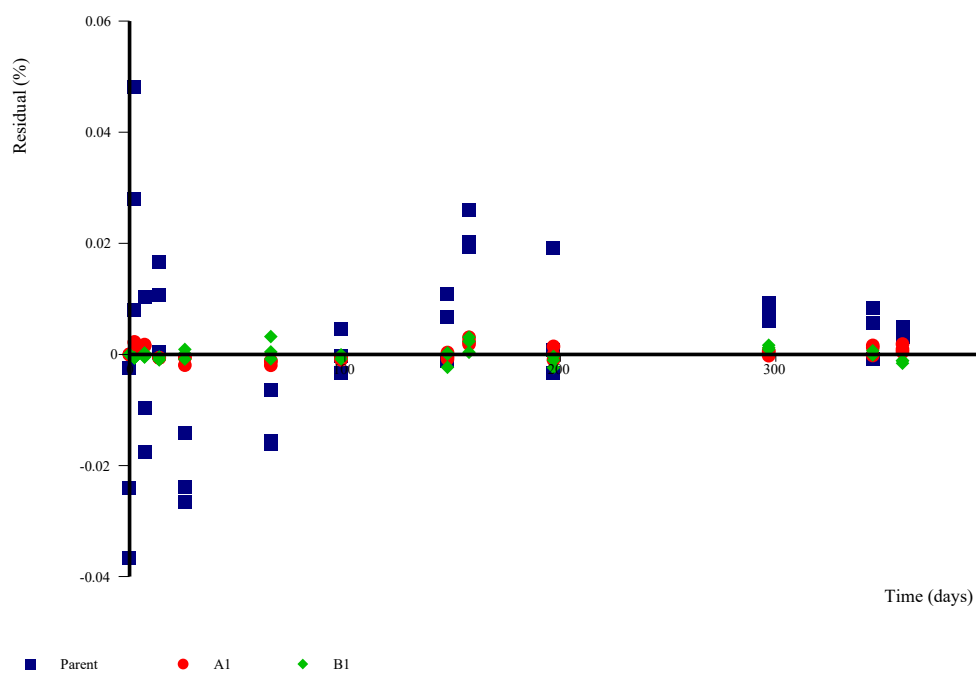
**Table B.8.1.2.1.2-29 Residues of inpyrfluxam and its metabolites in soil (mg/kg) determined at the Czech republic field site as presented by HSE**

<b>Sampling days</b>	<b>Normalised days</b>	<b>Plot</b>	<b>S2399</b>	<b>3'-OH-2840</b>	<b>1'-COOH-S-2840</b>
0	0	A	0.12072	0	0
0	0	B	0.099	0	0
0	0	C	0.0864	0	0
3	2.3	A	0.170	0.00241	0.0001
3	2.3	B	0.150	0.00115	0.0001
3	2.3	C	0.130	0.00257	0.0001
7	7	A	0.102	0.00270	0.0023
7	7	B	0.130	0.00250	0.00164
7	7	C	0.11	0.00247	0.00155
15	13.8	A	0.133	0.00115	0.00315
15	13.8	B	0.127	0.00115	0.00263
15	13.8	C	0.1167	0.00115	0.002679
28	25.7	A	0.0869	0.00115	0.00579
28	25.7	B	0.08413	0.00256	0.00651
28	25.7	C	0.0967	0.00252	0.00477
61	65.6	A	0.0877	0.00509	0.01142
61	65.6	B	0.078	0.00437	0.0074
61	65.6	C	0.0785	0.00518	0.00866
92	98.2	A	0.0789	0.00695	0.00812
92	98.2	B	0.0820	0.00718	0.0074
92	98.2	C	0.0868	0.00735	0.008054
182	147.6	A	0.078	0.00946	0.00487
182	147.6	B	0.0660	0.00830	0.00724
182	147.6	C	0.0739	0.00872	0.00747
265	157.6	A	0.0839	0.01231	0.00733
265	157.6	B	0.0847	0.01155	0.01005
265	157.6	C	0.09043	0.01107	0.0093
366	196.8	A	0.0515	0.00834	0.00363
366	196.8	B	0.0557	0.00926	0.00496
366	196.8	C	0.0741	0.01078	0.00565
455	296.7	A	0.0425	0.00803	0.00492
455	296.7	B	0.0446	0.00869	0.00567
455	296.7	C	0.0457	0.00890	0.00523
540	345	A	0.0291	0.00725	0.003206
540	345	B	0.0382	0.00906	0.003268
540	345	C	0.0356	0.00866	0.003952
629	358.8	A	0.0323	0.00813	0.00153

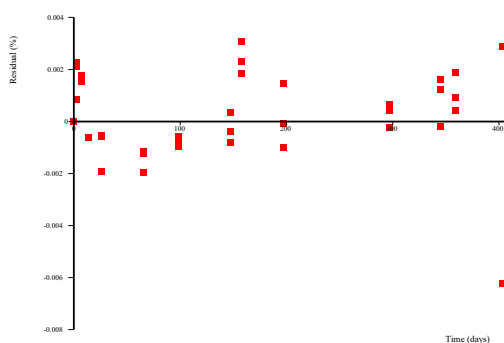
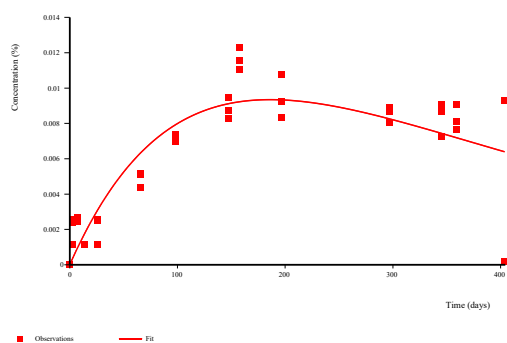
629	358.8	B	0.0332	0.00906	0.001975
629	358.8	C	0.0314	0.00763	0.002008
733	403.5	A	0.0002	0.00019	0.0002
733	403.5	B	0.0002	0.00019	0.0002
733	403.5	C	0.0328	0.00930	0.00229

Using the data presented from the Czech Republic trial site HSE has considered the kinetic fit using the CAKE 3.7 software with IRLS fitting and obtained the following fits for SFO-SFO kinetics.

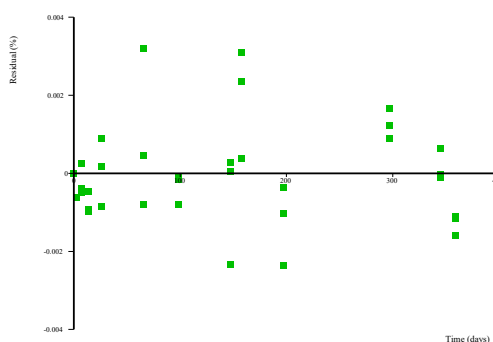
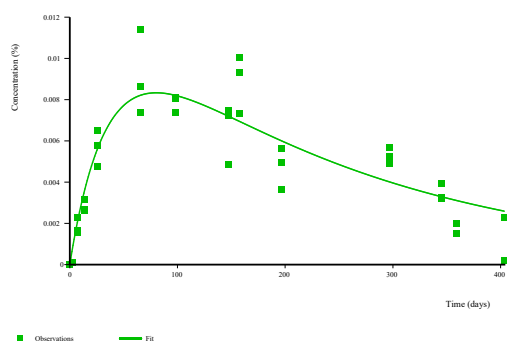




3'-OH-S-2840 :



1'-COOH-S-2840:



**Figure B.8.1.2.1.2-11: Czech Republic field data SFO-SFO – inpyrfluxam with metabolites, applicant assessment**



**Table B.8.1.2.1.2-30 Kinetic fitting outputs for the Czech Republic field site HSE assessment**

<b>Compound</b>	<b>Inpyrfluxam</b>	<b>3'OH-S-2840</b>	<b>1'COOH-S-2840</b>
Kinetic fit	SFO	SFO	SFO
Overall X <sup>2</sup> error%	24.8		
X <sup>2</sup> error (%)	14.8	19.8	18.6
Visual fit	Good	Acceptable	Acceptable
k value	0.004103	0.00687	0.0283
P value	4.6E-28	8.08E-8	4.07E-9
Statistical fit	Good	Acceptable	Acceptable
DT <sub>50</sub>	169	101	24.7
DT <sub>90</sub>	561	335	82.1
Ff (molar basis)	-	0.273	0.643

The HSE assessment of the data indicated an acceptable SFO fit for the parent data. It is noted that there was high variability in the final timepoints, however, this did not affect the fitting outcome. The use of the slightly adjusted time step normalised days and data handling gave slightly better statistical fits from the applicant however this is not considered to have a significant impact on the overall outcomes. The kinetic fitting is also considered acceptable for the metabolite compounds.

The following values are agreed for the Czech Republic field data:

**Table B.8.1.2.1.2-31 Endpoints for Czech Republic field data**

<b>Compound</b>	<b>Inpyrfluxam</b>	<b>3'OH-S-2840</b>	<b>1'COOH-S-2840</b>
Kinetic fit	SFO	SFO	SFO
DT <sub>50</sub> inpyrfluxam	169	101	24.7
DT <sub>90</sub>	561	335	82.1
Ff (molar basis)	-	0.273	0.643

Although the slight differences in data handling did not significantly alter the fitted parameters, the HSE values will be taken forward for the purposes of averaging alongside the values determined from the other sites.

### **Kinetic assessment for the Italian field site**

The field values for the Italian site presented by the applicant for use in the kinetic assessment are detailed in Table B.8.1.2.1.2-32 below. Residues are expressed as the sum of residues corrected as parent equivalents.

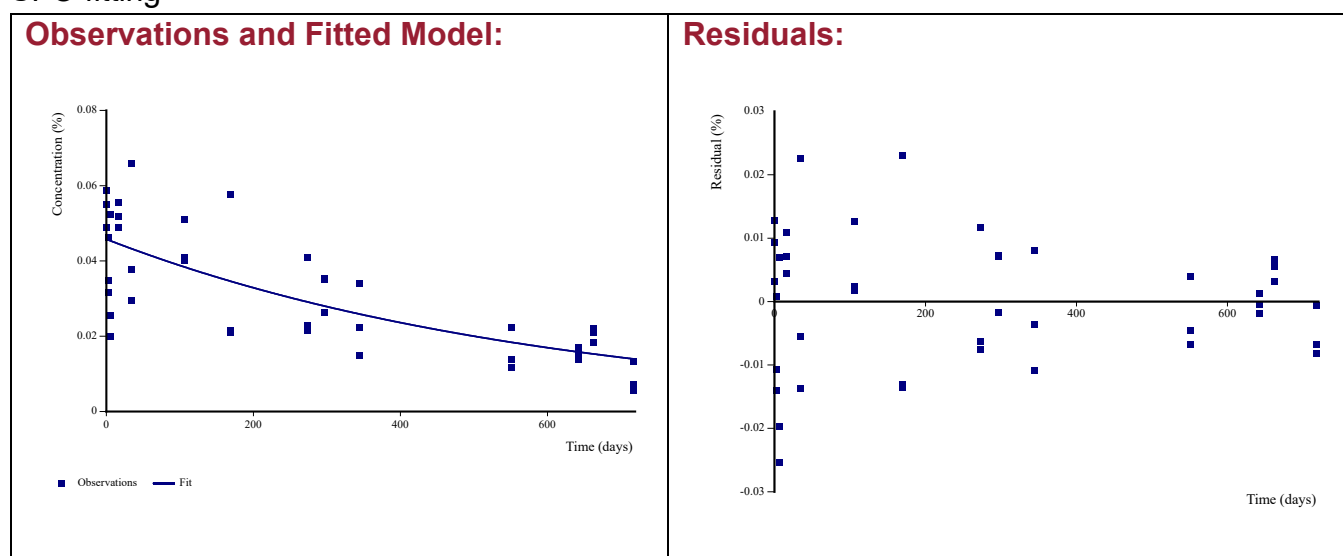
**Table B.8.1.2.1.2-32 Residues of inpyrfluxam and its metabolites in soil (mg/kg)  
– Italian site, applicant values**

Sampling Event (days)	Plot	compound		
		Inpyrfluxam	3'-OH- 2840	1'-COOH- 2840
0	A	0.0586	0	0
	B	0.0551	0	0
	C	0.049	0	0
3	A	0.0348	0	0
	B	0.0315	0	0
	C	0.0463	0	0
6	A	0.0523	0	0
	B	0.0199	0	0
	C	0.0256	0	0
14	A	0.0517	0	0
	B	0.049	0	0
	C	0.0554	0	0
28	A	0.0296	0.00115	0
	B	0.0377	0.00115	0
	C	0.0655	0.00305	0
60	A	0.0408	0.00259	0
	B	0.051	0.00258	0
	C	0.0401	0.00115	0
89	A	0.0577	0.00431	0.00177
	B	0.0211	0.00115	0
	C	0.0215	0.00115	0
180	A	0.0408	0.00407	0.00178
	B	0.0216	0.00270	0
	C	0.0229	0.00326	0
272	A	0.0351	0.00477	0
	B	0.0263	0.00340	0
	C	0.0353	0.00444	0
358	A	0.015	0.00272	0
	B	0.0223	0.00302	0
	C	0.034	0.00575	0
455	A	0.0117	0.00115	0
	B	0.0138	0.00232	0
	C	0.0223	0.00320	0
550	A	0.0139	0.00379	0

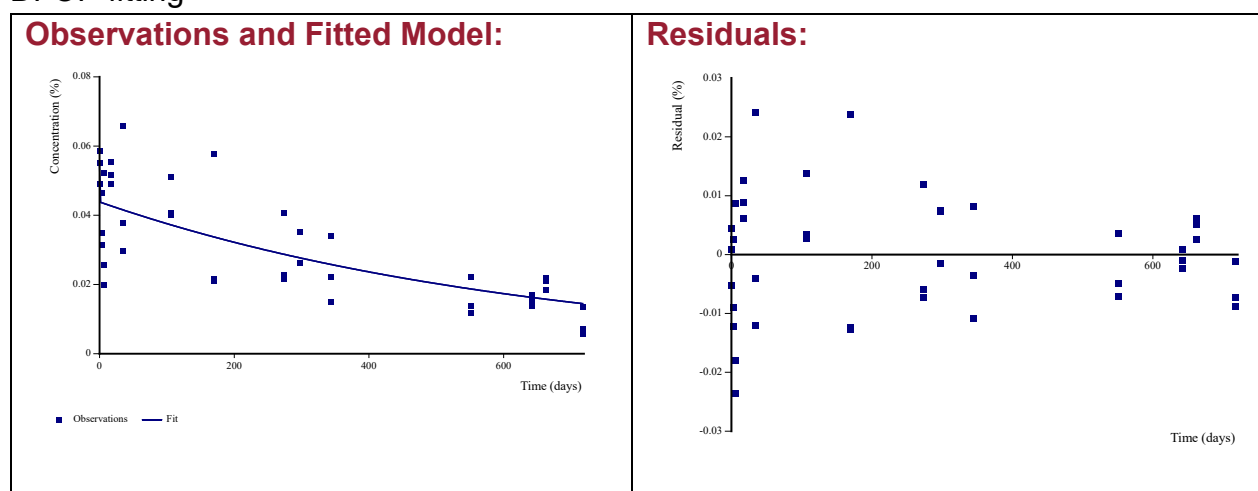
	B	0.0171	0.00425	0
	C	0.0153	0.00341	0
	A	0.0184	0.00415	0
637	B	0.0220	0.00529	0
	C	0.0209	0.00455	0
	A	0.0134	0.00391	0
728	B	0.00572	0.00115	0
	C	0.00718	0.00234	0
	A			

The applicant has presented the following kinetic fits using the CAKE 3.2 software and the data presented from the Italian trial site.

#### SFO fitting



#### DFOP fitting



**Figure B.8.1.2.1-12: Italy field site residue data kinetic fitting– inpyrfluxam alone**

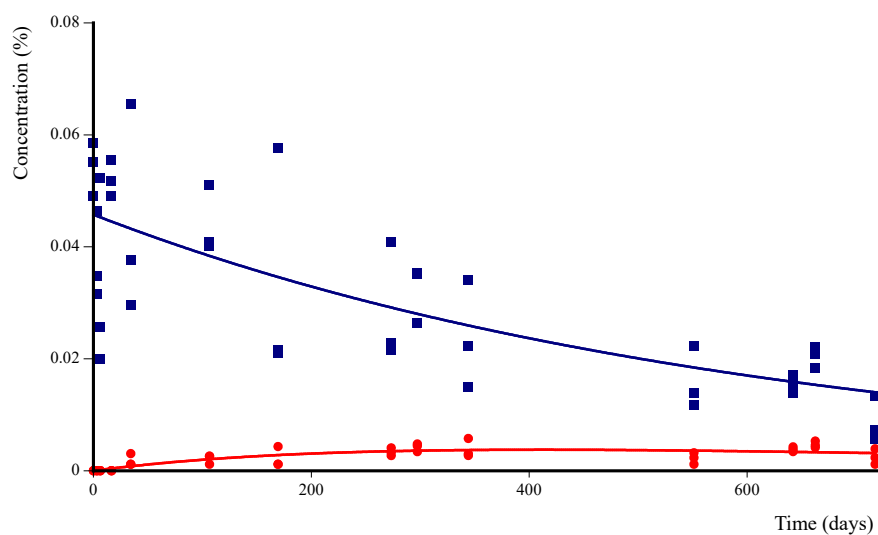
The  $\chi^2$  error value was comparable from both fittings. For the DFOP fitting the applicant noted that the t-test was non-significant for  $k_1$  and, therefore the DFOP fit was considered statistically unacceptable. However, HSE noted that there is little difference between SFO and DFOP in the visual fit, and  $DT_{90}$  values are comparable. Therefore in line with the FOCUS guidance and the desire to accept SFO for FOCUS modelling when the fit is good enough. It was concluded that the SFO fitting for the parent compound is acceptable. As noted above the applicant considered the levels of the metabolite 1'-COOH-S-2840 too low to generate robust fittings. Therefore they have only assessed the parent and 3'-OH-S-2840 fittings.

**Table B.8.1.2.1.2-33: Results of the kinetic determinations for data from the Italian site for inpyrfluxam (alone), applicant assessment.**

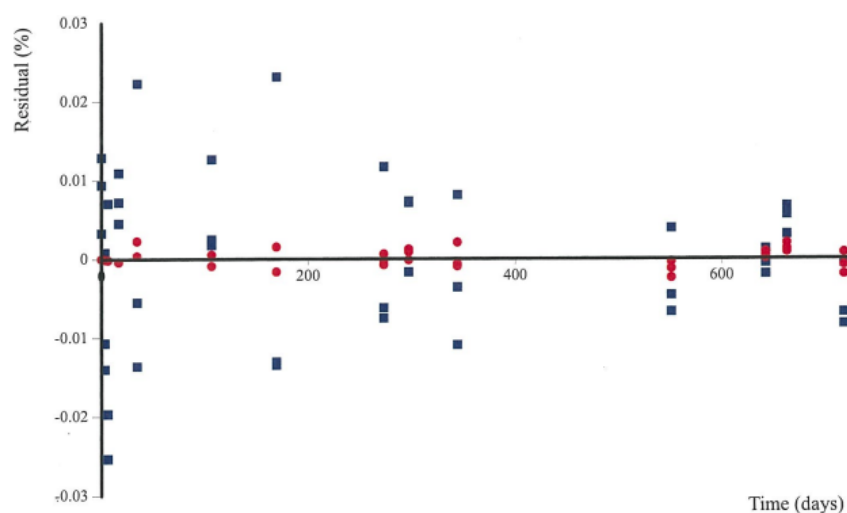
<b>Parameter</b>		
Model	SFO	DFOP
$\chi^2$ error (%)	14.9	14.4
$k$ (days <sup>-1</sup> )*	0.00165 (3.3x10 <sup>-7</sup> )	-
$k_1^*$ (days <sup>-1</sup> )	-	2.05 (0.363)
$k_2^*$ (days <sup>-1</sup> )	-	0.00154 (5.2x10 <sup>-9</sup> )
g value	-	0.192
Statistical fit	Good	Unacceptable
Visual fit	Acceptable	Acceptable
$DT_{50}$ (d)**	419	311 (449)
$DT_{90}$ (d)	1393	1354

\*P value from the t-test is given in brackets.

\*\* values in brackets represent slow phase.

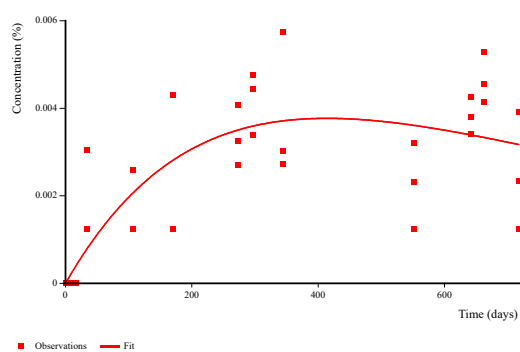


■ Parent    — Parent Fit    ● A1    — A1 Fit

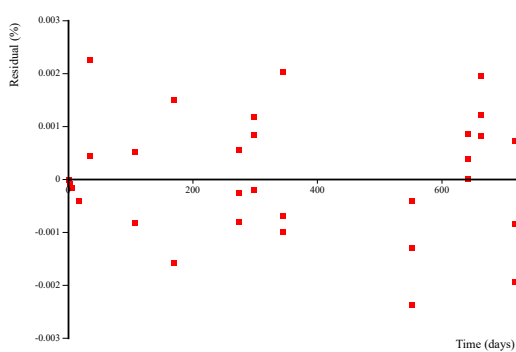


■ Parent    ● A1

### 3'-OH-S-2840



■ Observations    — Fit



**Figure B.8.1.2.1.2-13: Kinetic fit from Italy trial site data SFO-SFO – inpyrfluxam with metabolite 3'-OH-S-2840 (A1). Applicant assessment.**

**Table B.8.1.2.1.2-34 Applicant assessment of the SFO-SFO kinetic fit outputs for the Italian site**

<b>Substance</b>	<b>Inpyrfluxam</b>	<b>3'-OH-S-2840</b>
Overall $\chi^2$ error%	21	
$\chi^2$ error%	14.9	24.0
Visual fit	Good	Acceptable
Statistical fit	Acceptable	Acceptable
DT <sub>50</sub>	421	204
DT <sub>90</sub>	1400	678
Formation fraction	-	0.335

The applicant has noted that they consider that the visual fit for the metabolite shows a clear formation and decline and whilst there is some scatter effect due to the variability in the data points they consider that there is no bias of residuals and therefore they consider that the visual fit is acceptable. Regarding the statistical fits for the metabolite they note that the  $\chi^2$  error is >15% and consider that this is acceptable for field data and is due to the variability of the data values. Therefore they consider the statistical fit acceptable.

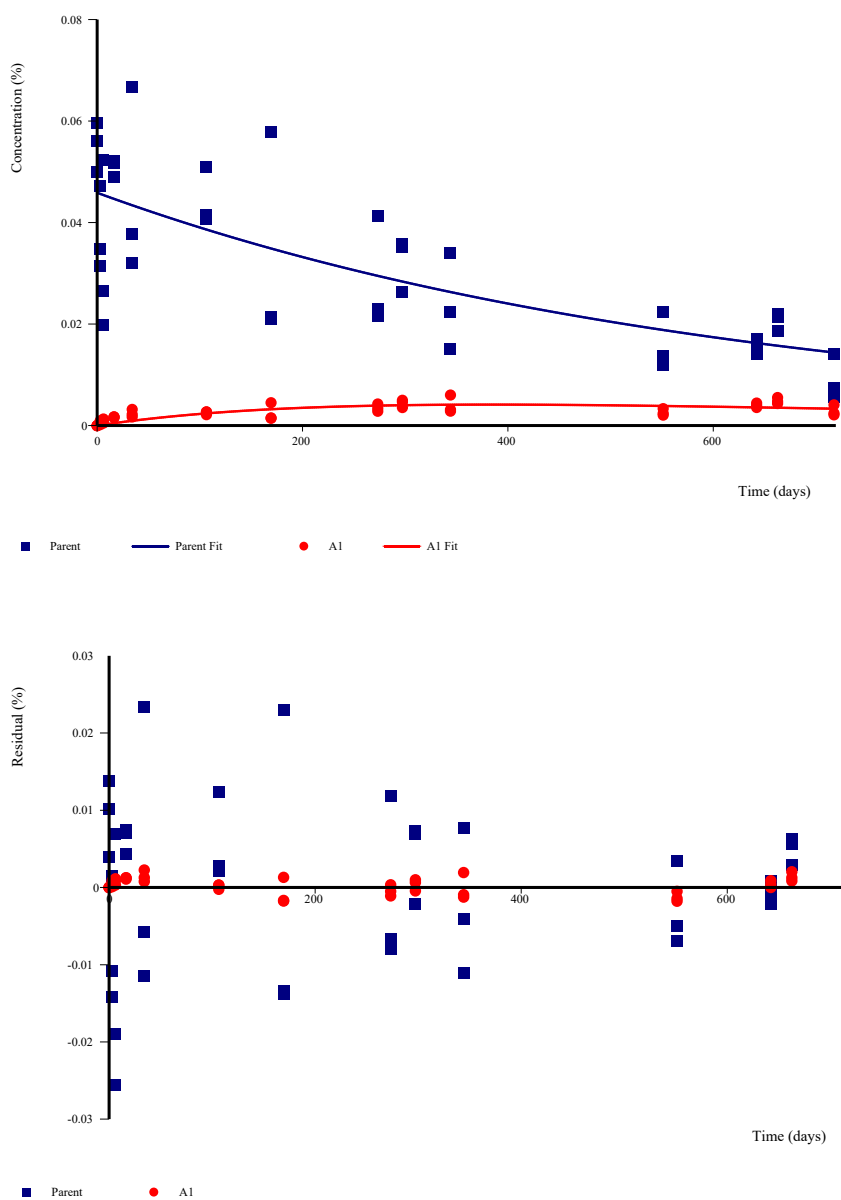
HSE has taken the measured values from the Italian trial site and presented day 0 levels of metabolite 3'-OH-S-2840 as parent equivalents (Table B.8.1.2.1.2-35). Based upon the data values provided HSE would agree with not assessing the 1'-COOH-S-2840 data for kinetic fitting from parent. As the levels measured were only > LOQ at three time points and hence determining a good fit for formation and decline would be inappropriate.

**Table B.8.1.2.1.2-35 Residues of inpyrfluxam and its metabolites in soil (mg/kg) – Italian field site as presented by HSE**

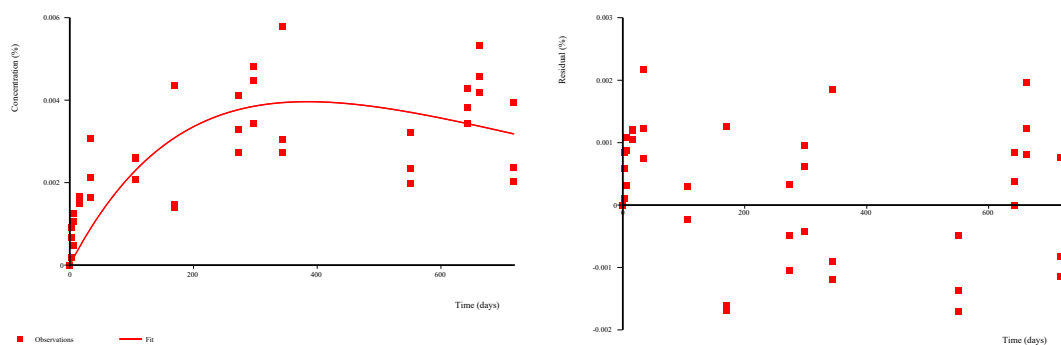
<b>Actual sampling Event (days)</b>	<b>Normalised day</b>	<b>Plot</b>	<b>Compound</b>		
			<b>Inpyrfluxam</b>	<b>3'-OH-S-2840</b>	<b>1'-COOH-S-2840</b>
0	0	A	0.0597	0	0
		B	0.0560	0	0
		C	0.0499	0	0
3	3.1	A	0.0348	0.000924	0
		B	0.0315	0.000672	0
		C	0.04713	0.000192	0
6	6.2	A	0.0523	0.001048	0

		B	0.0199	0.000484	0
		C	0.02644	0.00126	0
14	16.5	A	0.0517	0.001673	0.000217
		B	0.049	0.0015	0.00026
		C	0.0521	0.001644	0.000226
28	34.2	A	0.03197	0.001644	0.0008
		B	0.0377	0.002135	0.000784
		C	0.06679	0.003077	0.00123
60	106.5	A	0.0408	0.002606	0.001406
		B	0.051	0.002596	0.001241
		C	0.04147	0.002077	0.001224
89	169.4	A	0.0579	0.004346	0.002248
		B	0.0211	0.001471	0.000537
		C	0.0215	0.001394	0.000685
180	273.3	A	0.0414	0.004106	0.00217
		B	0.0216	0.002721	0.0007
		C	0.0229	0.003288	0.001187
272	297.3	A	0.0357	0.004808	0.001951
		B	0.0263	0.003423	0.000845
		C	0.0353	0.004471	0.001465
358	344.1	A	0.0152	0.00274	0.000219
		B	0.0223	0.003038	0.000841
		C	0.034	0.005798	0.001339
455	551.2	A	0.0119	0.00199	0.000222
		B	0.0138	0.002337	0.000484
		C	0.0223	0.003221	0.000938
550	642.2	A	0.0141	0.003817	0.000608
		B	0.0171	0.004279	0.0001
		C	0.0155	0.003433	0.0001
637	662.6	A	0.0186	0.004183	0.0001
		B	0.0220	0.005327	0
		C	0.02139	0.004587	0
728	717.5	A	0.01415	0.003942	0
		B	0.00572	0.002038	0
		C	0.00738	0.002356	0

Using the data presented from the Italian trial site HSE has considered the kinetic fit using the CAKE 3.7 software with IRLS fitting and obtained the following fit for SFO-SFO kinetics. The following kinetic fit was determined for parent inpyrfluxam and the 3'-OH-S-2840 metabolite.



## 3'-OH-2840



**Figure B.8.1.2.1.2-14 Kinetic fit from Italy trial site data SFO-SFO – inpyrfluxam with metabolite 3'-OH-S-2840 (A1). HSE assessment**



**Table B.8.1.2.1.2-36 Kinetic fitting outputs for the Italian field site HSE assessment**

Compound	Inpyrfluxam	3'OH-S-2840
Kinetic fit	SFO	SFO
Overall X <sup>2</sup> error%	20.6	
X <sup>2</sup> error (%)	14.8	23.8
Visual fit	Good	Acceptable
k value	0.001616	0.003926
P value	7.5E-8	1.37E-4
Statistical fit	Good	Acceptable
DT <sub>50</sub>	429	177
DT <sub>90</sub>	1430	586
ff	-	0.39

The HSE assessment of the data supports the applicant approach and outcomes and since HSE largely accepted the applicant data handling and time step normalisation, agrees with the proposed endpoints for the Italy field data

**Table B.8.1.2.1.2-37 Endpoints for Italy field data**

Compound	Inpyrfluxam	3'OH-S-2840
Kinetic fit	SFO	SFO
DT <sub>50</sub> inpyrfluxam	421	204
DT <sub>90</sub>	1400	678
Ff (molar basis)	-	0.34

### Kinetic assessment for the Spanish field site

The field values for the Spanish site presented by the applicant for use in the kinetic assessment are detailed in Table B.8.1.2.1.2-38 below. Residues are expressed as the sum of residues corrected as parent equivalents.

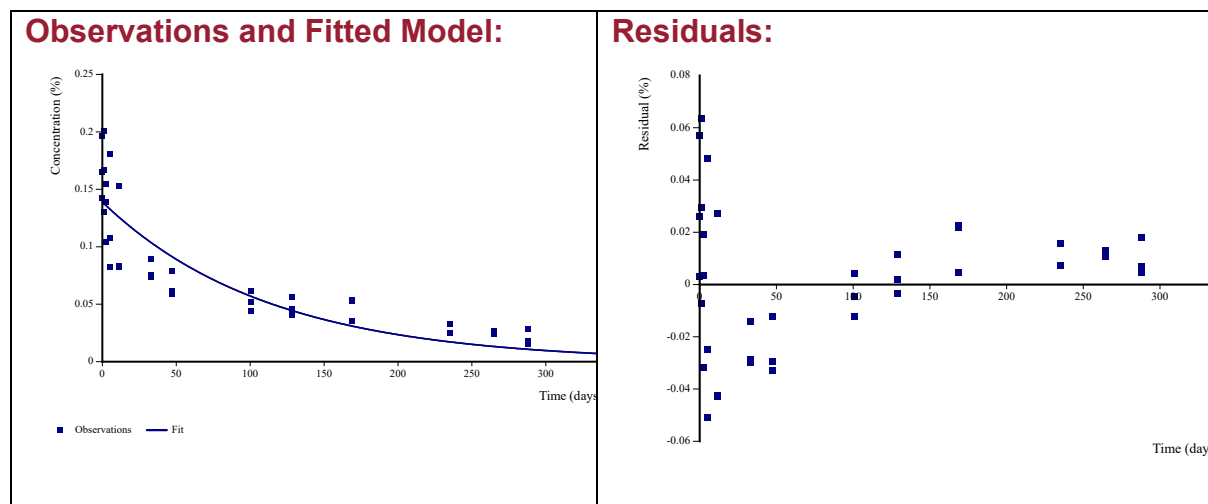
**Table B.8.1.2.1.2-38 Residues of inpyrfluxam and metabolites in soil– Spain site**

Sampling Event (days)	Normalised days	Plot	Soil layer (cm)		
			Inpyrfluxam	3'-OH-2840	1'-COOH-2840
0	0.0	A	0.196	0.00244	0
		B	0.165	0.00115	0
		C	0.142	0.00115	0
3	1.2	A	0.167	0.00305	0.00168

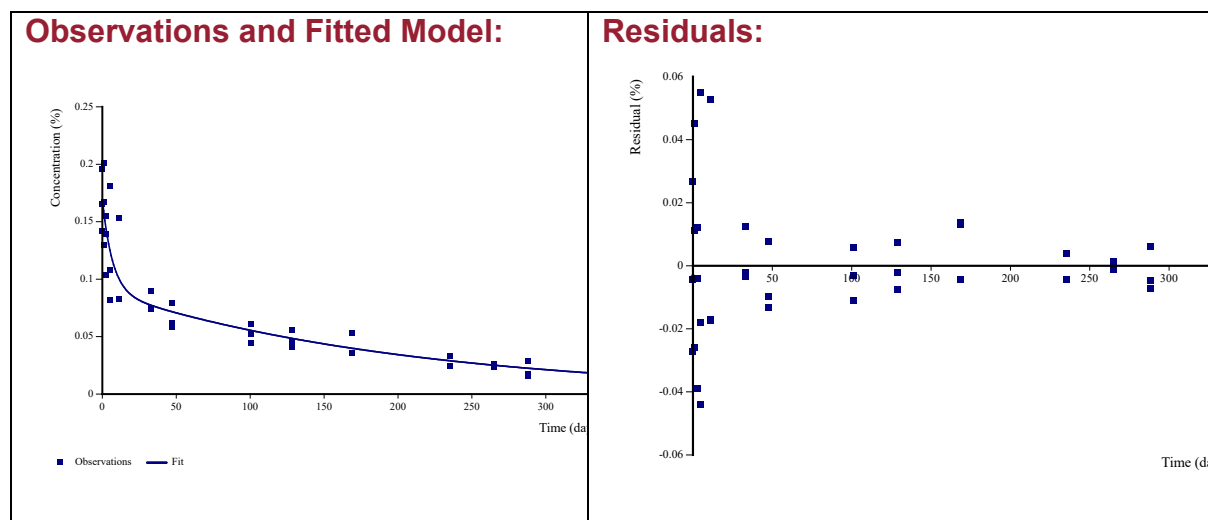
		B	0.13	0.00261	0.00172
		C	0.201	0.00351	0.00217
7	2.6	A	0.139	0.00115	0.00484
		B	0.104	0.00115	0.00401
		C	0.155	0.00395	0.00596
14	5.0	A	0.181	0.00808	0.01057
		B	0.082	0.00326	0.00612
		C	0.108	0.00273	0.00649
28	11.3	A	0.0831	0.00802	0.00778
		B	0.0828	0.00590	0.00864
		C	0.153	0.00716	0.01183
62	32.8	A	0.0897	0.01240	0.01311
		B	0.0751	0.00983	0.01380
		C	0.0739	0.00719	0.01163
89	47.3	A	0.0792	0.00992	0.01284
		B	0.0585	0.00942	0.01193
		C	0.0618	0.00680	0.01207
176	100.9	A	0.0611	0.01498	0.01683
		B	0.0444	0.01011	0.01216
		C	0.0522	0.00760	0.01549
266	128.6	A	0.0462	0.01536	0.01474
		B	0.041	0.01129	0.01262
		C	0.0559	0.01116	0.01778
361	168.8	A	0.0535	0.00349	0.01318
		B	0.053	0.00653	0.01562
		C	0.0357	0.00827	0.01606
454	235.1	A	0.0329	0.01193	0.01811
		B	0.0246	0.00690	0.01442
		C	0.0246	0.00636	0.01649
538	264.7	A	0.0239	0.00881	0.01122
		B	0.0264	0.00802	0.01348
		C	0.0259	0.00811	0.01230
629	288.1	A	0.0177	0.00775	0.01039
		B	0.0154	0.00482	0.00940
		C	0.0287	0.00777	0.01631
740	339.1	A	0.0200	0.00878	0.01194
		B	0.0133	0.00284	0.01145
		C	0.0190	0.00633	0.01127

The applicant has presented the following kinetic fits using the CAKE 3.7 software and the data presented from the Spanish trial site. Initial consideration of inpyrfluxam only data indicated an acceptable fit was determine for the DFOP fitting

### SFO



### DFOP



**Figure B.8.1.2.1.2-15 Spain field data kinetic fitting– inpyrfluxam alone**

The applicant noted that for the Spanish site the  $\chi^2$  value was significantly lower for the DFOP fit and in addition a significant result was noted for the t-test for both degradation rates. Therefore the DFOP fitting is accepted for the parent compound and HSE would agree with this approach as there was some indication of a systematic pattern in the residual plot for the SFO fit.

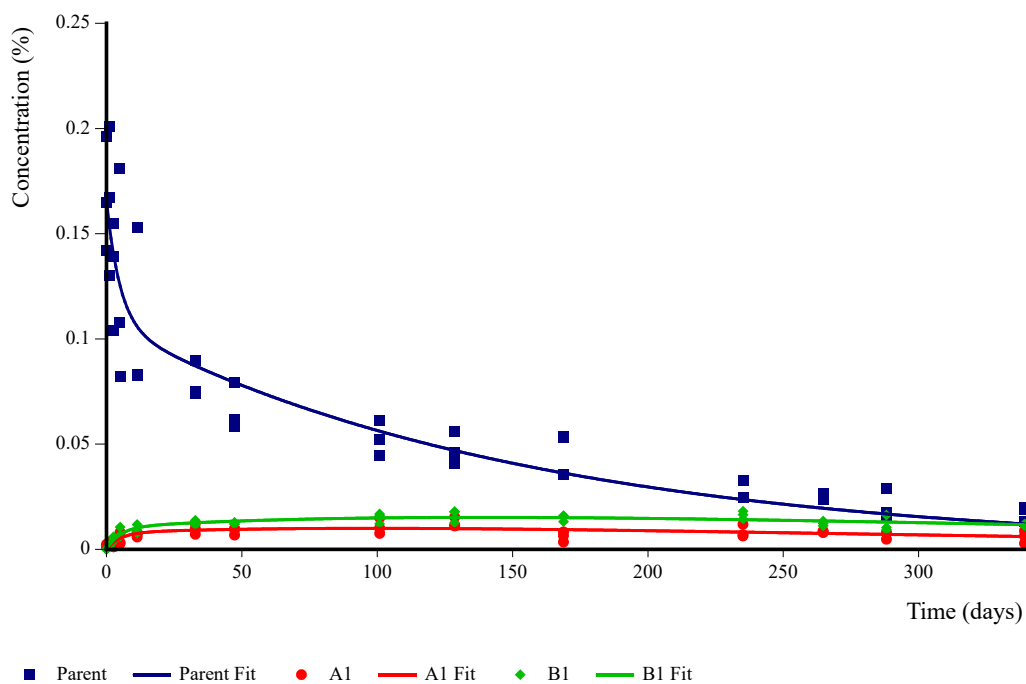
**Table B.8.1.2.1.2-39 Results of the kinetic determinations for inpyrfluxam (alone)**

<b>Parameter</b>	<b>Spanish Site</b>	
<b>Model</b>	<b>SFO</b>	<b>DFOP</b>
$\chi^2$ error (%)	18.0	5.69
k (days <sup>-1</sup> )*	0.00888 (1.5x10 <sup>-8</sup> )	
k <sub>1</sub> *(days <sup>-1</sup> )	-	0.1463 (0.014)
k <sub>2</sub> *(days <sup>-1</sup> )	-	0.00481 (4.9x10 <sup>-5</sup> )
G	-	0.469
Statistical fit	Good	Good
Visual fit	Poor	Good
DT <sub>50</sub> (d)**	78.1	21.3 (144)
DT <sub>90</sub> (d)	259	347

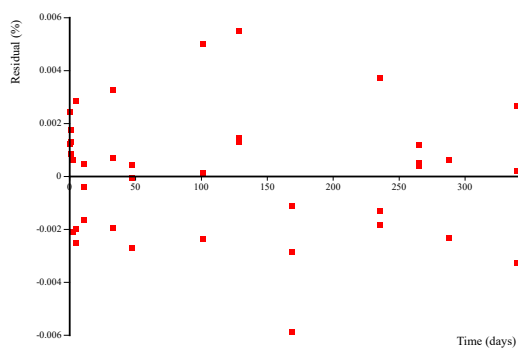
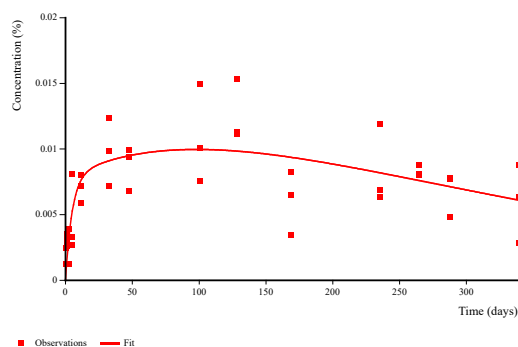
\*P value from the t-test is given in brackets.

\*\* values in brackets represent slow phase.

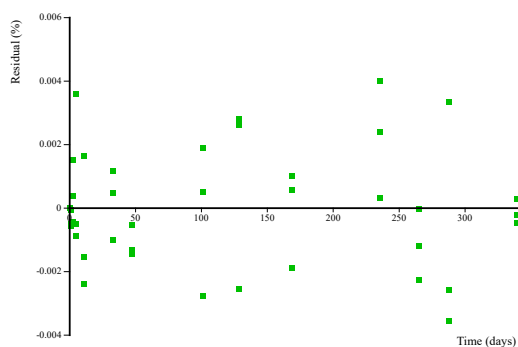
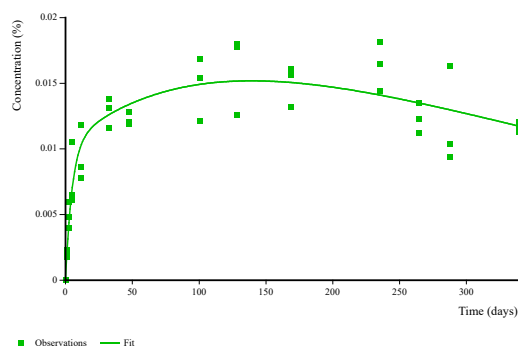
The applicant presented the following kinetic fits for inpyrfluxam and its metabolites.



## 3'-OH-S-2840



## 1'-COOH-S-2840



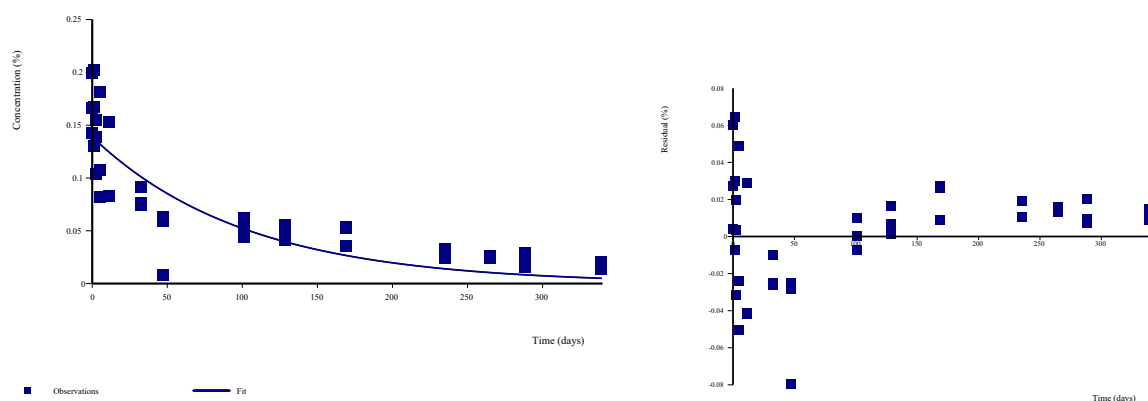
**Figure B.8.1.2.1.2-15 Kinetic fit from Spain trial site data SFO-SFO – inpyrfluxam with metabolites 3'-OH-S-2840 (A1) and 1'-COOH-S-2840 (B1), applicant assessment**

**Table B.8.1.2.1.2-40 Applicant assessment of the DFOP-SFO kinetic fit outputs for inpyrfluxam and metabolite data from the Spanish site**

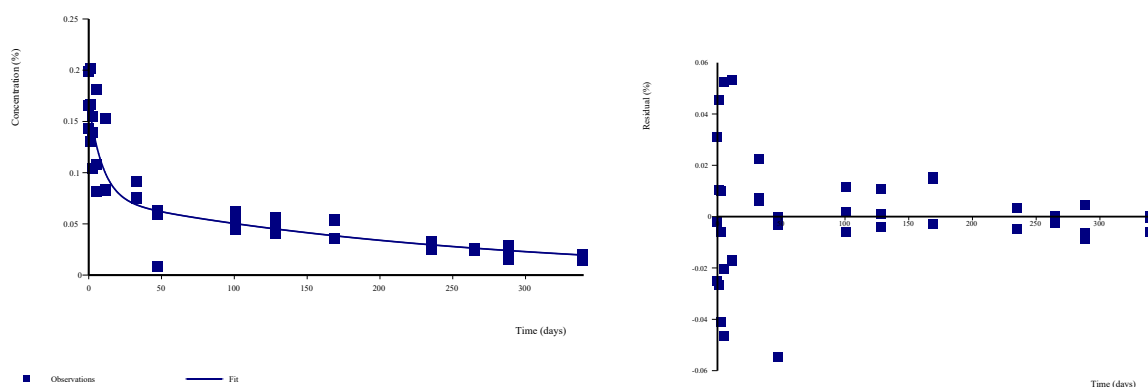
<b>Soil</b>	<b>Inpyrfluxam</b>	<b>3'OH-S-2840</b>	<b>1'COOH-S-2840</b>
<b>Parent kinetic</b>	DFOP	SFO	SFO
Overall $\chi^2$ error%	11.4		
$\chi^2$ error%	7.54	15.5	6.44
Visual fit	Good	Acceptable	Acceptable
Statistical fit	Good	Acceptable	Acceptable
DT <sub>50</sub> inpyrfluxam	<b>38 (111)</b>	<b>148</b>	<b>224</b>
ff		0.128	<b>0.169</b>

The applicant noted that the data from the Spanish site had a lower  $\chi^2$  using DFOP kinetics and a good statistical outcome compared to the SFO fitting therefore the DFOP fitting was accepted by the applicant. HSE has taken the residue values from the Spain trial site as presented by the applicant in Table B.8.1.2.1.2-38 for kinetic assessment. HSE has taken the day zero levels of metabolite 3'-OH-S-2840 and summed these as parent equivalents with the inpyrfluxam day zero values, this is an appropriate adjustment of the data for a field site study and a rapidly forming metabolite. It is noted that in the laboratory rate study this metabolite was identified as an impurity in the dosing solution so therefore was not added to parent levels for day zero. However this information is not detailed for the formulated product used to dose the field trials. It is further considered that the day zero levels were just at LOQ at one of the sites and therefore the impact on the kinetic fitting is expected to be negligible. It is noted that the applicant fixed the 3'-OH-S-2840 level to zero in their kinetic fitting.

Using the data presented from the Spain trial site HSE has considered the kinetic fit for the parent compound inpyrfluxam using the CAKE 3.7 software with IRLS fitting. The outcomes are detailed below. Based upon this HSE would agree with the fitting of the parent substance as a DFOP fit for this soil. The outcome of the fitting is detailed in Figure B.8.1.2.1.2-16 and Table B.8.1.2.1.2-41 below.



### Spain site DFOP fitting for inpyrfluxam



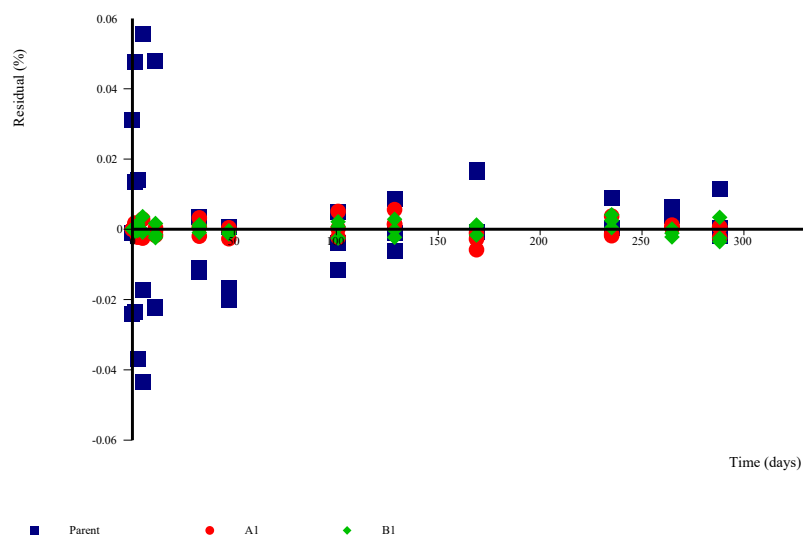
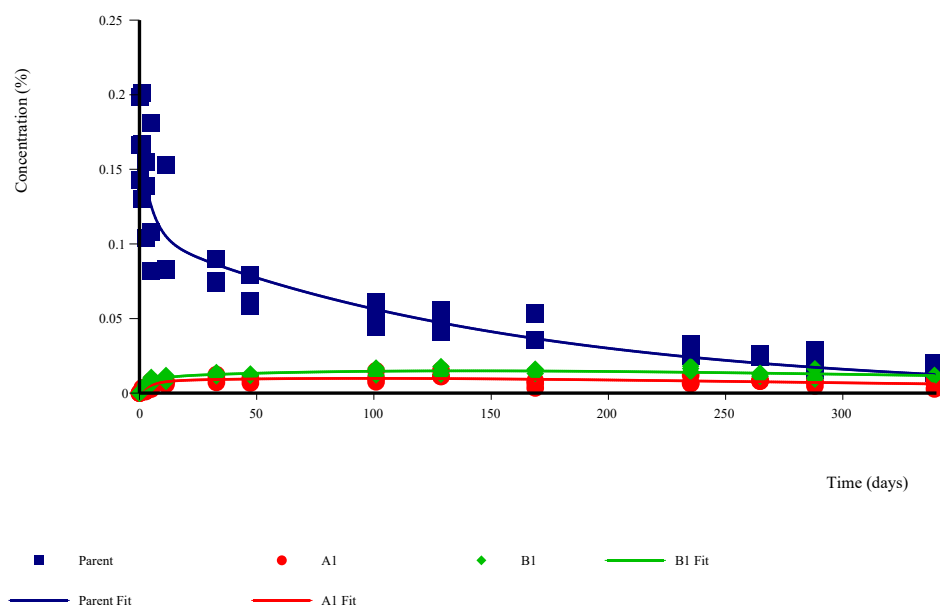
**Figure B.8.1.2.1-16 Spain site SFO fitting for inpyrfluxam (alone). HSE assessment.**

**Table B.8.1.2.1.2-41 Results of the kinetic determinations for inpyrfluxam (alone) from the Spanish trial site**

Parameter		
Model	SFO	DFOP
$\chi^2$ error (%)	21.7	9.47
k (p value)	0.00973 (2.6E-7)	-
k1 (p value)	-	0.103 (0.0137)
k2 (p value)	-	0.00393 (0.00266)
Statistical fit	Acceptable	Acceptable
Visual fit	Poor	Good
DT <sub>50</sub> (d)	71.2	18.2 overall 6.73 fast phase 177 slow phase

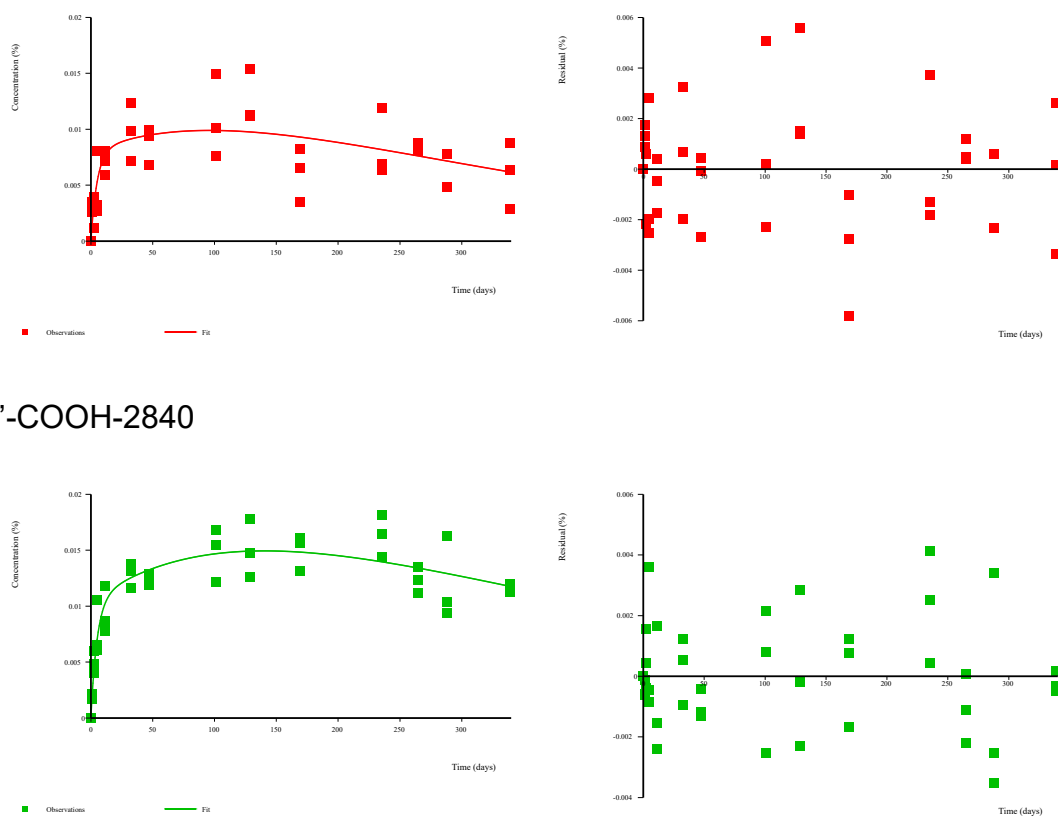
DT <sub>90</sub> (d)	237	380
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HSE agrees with the applicant that the  $\chi^2$  value is significantly lower for the DFOP fit and the visual fit is much improved. Therefore, HSE agree with the use of DFOP kinetics for the parent substance in the kinetic assessment of the metabolite endpoints.



3'-OH-S-2840





1'-COOH-2840

**Figure B.8.1.2.1.2-17 Spain site SFO fitting for inpyrfluxam (alone). HSE assessment.**

**Table B.8.1.2.1.2-42 Results of the kinetic determinations for inpyrfluxam from the Spanish trial site**

<u>Parameter</u>	<u>Inpyrfluxam</u>	<u>3'OH-S-2840</u>	<u>1'-COOH-2840</u>
<i>Model</i>	DFOP	SFO	SFO
Overall $\chi^2$ error (%)	11.4		
$\chi^2$ error (%)	7.55	15.5	6.44
k (p value)	-	0.00465 (1.94E-6)	0.00308(2.8E-7)
k <sub>1</sub> (p value)	0.1983 (7.1E-6)	-	-
k <sub>2</sub> (p value)	0.006287 (9.6E-11)	-	-
g	0.37	-	-
Statistical fit	Acceptable	Acceptable	Acceptable
Visual fit	Good	Acceptable	Acceptable
DT <sub>50</sub> (d)	37.5 overall 3.5 fast phase 110 slow phase	149	225
DT <sub>90</sub> (d)	293	495	748

Formation fraction	-	0.13	0.17
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It is concluded for the Spain site that slight differences in data handling did not significantly alter the fitted parameters and the values proposed by the applicant can be used for the purposes of averaging alongside the values from the other sites.

## Summary

The kinetic assessment of the EU field data for the determination of modelling endpoints as presented by the applicant has been assessed by HSE. The approaches taken are in line with the current FOCUS guidance and the outcomes have been validated by HSE using the CAKE 3.7. software. Slight differences in handling of the data and outcomes of the timestep normalisation had a small impact on the fitting outcomes. For the German field site data the difference in data handling and kinetic fitting approaches and decisions on the acceptability of sequential fitting for the 1'COOH-S-2840 metabolite resulted in difference in the endpoints determined and HSE values will be taken forward. For the Czech Republic site slight differences in data handling did not significantly alter the fitted parameters and the HSE values will be taken forward. The HSE determined values for the Germany and Czech Republic sites will be taken forward for the purposes of averaging alongside the applicant values from the Italy and Spain field sites, where HSE was able to closely replicate the applicant values. The following degradation values as determined in the EU field studies can be used in the environmental assessment (Table B.8.1.2.1.2-43).

**Table B.8.1.2.1.2-43 Results of the kinetic determinations for inpyrfluxam with its metabolites when normalised to 20°C and pF2**

Soil	Germany	Czech Republic	Italy	Spain	Ontario
	HSE values	HSE values	Applicant values	Applicant values	Applicant values
<b>Parent kinetic</b>	SFO	SFO	SFO	DFOP	SFO
Overall $\chi^2$ error%	28.2	24.8	24.7	11.4	-
<b>Inpyrfluxam</b> $\chi^2$ error%	16.7	14.8	14.9	7.54	19.5
Visual fit	Good	Good	Good	Good	Good
Statistical fit	Good	Good	Acceptable	Good	Acceptable
DT <sub>50</sub>	<b>78.8</b>	<b>169</b>	<b>421</b>	38 (overall) 3.51 (fast phase) 111 (slow phase)	<b>104</b>

				g value 0.37	
DT <sub>90</sub> inpyrfluxam	262	561	1400	295 (overall)	344
<b>3'OH-S-2840</b> $\chi^2$ error%	18.2	19.8	24.0	15.5	-
Visual fit	Acceptable	Acceptable	Acceptable	Acceptable	-
Statistical fit	Acceptable	Acceptable	Acceptable	Acceptable	-
DT <sub>50</sub>	<b>96.6</b>	<b>101</b>	<b>204</b>	<b>149</b>	-
DT <sub>90</sub>	321	335	678	495	-
ff	0.18	0.27	0.34	0.13	-
<b>1'COOH-S-2840</b> $\chi^2$ error%	14.4	18.6	-	6.44	-
Visual fit	Acceptable	Acceptable	-	Acceptable	-
Statistical fit	Acceptable	Acceptable	-	Acceptable	-
DT <sub>50</sub>	<b>75.4</b>	<b>24.7</b>	-	<b>224</b>	-
DT <sub>90</sub>	250	82.1		744	-
ff	0.77	0.64	-	0.17	-

For inpyrfluxam at the Spanish site, the DT<sub>90</sub>/3.32 is 88.86 days. This can then be compared alongside DegT<sub>50</sub> values for SFO data sets. An alternative choice could have been the slow phase DegT<sub>50</sub> value for biphasic data sets. Since there will often be a mixture of SFO and biphasic data sets, this would generate an artificial discrepancy between SFO and biphasic data sets. Therefore, the pseudo SFO DegT<sub>50</sub> is preferred as it provides a better representation of the overall degradation behaviour including the fast and slow phases.

### Consideration of non-normalised degradation for inpyrfluxam from the field study sites.

The applicant has presented kinetic fitting of the non-normalised data for the purposes of determining trigger and persistence endpoints for inpyrfluxam. HSE consider that the field study data from sites in Germany, Czech Republic, Italy, Spain and Ontario (USA) are considered acceptable for use in the kinetic assessment. The applicant has used the CAKE 3.7 software and IRLS fitting to determine endpoints and a summary is provided in the table below:

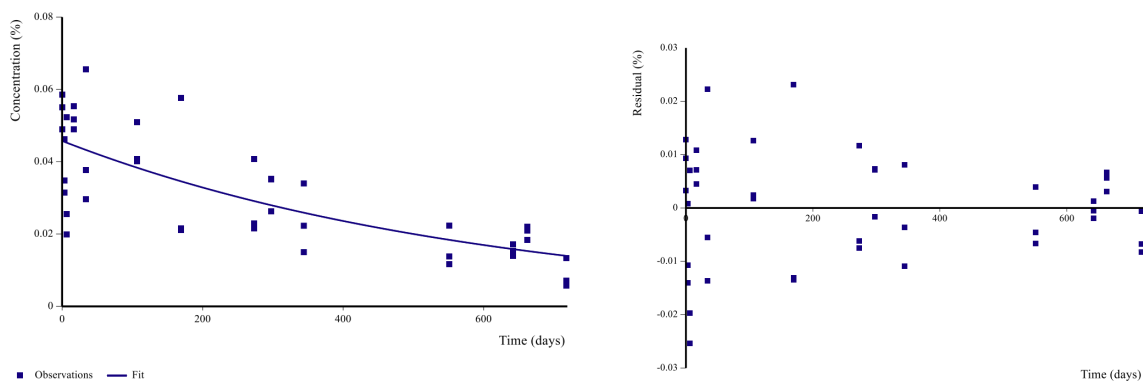
**Table B.8.1.2.1.2-44 Applicant values for triggering endpoints, using non-normalised data. Proposed endpoint for each site indicated with (T)**

Site/Study	Kinetic Model	M <sub>0</sub>	Parameter (k, k <sub>1</sub> , k <sub>2</sub> , g, t <sub>b</sub> , α, β)	Chi-sq error	Prob >t	DT <sub>50</sub> (days)	DT <sub>90</sub> (days)
Ontario/ [REDACTED] 2017b	SFO	0.0481	k: 0.0152	28.5	0.000605	45.7	152
	FOMC	0.0637	α: 0.317 β: 1.39	9.24	n.r. n.r.	11	1995
	<b>DFOP (T)</b>	0.0616	k <sub>1</sub> : 0.157 k <sub>2</sub> : 0.00257 g: 0.596	7.85	0.0315 0.0231 n.r.	<b>10.9</b>	543
German/ [REDACTED] 2018	<b>SFO (T)</b>	0.126	k: 0.00593	19	4.6E-08	<b>117</b>	388
	FOMC	0.13	α: 2.09 β: 244	18.7	n.r. n.r.	95.8	489
	DFOP	0.154	k <sub>1</sub> : 3.53 k <sub>2</sub> : 0.0052 g: 0.244	16.4	0.49 1.02E-08 n.r.	79.6	389
Czech / [REDACTED] 2018	<b>SFO (T)</b>	0.114	k: 0.00215	16.1	4.7E-11	<b>322</b>	1069
	FOMC	0.115	α: 90.5 β: 4.1E+04	16.7	n.r. n.r.	316	1058
	DFOP	0.125	k <sub>1</sub> : 0.0431 k <sub>2</sub> : 0.00177 g: 0.204	16	0.209 1.27E-05 n.r.	263	1173
Italian/ [REDACTED] 2018	<b>SFO (T)</b>	0.0447	k: 0.00181	15.2	7.51E-07	<b>383</b>	1272
	FOMC	0.0448	α: 12.1 β: 6402	15.7	n.r. n.r.	379	1347
	DFOP	0.0542	k <sub>1</sub> : 3.56 k <sub>2</sub> : 0.00168 g: 0.211	14.4	0.475 5.21E-09 n.r.	271	1227
Spanish [REDACTED], 2018	SFO	0.14	k: 0.00463	18.8	5.86E-08	150	498
	FOMC	0.169	α: 0.513 β: 18	6.74	n.r. n.r.	51.5	1584
	<b>DFOP (T)</b>	0.168	k <sub>1</sub> : 0.0458 k <sub>2</sub> : 0.0021 g: 0.513	5.78	0.0128 0.000513 n.r.	<b>47.3</b>	753

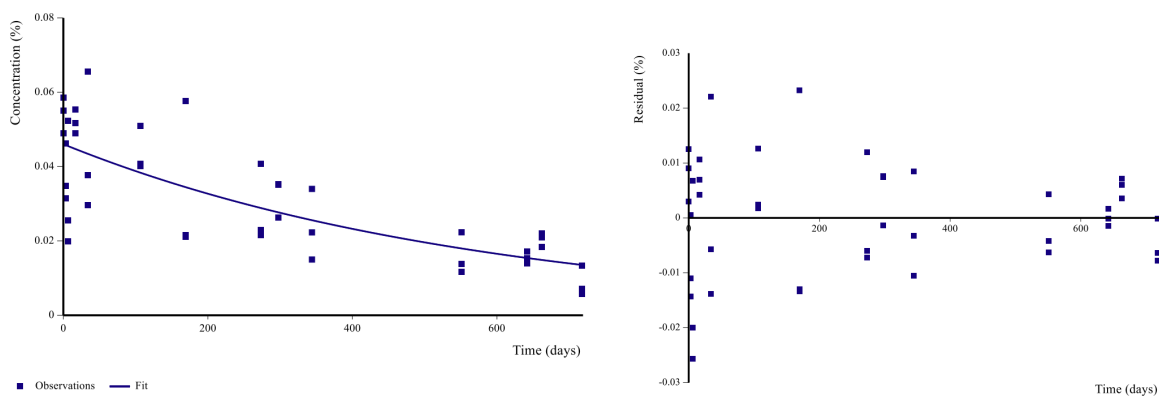
Using the determined field residue values as detailed in section B.8.1.2.1 with the sampled field dates HSE has considered the kinetic fitting presented by the applicant to determine trigger and persistence endpoints. The applicant has used the CAKE 3.7 software with IRLS fitting. HSE has validated the outcomes as detailed above

and would agree with the applicant values with the Italy site data giving the longest non-normalised values for use as triggering endpoint. This value may also be used in PECsoil calculations, including accumulation calculations. The fitting outcomes for the Italy site data are detailed below.

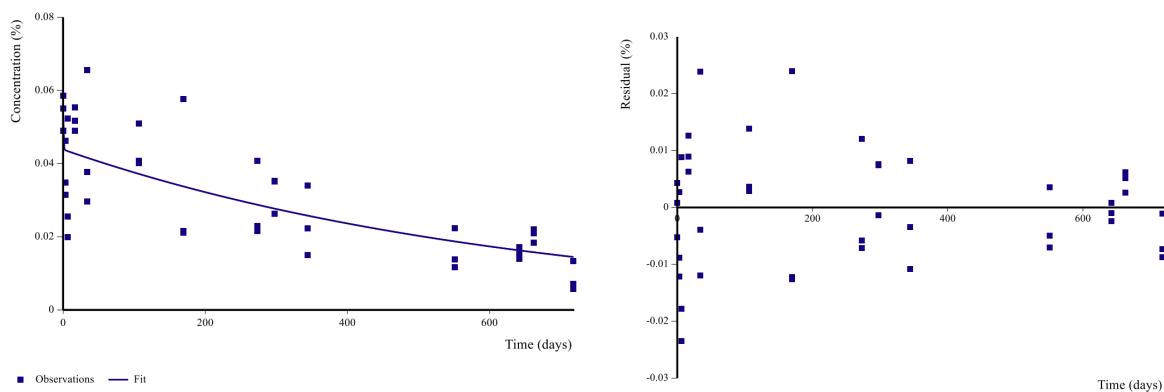
## SFO



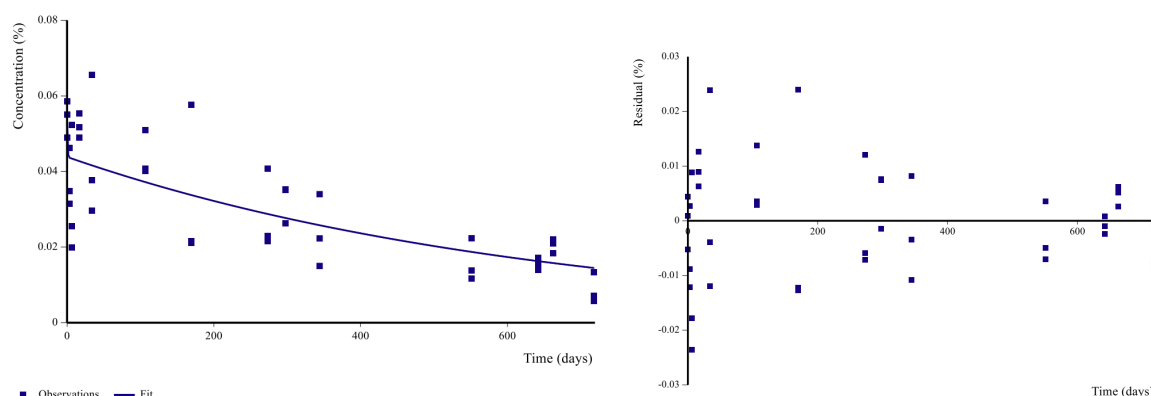
## FOMC



## DFOP



## HS



**Figure B.8.1.2.1-18 Spain site SFO fitting for inpyrfluxam (alone). HSE assessment.**

The longest non-normalised  $DT_{50}$  for use in trigger assessment is 383 days (SFO) and the longest  $DT_{90}$  associated with this SFO fit is 1272. As the  $DT_{90}$  value exceeds 365 days at more than one field site the need to address soil accumulation is triggered.

In consideration of persistence/triggering endpoints for metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 it is noted that non-normalised kinetic fittings are not available from the field data for these compounds. It is therefore proposed that the degradation endpoints from the laboratory studies be used to consider persistence/triggering endpoints for these substances, which are detailed in Table 8.1.1.2.2-04. For metabolite 3'-OH-S-2840 this would result in a longest non-normalised  $DT_{50}$  for use in trigger assessment of 369 days (SFO) and the longest  $DT_{90}$  associated with this SFO fit is 1226 – this value was noted to be longer than the normalised values available from the field dissipation studies. For metabolite 1'-COOH-S-2840 this would result in a longest  $DT_{50}$  for use in trigger assessment of 148 days (SFO) and the longest  $DT_{90}$  associated with this SFO fit is 491 – this value is noted to be shorter than the longest normalised value from the field dissipation studies (224 d). For both metabolites the  $DT_{90}$  value from the laboratory exceeds 365 days in more than one soil type and the need to address soil accumulation is therefore triggered. For metabolite 1'-COOH-S-2840 HSE concludes that using the longest  $DT_{50/90}$  value from the laboratory will be sufficiently conservative for the purposes of calculating  $PEC_{soil}$ , when combined with the higher levels of formation seen in the lab compared to the field.

**B.8.1.2.2. Soil accumulation studies**

<b>Data Point:</b>	KCA 7.1.2.2.2/01 KCA 7.1.2.2.2/02
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2018 2023
<b>Report Title:</b>	Soil accumulation study after application of S-2399 at four different locations in Europe – 2016/2021 (Interim Report)  Soil accumulation study after application of S-2399 at four different locations in Europe – 2016/2021 (Final Report)
<b>Study number</b>	TPR-0089 TPR-0090
<b>Guideline(s) followed in study:</b>	Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market
<b>GLP?</b>	Yes
<b>Deviations</b>	<p><b>HSE accepts this study overall</b></p> <ul style="list-style-type: none"> <li>• Soils were characterised only to a depth of 30 cm. However, no quantifiable residues were present below 20 cm and therefore this is acceptable.</li> <li>• For some soil residue samples, the lowermost soil section analysed contained detectable residues. However, these were always &lt;LOQ and therefore HSE accepts this deviation.</li> <li>• Application verification results were highly variable and on average lower than the target application rate. However, they were higher than that of the representative product use pattern in all cases and therefore HSE accepts the rate achieved.</li> <li>• During transport of frozen soil cores, minor temperature deviations above -18 °C were observed but samples remained hard frozen so HSE accepts this deviation.</li> <li>• A small number of individual procedural recoveries during the analytical phase were &gt;110%, although</li> </ul>

	<p>remained &lt;120%. The mean recoveries were all in the acceptable range 70-110% and therefore HSE accepts that this is unlikely to significantly impact the study outcome.</p> <ul style="list-style-type: none"> <li>In the CZ02 trial the storage temperature was above -18 °C on a number of occasions with a maximum temperature of -14.9 °C being reached. It is noted that all samples remained hard frozen so HSE accepts this deviation.</li> </ul>
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## INTRODUCTION

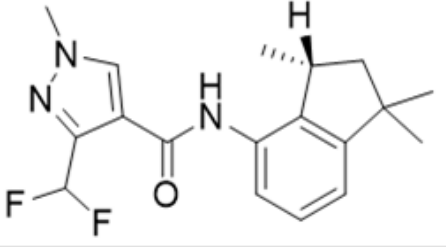
The accumulation of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B was assessed in soil under field conditions at four EU sites. A 37.3% w/w SC formulation of inpyrfluxam was applied via a manual boom sprayer to bare (uncropped) soil at a nominal rate of 200 g a.s./ha in May/early June 2016, 2017 and 2018.

The study was performed in parallel with a field dissipation study (██████████ 2018a; Soil dissipation study after application of inpyrfluxam at four different locations in Europe) at the same field sites.

## MATERIAL AND METHODS

### Materials

**Table B.8.1.2.2-01 Properties of the test item**

<b>Formulation name</b>	<b>S-2399 40 SC</b>
Main use	Fungicide
Formulation type	SC
Active ingredient	Inpyrfluxam
Chemical structure	
Chemical name	<i>N</i> -[(3 <i>R</i> )-2,3-dihydro-1,1,3-trimethyl-1 <i>H</i> -inden-4-yl]-1-methyl-3-(difluoromethyl)-1 <i>H</i> -pyrazole-4-carboxamide



	3-(difluoromethyl)- <i>N</i> -[( <i>R</i> )-2,3-dihydro-1,1,3-trimethyl-1 <i>H</i> -inden-4-yl]-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide (IUPAC)
CAS number	1352994-67-2
Nominal concentration	40 % w/v, 400 g/L
Nominal density	1.1g/mL as mentioned in the SDS
<b>Batch number</b>	<b>AJ11-10L601</b>
Sumitomo Code Recipe	AJ11-10
Expiry date	June 16, 2018
Actual concentration ( <b>total isomer content</b> )	38.61 % +/- 0.32 w/w or 386.1 +/- 3.2 g/kg or 422.3 +/- 3.5 g/L
Actual concentration ( <b>inpyrfluxam content (calculation)</b> )	37.31 % +/- 0.31 w/w or 373.1 +/- 3.1 g/kg or 408.1 +/- 3.4 g/L
Actual density	1.0938 g/mL
Certificate of analyses	SUMITOMO / FO 24239 / Ch.6547 / 2016 B dated 15/07/2016

### Field sites

Four sites were selected in Germany, Czech Republic, Italy and Spain. These are same locations used for the European field dissipation study [REDACTED] 2018a. Therefore, the same site characteristics apply and HSE accepts these sites as suitable.

The selected sites were not liable to erosion or flooding, were not stony or overshadowed by trees and had no appreciable slope. The sites had not been cultivated for crops in several years and had not been treated with any of the SDHI family of products (boscalid, fluxapyroxad, bixafen, penthiopyrad, penflufen, sedaxane, isopyrazam and benzovindiflupyr) in the years 2013-2016 or soil disinfectants within the previous 3 years.

Soils were characterised only to a depth of 30 cm. HSE does note that in a small number of cases residues (always <LOQ) were detected in the 30-40 cm layer. However, as quantifiable residues remained exclusively in the top 30 cm (and <LOQ in 20-30 cm layer), the characterisation depth is considered acceptable in this case.

**Table B.8.1.2.2-02 Chemical and physical characterisation of the soils used in the study**

	<b>267-2016 GE01</b>	<b>267-2016 CZ02</b>	<b>267-2016 IT03</b>	<b>267-2016 SP04</b>
Country (Region)	Germany (North Rhine-Westphalia)	Czech Republic (Stredni Morava)	Italy (Emilia Romagna)	Spain (Ourense)

Corresponding field trial number Of the present study	267-2016 GE01	267-2016 CZ02	267-2016 IT03	267-2016 SP04
GPS coordinates				
Altitude (m)	17	180	8	624
Sample Reference (*)	BIO F3	ATC F6	AGR F7	TRI F5
pH (water)	6.5	7.9	8.1	5.0
pH (0.01M CaCl <sub>2</sub> )	6.0	7.4	7.5	4.4
Organic Matter %	3.29	3.87	1.39	4.42
Organic Carbon %	1.91	2.24	0.81	2.57
Cation Exchange Capacity meq/100g	17.4	27.6	9.2	16.3
Water Holding Capacity at pF2 %	37.7	44.1	21.7	44.7
Sand %	50	12	63	43
Silt %	24	40	21	37
Clay %	26	48	16	20
Textural Class USDA Classification	Sandy Clay Loam	Clay/Silty Clay	Sandy Loam	Loam
Microbial biomass (mg C/kg)** Prior to application (7 to 0 days before)	957	846	250	282
365 days (± 7 days) after application	386	700	89	245
730 days (± 14 days) after application	497	472	198	263
% Biomass carbon as % TOC** Prior to application (7 to 0 days before)	3.06	3.69	5.10	1.01
365 days (± 7 days) after application	2.80	2.87	1.60	0.97
730 days (± 14 days) after application	1.71	2.06	3.30	1.10

All results on a dry soil basis

(\*) Sample Reference of the study CEMS-7522

(\*\*) On a dry soil basis

**Table B.8.1.2.2-03 Previous use of the trial sites**

	<b>Crop (2015)</b>	<b>Field status at application (2016)</b>
Germany	Grass (since 2013)	Bare soil
Czech Republic	Alfalfa (since 2013)*	Bare soil
Italy	Alfalfa (since 2012)	Bare soil
Spain	Potato (2015)*	Bare soil

\*Also see Table B.8.1.2.2-04 below

No fertilizers were applied at any site and there no irrigation was used. Previous pesticide use was supplied for all 4 trial sites.

- At Trial site 1 (Germany), no plant protection products were applied between 2013 and the start of the trial. From June 2016 through to May 2018 glyphosate containing products were applied on 10 occasions, presumably to clear the site prior to application and to keep the plots clear of weeds during the trial. 'Summax' a product containing flumioxazin was also applied on one occasion in March 2017; this is also a herbicide.
- At Trial site 3 (Italy), no plant protection products were applied between 2012 and 2015. Glyphosate containing products were applied on 12 occasions between June 2016 and July 2018.
- The pesticide histories of plots 2 (Czech Republic) and 4 (Spain) were more complicated. The histories of these plots are summarised below.

**Table B.8.1.2.2-04 Site use and plant protection products applied at sites 2 and 4 (Czech Republic and Spain)**

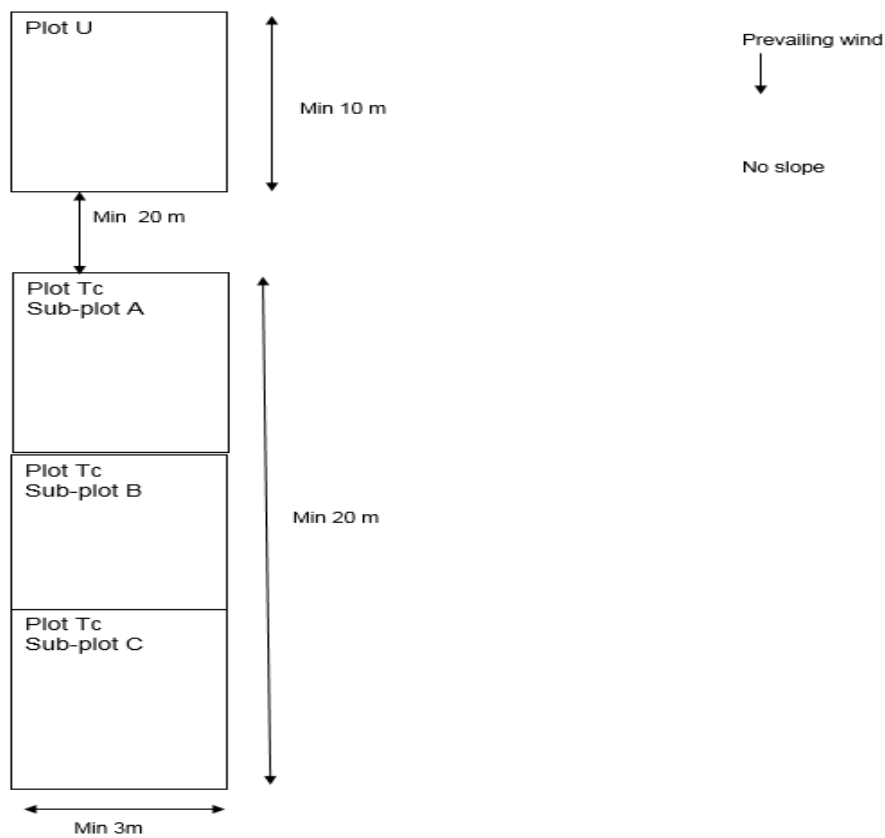
<b>Year</b>	<b>Crop</b>	<b>Active Substance</b>
Trial site 2 – Czech Republic		
2010	Winter wheat	Aminopyralid, florasulam, pyroxsulam 50 g/kg, chlormequat-chloride, quinoxifen, prochloraz, propiconazole, prothioconazole, tebuconazole
2011	Sugar beet	Desmedipham, phenmedipham, Ethofumesate, propaquizafop, clopyralid, metamitron, desmedipham, phenmedipham, triflusal, flusulfuron-methyl, desmedipham, phenmedipham, chloridazon, propaquizafop, flusilazole, carbendazim, flutriafol, thiophanate methyl, chlorpyrifos, cypermethrin
2012	Spring barley	Trinexapac-ethyl, 2,4-D, aminopyralid, florasulam, fenpropidin, propiconazole, pinoxaden, cypermethrin, cyproconazole, propiconazole, pinoxaden, cypermethrin, cyproconazole
2013	Alfalfa	None
2014	Alfalfa	Thiocluprid
2015	Alfalfa	Thiocluprid, diquat
2016	Bare soil	Glyphosate

2017	Bare soil	Glyphosate
2018	Bare soil	Glyphosate
Trial site 4 - Spain		
2011	Potato	Chlorpyrifos, linuron, cymoxanil - folpet - fosetyl aluminium, mancozeb + dimethomorph 695 wp, cymoxanil + mancozeb, dibromide
2012	Wheat	Pyrethrin
2013	Potato	Chlorpyrifos, linuron, cymoxanil - folpet - fosetyl aluminium, mancozeb + dimethomorph, cymoxanil + mancozeb, dibromide
2014	Wheat	Pyrethrin
2015	Potato	Chlorpyrifos, linuron, cymoxanil - folpet - fosetyl aluminium, mancozeb + dimethomorph, cymoxanil, dibromide
2016	Not applicable	Glyphosate
2017	Not applicable	Glyphosate
2018	Not applicable	Glyphosate-ammonium, glyphosate

No sites had been treated with any SDHI-group fungicide during the preceding three years and all were in agricultural production (grass at German site, alfalfa at Czech and Italian sites, potato at Spanish site). The sites were maintained as bare soil during the study by the application of glyphosate, flumioxazin and glufosinate-ammonium for weed control.

#### *Site layouts*

The sites were mechanically cleared of all vegetation and subsequently kept free of weeds via herbicide application (glyphosate) at recommended commercial rates. Each trial site was divided into one main untreated plot (U) and one treated plot (Tc); the treated plot was divided into 3 sub-plots (TcA, TcB and TcC). Each of the sub-plots was then divided by the number of scheduled sampling events (sub-sub-plots). Ten soil cores were collected at each sampling date. The untreated plot was separated from the treated plots by a buffer zone  $\geq 20$  m to prevent contamination from application of the test item. The design of the study sites is shown below:



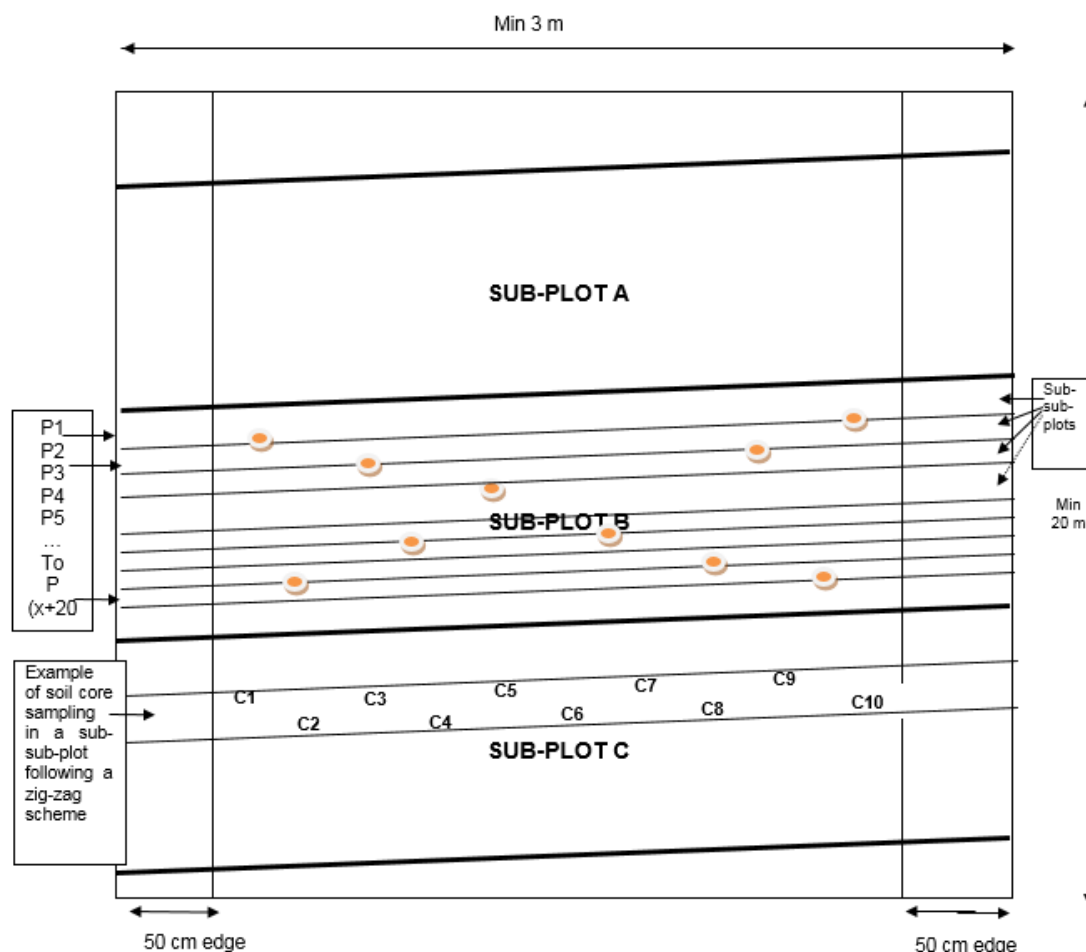
**Figure B.8.1.2.2-01 Setup at the trial sites**

**C1 to C10** = 10 cores per sampling date and per sub-plot = a total of 30 cores per treated plot and per sampling date.

**P1 to P(x+20)** = Sampling Dates per sub-plot A, B or C. (15 sampling events + about 5 margin security event).

In other words: each subplot is made of about 15+5 sub-sub-plots.

● = 10 Petri Dishes per subplot, to place between the sub-subplots.



**Figure B.8.1.2.2-02 Setup of a treated plot**

#### *Application of test item*

The test item was applied to bare soil in the treated plots uniformly with a hand-carried boom sprayer, once yearly at a target dose of 200 g a.s./ha (0.5 L/ha formulated product). Applications took place in May or early June of 2016, 2017, and for the Czech Republic and Italian sites, 2018. The Spanish and German studies were terminated after the 2017 application due to low residues.

Application rates were verified by placement of 10 glass petri dishes (10.8 cm diameter) filled with sieved soil ( $20 \pm 0.2$  g) on the soil surface on each sub-plot (30 per field site). Petri dishes were placed prior to spraying. As shown in the figure above, the petri dishes were placed between sub-sub plots so as not to interfere with the area where soil cores would be sampled from. The petri dishes were sealed after

spraying and shipped frozen < -18 °C. Residues were analysed as for soil core samples. The results of the application verification are shown below in the Results section.

For the Czech Republic site, one petri dish was not opened prior to the first application spraying in subplot A and therefore there were only 9 petri dishes for this application verification. HSE agrees with the applicant that this is still sufficient enough to be representative.

## STUDY DESIGN AND METHODS

### *Experimental Conditions*

Weather data during the trials was collected from weather stations placed inside the trial field. Historical weather data was taken from institutional weather stations at a maximum distance of 40 km from the field sites.

**Table B.8.1.2.2-05 Temperature and rainfall data (historical and during trial) for German field site**

<b>Month</b>	<b>Average minimum air temperature Current year (1) (°C)</b>	<b>Average minimum air temperature Historical (2) (°C)</b>	<b>Average maximum air temperature Current year (1) (°C)</b>	<b>Average maximum air temperature Historical (2) (°C)</b>	<b>Rainfall Current year (1) (mm)</b>	<b>Rainfall Historical (2) (mm)</b>
May 16 (1)	12.3	7.6	19.2	18.9	57.6	53.7
June 16	14.4	10.3	21.8	21.5	207.8	69.1
July 16	14.3	13.1	24	24	44.2	91.2
Aug 16	13	12.3	23.6	23.8	56.8	95.6
Sept. 16	12	9.8	23.6	19.9	12.4	69.2
Oct 16	7	7.2	13.2	15.5	51	68.6
Nov 16	2.8	4.3	8.2	10.5	70.4	56.8
Dec 16	1.9	3	7.1	8.1	23.2	94.1
Jan 17	-1.5	1.1	3.6	6.1	43.6	83.4
Feb 17	2.7	-0.2	8.1	6.3	64.6	52.9
Mar 17	4.5	1.6	13.3	10.9	63	38.8
April 17	3.1	4.1	13.1	14.9	16.6	53.3
May 17	10.9	8.6	21.4	18.7	43.8	62.1
June 17	13.7	11	23.8	21.4	32.4	92.7
July 17	14.1	13.7	23.2	24.5	89.4	78.8
Aug 17	12.8	12.4	22.8	24	37.60	81.8
Sept 17	10.3	10	18.5	20.4	99.40	63.6
Oct 17	10.2	7.3	16.3	14.9	42.40	66.4
Nov 17	4.2	4.2	9.4	9.9	62.00	69.6

Dec 17	2.8	2.8	6.4	8	83.20	72.6
Jan 18	3.6	0.5	7.7	5.5	80.70	72.8
Total rainfall (deviation to Norm):					1224.5 (- 20%)	1487.1

(1) Average monthly temperature and rainfall data for 2016 were calculated for the entire months (with exception of data for May 2016 calculated from the trial start date of 18<sup>th</sup> May)

(2) Norm is the 5-previous year average calculated for entire months.

**Table B.8.1.2.2-06 Temperature and rainfall data (historical and during trial) for Czech field site**

Month	Average min air temp Current year (°C) (1)	Average min air temp Historical (2) (°C)	Average max air temp Current year (°C) (1)	Average max air temp Historical (2) (°C)	Rainfall Current year (1) (mm)	Rainfall Historical (2) (mm)
May 16	9	8.3	21.2	20	52.6	69
June 16	12.4	11.7	26.3	23.3	83.8	86
July 16	14	12.8	27.7	25.1	172.4	82
Aug 16	12	12.5	25.4	24.6	52.6	78
Sept. 16	10.8	9.4	24.8	20.6	42.8	50
Oct 16	5	4.9	12.7	14.7	85	40
Nov 16	1.5	1.3	7.7	7.4	50.4	46
Dec 16	-3.7	-2.4	2.4	2.7	12	38
Jan 17	-9.4	-4.9	-1.5	0.9	8.2	31
Feb 17	-1.5	-3.2	5.3	3.6	33.6	30
Mar 17	1.7	0	13.4	8.9	25	32
April 17	3.7	4	13.9	15	72.4	43
May 17	8.6	8.3	21.8	20	36	69
June 17	12.3	11.7	27.3	23.3	45.6	86
July 17	13.7	12.8	27.7	25.1	79.6	82
Aug 17	14.2	12.5	29.1	24.6	55	78
Sept 17	10.2	9.4	19	20.6	115.2	50
Oct 17	6.2	4.9	15.4	14.7	124	40
Nov 17	1.6	1.3	8	7.4	45.8	46
Dec 17	-1.9	-2.4	4.4	2.7	49.6	38
Jan 18	-0.2	-4.9	5.2	0.9	43.6	31
Feb 18	-4.5	-3.2	0.6	3.6	16.6	30
Mar 18	-2.6	0	7.1	8.9	19	32
April 18	7.6	4	20.9	15	33.4	43
May 18	11.4	8.3	24.8	20	65.8	69



Total rainfall (deviation to Norm):					1420.0 (+ 8%)	1319
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(1) Average monthly temperature and rainfall data for 2016 were calculated for the entire months

(2) Norm is the 30 year average (1981-2010) calculated for entire months

**Table B.8.1.2.2-07 Temperature and rainfall data (historical and during trial) for Italian field site**

Month	Average min air temp Current year (1) (°C)	Average min air temp Historical (2) (°C)	Average max air temp Current year (1) (°C)	Average max air temp Historical (2) (°C)	Rainfall Current year (1) (mm)	Rainfall Historical (2) (mm)
May 16	11.5	10.9	23.2	24.2	93.8	65.4
June 16	15.2	15.1	27.9	29.2	82.6	47.2
July 16	17.9	17.6	32.1	31.9	36.2	38.3
Aug 16	15.7	17.4	30.3	32.3	53.4	30
Sept. 16	14.2	14.3	28	26.9	50	52.2
Oct 16	8.7	10.2	18	20.1	94.6	75.4
Nov 16	5	5.9	12.4	13.8	83.2	64.1
Dec 16	-0.5	0.4	7.2	8.3	39.4	24.9
Jan 17	-4.1	-0.6	5.9	7.9	4	44.1
Feb 17	1.8	0.2	10.9	9.1	68.8	110.6
Mar 17	4	3.4	19.1	15.6	12.2	70
April 17	7.1	7.5	21.2	19.8	28.8	65.1
May 17	11.5	11.1	24.6	23.7	98.4	77.3
June 17	16.4	15.1	31.3	29	34	53.8
July 17	17.5	17.9	32.4	32.3	18.8	34.9
Aug 17	17.9	17	33.2	31.6	24	40.7
Sept 17	12.7	13.9	24.3	26.6	98.6	56.4
Oct 17	8.9	10.6	21.6	19.6	3.6	85
Nov 17	3.7	6.2	12.5	13.8	139.2	74.4
Dec 17	-1.1	0.5	8.2	8	24	28.6
Jan 18	0.7	-0.8	9.5	7.8	10.4	42.9
Feb 18	-0.2	1.6	6.7	10.1	172.8	116.1
Mar 18	2.6	3.6	11.7	15.5	77.4	72.2
April 18	8.9	7.6	21.9	20.3	14	54.1
May 18	13.6	11.3	24.9	23.8	98	82.4
Total rainfall (deviation to Norm):					1460.2 (- 3%)	1506.1

n to Norm):						
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(1) Average monthly temperature and rainfall data for 2016 were calculated for the entire months. (2) Norm is the 5-previous year average calculated for entire months

**Table B.8.1.2.2-08 Temperature and rainfall data (historical and during trial) for Spanish field site**

Month	Average min air temp Current year (°C) (1)	Average min air temp Historical (2) (°C)	Average max air temp Current year (°C) (1)	Average max air temp Historical (2) (°C)	Rainfal l Curren t year (1) (mm)	Rainfall Historica l (2) (mm)
June 16	9.9	10.6	27.9	26.4	0	30
July 16	10.4	13.3	28.9	29.1	0	16.9
Aug 16	9.5	14	29.6	28.5	0	22.8
Sept. 16	7.7	12.5	23.7	27.9	38.6	36.1
Oct 16	6.3	9.6	19.8	21.5	22.2	75.7
Nov 16	3.3	5.9	12.7	14.7	90.2	78.9
Dec 16	1.3	3.4	11.9	12.8	26.8	79.8
Jan 17	2.8	5.2	13.9	13.8	120.4	123.1
Feb 17	6.7	4.5	16.7	10.9	208.8	84.9
Mar 17	7.4	6.2	18.5	14.9	119	71.8
April 17	2.3	8.1	21.8	16.9	0.6	67.6
May 17	7.5	10.1	21.8	20.6	80	54.1
June 17	11.4	13.8	26.4	23.7	33.2	34.4
July 17	19	17.3	28.3	26.4	10.2	16.4
Aug 17	18.7	17.2	28.3	25.2	14.6	26.6
Sept 17	14.6	14.6	23.5	24.6	0.4	44.5
Oct 17	13.2	10.9	24.2	18.9	26.4	225
Nov 17	7.1	7.3	14	13.7	64.2	97.6
Dec 17	5.4	4.7	10.6	11.4	110.6	85.8
Jan 18	1.2	4.9	10.4	14.2	76.6	147.1
Feb 18	-0.9	5.2	9.4	12.6	47	126.7
Mar 18	2	6.3	10.1	16	240	95.6
April 18	4.5	7.3	15.9	19.3	71.2	44.6
May 18	5.6	9.8	19.5	21.9	57.6	57.1
Total rainfall (deviatio n to Norm):					1458.6 (- 16%)	1743.1

(1) Average monthly temperature and rainfall data for 2016 were calculated for the entire months (2) Norm is the 5-previous year average calculated for entire months

### Sampling

Soil samples (cores) from each test plot were taken on day 0 (both immediately prior to and following (within 3 hours) application), 120, 240 and immediately before the next application. The provided interim report provides analysed data up to 240 days after the second application (2017). At each sampling time, ten soil cores per subplot were taken (sampling depth to 30 cm on day 0 and to 90 cm at all other sampling dates). At the Italian site, for the sampling event 240 days following the second application, due to poor winter conditions at the field site sampling in subplot A was only able to be carried out to 60 – 75 cm rather than 90 cm. However, this is acceptable as residues >LOQ were not observed below 20 cm depth and full cores were sampled from subplots B and C.

Zero contamination soil sampling equipment with acetate tubes were used for collecting soil cores, 5 cm external diameter of corer. Untreated specimens were collected first and those specimens were stored separately from the treated ones. After cores have been taken from the treated plot, the sampling holes were filled with soil collected from uncontaminated areas to prevent wash off of treated soil to lower depths.

Samples were frozen at the site and transported frozen < -18 °C. It is noted that, on the shipments from the Spanish site on 18<sup>th</sup> October and 19<sup>th</sup> October 2016, temperatures raised to -13 °C and -16 °C respectively. In addition in the shipments from the Czech trial the storage temperature was above -18 °C on a number of occasions with a maximum temperature of -14.9 °C being reached. The reason for this is unknown. However, the period of temperature deviation was noted as being short in duration, a value of less than 18 hours is stated for the Spanish site samples, and in both cases the samples remained hard frozen. Therefore HSE agrees that the study was not adversely impacted.

At the analytical facility frozen cores were then cut into 10cm segments and corresponding depth segment mixed. All soil for which results are presented in the interim study was analysed within 532 days of sampling. The analysis in the final report for the Czech and Italian sites was conducted within 698 days for the samples up to 30cm depth and up to 1363 days for the deeper horizons of 40-50 cm which included 5 samples from the Czech site and one at the Italian site. Storage stability in soil under frozen conditions has been demonstrated for a period of 24 months for SE-2399 and its metabolites (see section B.8.1.4 Annex Point KCA 7.1.2.2.1/09 and 10). In addition the applicant refers to a further stability study in which stability of the inpyrfluxam and its metabolites were determined in the dark at temperature of -7 °C for 7 days.

Validation study S16-05522 (TPA-0043 (KCA 4.1.2/03) indicated that the analytes were not stable in the final sample extracts for 8 days when stored refrigerated at 1-10 °C in the dark. Therefore, the extracts here were ideally analysed within 24 h after extraction. If this period was extended to 48 h, the stability was confirmed with

procedural recoveries which were also analysed only after a 48 h period and were always within an acceptable range between 70 – 110%.

### *Description of analytical procedures*

Soil was analysed for residues of inpyrfluxam, 3'-OH-S-2840, 1'-COOH-S-2840A, and 1'-COOH-S-2840B using the validated method summarised at B.5.1.2.1. in the Volume 3CA – B.5 Methods of Analysis (Annex Point KCA 4.1.2/01). Soil samples were extracted twice with an acetone/water (4/1, v/v) mixture, followed by a further extraction with acetone/0.5 M HCl (4/1, v/v). After filtration through celite, an aliquot of the above acetone/water/HCl mixture was extracted with ethyl acetate and an aliquot of the organic phase dried using a rotary evaporator. Dried residues were reconstituted with methanol, followed by water in a ratio of 1/3 (v/v).

Samples were analysed using LC-MS/MS, using 2 methods which are both summarised at B.5.1.2.1(Annex Point KCA 4.1.2/03). The second method exchanged the chromatographic column in order to improve the consistency of response peak shape. The LOQ for inpyrfluxam and 3'-OH-S-2840 was 0.002 mg/kg wet soil whilst that for 1'-COOH-S-2840A, and 1'-COOH-S-2840B was 0.001 mg/kg wet soil. The LOD for inpyrfluxam and 3'-OH-S-2840 was 0.0004 mg/kg wet soil whilst that for 1'-COOH-S-2840A, and 1'-COOH-S-2840B was 0.0002 mg/kg wet soil. The nominal application rate of 200 g a.s./ha for inpyrfluxam is equivalent to 0.133 mg/kg, meaning the LOD of inpyrfluxam is equivalent to 0.3 % nominal application rate of the active substance and the LOQ is 1.5 %. This is acceptably sensitive to HSE.

Procedural recoveries were undertaken for each analyte on soil samples spiked at concentrations equivalent to the LOQ, 10 LOQ, 25 LOQ, 50 LOQ and 250 LOQ. Mean recoveries at each fortification level were in the acceptable range of 70 - 110 % for each analyte. Some individual recovery measures exceeded 110%, for all analytes at the concentrations of LOQ and 10 LOQ, however all recoveries were still below 120%. Additionally, HSE has examined the recovery data and notes that only occasional replicates are outside of the 70-110 % range. As only a very small number of replicates are >110 % and always <120 %, it is agreed that this deviation has not affected the outcome of the study.

## **RESULTS AND DISCUSSION**

### **Application verification**

The nominal application rate was 200 g a.s./ha. This is higher than the proposed application rate of 90 g a.s./ha for the representative product. The application rate verification results are shown below.

**Table B.8.1.2.2-09 Analytical results of the concentration of inpyrfluxam in the petri dishes**

Site	Application number	Plot	Residue (g/ha based on wet soil)*	% of 200 g/ha target
Germany	1	TcA	175	87.5
		TcB	126	63
		TcC	126	63
	2	TcA	241	120.5
		TcB	173	86.5
		TcC	149	74.5
Czech Republic	1	TcA	170	85
		TcB	145	72.5
		TcC	151	75.5
	2	TcA	135	67.5
		TcB	240	120
		TcC	206	103
	3	TcA	184	92
		TcB	114	57
		TcC	174	87
	4	TcA	149	74.5
		TcB	205	102.5
		TcC	156	78
	5	TcA	160	80
		TcB	141	70.5
		TcC	106	53
Italy	1	TcA	193	96.5
		TcB	166	83
		TcC	191	95.5
	2	TcA	158	79
		TcB	163	81.5
		TcC	163	81.5
	3	TcA	185	92.5
		TcB	195	97.5
		TcC	181	90.5
	4	TcA	214	107
		TcB	190	95
		TcC	148	74
	5	TcA	165	82.5
		TcB	101	50.5
		TcC	152	76
Spain	1	TcA	233	116.5
		TcB	264	132
		TcC	199	99.5
	2	TcA	207	103.5
		TcB	191	95.5
		TcC	154	77
Mean average	-	-	172	86.0

\*Data corrected for daily recoveries on level 5 mg/kg or 10 mg/kg

The results for the petri dish specimens indicate that between 50.5 and 120.5 % of the target amount was deposited on the soil surface, which is a large variation. The mean average application rate was 180.0 g a.s./ha,  $\pm 84$  g a.s./ha or  $90.0 \pm 30.5$  % of the target. Although the rates were very variable, the proposed application rate of the representative product is 1 x 90 g a.s./ha, and so even the lowest application rate measured (126 g a.s./ha) is still greater than the proposed rate. Therefore, HSE accepts the application rate achieved by the applicant.

## Residues

No residues were detected in the untreated specimens analysed as part of this phase of the study. The full residues results are shown in Table B.8.1.2.2-10 to B.8.1.2.2-13 below.

**German site:** Residues of inpyrfluxam in treated soil specimens ranged between 0.182 mg/kg and 0.127 mg/kg dry soil after A1 and were between 0.0457 mg/kg and 0.0357 mg/kg dry soil at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizon. For the same sampling range, residues between 0.00273 mg/kg dry soil and < LOD were detected in the 10-20 cm horizons and residues between < LOQ and < LOD were detected in the 20-30 cm horizons. Residues of 3'-OH-S-2840 in treated soil specimens were < LOQ (between 0.00108 mg/kg dry weight and 0.000841 mg/kg dry weight) after Application 1 and were between 0.00808 mg/kg and 0.00632 mg/kg dry soil at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizons. For the same sampling range, residues < LOD were detected in the 10-20 cm horizons and residues < LOD were detected in the 20-30 cm horizons. Residues of 1'-COOH-S-2840A in treated soil specimens were < LOD after Application 1 and were between 0.00157 mg/kg dry soil and < LOQ at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizon. For the same sampling range, no residues were detected in the 10-20 cm and 20-30 cm horizons (< LOD). Residues of 1'-COOH-S-2840B in treated soil specimens were < LOD after Application 1 and were between 0.00249 mg/kg and 0.00194 mg/kg dry soil at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizons. For the same sampling range, no residues were detected in the 10-20 cm and 20-30 cm horizons (< LOD). Analysis will not be continued beyond 120 ( $\pm 3$ ) days after A2.

**Czech site:** Residues of inpyrfluxam in treated soil specimens after A1 ranged between 0.114 mg/kg and 0.155 mg/kg dry soil in the 0-10 cm horizon, and were detected up to 0.0044 mg/kg in the 10-20 cm horizon and at < LOQ in the 20-30 cm horizon. These declined 365 days later to 0.0395 - 0.0532 mg/kg in the 0-10 cm horizon, < LOQ in the 10-20 cm horizon and < LOD in the 20-30 horizons. At the Czech site the study was continued for 5 annual applications at the nominal rate of 200 g a.s./ha and the residual levels in soil 365 days after A5 were at 0.0567 and 0.0765 mg/kg at 0-10 cm, < LOQ at 10-20 cm and < LOD in two out of the three plots tested. The third plot had < LOQ residues at 20-30 cm depth and < LOD at the 30-40 depth. Quantifiable residues of 3'-OH-S-2840 in treated soil specimens were determined at 120 days after the first application ranging from 0.00498 mg/kg to 0.00532 mg/kg. Levels present at 365 days after A1 were at 0.00619 – 0.00797

mg/kg. At the end of the 5 year study levels of 3'-OH-S-2840 were at 0.0189 – 0.0230 mg/kg at the 10-20 cm horizon. The maximum levels indicated were at 120 days after A5 at 0.028 mg/kg. Residues at <LOQ were determined in the 10-20 cm horizon in some plots and at < LOD were detected in the 20-30 cm horizons. Quantifiable residues of 1'-COOH-S-2840A in treated soil specimens were determined at 120 days after the first application ranging from 0.00177 – 0.00205 mg/kg. Levels present at 365 days after A1 were  $\leq$  0.00134 mg/kg. At the end of the five year study levels were at <LOQ in all samples at the 0-10 cm horizon and <LOD at all other horizons tested. Quantifiable residues of 1'-COOH-S-2840B in treated soil specimens were determined at 120 days after the first application ranging from 0.00194 – 0.00223 mg/kg. Levels present at 365 days after A1 were  $\leq$  0.00159 mg/kg. At the end of the five year study levels were at <LOQ in all samples at the 0-10 cm horizon and <LOD at all other horizons tested.

**Italian site:** Residues of inpyrfluxam in treated soil specimens after A1 ranged between 0.069 mg/kg and 0.0999 mg/kg dry soil in the 0-10 cm horizon, and were detected up to 0.0368 mg/kg in the 10-20 cm horizon and at < LOD in the 20-30 cm horizon. These declined 365 days later to 0.0137 - 0.0225 mg/kg in the 0-10 cm horizon, <LOQ in the lower horizons (10-30 cm). At the Italian site the study was continued for 5 annual applications at the nominal rate of 200 g a.s./ha and the residual levels in soil 365 days after A5 were at 0.0176 to 0.0224 mg/kg at 0-10 cm and <LOD in the lower horizons (10-30 cm). Quantifiable residues of 3'-OH-S-2840 in treated soil specimens were determined at 120 days after the first application ranging from 0.00245 mg/kg to 0.00476 mg/kg. Levels present at 365 days after A1 were at 0.00351 – 0.00426 mg/kg. At the end of the 5 year study levels of 3'-OH-S-2840 were at 0.00647 – 0.00995 mg/kg at the 0-10 cm horizon and <LOD at all other horizons (10-30 cm). The maximum residues of 0.0116 mg/kg were determined at 120 days after A4. Quantifiable residues of 1'-COOH-S-2840A in treated soil specimens were determined in one plot at 120 days after the fourth application, with levels at 0.00240 mg/kg. Levels at all other timepoints and horizons were at <LOQ. At the end of the five year study levels were at <LOQ in all samples at the 0-10 cm horizon and <LOD at all other horizons tested. Quantifiable residues of 1'-COOH-S-2840B in treated soil specimens were determined in one plot at 120 days after the first application with levels at 0.00195 mg/kg in the 10-20 cm horizon. And again at 120 days after the fourth application at 0.00185 mg/kg in the 0-10 cm horizon. At all other timepoints and horizons levels were at <LOQ. . At the end of the five year study levels were at <LOQ in all samples at the 0-10 cm horizon and <LOD at all other horizons tested.

**Spanish site:** Residues of inpyrfluxam in treated soil specimens ranged between 0.217 mg/kg and 0.0904 mg/kg dry soil after A1 and were between 0.0183 mg/kg and 0.00849 mg/kg dry soil at 120 ( $\pm$ 3) days after A2 within the 0-10 cm horizons. For the same sampling range, residues between 0.00288 mg/kg dry soil and < LOD were detected in the 10-20 cm horizons and residues between < LOQ and < LOD were detected in the 20-30 cm horizons. Residues of 3'-OH-S-2840 in treated soil

specimens were < LOQ (between 0.00246 mg/kg dry weight and 0.000940 mg/kg dry weight) after Application 1 and were between 0.00370 mg/kg dry soil and < LOQ at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizons. For the same sampling range, residues < LOD were detected in the 10-20 cm horizons and residues between < LOQ and < LOD were detected in the 20-30 cm horizons. Residues of 1'-COOH-S-2840A in treated soil specimens were < LOD after Application 1 and were between 0.00197 mg/kg and 0.00131 mg/kg dry soil at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizons. For the same sampling range, residues between < LOQ and < LOD were detected in the 10-20 cm horizons and residues between < LOQ and < LOD were detected in the 20-30 cm horizons. Residues of 1'-COOH-S-2840B in treated soil specimens were < LOD after Application 1 and were between 0.00379 mg/kg and 0.00242 mg/kg dry soil at 120 ( $\pm 3$ ) days after A2 within the 0-10 cm horizons. For the same sampling range, residues between < LOQ and < LOD were detected in the 10-20 cm horizons and residues < LOD were detected in the 20-30 cm horizons. Analysis will not be continued beyond 120 ( $\pm 3$ ) days after A2.

HSE notes that for some samples (e.g. German site, day 365 after second application, inpyrfluxam residues), the bottom layer of analysed soil still had detectable residues. However, these residues were always <LOQ, so while it would be prudent to analyse further depths until a layer with no detectable residues was reached, HSE accepts this deviation as no quantifiable residues were present below 10-20 cm.

**Table B.8.1.2.2-10: Residues of inpyrfluxam and its metabolites in soil (mg/kg dry soil) – German site**

Sampling Event	Residues in German soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
0-day after 1 <sup>st</sup> APP	A	0-10	0.182	< LOQ	< LOD	< LOD
		10-20	0.00273	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
	B	0-10	0.160	<LOQ	< LOD.	< LOD.
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
	C	0-10	0.127	< LOQ	< LOD	< LOD
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
120-day after 1 <sup>st</sup> APP	A	0-10	0.0411	0.009	0.00129	0.00279
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	B	0-10	0.0280	0.00449	<LOQ	0.0014
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	C	0-10	0.0401	0.00884	<LOQ	0.00276
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD



Sampling Event	Residues in German soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
240-day after 1 <sup>st</sup> APP	A	0-10	0.0126	0.00489	< LOD	< LOQ
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	B	0-10	0.0189	0.00543	< LOD	<LOQ
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	C	0-10	0.0109	0.00277	< LOD	<LOQ
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
362-day after 1 <sup>st</sup> app (Just before 2 <sup>nd</sup> App)	A	0-10	0.0188	0.00788	< LOQ	< LOQ
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	B	0-10	0.0162	0.00505	<LOQ	<LOQ
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	C	0-10	0.0242	0.00921	<LOQ	<LOQ
		10-20	<LOD	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
362-day after 1 <sup>st</sup> (Just after 2 <sup>nd</sup> app)	A	0-10	0.109	0.00869	<LOQ	<LOQ
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	B	0-10	0.0734	0.00555	<LOD	<LOQ
		10-20	<LOD	< LOD	< LOD	< LOQ
		20-30	<LOD	< LOD	< LOD	< LOD
	C	0-10	0.119	0.00622	<LOQ	<LOQ
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
120-day after 2 <sup>nd</sup> APP	A	0-10	0.0410	0.00632	0.00145	0.00249
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	B	0-10	0.0457	0.00808	0.00157	0.00246
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD
	C	0-10	0.0357	0.00684	< LOQ	0.00194
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	< LOD	< LOD

**Table B.8.1.2.2-11: Residues of inpyrfluxam and its metabolites in soil (mg/kg dry soil) – Czech site**

Sampling Event	Residues in Czech soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
0-day after 1 <sup>st</sup> APP	A	0-10	0.137	< LOQ	< LOD	< LOD
		10-20	0.00397	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
	B	0-10	0.114	<LOQ	< LOD.	< LOD.
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
	C	0-10	0.155	< LOQ	< LOD	< LOD
		10-20	0.0044	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
120-day after 1 <sup>st</sup> APP	A	0-10	0.0574	0.00532	0.00205	0.00217
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOD	< LOD	<LOD	<LOD
	B	0-10	0.0528	0.00498	0.00185	0.00223
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0566	0.00521	0.00177	0.00194
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
240-day after 1 <sup>st</sup> APP	A	0-10	0.103	0.0125	0.0604	0.00185
		10-20	0.00500	<LOQ	<LOQ	<LOQ
		20-30	<LOQ	< LOD	<LOQ	<LOQ
		30-40	< LOQ	<LOD	<LOQ	<LOQ
	B	0-10	0.116	0.0103	0.00149	0.00183
		10-20	0.00873	<LOQ	0.00134	0.00162
		20-30	< LOQ	< LOD	<LOQ	<LOQ
		30-40	<LOD	<LOD	<LOQ	<LOQ
	C	0-10	0.127	0.0143	0.00197	0.00221
		10-20	0.0123	0.00257	0.00192	0.00177
		20-30	<LOQ	< LOD	<LOQ	<LOQ
		30-40	<LOQ	<LOD	<LOQ	<LOQ
365-day after 1 <sup>st</sup> app (just before 2 <sup>nd</sup> App)	A	0-10	0.0532	0.00797	0.00137	0.00159
		10-20	<LOQ	< LOD	< LOD	<LOQ
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.0433	0.00619	<LOQ	0.00139
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0395	0.00664	<LOQ	<LOQ
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
365-day after 1 <sup>st</sup> app	A	0-10	0.183	0.00745	0.00134	0.00146
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
		30-40	<LOD	< LOD	< LOD	< LOD

Sampling Event	Residues in Czech soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
(just after 2 <sup>nd</sup> app)	B	0-10	0.143	0.00755	0.00130	0.00145
		10-20	<LOQ	< LOD	< LOD	< LOQ
		20-30	<LOQ	< LOD	< LOD	<LOQ
		30-40	<LOD	< LOD	< LOD	< LOD
	C	0-10	0.182	0.00629	<LOQ	<LOQ
		10-20	<LOQ	< LOD	< LOD	< LOD
		20-30	<LOQ	< LOD	< LOD	< LOD
		30-40	<LOD	< LOD	< LOD	< LOD
120-day after 2 <sup>nd</sup> APP	A	0-10	0.102	0.0099	0.00311	0.00369
		10-20	<LOQ	< LOD	<LOQ	<LOQ
		20-30	<LOQ	< LOD	< LOD	< LOD
	B	0-10	0.110	0.0105	0.00382	0.00417
		10-20	< LOD	< LOD	<LOQ	<LOQ
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.109	0.0110	0.00304	0.00366
		10-20	<LOQ	< LOD	<LOQ	<LOQ
240-day after 2 <sup>nd</sup> APP		20-30	< LOD	< LOD	< LOD	< LOD
	A	0-10	0.0881	0.0129	0.00135	0.00168
		10-20	0.00297	<LOQ	0.00129	0.00159
		20-30	<LOQ	<LOD	<LOQ	<LOQ
	B	0-10	0.0812	0.0104	<LOQ	<LOQ
		10-20	<LOQ	< LOD	<LOQ	<LOQ
		20-30	< LOD	< LOD	<LOQ	<LOQ
	C	0-10	0.0629	0.0133	<LOQ	0.00172
365 days after 2 <sup>nd</sup> APP		10-20	<LOQ	< LOD	<LOQ	0.00136
		20-30	< LOD	< LOD	<LOQ	<LOQ
	A	0-10	0.0817	0.0122	0.00235	0.00284
		10-20	<LOQ	<LOD	<LOD	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0692	0.0126	0.00203	0.00244
		10-20	<LOD	<LOD	<LOD	<LOQ
Just after 3 <sup>rd</sup> app		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0575	0.0109	0.00190	0.00218
		10-20	<LOQ	<LOD	<LOD	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	A	0-10	0.180	0.00811	0.00194	0.00216
		10-20	0.00345	<LOD	<LOD	<LOQ
		20-30	0.00303	<LOD	<LOD	<LOD
Just after 3 <sup>rd</sup> app		30-40	<LOQ	<LOD	<LOD	<LOD
		40-50	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.159	0.0149	0.00293	0.00338
		10-20	0.00278	<LOD	<LOD	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD

Sampling Event	Residues in Czech soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
	C	0-10	0.176	0.0124	0.00223	0.00252
		10-20	0.00337	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOQ	<LOD	<LOD	<LOD
		40-50	<LOD	<LOD	<LOD	<LOD
120d after 3 <sup>rd</sup> app	A	0-10	0.0714	0.0108	0.00287	0.00337
		10-20	0.00547	<LOQ	<LOQ	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0499	0.00967	0.0027	0.0033
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.063	0.0101	0.00189	0.00217
		10-20	0.00356	<LOQ	<LOQ	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOQ	<LOD	<LOD	<LOD
		40-50	<LOD	<LOD	<LOD	<LOD
240 d after 3 <sup>rd</sup> App	A	0-10	0.0557	0.0127	0.00248	0.00313
		10-20	0.00274	<LOQ	<LOQ	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0752	0.0151	0.003	0.0038
		10-20	0.00290	<LOQ	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0843	0.0176	0.00298	0.00378
		10-20	0.00550	<LOQ	<LOQ	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
365 days after 3 <sup>rd</sup> app	A	0-10	0.0292	0.00502	<LOQ	<LOQ
		10-20	0.00529	<LOQ	<LOD	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0323	0.00539	<LOD	<LOQ
		10-20	0.00536	<LOQ	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0300	0.00588	<LOQ	<LOQ
		10-20	0.00586	<LOQ	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
Just after 4 <sup>th</sup> App	A	0-10	0.0952	0.00944	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	0.00496	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0757	0.00747	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	0.00285	<LOD	<LOD	<LOD
		30-40	<LOQ	<LOD	<LOD	<LOD

Sampling Event	Residues in Czech soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
		40-50	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0905	0.00970	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	0.00498	<LOD	<LOD	<LOD
		30-40	<LOQ	<LOD	<LOD	<LOD
		40-50	<LOD	<LOD	<LOD	<LOD
120 days after 4 <sup>th</sup> app	A	0-10	0.0690	0.0133	0.00216	0.00268
		10-20	<LOQ	<LOD	<LOQ	<LOQ
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0768	0.0186	0.00259	0.00302
		10-20	<LOQ	<LOD	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0739	0.0146	0.00203	0.00237
		10-20	0.00276	<LOQ	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
240 days after 4 <sup>th</sup> app	A	0-10	0.0840	0.0209	<LOQ	0.00192
		10-20	<LOD	<LOD	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0693	0.00265	<LOQ	0.00145
		10-20	0.00302	<LOQ	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0504	0.0171	<LOQ	<LOQ
		10-20	0.00269	<LOQ	<LOD	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
365 days after 4 <sup>th</sup> app	A	0-10	0.0454	0.0140	0.00135	0.00166
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0677	0.0212	0.00191	0.00237
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	<LOD	<LOD	<LOD	<LOQ
		10-20	0.00361	<LOQ	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
Just after App 5	A	0-10	0.158	0.0144	0.00176	0.00195
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	0.00494	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.184	0.0160	0.00157	0.00181
		10-20	0.00266	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.101	0.009	>LOQ	0.00128
		10-20	0.00303	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	A	0-10	0.0669	0.0196	0.0034	0.0036

Sampling Event	Residues in Czech soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
120 days after App 5		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0903	0.0221	0.00315	0.00344
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0751	0.028	0.0035	0.0035
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
240 days after App 5	A	0-10	0.0828	0.0273	<LOQ	0.00142
		10-20	0.00304	<LOQ	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0567	0.0182	<LOQ	0.00142
		10-20	<LOQ	<LOQ	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0698	0.0216	<LOQ	<LOQ
		10-20	<LOQ	<LOQ	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
365 days after App 5	A	0-10	0.0693	0.0189	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0765	0.0230	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0567	0.0192	<LOQ	<LOQ
		10-20	<LOQ	<LOQ	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD

**Table B.8.1.2.2-12: Residues of inpyrfluxam and its metabolites in soil (mg/kg dry soil) – Italian site**

Sampling Event	Residues in Italian soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
0-day after 1 <sup>st</sup> APP	A	0-10	0.0723	< LOQ	< LOD	< LOD
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.069	<LOQ	< LOD.	< LOD.
		10-20	0.0368	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0999	< LOQ	< LOD	< LOD
		10-20	0.00509	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
120-day after 1 <sup>st</sup> APP	A	0-10	0.0561	0.00476	< LOQ	0.00195
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	<LOQ	< LOD	< LOD	< LOD
	B	0-10	0.0181	0.00245	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0285	0.00342	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
240-day after 1 <sup>st</sup> APP	A	0-10	0.0309	0.00314	< LOQ	< LOQ
		10-20	< LOQ	< LOD	< LOQ	< LOQ
		20-30	< LOQ	< LOD	< LOD	< LOD
	B	0-10	0.0174	0.00375	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0213	0.00287	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOQ	< LOQ
		20-30	< LOD	< LOD	< LOD	< LOD
365 day after 1 <sup>st</sup> app (Just before 2 <sup>nd</sup> App)	A	0-10	0.0225	0.00359	< LOD	< LOQ
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	<LOQ	< LOD	< LOD	< LOD
	B	0-10	0.0137	0.00351	< LOD	< LOD
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0199	0.00426	< LOD	< LOD
		10-20	< LOD	< LOD	< LOD	< LOD
365 day after 1 <sup>st</sup> app (just after 2 <sup>nd</sup> app)	A	0-10	0.0709	0.00460	< LOD	< LOQ
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.0938	0.00431	< LOD	< LOD

Sampling Event	Residues in Italian soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	< LOQ	< LOD	< LOD	< LOD
	C	0-10	0.0981	0.00560	< LOD	< LOD
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	< LOD	< LOD	< LOD	< LOD
120-day after 2 <sup>nd</sup> APP	A	0-10	0.0291	0.00451	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.0182	0.00256	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0207	0.00270	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
240-day after 2 <sup>nd</sup> APP	A	0-10	0.0257	0.00506	< LOD	< LOQ
		10-20	< LOQ	< LOQ	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
	B	0-10	0.0255	0.00315	< LOD	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0337	0.00707	< LOD	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
365 days after App2	A	0-10	0.0161	0.00390	<LOD	<LOD
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0107	0.00360	<LOD	<LOD
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0102	0.00370	<LOD	<LOD
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
Just after App 3	A	0-10	0.0358	0.00236	<LOD	<LOD
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOQ	<LOD	<LOD	<LOD
		40-50	<LOQ	<LOD	<LOD	<LOD
		50-65	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0698	0.00632	<LOD	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD



Sampling Event	Residues in Italian soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
		30-40	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0397	0.00499	<LOD	<LOD
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
120 days after App 3	A	0-10	0.0154	0.00404	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOQ	<LOD	<LOD	<LOD
		40-50	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0489	0.0115	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0205	0.00483	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
240 days after App3	A	0-10	0.0121	0.00376	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0182	0.00628	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0177	0.00637	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
365 days after App 3	A	0-10	0.0470	0.00419	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0129	0.00410	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0135	0.00613	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
Just after App4	A	0-10	0.0119	0.00324	<LOD	<LOQ
		10-20	<LOQ	<LOQ)	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0506	0.00548	<LOQ	<LOQ
		10-20	0.000465	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0211	0.00682	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
120 days after App4	A	0-10	0.0171	0.00406	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD

Sampling Event	Residues in Italian soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
	B	0-10	0.0226	0.00716	<LOQ	<LOQ
		10-20	<LOQ	<LOQ	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0442	0.0116	0.00240	0.00185
		10-20	<LOD	<LOD	<LOQ	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
240 days after App 4	A	0-10	0.0288	0.00547	<LOD	<LOD
		10-20	0.00267	<LOQ	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0201	0.00537	<LOD	<LOD
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOQ	<LOD	<LOD	<LOD
		30-40	<LOD	<LOD	<LOD	<LOD
		40-50	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0211	0.00615	<LOD	<LOD
		10-20	<LOQ	<LOQ	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
365 days after App4	A	0-10	0.0157	0.00500	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0111	0.00432	<LOD	<LOD
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0200	0.00635	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
Just after App 5	A	0-10	0.0229	0.00309	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0167	0.00337	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0522	0.0079	<LOQ	<LOQ
		10-20	<LOQ	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
120 days after App 5	A	0-10	0.0186	0.00497	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0307	0.00763	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0210	0.00629	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	A	0-10	0.0270	0.00916	<LOQ	<LOQ
		10-20	<LOQ	<LOQ	<LOQ	<LOQ

Sampling Event	Residues in Italian soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
240 days after App5		20-30	<LOD	<LOD	<LOQ	<LOD
		30-40	<LOD	<LOD	<LOQ	<LOQ
		40-50	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0196	0.00604	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOQ	<LOQ
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0300	0.0101	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
365 days after App 5	A	0-10	0.0176	0.00647	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	B	0-10	0.0193	0.00686	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD
	C	0-10	0.0224	0.00995	<LOQ	<LOQ
		10-20	<LOD	<LOD	<LOD	<LOD
		20-30	<LOD	<LOD	<LOD	<LOD

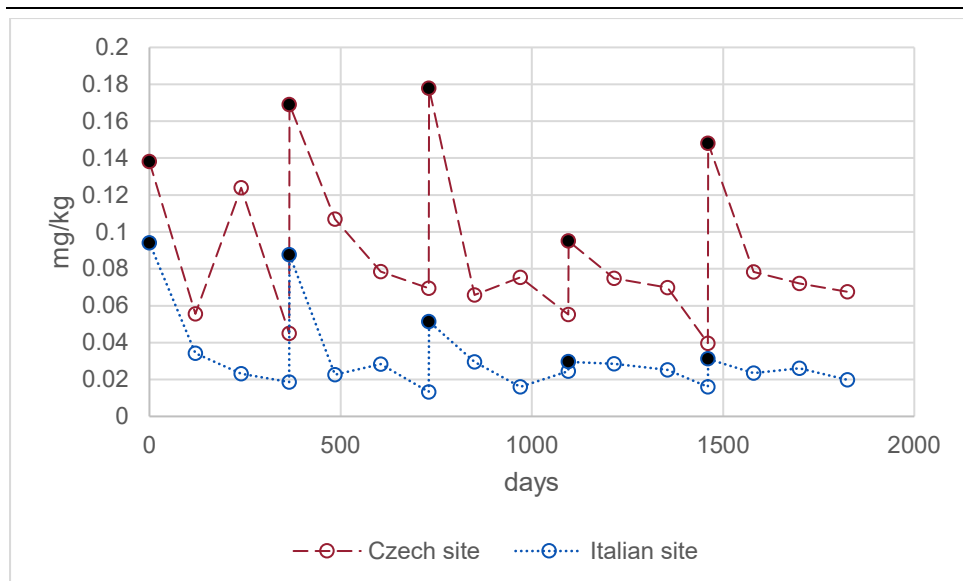
**Table B.8.1.2.2-13: Residues of inpyrfluxam and its metabolites in soil (mg/kg dry soil) – Spanish site**

Sampling Event	Residues in Spanish soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
0-day after 1 <sup>st</sup> APP	A	0-10	0.217	< LOQ	< LOD	< LOD
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.0904	< LOQ	< LOD	< LOD
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.112	< LOQ	< LOD	< LOD
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
120-day after 1 <sup>st</sup> APP	A	0-10	0.0125	0.00264	< LOQ	0.00152
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.0156	0.00521	0.00153	0.00222
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.00975	< LOQ	< LOQ	< LOQ
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
240-day after 1 <sup>st</sup> APP	A	0-10	0.00967	0.00262	< LOQ	0.00191
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.00556	< LOQ	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.00801	0.00278	< LOQ	0.00174
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
365 day after 1 <sup>st</sup> app (just before 2 <sup>nd</sup> App)	A	0-10	0.00729	0.00282	< LOQ	0.00242
		10-20	< LOD	< LOD	< LOD	< LOQ
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.0046	< LOQ	< LOQ	< LOQ
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.00579	< LOQ	< LOQ	0.00181
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
365 day after 1 <sup>st</sup> app	A	0-10	0.0959	0.0037	< LOQ	0.00140
		10-20	0.00288	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	< LOD	< LOD	< LOD	< LOD

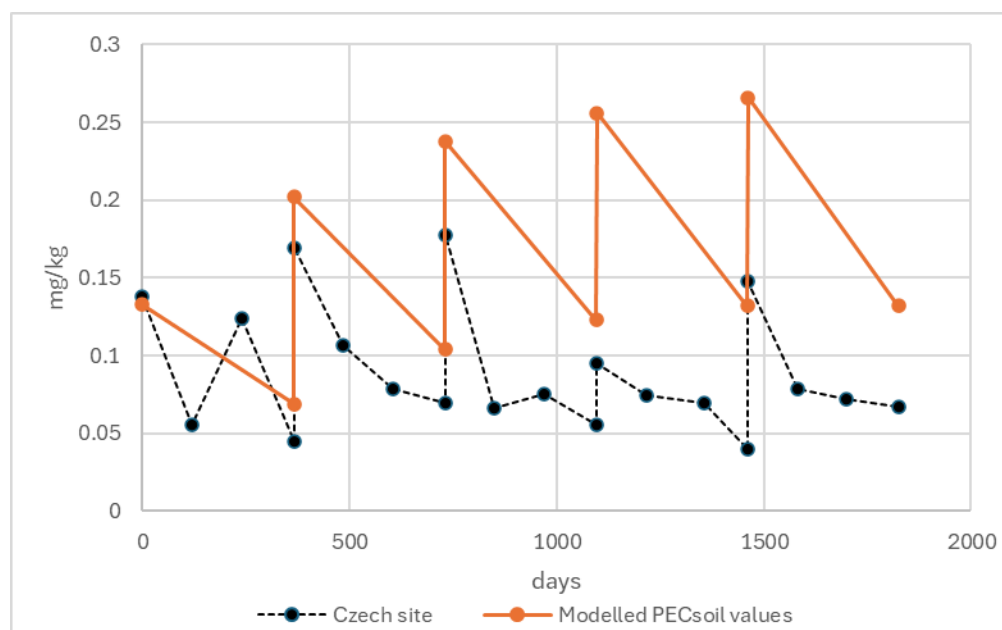
Sampling Event	Residues in Spanish soil expressed as mg/kg					
	Plot	Depth (cm)	Inpyrfluxam	3'-OH-S-2840	1'-COOH-S-2840A	1'-COOH-S-2840B
(just after 2 <sup>nd</sup> app)	B	0-10	0.0666	0.00494	< LOD	0.00220
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	< LOQ	< LOD	< LOD	< LOD
	C	0-10	0.0620	0.00460	0.00161	0.00258
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOQ	< LOD	< LOD	< LOD
		30-40	< LOQ	< LOD	< LOD	< LOD
120-day after 2 <sup>nd</sup> APP	A	0-10	0.0183	0.00318	0.00197	0.00379
		10-20	< LOQ	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	B	0-10	0.00849	< LOQ	0.00131	0.00242
		10-20	< LOD	< LOD	< LOD	< LOD
		20-30	< LOD	< LOD	< LOD	< LOD
	C	0-10	0.0129	0.00370	0.00189	0.00366
		10-20	< LOQ	< LOD	< LOQ	< LOQ
		20-30	< LOQ	< LOD	< LOQ	< LOD
		30-40	< LOD	< LOD	< LOD	< LOD

## CONCLUSION

The average soil residues at each time point have been plotted for the Italian and Czech sites (Figure B.8.1.2.2-03). The data indicate that after 5 annual applications the soil residues levels are approx 30% and 50 % of the original dose at the Italian and Czech sites respectively. In both sites the levels of residue determined decline after application. It is noted that the levels detected in soil are low just after the fourth application for both sites. The reason for this is not clear from the study, and consideration of the petri dish samples in Table B.8.1.2.2-09 do not indicate a significant dosing issue with these sites. It is suggested this would indicate a data handling or analysis error with the samples from this time point. However in terms of assessing accumulation the decline in residues following application is apparent within each annual period. The residual levels at the end of each 365 day period are not indicating significant accumulation above that which would be estimated with standard modelling approaches (Figure B.8.1.2.2-04). Based upon the proposed longest non-normalised DT<sub>50</sub> in soil for inpyrfluxam of 383 days (Table B.8.1.2.1.2-44). Standard modelling approaches would indicate a plateau is reached after 8 years.



**Figure B.8.1.2.2-03: Average measured soil residues at the Czech and Italian field sites. Solid points indicate residues level immediately after an annual application of inpyrfluxam to soil has taken place**



**Figure B.8.1.2.2-04 Comparison of the residue levels determined in the soil accumulation study at the Czech site with the modelled PECsoil values for a comparable application which is 200 g/ha with a 10 cm depth.**

## CONCLUSION

At the end of year 1 at the German and Spanish sites, ca. 5-20% of the day 0 residues of inpyrfluxam were remaining, and so these experiments were terminated as there was no significant carryover of residues between years (also noting that the degree of carry-over was greater in the other two sites that were continued beyond year 1). At the Czech and Italian sites there was some carryover of inpyrfluxam

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residues at the end of the first year (ca 20-40% remaining) and hence these studies were extended. In continuing with the two sites that had highest levels of carry over the applicant has met the data requirement for two soils to be considered in different geographical areas for field accumulation.

The soil residue analysis following five annual applications to soil indicate that the residues remaining are at approx. 30-50% of the initial application rate and, with the exception of application four at the Italian site, residue levels decline following application to soil. The residue levels determined conclude that the substance is not accumulating in the field at a rate that is over and above that considered for a substance with a longest non-normalised DT<sub>50</sub> in the field at 383 days. The modelling approaches would indicate a plateau formation following at 8 years of application which is beyond the duration of this study. Therefore soil accumulation in the risk assessment can be addressed by standard modelling approaches and the generation of a specific accumulation factor in soil is not required.

The metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B are not accumulating, with low levels at 365 days after application and most plot detections at <LOQ at study end.

HSE accepts this study for use in the risk assessment of inpyrfluxam.

### B.8.1.3. Selection of laboratory and field endpoints for modelling purposes

Following the evaluation of the laboratory and field studies, HSE considered the differences in degradation rates observed in the laboratory and field studies for inpyrfluxam, 3' OH S 2840, and 1' COOH S 2840. To do this, HSE collated the laboratory aerobic degradation study DegT<sub>50</sub>'s and compared these with the field study DegT<sub>50</sub>'s using the EFSA DegT<sub>50</sub> Endpoint Selector tool [EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT<sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662]. Laboratory studies of inpyrfluxam were conducted at 20°C, and at a higher water content than pF2. Resultantly, no normalisation was required. Metabolite laboratory studies were also performed at 20°C and pF2 and therefore did not require normalisation either. All field studies have also been normalised to 20°C and pF2.

#### Inpyrfluxam

Following the EFSA guidance (EFSA Journal 2014;12(5):3662), due to a geomean Laboratory DegT<sub>50</sub> value  $\geq 240$  days, and  $\geq 4$  inpyrfluxam active substance field studies being provided, the geomean of the field studies was used to determine the final DegT<sub>50</sub> value.

The degradation endpoint for use in the Spanish soil for the parent substance inpyrfluxam is 88.86 days based on the DT<sub>90</sub> value divided by 3.32. This can then be compared alongside DegT<sub>50</sub> values for SFO data sets. An alternative choice could have been the slow phase DegT<sub>50</sub> value for biphasic data sets. Since there will often be a mixture of SFO and biphasic data sets, this would generate an artificial discrepancy between SFO and biphasic data sets. Therefore, the pseudo SFO DegT<sub>50</sub> is preferred as it provides a better representation of the overall degradation behaviour including the fast and slow phases.

**Table B.8.1.3-1: Modelling endpoints derived from laboratory and field studies of inpyrfluxam used to select the final modelling endpoints.**

Laboratory modelling DT <sub>50</sub> 's (d)	Field modelling DT <sub>50</sub> 's (d)
Inpyrfluxam	Inpyrfluxam
121	78.8
1000 <sup>a</sup>	169
1000 <sup>a</sup>	421
1000 <sup>a</sup>	88.86 <sup>b</sup>
	104
Lab geomean: 589.8	Field geomean: <b>139.0</b>

<sup>a</sup>Pseudo-SFO-DT<sub>50</sub> derived from DFOP slow phase (k<sub>2</sub>) value



<sup>b</sup>Pseudo-SFO DT<sub>50</sub> derived from the DFOP DT<sub>90</sub>/3.32

### 3'-OH-S-2840

Following the EFSA guidance (EFSA Journal 2014;12(5):3662), due to a geomean Laboratory DegT<sub>50</sub> value  $\geq 240$  days, and  $\geq 3$  3'-OH-S-2840 metabolite field studies being provided, the geomean of the field studies was used to determine the final DegT<sub>50</sub> value.

**Table B.8.1.3-2: Modelling endpoints derived from laboratory and field studies of 3'-OH-S-2840 used to select the final modelling endpoints.**

Laboratory modelling DT <sub>50</sub> 's (d)	Field modelling DT <sub>50</sub> 's (d)
3'-OH-S-2840	3'-OH-S-2840
369	96.6
303	101
276	204
	148
Lab geomean: 313.7	<b>Field geomean: 131.0</b>

### 1'-COOH-S-2840

The EFSA guidance (EFSA Journal 2014;12(5):3662) was used to determine a final DegT<sub>50</sub> value. As the geomean Laboratory DegT<sub>50</sub> value is  $< 240$  days, the null hypothesis that the lab and field studies give statistically indistinguishable endpoints was tested. The test rejected the null hypothesis (Student's t-test,  $t = 1.54$ ,  $\alpha = 0.25$ ), and showed that field studies produced shorter DegT<sub>50</sub> values than laboratory studies. As  $\geq 3$  field DegT<sub>50</sub> values were present for this metabolite, these were used to determine the modelling endpoint.

**Table B.8.1.3-3 Modelling endpoints derived from laboratory and field studies of 1'-COOH-S-2840 used to select the final modelling endpoints.**

Laboratory modelling DT <sub>50</sub> 's (d)	Field modelling DT <sub>50</sub> 's (d)
1'-COOH-S-2840	1'-COOH-S-2840
91.3	75.4
187 <sup>a</sup>	24.7
148	224
207 (parent-dosed)	
840 (parent-dosed)	
Lab geomean: 213.1	<b>Field geomean: 74.7</b>

<sup>a</sup>DFOP fit, therefore DT<sub>90</sub>/3.32 used

## Selection of a model

Overall modelling endpoints for inpyrfluxam includes endpoints from both SFO and DFOP models. Due to the presence of at least one DFOP fit, when all endpoints are averaged the overall average will also be DFOP, even when only a single soil in the database was fitted according to biphasic kinetics. Implementing DFOP kinetics in the exposure assessment (e.g. FOCUS groundwater modelling) can significantly complicate the assessment, especially when both parent and metabolite potentially require biphasic kinetics to be implemented. Even when the average behaviour is biphasic, HSE consider it is possible that behaviour can still be adequately described by an overall pseudo SFO fit. Therefore HSE conducted further analysis to support a decision whether the overall behaviour of inpyrfluxam can be adequately described by an SFO model, or if use of a DFOP model is necessary.

The first step in the analysis was to calculate average parameters from all fits (SFO and DFOP). To do this the separate DFOP fast and slow phase rate constants were averaged with the single SFO rate constants to determine separate geomean values for fast and slow phases. The DFOP *g* value was taken from the soils fitted with DFOP kinetics.

An assessment of the extent of biphasic degradation seen with the average parameters can be determined by comparing the ratio between the average DFOP DT<sub>90</sub> and average DFOP DT<sub>50</sub>. The further this value deviates from 3.32 (the standard ratio between an SFO DT<sub>90</sub> and SFO DT<sub>50</sub>) provides some quantification of the extent of the biphasic fit of the average parameters. A further visual assessment is performed by comparing the average DFOP fit with a pseudo SFO fit derived from the average DFOP DT<sub>90</sub>/3.32. This is illustrated below and a qualitative assessment made over the similarity in fit between the DFOP and pseudo-SFO fit.

## Inpyrfluxam

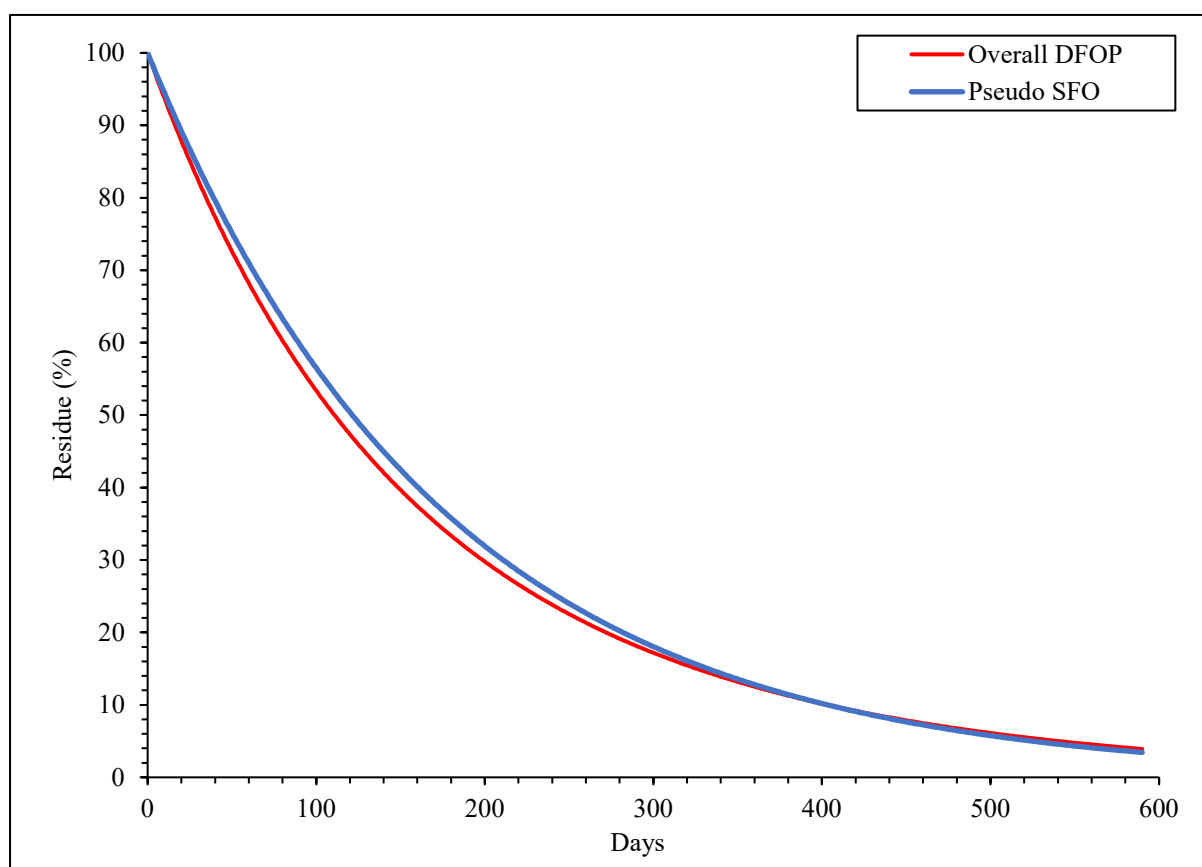
Only field studies were considered for the endpoint determination for inpyrfluxam based on the assessment on line with the EFSA DegT<sub>50</sub> guidance (2014) above. This consisted of four SFO model fits, and one DFOP model fit (for the Spain site). The degradation parameters for deciding between an overall SFO or DFOP model are given below in Table B.8.1.3-4.

**Table B.8.1.3-4 Soil degradation parameters for selecting overall kinetic model for inpyrfluxam**

<b>Soil</b>	<b>Fast phase <math>k_1</math> DT<sub>50</sub> (days)</b>	<b>Slow phase <math>k_2</math> DT<sub>50</sub> (days)</b>	<b><i>g</i> value</b>	<b>Kinetic</b>
Germany	78.8	78.8	-	SFO
Czech Republic	169.0	169.0	-	SFO
Italy	421.0	421.0	-	SFO
Spain	3.5	111.0	0.37	DFOP
Ontario	104.0	104.0	-	SFO

<b>geomean</b>	72.8	145.3	-	-
<b>arithmetic mean</b>	-	-	<b>0.37</b>	-

Based on the geomean fast phase and slow phase  $DT_{50}$ 's above and the single  $g$  value, this would equate to a DFOP fit with an overall  $DT_{50}$  of 110.9 d and overall  $DT_{90}$  of 403.3 d. This gives an overall  $DT_{90}/DT_{50}$  ratio value of 3.6. This is noted to be close to the standard SFO ratio of 3.32, suggesting that the average DFOP fit is quite close to following SFO kinetics. In addition as noted above, behaviour in 4 out of the 5 field soils was adequately described by SFO kinetics, suggesting that in the fields that were tested biphasic behaviour was not the norm and that the overall average behaviour is likely to be closer to SFO. A pseudo SFO model was then produced based on the average DFOP fit, being based on the DFOP  $DT_{90}/3.32$  (ie  $403.3/3.32 = 121.4$  d). This allows a further visual comparison between the pseudo SFO model and the overall DFOP model (see Figure B.8.1.3-1) and can further help the decision over whether the average behaviour can be described by an SFO model.



**Figure B.8.1.3-1 Comparison of the averaged DFOP degradation model of inpyrfluxam, and an SFO approximation**

The averaged degradation profile of inpyrfluxam, which was biphasic, was found to be approximated suitably by the pseudo SFO model. Therefore in the opinion of HSE the pseudo-SFO equation can be used for subsequent FOCUS modelling without significantly impacting the modelling of subsequent metabolites. This resulted in the use of a value of 121.4 d in FOCUS modelling. HSE notes that the pseudo SFO value

selected is similar to the actual average DFOP DT<sub>50</sub> of 110.9 (and both models had the same DT<sub>90</sub>).

### 3'-OH-S-2840

As only SFO models were used to describe degradation kinetics, an average of SFO can be taken forward for FOCUS modelling, with a DT<sub>50</sub> of 131 days.

### 1'-COOH-S-2840

As only SFO models were used to describe degradation kinetics, an average of SFO can be taken forward for FOCUS modelling, with a DT<sub>50</sub> of 74.7 days.

**Table B.8.1.3-5 Summary of final modelling endpoints in soil**

<b>Compound</b>	<b>DegT<sub>50</sub> endpoint (d)</b>	<b>Source</b>
inpyrfluxam	121.4	SFO approximation of DFOP Field DegT <sub>50</sub> geomean
3'-OH-S-2840	131.0	Field DegT <sub>50</sub> geomean
1'-COOH-S-2840	74.7	Field DegT <sub>50</sub> geomean

#### **B.8.1.4. Storage stability**

<b>Reference:</b>	KCA 7.1.2.2.1/09
<b>Report Title:</b>	S-2399: Freezer Storage Stability of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Soils
<b>Author(s) &amp; Year:</b>	██████ 2017
<b>Document No</b>	TPR-0064
<b>Guideline(s):</b>	OCSP 860.1380
<b>Deviations:</b>	No
<b>GLP?</b>	Yes
<b>Acceptability:</b>	Yes
<b>Study relied upon:</b>	Yes

The purpose of this study was to determine the freezer storage stability of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in soil. Currently, there is no guidance available to assess storage stability of residues in soil, therefore the principles outlined in OECD 506 (Stability of Pesticide Residues in Stored Commodities) has been applied. The study was conducted in accordance with OCSPP 860.1380, but has been assessed to OECD 506.

The storage stability study was conducted to support residue testing of terrestrial field soil dissipation studies. As only the Ontario, Canada (VP-38593) dissipation study is considered representative of European conditions and has been used for risk assessment purposes, the storage stability of inpyrfluxam and its metabolites in the other locations are given for additional demonstration of stability only.

### *Materials and methods*

The frozen storage of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B was studied in soil subsamples for 19.5 or 24 months.

Soil samples from Washington (VP-38546), Mississippi (VP-38553), California (VP-38586), Ontario, Canada (VP-38593) and North Dakota (VP-38603) were stored at ca. -20 °C in the dark until fortification and extraction. A sample weight of 10 g was transferred into a 50 mL centrifuge tube and fortified at 0.1 mg/kg (10x LOQ) with a 10.0 mg/mL acetone solution of the respective test items. The inpyrfluxam storage stability samples were prepared separately from the metabolite-fortified samples. The centrifuge tubes were closed and placed into the deep freezer.

After fortification, all soil storage stability samples were frozen and maintained frozen until removed for analysis. 3 samples (2 fortified and 1 fresh) per analyte was prepared for each sampling time point.

After time intervals of approximately 6 – 6.5, 13.5, 19.5 and 24 months, samples were analysed.

Analysis was done using the method from Report No. TPA-0028 (method RM-50S). Full details and validation data for this method can be found in Vol. 3CA B5.1.2.1. The method limit of quantitation (LOQ) is 0.01 mg/kg for all analytes (all LOQ values based on wet soil basis in soil). Extracts were stored for a maximum of 86 days. Despite this being a long period to store analytical extracts prior to analysis, stability of residues in sample extracts has been satisfactorily addressed, as 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.

### *Results and discussion*

The recoveries of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B from soil after the various storage periods are summarised in Table B.8.1.4-1.

Mean procedural (fresh) recoveries were generally between 70 - 110% for each analyte at each storage timepoint. Some results varied from this, but none from the Ontario, Canada (VP-38593) samples, relevant to risk assessment. Most results which did not meet acceptable criteria in accordance with OCSPP 860.1380 (70 - 120%) were reported as 'N/A'. The mean recovery is a % of the fortification; it was not corrected for procedural recovery.

### Conclusion

Inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B are considered stable (recovery typically >70%) in soil when stored frozen at ca. -20°C for a period of 19.5 (Washington and Mississippi,) or 24 months (California, North Dakota and Ontario, Canada).

For the storage stability study on the Ontario, Canada (VP-38593) soil samples, inpyrfluxam is found at 52.0 and 46.0% at 24 months of storage and therefore appears to have declined by ~50%. It is still considered sufficiently stable over 24 months frozen storage, as the corresponding procedural recovery is notably lower than for the earlier time-points (78.9% compared to 90.4 - 109%). Whilst some decline in stability has been observed, it is not expected to be as unstable as the recoveries demonstrate and is considered sufficiently stable for risk assessment purposes.

For a number of the analytes at different locations, there is a drop in stability (<70% stored recovery) at the middle timepoints. As the stored recoveries at later time points are >70%, stability can be assumed for the length of the storage stability study.

**Table B.8.4.1-1 Storage stability of 2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in stored Washington (VP-38546) soil**

Time (days)	% Recovery ( 0.1 mg/kg Fortification )			Mean % Recovery
	Fresh Fortified	Stored Sample (SS1)	Stored Sample (SS2)	
Inpyrfluxam				
0	95.2	104.7	102.9	103.8
180	88.5	97.5	111.0	104.3
197	91.4	86.3	85.3	85.8
407	95.1	99.2	108.3	103.8
585	111.0	117.0	119.0	118.0
1'-COOH-S-2840-A				
0	98.7	106.0	102.4	104.2
180	77.3	89.3	89.5	89.4

197	90.3	86.1	92.0	89.1
407	107.5	107.4	104.6	106.0
585	98.8	106.8	98.7	102.8
<b>1'-COOH-S-2840-B</b>				
0	90.5	95.0	92.1	93.6
180	N/A	N/A	N/A	N/A
197	81.9	62.8	61.0	61.9
407	99.2	85.7	82.9	84.3
585	96.9	94.2	86.6	90.4
<b>3'-OH-S-2840</b>				
0	87.0	93.1	91.2	90.4
180	N/A	N/A	N/A	N/A
197	78.4	78.8	76.5	99.0
407	98.7	106.4	107.8	108.5
585	85.9	89.0	85.8	101.7

**Table B.8.4.1-2 Storage stability of 2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in stored Mississippi (VP-38553) soil**

Time (days)	% Recovery (0.1 mg/kg Fortification )			Mean % Recovery
	Fresh Fortified	Stored Sample (SS1)	Stored Sample (SS2)	
Inpyrfluxam				
0	112.0	123.0	120.0	121.5
176	97.7	93.6	105.1	99.4
196	93.8	107.4	101.4	104.4
409	94.1	89.7	88.8	89.3
582	110.0	121.0	120.0	120.5
1'-COOH-S-2840-A				
0	104.9	99.7	95.0	97.4
176	74.9	92.0	90.9	91.5
196	87.5	93.5	91.3	92.4
409	101.8	103.5	99.3	101.4
582	95.0	125.0	115.0	120.0
1'-COOH-S-2840-B				
0	94.9	89.1	86.4	87.8
176	68.2	63.1	63.3	63.2

196	81.3	68.1	65.3	66.7
409	95.1	85.1	83.5	84.3
582	98.6	98.1	101.0	99.6
<b>3'-OH-S-2840</b>				
0	91.1	82.7	78.5	80.6
176	73.4	85.7	84.8	85.3
196	72.0	70.5	65.5	68.0
409	86.8	99.4	87.6	93.5
582	98.1	96.6	95.1	95.9

**Table B.8.4.1-3 Storage stability of 2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in stored California (VP-38586) soil**

Time (days)	% Recovery (0.1 mg/kg Fortification )			Mean % Recovery
	Fresh Fortified	Stored Sample (SS1)	Stored Sample (SS2)	
Inpyrfluxam				
0	96.7	108.6	100.2	104.4
408	92.8	88.4	92.5	90.5
582	102.0	103.4	102.4	102.9
725	76.7	38.0	75.8	56.9
1'-COOH-S-2840-A				
0	99.4	89.4	94.1	91.8
408	90.1	106.5	105.6	106.1
582	107.4	109.9	109.8	109.9
725	95.2	88.7	96.9	92.8
1'-COOH-S-2840-B				
0	99.1	92.4	100.7	96.6
408	78.0	83.0	76.7	79.9
582	96.7	95.6	91.3	93.5
725	98.2	83.4	92.0	87.7
3'-OH-S-2840				
0	86.3	79.4	84.4	81.9
408	92.9	99.3	97.9	98.6
582	82.1	92.1	94.5	93.3
725	84.0	86.0	75.6	80.8



**Table B.8.4.1-4 Storage stability of 2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in stored Ontario, Canada (VP-38593) soil**

Time (days)	% Recovery (0.1 mg/kg Fortification)			Mean % Recovery
	Fresh Fortified	Stored Sample (SS1)	Stored Sample (SS2)	
Inpyrfluxam				
0	96.2	100.4	89.9	95.2
190	90.4	94.2	110.0	102.1
406	97.1	96.5	108.8	102.7
582	109.0	112.0	109.3	110.7
724	78.9	52.0	46.0	49.0
1'-COOH-S-2840-A				
0	90.1	107.8	113.0	110.4
190	83.2	95.1	102.5	98.8
406	98.9	94.1	112.0	103.1
582	104.1	109.6	109.5	109.6
724	111.0	106.3	79.1	92.7
1'-COOH-S-2840-B				
0	98.4	103.6	107.4	105.5
190	73.2	72.9	75.8	74.4
406	88.2	73.0	80.8	76.9
582	99.4	88.2	88.6	88.4
724	104.3	81.2	73.6	77.4
3'-OH-S-2840				
0	74.8	92.8	89.2	91.0
190	69.6	82.4	77.3	79.9
406	87.1	87.0	97.4	92.2
582	91.6	104.2	102.2	103.2
724	89.3	82.9	78.7	80.8

**Table B.8.4.1-5 Storage stability of 2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in stored North Dakota (VP-38603) soil**

Time (days)	% Recovery (0.1 mg/kg Fortification)			Mean % Recovery
	Fresh Fortified	Stored Sample (SS1)	Stored Sample (SS2)	
Inpyrfluxam				
0	108.8	105.1	104.2	104.7
191	90.5	99.6	102.7	101.2
405	96.5	101.1	103.6	102.4
582	99.0	105.1	117.0	111.1
727	73.7	74.1	64.4	69.3
1'-COOH-S-2840-A				
0	98.6	87.5	69.5	78.5
191	N/A	N/A	N/A	N/A
405	74.5	91.1	97.0	94.1
582	94.6	107.6	102.9	105.3
727	96.7	94.9	88.4	91.7
1'-COOH-S-2840-B				
0	84.6	79.9	66.2	73.1
191	N/A	N/A	N/A	N/A
405	65.0	55.1	68.2	61.7
582	84.9	79.7	77.6	78.7
727	76.6	68.7	76.9	72.8
3'-OH-S-2840				
0	97.3	85.3	87.2	86.3
191	79.5	90.4	95.2	92.8
405	96.9	94.9	109.9	102.4
582	93.8	105.9	104.7	105.3
727	97.6	100.9	98.9	99.9

<b>Reference:</b>	KCA 7.1.2.2.1_10
<b>Report Title:</b>	Storage Stability of S-2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S- 2840A and 1'-COOH-S-2840B in Soil under Deep Frozen Conditions
<b>Author(s) &amp; Year:</b>	██████ 2018
<b>Document No</b>	TPR-0088
<b>Guideline(s):</b>	OECD 506, 2007; OECD Guideline for the Testing of Chemicals – Stability of Pesticide Residues in Stored Commodities 7032/VI/95, Appendix H of the Commission of the European Communities SANCO/3029/99 rev. 4, 10/07/2000
<b>Deviations:</b>	No
<b>GLP?</b>	Yes
<b>Acceptability:</b>	Yes
<b>Study relied upon:</b>	Yes

The purpose of this study was to determine the freezer storage stability of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in soil. Currently, there is no guidance available to assess storage stability of residues in soil, therefore the principles outlined in OECD 506 (Stability of Pesticide Residues in Stored Commodities) has been applied.

#### *Materials and methods*

The frozen storage of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B were studied in soil subsamples for up to 24 months.

Soil samples (Soil ATC F6 from Czech Republic) were stored at  $\leq -18^{\circ}\text{C}$  in the dark until fortification and extraction. A sample weight of 5 g was transferred into a 50 mL centrifuge tube and was fortified at 0.02 mg/kg (10x LOQ) for inpyrfluxam and 3'-OH-S-2840 and at 0.01 mg/kg (10x LOQ) for 1'-COOH-S-2840A and 1'-COOH-S-2840B with the test items. The test items inpyrfluxam and 3'-OH-S-2840 were spiked individually, while 1'-COOH-S-2840A and 1'-COOH-S-2840B were spiked together. A standard solution of each test item was distributed dropwise to the thawed sample with a solvent volume not exceeding a total of 2 % of the sample extraction volume per sample. The solvent was allowed to evaporate for approx. 10 min. After that procedure the centrifuge tube was closed and placed into the deep freezer.

After fortification, all soil storage stability samples were frozen and maintained frozen until removed for analysis. At least 3 samples (2 fortified and 1 fresh) per analyte was prepared for each sampling time point.

After time intervals of approximately 1, 3, 6, 9, 12, 18 and 24 months, samples were analysed.

Analysis was done using the method from Report No. TPA-0043, validated on Soil ATC F6 from the Czech Republic. Full details and validation data for this method can be found in Vol. 3CA B5.1.2.1. The method limit of quantitation (LOQ) is 0.002 mg/kg for inpyrfluxam and 3'-OH-S-2840 and 0.001 mg/kg for 1'-COOH-S-2840A and for 1'-COOH-S-2840B (all LOQ values based on wet soil basis in soil). Extracts were stored for a maximum of 1 days and their stability confirmed by the procedural recoveries.

### *Results and discussion*

The recoveries of inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B from soil after the various storage periods are summarised in Table B.8.1.4-6.

Mean procedural (fresh) recoveries were between 70 - 110% for each analyte at every storage timepoint.

### *Conclusion*

Inpyrfluxam and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B are considered stable (recovery >70%) in soil when stored frozen at  $\leq -18^{\circ}\text{C}$  for a period of at least 24 months.

**Table B.8.1.4-6 Storage stability of 2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in stored samples of soil**

Storage Period	Procedural (fresh) Recoveries		Storage Samples			Percentage recovered (mean)
	mg/kg	%	Single Values (mg/kg)	Single Values (%)	Mean (%)	
Inpyrfluxam: Fortification Level: 0.02 mg/kg						
0 months	0.0194	97	0.0190, 0.0187, 0.0195	95, 94, 98	96	99
1 months	0.0211	106	0.0211, 0.0201	106, 101	104	98
3 months	0.0185	93	0.0161, 0.0158	81, 79	80	86
6 months	0.0206	103	0.0179, 0.0178	90, 89	90	87
9 months	0.0206	103	0.0181, 0.0187	91, 94	93	90
12 months	0.0202	101	0.0173, 0.0176	87, 88	88	87
18 months	0.0210	105	0.0183, 0.0188	92, 94	93	89

24 months	0.0224	112	0.0217, 0.0230	109, 115	112	100
<b>3'-OH-S-2840: Fortification Level: 0.02 mg/kg</b>						
0 months	0.0202	101	0.0180, 0.0192, 0.0194	90, 96, 97	94	93
1 months	0.0209	105	0.0194, 0.0192	97, 96	97	92
3 months	0.0176	88	0.0157, 0.0152	79, 76	78	88
6 months	0.0206	103	0.0177, 0.0180	89, 90	90	87
9 months	0.0227	114	0.0221, 0.0221	111, 111	111	97
12 months	0.0206	103	0.0181, 0.0181	91, 91	91	88
18 months	0.0211	106	0.0194, 0.0193	97, 97	97	92
24 months	0.0221	111	0.0196, 0.0195	98, 98	98	88
<b>1'-COOH-S-2840A: Fortification Level: 0.01 mg/kg</b>						
0 months	0.0104	104	0.0103, 0.0103, 0.0102	103, 103, 102	103	99
1 months	0.0102	102	0.00928, 0.00904	93, 90	92	90
3 months	0.0099 5	100	0.00876, 0.00866	88, 87	88	88
6 months	0.0098 1	98	0.00894, 0.00872	89, 87	88	90
9 months	0.0116	116	0.0101, 0.00997	101, 100	101	87
12 months	0.0103	103	0.00871, 0.00871	87, 87	87	84
18 months	0.0105	105	0.00891, 0.00918	89, 92	91	87
24 months	0.0104	104	0.00945, 0.00860	95, 86	91	87
<b>1'-COOH-S-2840B: Fortification Level: 0.01 mg/kg</b>						
0 months	0.106	106	0.0104, 0.0106, 0.0105	104, 106, 105	105	99
1 months	0.0098 1	98	0.0101, 0.00991	101, 99	100	102
3 months	0.0098 1	98	0.00880, 0.00877	88, 88	88	90
6 months	0.0098 1	98	0.00955, 0.00932	96, 93	95	97
9 months	0.0100	100	0.00985, 0.00976	99, 98	99	99
12 months	0.0099 6	100	0.00863, 0.00896	86, 90	88	88
18 months	0.0105	105	0.00868, 0.00888	87, 89	88	84
24 months	0.0102	104	0.00988, 0.00908	99 91	95	93

**B.8.1.5. Adsorption and desorption in soil****B.8.1.5.1. Adsorption and desorption in soil of the active substance**

<b>Reference:</b>	KCA 7.1.3.1.1/01
<b>Report Title:</b>	[ <sup>14</sup> C] S-2399: Adsorption/Desorption in Soil
<b>Author(s) &amp; Year:</b>	██████ & ██████ 2016
<b>Document No</b>	TPM-0018, 3201088
<b>Substance used:</b>	[phenyl- <sup>14</sup> C] inpyrfluxam (Batch No. CFQ41803; Radiochemical Purity: ≥ 99.0%)
<b>Guideline(s):</b>	OECD Test Guideline No. 106 (2000) OECD 106 checklist (2017)
<b>Deviations:</b>	HSE has evaluated the adsorption and desorption of test substance in soil in accordance with the OECD 106 guidelines. Major and minor deviations found are listed in the table below.
<b>GLP?</b>	Yes
<b>Acceptability:</b>	Yes
<b>Study relied upon:</b>	Yes

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Brierlow soil was collected from a depth of up to 23 cm, greater than the maximum depth of 20 cm required by the guidelines.	Minor deviation. Organic carbon content of these soils exceed 0.3 %, and therefore should not impact the correlation between organic carbon and sorption.
Cultivation history of the sampling sites for all soils are not stated.	Minor deviation. Land use and pesticide histories have been provided. Also applicant has confirmed that no

	pesticides were used on the sites for the last 5 years prior to collection.
Pesticide history for soil sampling sites provided, but not information regarding other forms of contamination such as fertilisers, biological additions of accidental contamination.	Minor omission. These substances may alter soil properties and microbial communities, however soils were characterised prior to study start and study is investigating the physicochemical processes of adsorption and not biodegradation.
Environmental conditions of the soil samples during transportation were not reported, nor the containers they were stored in.	Minor omission. Soil properties relevant to sorption are not expected to be significantly altered during transport.
Not stated if the stock & application solutions were prepared immediately before use or were stored, and if so the storage conditions and duration.	Minor omission as the stability of the test item in the test system was verified and mass balances were > 90 %.
The volume of each aliquot taken for LSC was 4 % (1 mL) of the total volume (25 mL) for each timepoint rather than 1 % specified in the OECD 106 guidelines.	Minor deviation not thought to affect study outcomes, as this was performed in the preliminary test.
No soil matrix blank samples were analysed, and so the results are not corrected for these.	Minor deviation as the soil history is known, recoveries are acceptable, and the test item was radiolabelled.
Applicant stated mass balances were > 90 % but did not subtract non-extractable residues.	Minor deviation as the Soils passed the preliminary soil stability test in line with OECD 106.
Minor adsorption to Teflon® test vessels occurred over 24 hours.	Minor deviation. Decrease in recovery after the 24 h shaking period was very slight, therefore adsorption was negligible (if at all, as this may be attributable to other abiotic processes).

The 95 % confidence intervals of the adsorption $K_f$ and $1/n$ values are not reported	Minor omission. HSE has calculated these values and added them to the summary on Table B.8.1.4.1-28.
Plots of the residuals were not supplied by the applicant for the adsorption isotherms.	Minor omission. The adsorption isotherms, linear plot parameters and derived adsorption values derived by HSE closely matched those provided by the applicant. Therefore the plot of residuals were provided by HSE.

### HSE conclusion on deviations

Deviations are not considered by HSE to void the validity of the study, and therefore further clarification is not required.

## INTRODUCTION

The adsorption and desorption characteristics of [ $^{14}\text{C}$ ] inpyrfluxam were determined in seven soils in a laboratory batch equilibrium experiment. Seven soils were used - four soils from the UK: Brierlow (silt loam), Kenslow (loam), Clipstone (loamy sand) and Hareby (clay loam); two soils from the US: LAD-SCL-PF (clay or clay loam) and Atwater (sandy loam), and one soil from Japan: Ibaraki (sandy loam). The adsorption phase of the study was carried out at soil ratios 1:10, 1:5 and 1:2 in the dark at  $20 \pm 2$  °C for 48 hours using pre-equilibrated air-dried soils. Nominal concentrations of [ $^{14}\text{C}$ ] inpyrfluxam were 1.0, 0.3, 0.1, 0.03, 0.01 mg/L.

HSE performed quality checks as part of confirming the acceptability of the study conduct and of the endpoints reported by the applicant. These were based on the OECD 106 evaluators checklist published by EFSA (2017). Quality checks covered the study conduct, the suitability of analytical methods and data handling. The study followed the OECD guideline 106 and was conducted to GLP.

## MATERIAL AND METHODS

### I. Test materials and reference items

The applicant used phenyl labelled [ $^{14}\text{C}$ ] inpyrfluxam and details are summarised in Table B.8.1.4.1-01 below. HSE confirms that the position of the radiolabel is suitable for this study as it is placed in a ring structure within the most stable part of the compound.



All application solutions of [ $^{14}\text{C}$ ] inpyrfluxam were prepared from repurified stock solution and the concentration of [ $^{14}\text{C}$ ] inpyrfluxam in solution was determined by Liquid Scintillation Counting (LSC). The radiochemical purity of [ $^{14}\text{C}$ ] inpyrfluxam in the stock solution was checked in two chromatographic systems (High Performance Liquid Chromatography, HPLC and Thin Layer Chromatography, TLC) prior to use and was specified as  $\geq 98.0\%$ .

**Table B.8.1.5.1-01 Summary of the properties of the test item, [ $^{14}\text{C}$ ] inpyrfluxam used in this study**

<b>Test item</b>	[Phenyl- $^{14}\text{C}$ ] inpyrfluxam (PHE-label)
<b>Lot/ Batch</b>	CFQ41803
<b>Specific activity</b>	4.51 GBq/mmol (13.38 MBq/mg)
<b>Molecular weight</b>	333.38 (non-radiolabelled), 337.3 (radiolabelled)
<b>Radiochemical Purity:</b>	$\geq 98.0\%$
<b>CAS#</b>	Not assigned
<b>Stability of compound</b>	Not stated
<b>Chemical structure</b>  *Denotes $^{14}\text{C}$ radiolabel position	

Application solutions were prepared by evaporating portions of the stock solution to dryness under nitrogen and dissolving the residue in 0.01 M calcium chloride ( $\text{CaCl}_2$ ) solution.

## II. Soils

The adsorption behaviour of seven soils (four UK soils, two soils from the US and one soil from Japan) was determined in this study. HSE notes that soils in the Ibaraki prefecture are classed as Andosols by the Food and Agriculture Organization (FAO)-UNESCO Soil Map of the World. Andosols are a type of volcanic soil. HSE highlights the concerns outlined in the OECD 106 guideline regarding volcanic soils, particularly relation to the content of amorphous iron and aluminium oxides. The applicant did not characterise the soil in respect of the level of these minerals, therefore the impact on the adsorption/ desorption of the test substance is unknown and HSE is inclined to exclude results from the Ibaraki soil in the calculation of mean values of adsorption endpoints. Nevertheless, the impact of excluding this soil was considered in the annex

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of Table B.8.1.4.1-28, and the adsorption behaviour of inpyrfluxam in the Ibaraki has been reported for clarity.

The other six soils covered a pH range (CaCl<sub>2</sub>) from 5.5 to 8.1 and six different USDA textural classes: sandy loam, silt loam, loam, loamy sand, clay loam and clay. The soil characteristics are in line with OECD 106 guidelines, which suggest a pH (measured in CaCl<sub>2</sub>) range of 5-8, clay content ranging from <10 % to 80 %, and organic carbon content within the range of 0.3 to 4 % (the soils used in this study were 0.3 % to 3.8 %).

Details of the physical and chemical properties are given in Table B.8.1.4.1-02. HSE notes that the cultivation history of the sampling sites for all soils are not stated; however the land use and pesticide histories have been provided and the applicant has confirmed that no pesticides were used on the sites for the last 5 years prior to collection.

HSE also notes that no information regarding other forms of contamination were provided, such as fertilisers, biological additions or accidental contamination. These substances can affect soil properties (e.g. pH, organic matter content or cation exchange capacity), and in turn adsorption behaviour of the test substance. It may also alter the microbial communities in the soil. However, the soils were characterised with respect to these properties prior to study initiation. Furthermore, the study is investigating the physicochemical processes of adsorption and not biodegradation. Therefore, this is thought to be a minor deviation which does not impact study outcomes, particularly as the test substance was found to be stable for the duration of the adsorption equilibrium period.

Five out of the six soils were collected from the top 0-20 cm layer of the A horizon, however Brierlow soil was collected from a depth of up to 23 cm. The OECD 106 guidelines state samples should be collected from only the A horizon up to a maximum depth of 20 cm. Given the organic carbon (OC) content of these soils exceed 0.3 %, HSE considers this a minor deviation that will not impact the overall study.

All soils were sieved through a 2 mm sieve, thoroughly mixed, air-dried and stored in the dark at room temperature (15.0 to 30.0°C) prior to use, and the soils were stored for < 3 years. HSE notes that the applicant did not report environmental conditions of the soil samples during transportation nor the containers they were stored in. However, this is a minor deviation as the soil properties which are relevant to sorption are not expected to be significantly altered during transport.

**Table B.8.1.5.1-02 Characteristics of test soils used in the adsorption study of [<sup>14</sup>C] inpyrfluxam**

<b>Property</b>	<b>Brierlow</b>	<b>Kenslow</b>	<b>Clipstone</b>	<b>Hareby</b>	<b>LAD-SCL-PF</b>	<b>Atwater</b>	<b>Ibaraki*</b>
<b>Sampling location</b>	Derbys hire, UK	Derbys hire, UK	Nottingham shire, UK	Lincolns hire, UK	Wyoming, USA	California, USA	Ibaraki Prefecture, Japan
<b>Sampling depth</b>	12-23 cm	10-20 cm	5-15 cm	4-10 cm	0-15.2 cm	0-7.6 cm	> 10 cm
<b>Soil texture<sup>a</sup></b>	Silt loam	Loam	Loamy sand	Clay loam	Clay or clay loam	Sandy loam	Sandy loam
<b>% Clay (&lt;2 µm)</b>	16	14	6	34	40	15	19
<b>% Silt (&lt;50-2 µm)</b>	55	44	8	33	25	13	22
<b>% Sand (2000-50 µm)</b>	29	42	86	33	35	72	59
<b>pH (0.01 M CaCl<sub>2</sub>)</b>	6.1	5.5	5.5	7.4	8.1	7.0	7.3
<b>Organic carbon (%)</b>	2.4	3.8	1.2	1.6	0.9	0.3	2.6
<b>CEC (meq/100 g soil)</b>	20.5	20.2	7.8	12.2	30.2	10.3	47.6
<b>Moisture at pF 0 (w/w %)</b>	61.4	68.2	INP	INP	60.3	INP	INP
<b>Moisture at pF 2 (w/w %)</b>	35.8	42.3	INP	INP	36.2	INP	INP
<b>Soil classification</b>	Inceptisol	Inceptisol	Entisol	Inceptisol	INP	INP	INP
<sup>a</sup> USDA soil classification system INP Information not provided *Ibaraki soil presented for information purposes only							

### III. Preparation of Test Units

Prior to dispensing the soils, the moisture contents of the stored soils were determined by drying a sample of each at approximately 105 °C. The wet : dry weight ratio was calculated and this allowed the dry weight equivalent of the soil to

be calculated from the wet weight. All calculations were based upon soil dry weight. HSE accepts that soil moisture content has been accounted for in subsequent calculations.

For all Tiers the soils were weighed into pre-weighed Teflon® centrifuge tubes and were equilibrated by shaking with 0.01 M CaCl<sub>2</sub> solution overnight before the day of treatment. The volumes and weights of soil used depended on the intended ratio (Table B.8.1.5.1-03).

**Table B.8.1.5.1-03 Mass of soil and volume of CaCl<sub>2</sub> required for each ratio**

<b>Intended Ratio (w/v)</b>	<b>Weight of dry soil (g)</b>	<b>Volume (mL) of 0.01 M CaCl<sub>2</sub> solution*</b>
1:2	12.5	22.5
1:5	5	22.5
1:10	2.5	22.5

\*Volumes were 2.5 mL less than those required to obtain the correct ratio to allow for the 2.5 mL of application solution that would be added.

#### **IV. Preparation of stock and application solutions**

A stock solution of [<sup>14</sup>C] inpyrfluxam (0.5 mL) was prepared by evaporating in nitrogen and dissolving in 5 mL acetonitrile. The concentration was determined by LSC as 0.83 mg/mL, however, the purity of [<sup>14</sup>C] inpyrfluxam in this stock solution was < 98 % and therefore required repurification by HPLC.

This stock solution was concentrated under nitrogen (2 mL), combined with water (6.3 mL) and repeatedly injected on to HPLC. After isolated regions of the chromatogram corresponding to inpyrfluxam were isolated and combined, the concentration of [<sup>14</sup>C] inpyrfluxam in solution was determined by LSC as 0.293 mg/mL. The radiochemical purity of [<sup>14</sup>C] inpyrfluxam in this stock solution (named Repurified Stock Solution 1 or SS1) was checked in two chromatographic systems (HPLC and TLC) prior to use.

Radiochemical purity values were found to be ≥ 98 %. Example chromatograms were presented by the applicant and HSE has verified that these demonstrate high purity. Application solutions then were prepared by evaporating portions of Stock Solution 1 to dryness under nitrogen and dissolving the residue in 0.01 M CaCl<sub>2</sub> solution. When reconstituted with CaCl<sub>2</sub>, the concentration of acetonitrile was < 1 % of the total volume.

The total amount of radioactivity in the solution was determined by LSC and the concentration of [<sup>14</sup>C] inpyrfluxam was calculated from the specific activity.

It is not stated if the stock and application solutions were prepared immediately before use or were stored, and if so the storage conditions and duration were not provided. However, the stability of the test item in the test system was verified. HSE deems this to be a minor omission as the stability of the test item in the test system was verified and mass balances were > 90 % for all soils.

## STUDY DESIGN

The adsorption to test vessels, ratio of soil to aqueous phase test, equilibrium time determination and isotherm tests were conducted in the dark at  $20 \pm 2$  °C.

### I. Preliminary Investigations

In the preliminary investigations, experiments were conducted to determine the conditions to be used in the definitive study.

A test was performed to determine whether [ $^{14}\text{C}$ ] inpyrfluxam was soluble at 10 µg/mL in  $\text{CaCl}_2$  solution (0.01 M). This was performed by dissolving [ $^{14}\text{C}$ ] inpyrfluxam in 0.01 M  $\text{CaCl}_2$  using sonication and assaying the concentration achieved by LSC.

A test was performed to determine whether [ $^{14}\text{C}$ ] inpyrfluxam adsorbed to Teflon® vessels from a 0.01 µg/mL  $\text{CaCl}_2$  solution (0.01 M). This was performed by adding 0.01 M  $\text{CaCl}_2$  to duplicate vessels containing no soil (control samples). Vessels were shaken for 24 hours and the solution re-assayed. The amount of radioactivity lost from the solution indicates the amount of test substance adsorbed onto the surface of the vessel.

Investigations of appropriate soil : solution ratios were conducted using a treatment concentration of 0.1 µg/mL. Ratios of 1:5 w/v and 1:10 w/v were tested by preparing appropriate weights of soil and volumes of 0.01 M  $\text{CaCl}_2$  and shaking continuously for 24 hours ensuring that soil remains in suspension. After 24 hours the soil : solution mixtures were centrifuged and percentage adsorption was determined. The purpose of this test was to find a ratio of soil to aqueous phase such that > 20 % and preferably > 50 % solute is adsorbed into the soil. The results of this test were used to decide on the soil : aqueous ratios to be used in the remaining tests. Further investigations of soil : solution ratios were conducted in Atwater soil with a ratio of 1:2 w/v.

An adsorption equilibrium time test was performed on all soils at a 1:10, 1:5 and 1:2 w/v ratio and at a test substance concentration of 0.1 µg/mL. The purpose of this was to determine the adsorption equilibrium time for the definitive test. The serial method (in duplicate) was used, and the mixtures were shaken for a period of 48 hours. At sampling times of 3, 6, 24 and 48 hours, the vessels were removed and centrifuged (5000 g, 13, 15 or 34 minutes). HSE notes that OECD 106 requirements for centrifugation time are dependant on soil density which has not been provided. HSE notes that if centrifugation times are insufficient for full phase separation, this

will result in more test substance being detected in the liquid phase, and results in lower, more conservative sorption values. HSE therefore does not view this as a major deviation.

Aliquots were removed (1 mL) and were analysed by LSC. The volume of the aliquots was then replaced with fresh 0.01M CaCl<sub>2</sub> and the units were placed back on the shaker until the next sampling time. HSE notes that the volume of each aliquot taken for LSC was 4 % (1 mL) of the total volume (25 mL) for each timepoint rather than 1 % specified in the OECD 106 guidelines, which would affect the soil: solution ratio. The applicant states this would not have any impact on results from the isotherm test used to determine the adsorption coefficients. Considering this was performed in the preliminary test, HSE accepts that this is a minor deviation which does not affect study outcomes.

The stability test was performed on both replicate 48-hour samples per soil from the adsorption equilibrium time test. After removal of the adsorption supernatant, acetone (20 mL) was added to each unit, shaking for 20 minutes. The supernatant was separated by centrifugation (5000 g, 10 minutes). The extraction was repeated a further two times and extracts were pooled.

Due to poor extraction with Clipstone and Atwater soils, these were extracted further; once with acetone and three times with acetone: 0.5 M HCl (4:1 v/v). The adsorption supernatants and extracts were directly analysed by HPLC to determine recovery of radioactivity in each sample as well as the stability of [<sup>14</sup>C] inpyrfluxam, respectively.

A desorption equilibrium time test was performed on all soils at 0.1 µg/mL for periods of up to 72 hours using an adsorption equilibrium time of 48 hours.

## II. Definitive test

A summary of the definitive test can be seen in Table B.8.1.5.1-04 below. The aim of this experiment was to assess the influence of the concentration of inpyrfluxam on the extent of adsorption. The study was conducted using the 'indirect method' as only the aqueous phase was analysed and Freundlich isotherms produced.

**Table B.8.1.5.1-04 Summary of the test conditions used for the definitive test**

<b>Parameter</b>		<b>Description</b>
Soil		Air-dried, passed through 2 mm sieve prior to use
Sample weight		12.5 g for Atwater soil, 5 g for LAD-SCL-PF soil and 2.5 g for all other soils (dry weight) per replicate
Equilibrium solution		0.01 M CaCl <sub>2</sub> solution (22.5 mL)
Number of replicates		2

Test apparatus		Teflon® tubes
	Identity of solvent	0.01 M CaCl <sub>2</sub>
	Volume of test solution used/treatment	2.5 mL
Test item application	Application method	Pipette
	Evaporation of application solvent	Not applicable
Test item concentration	Nominal application rates (µg ai/mL)	0.01, 0.03, 0.1, 0.3 and 1 µg/mL
		1:2 (w/v) for Atwater soil
Soil : solution ratio		1:5 (w/v) for LAD-SCL-PF soil
		1:10 (w/v) for Brierlow, Kenslow, Clipstone, Hareby and Ibaraki soils
Indication of test item adsorbing to walls of test apparatus		None
	Temperature (°C)	20 ± 2°C
Adsorption and desorption equilibrium conditions	Time	48 hours adsorption and 24 hours desorption
	Continuous darkness (Yes/No)	Yes
	Shaking method	Reciprocating shaker
Method of separation of supernatant		Centrifugation
	Speed	5000 <i>g</i>
Centrifugation	Duration	13, 15 and 34 minutes
	Method of separating supernatant	Decanting

HSE notes that the acceptability of centrifugation conditions against OECD 106 guidelines can only be evaluated when soil density is known, which is not the case. As addressed above however, incomplete separation of phases will result in a higher concentration of test substance in the liquid phase when the indirect method is used, as it is here. This resultantly gives lower, more conservative sorption values. HSE therefore does not consider this as a major deviation.

All adsorption samples were shaken for 48 hours then centrifuged (5000 *g*) at ambient temperature. The adsorption supernatants were removed from each sample and the pH values of the supernatants were determined. They are presented in Table B.8.1.4.1-26.

Fresh 0.01 M CaCl<sub>2</sub> solution (25 mL) was added and the soil was mixed with the solution to re-suspend it. Samples were then shaken for a further 24 hours to obtain desorption supernatants. Radioactivity in desorption supernatants was determined by LSC.

A radioactive mass balance determination was performed on the samples for each soil at the highest concentration. Following desorption, acetone (25 mL) was added to the soil residues, samples were centrifuged (5000 g) and supernatants removed. Radioactivity in the rinse was determined by LSC. Residues were air dried, ground and combusted.

HSE notes that no soil matrix blank samples were analysed, and so the results are not corrected for these as recommended by OECD 106 guidance. This is regarded as a minor deviation as the soil history is known, recoveries are acceptable, and the test item was radiolabelled.

### III. Analytical Methods

HPLC was used for the determination of radiochemical purity and sample analysis. Specifically, both aqueous supernatants and soil extracts obtained during and after equilibration were analysed by HPLC with radio-detection. Samples were chromatographed with non-radiolabelled inpyrfluxam.

TLC was used for the determination of radiochemical purity. Samples were co-chromatographed with non-radiolabelled inpyrfluxam. Radiolabelled compounds were detected by preparation of a radioluminogram of the TLC plate using an image analyser and non-radiolabelled compounds were visualised by irradiation with UV light.

LSC was used to determine radioactive mass balance, quantification of [<sup>14</sup>C] inpyrfluxam in stock and application solutions, as well as radioactivity in adsorption and desorption supernatants. Where combustion of residues was required to determine non-extractable residues (NERs), samples were ground and combusted in oxygen using a Harvey Biological Sample Oxidiser. For the purpose of mass balance the limit of detection (LOD) was taken as 1.5 times the background radioactivity.

HSE notes that the applicant did not initially provide limits of detection nor quantification for HPLC-RAM, TLC and LSC. Furthermore, the LOD for LSC was taken as 1.5 times the background radioactivity, but the background level is not stated in the study report. This was requested from the applicant in a Request for Additional Information (RAI) and subsequently provided.

The LOD for HPLC-RAM was  $\leq 0.9$  % AR and the LOQ value is regarded as equivalent to LOD ( $\leq 0.9$  % AR) in the context of radioactivity analysis. For LSC, the LOD was  $\leq 0.2$  % AR (1.5 x background) and that the LOQ value is regarded as



equivalent. The applicant did not provide LOD and LOQ values for TLC as they state that this was used for supporting analysis only.

The OECD 106 evaluators checklist states that the LOQ should be at least two orders of magnitude below the lowest nominal concentration; the lowest test concentration is 0.01 µg/mL so the LOQ should be 0.0001 µg/mL or lower. HSE accepts that the LOQ is more than two orders of magnitude lower than the lowest nominal test concentration as two orders of magnitude below the 100 % applied radioactivity would be 1 % AR, and the calculated LOQ values are less than this.

#### **IV. Calculations**

The applicant calculated the adsorption and desorption coefficients ( $K_d$  and  $K_{d(des)}$  respectively) based on the concentration of [ $^{14}C$ ] inpyrfluxam in the soil and aqueous phase extracts. The organic carbon normalised coefficients for adsorption and desorption ( $K_{oc}$  and  $K_{oc(des)}$  respectively) were then calculated.

The calculations took into account the volume of entrained solution associated with the soil pellet at the end of each stage. As the weights for individual samples were not provided in the study report, HSE has used the concentrations in the soil provided by the applicant for the calculation of the adsorption and desorption coefficients.

HSE has checked the calculations and corresponding values provided for  $K_d$  and  $K_{oc}$  using the OECD 106 spreadsheet and confirms all values, with minor differences due to rounding errors. HSE Freundlich isotherms and residuals have been presented, rather than the applicants however, as the applicant has not provided graphs of the residuals.

### **RESULTS**

#### **I. Mass balance**

The total recovery was derived by summing the recovery of radioactivity from the adsorption supernatant, the desorption supernatant, the acetone wash and the portion unextracted from soil from the definitive adsorption equilibrium test. Only the highest concentration was tested for each soil. The recovery of the applied radioactivity was always > 90 %, ranging between 91.0 to 97.1 %. Therefore, HSE considers that the mass balances demonstrate there was no significant loss of radioactivity from the systems during testing.

It is noted, however, that the mass balances provided by the applicant includes the products from combustion. As per the OECD 106 Evaluators Checklist, mass balance in OECD 106 studies is defined as ‘the percentage of test substance which can be analytically recovered after the test versus the nominal amount at the beginning of the

test'. 'Analytically recovered' refers to the test substance that can be recovered through extractions and does not include NER combustion analysis.

Two soils have < 90% radioactivity without NER included, namely Atwater (mean of 84.05 %) and LAD-SCL-PF (mean of 88.5 %), are considered further in the 'Adsorption checklist' section below.

**Table B.8.1.5.1-05 Radioactive mass balance in all soils after definitive test**

Soil	Adsorption Supernatant	Recovery of Radioactivity (%)				Total mass balance including NER
		Desorption Supernatant	Acetone Wash	Mass balance	NER	
Brierlow	30.8	19.7	41.0	91.5	4.0	95.5
	31.1	19.9	40.5	91.5	4.4	95.9
Kenslow	28.0	18.5	46.0	92.5	4.6	97.1
	34.7	16.8	41.0	92.5	4.3	96.8
Clipstone	46.0	18.9	26.5	91.4	2.3	93.7
	43.1	19.4	28.7	91.2	3.2	94.4
Hareby	44.6	21.9	26.6	93.1	2.7	95.8
	44.1	22.2	26.3	92.6	2.7	95.3
LAD-SCL-PF	39.0	22.9	26.0	87.9	3.1	91.0
	41.7	21.7	25.7	89.1	3.3	92.4
Atwater	42.5	22.6	20.4	85.5	8.7	94.2
	46.5	21.1	15.0	82.6	7.7	90.3
Ibaraki*	31.5	19.9	37.7	89.1	6.0	95.1
	29.7	19.5	39.4	88.6	6.0	94.6

\*Ibaraki soil presented for information purposes only (results not included in overall mean endpoints)

The results of the preliminary investigations are summarised in sections II to VI below.

## II. Solubility test

Solubility of [<sup>14</sup>C] inpyrfluxam was assessed in 0.01 M CaCl<sub>2</sub> solution at a nominal concentration of 10 µg/mL. The recovery of radioactivity was assessed in solution after 24 hours. Recovery in solution was as follows:

**Table B.8.1.5.1-06 Solubility test of [<sup>14</sup>C] inpyrfluxam in 0.01 M CaCl<sub>2</sub> solution at a nominal concentration of 10 µg/mL**

<b>Concentration (µg/mL)</b>	<b>Replicate</b>	<b>% Recovery of radioactivity in Solution after 24 hours</b>
10	1	95.8
10	2	94.0

The results that [<sup>14</sup>C] inpyrfluxam was readily soluble in 0.01 M CaCl<sub>2</sub> solution of the concentration tested after 24 hours.

HSE notes that the applicant should have taken into account physical-chemical data including solubility, hydrolytic stability or the pKa. However, studies submitted in Section B.1 demonstrate that the solubility of the parent does not change across the environmentally relevant pH range. Similarly, there is no hydrolysis of inpyrfluxam at 50 °C and pH 4, 7 or 9. Therefore, HSE notes this as a minor deviation from OECD 106 which is not considered to impact the study outcome.

### III. Adsorption to test vessels

Radioactivity in solution was assessed before and after shaking for 24 hours. Results were as follows:

**Table B.8.1.5.1-07 Adsorption to containers: tested in duplicate Teflon® vessels at a nominal test substance concentration of 0.01 µg/mL in 0.01 M CaCl<sub>2</sub>**

<b>Assay time</b>	<b>Replicate A (% AR)</b>	<b>Replicate B (% AR)</b>
Before 24 h shake	96.0	96.2
After 24 h shake	95.2	95.0

The applicant stated that no adsorption to Teflon® test vessels occurred over 24 hours. HSE notes that there was a decrease in recovery after the 24 h shake in both replicates. However, the decrease is very slight (0.8 and 1.2 % AR) and may be attributable to abiotic degradation of the test substance rather than adsorption to the test vessel. The applicant could have identified which mechanism led to the decrease by washing the walls of the test vessel with solvent, however this was not completed. Nevertheless, HSE accepts that any adsorption to the test vessels is insignificant and should not affect study outcomes.

### IV. Stability test

The stability test was performed on both replicate 48-hour samples per soil from the adsorption equilibrium time test (as part of the preliminary test). Extraction of radioactivity (> 90%) was acceptable, with the exception of Clipstone and Atwater soils. These soils were extracted further.

The adsorption supernatants were directly analysed by HPLC. Extracts were pooled prior to concentrating (extract 3), then analysed by HPLC to determine stability.

HSE accepts that [ $^{14}\text{C}$ ] inpyrfluxam was stable based on > 90% recovery of [ $^{14}\text{C}$ ] inpyrfluxam in extracts after the 48 hour adsorption equilibrium. The high recovery confirms that the extraction procedure was efficient and would be suitable for the isotherms test.

## V. Soil:Solution Ratio test

The percent of applied [ $^{14}\text{C}$ ] inpyrfluxam present in the supernatant in duplicate following 24-hour mixing at soil to aqueous phase ratios of 1:5 w/v and 1:10 w/v are presented below in Table B.8.1.5.1-08 and B.8.1.5.1-09.

**Table B.8.1.5.1-08 Percent of applied [ $^{14}\text{C}$ ] inpyrfluxam present in the supernatant following 24-hour mixing at soil to aqueous phase ratios of 1:10 w/v**

Ratio (w/v): 1:10				
Soil	% AR in supernatant	% AR in soil (by calculation)	$K_d$ (mL/g)	$K_{oc}$ (mL/g)
Brierlow	34.5	65.5	19.03	793
	34.4	65.6	18.75	781
Kenslow	31.3	68.7	21.63	569
	31.6	68.4	21.47	565
Clipstone	48.0	52.0	10.23	853
	50.1	49.9	9.62	801
Hareby	48.1	51.9	10.43	652
	48.5	51.5	10.47	654
LAD-SCL-PF	67.8	32.2	4.69	521
	65.5	34.5	5.25	583
Atwater	86.6	13.4	1.49	497
	88.6	11.4	1.26	421
Ibaraki	36.5	63.5	17.23	663
	36.5	63.5	17.22	662

\*Ibaraki soil presented for information purposes only (results not included in overall mean endpoints)

**Table B.8.1.4.1-09 Percent of applied [ $^{14}\text{C}$ ] inpyrfluxam present in the supernatant following 24-hour mixing at soil to aqueous phase ratios of 1:5 w/v**

Ratio (w/v): 1:5				
Soil	% AR in supernatant	% AR in soil (by calculation)	$K_d$ (mL/g)	$K_{oc}$ (mL/g)
Brierlow	20.5	79.5	19.20	800
	20.5	79.5	19.39	808
Kenslow	18.3	81.7	22.39	589

	36.0	64.0	8.83	232
Clipstone	35.9	64.1	8.73	728
	32.3	67.7	10.11	843
Hareby	30.8	69.2	11.2	700
	31.1	68.9	10.95	684
LAD-SCL-PF	50.3	49.7	4.87	541
	49.3	50.7	5.09	565
Atwater	75.3	24.7	1.63	542
	71.9	28.1	1.90	632
Ibaraki	23.3	76.7	16.61	639
	22.9	77.1	17.01	654

\*Ibaraki soil presented for information purposes only (results not included in overall mean endpoints)

OECD guidelines stipulate that the adsorbed percentage should be > 20 % and preferably > 50 %. Based on these results, the following ratios were chosen by the applicant for further testing:

1:10 w/v ratio for soils Brierlow, Kenslow, Clipstone, Hareby and Ibaraki.

1:5 w/v ratio for LAD-SCL-PF soil.

Adsorption of [<sup>14</sup>C] inpyrflumax to soils at these ratios were between 49.7 and 68.7 %, therefore HSE agrees that the ratios used are acceptable.

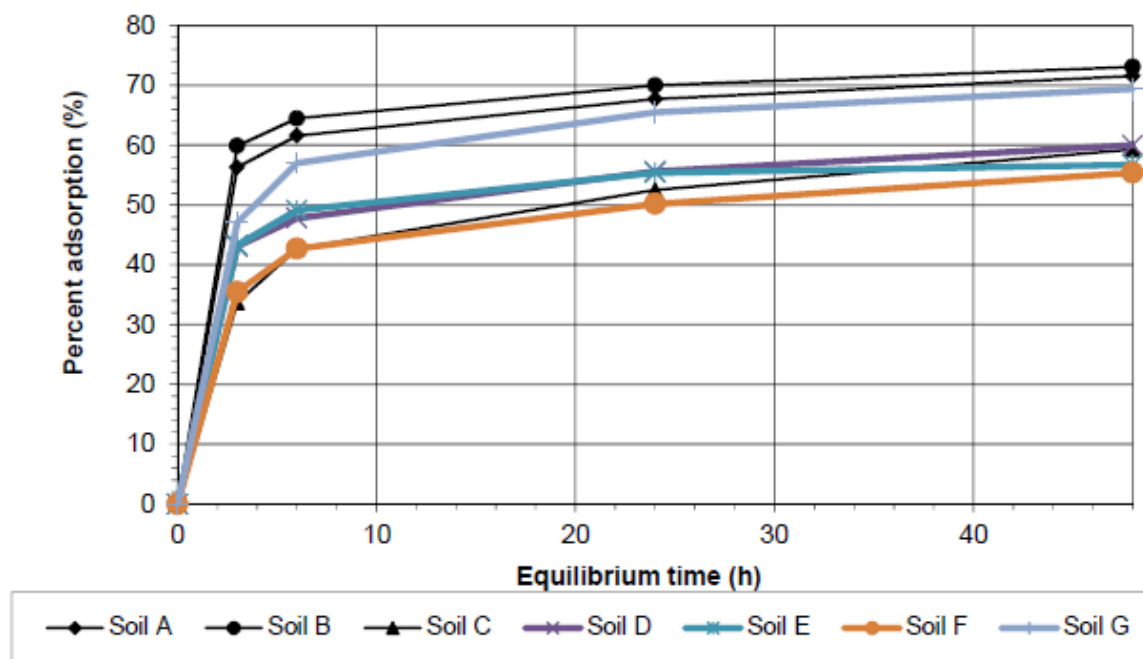
The percent of applied [<sup>14</sup>C] inpyrflumax adsorbed for Atwater soil for both ratios were < 30% therefore an additional test at 1:2 w/v ratio was performed. The results are presented below. HSE accepts that this ratio was acceptable for Atwater soil as the average adsorption was 44%.

**Table B.8.1.5.1-10 Additional test at 1:2 w/v ratio for Atwater soil due to < 30 % adsorption in soil at 1:10 and 1:5 w/v**

<b>Ratio (w/v): 1:2</b>				
<b>Soil</b>	<b>% AR in supernatant</b>	<b>% AR in soil (by calculation)</b>	<b>K<sub>d</sub> (mL/g)</b>	<b>K<sub>oc</sub> (mL/g)</b>
Atwater	55.9	44.1	1.6	520
	56.1	43.9	1.55	518

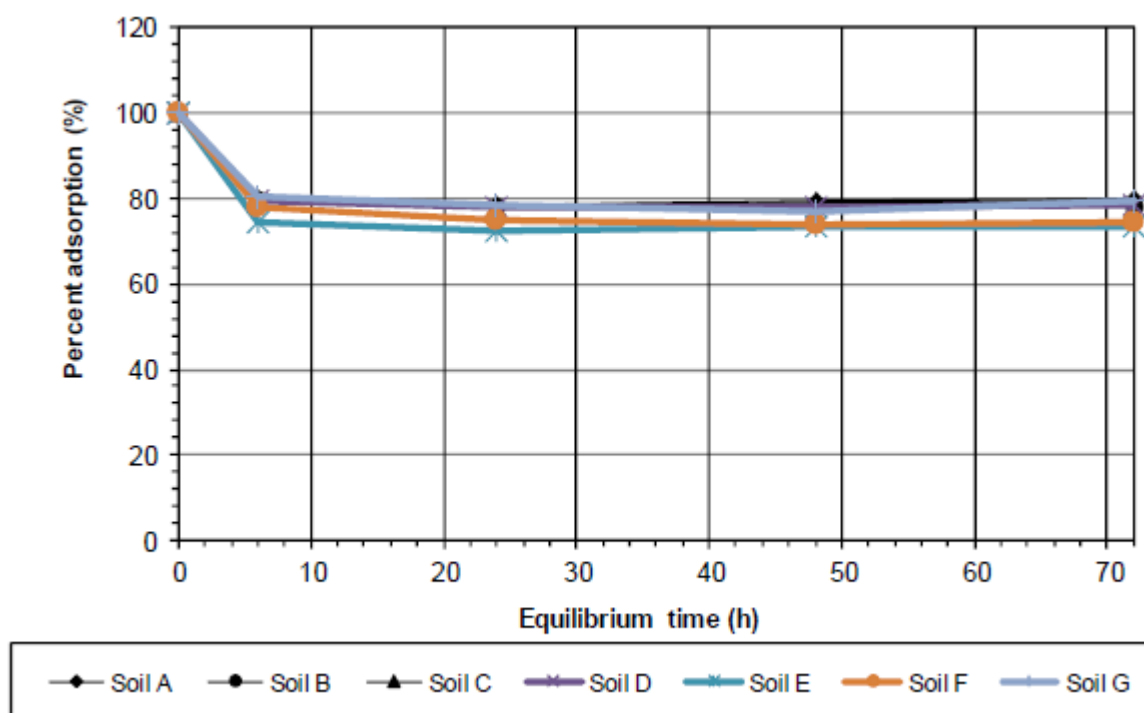
## VI. Adsorption/ desorption equilibrium time determinations

Figure B.8.1.5.1-01 shows the percentage adsorption over time for the 48 hour period. HSE notes that the percentage adsorption was still rising between 24 and 48 hours in all soils. However, as the equilibrium curves have flattened after 24 hours, an equilibrium time of 48 hours is accepted. Furthermore, the stability of the test substance was demonstrated only in 48 hours, not the total experimental time of 72 hours (adsorption and desorption combined). Therefore, 48 hours is accepted as a compromise between equilibration and possible instability.



Soil A = Brierlow. Soil B = Kenslow. Soil C = Clipstone. Soil D = Hareby. Soil E = LAD-SCL-PF. Soil F = Atwater. Soil G = Ibaraki.

**Figure 8.1.5.1-01 Graph showing adsorption equilibrium time for all soils**



**Figure B.8.1.5.1-02 Graph showing desorption equilibrium time for all soils**

For desorption, a plateau was reached at 24 hours, therefore 24 hours was chosen as the desorption equilibrium time for the definitive test.

## Definitive Test

Concentrations of [ $^{14}\text{C}$ ] inpyrfluxam in the supernatants and adsorbed to the soils are shown in Tables B.8.1.5.1-11 to B.8.1.5.1-23. Weights ( $\mu\text{g}$ ) and proportions of [ $^{14}\text{C}$ ] inpyrfluxam that were adsorbed and desorbed are shown in Tables B.8.1.4.1-12 to B.8.1.4.1-24.

Values for adsorption partition coefficients ( $K_d$ ) per soil were in the range 1.5 to 27 L/kg and  $K_{oc}$  values were in the range 424 to 1056 L/kg. When corrected for the organic carbon content of the soils, the resultant  $K_{foc}$  values for adsorption, ranged from 500 to 891 L/kg.

The graphical fits of the Freundlich equation presented in Figures B.8.1.5.1-03 to B.8.1.5.1-09 below and are based on the standard linear regression from using log-log transformed data alongside residual plots. It is noted that the applicant presented the Freundlich adsorption and desorption on the same plot. While HSE was closely able replicate the isotherms, linear plot parameters and derived adsorption values, it is preferred to have separate plots for clearer validation, therefore the graphs presented are plotted by HSE. Plots of the residuals were not supplied by the applicant and have been provided by the HSE for adsorption isotherms (Figures B.8.1.5.1-03 to B.8.1.5.1-09). Goodness of fit has not been assessed by HSE for the desorption phase as these values are not used in the exposure assessment.

**Table B.8.1.5.1-11 Concentration of [ $^{14}\text{C}$ ] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with Brierlow soil for adsorption and desorption**

	Adsorption			Desorption		
Nominal application rate ( $\mu\text{g/mL}$ )	Adsorption $C_e$ ( $\mu\text{g/mL}$ )	Weight ads in soil ( $\mu\text{g}$ )	X/m ( $\mu\text{g/g}$ )	Desorption $C_1$ ( $\mu\text{g/mL}$ )	Weight des in soil ( $\mu\text{g}$ )	$X_1/m$ ( $\mu\text{g/g}$ )
1	0.3407	16.8116	6.7166	0.2034	12.0671	4.821
1	0.3432	16.6821	6.6542	0.2048	11.9	4.7467
<b>Mean</b>	<b>0.342</b>	<b>16.7468</b>	<b>6.6854</b>	<b>0.2041</b>	<b>11.9835</b>	<b>4.7839</b>
0.3	0.0961	5.1472	2.0655	0.0589	3.7854	1.519
0.3	0.0973	5.1171	2.051	0.0614	3.6823	1.4759
<b>Mean</b>	<b>0.0967</b>	<b>5.1322</b>	<b>2.0582</b>	<b>0.0602</b>	<b>3.7338</b>	<b>1.4974</b>
0.1	0.0297	1.6304	0.6517	0.0188	1.1937	0.4771
0.1	0.0309	1.6346	0.6513	0.0187	1.1935	0.4755
<b>Mean</b>	<b>0.0303</b>	<b>1.6325</b>	<b>0.6515</b>	<b>0.0188</b>	<b>1.1936</b>	<b>0.4763</b>
0.03	0.0095	0.5379	0.2151	0.0063	0.3911	0.1564
0.03	0.0099	0.5377	0.2145	0.0062	0.3936	0.157

<b>Mean</b>	<b>0.0097</b>	<b>0.5378</b>	<b>0.2148</b>	<b>0.0062</b>	<b>0.3923</b>	<b>0.1567</b>
0.01	0.0031	0.1977	0.0788	0.002	0.1487	0.0593
0.01	0.0033	0.1998	0.0798	0.0021	0.1496	0.0598
<b>Mean</b>	<b>0.0032</b>	<b>0.1987</b>	<b>0.0793</b>	<b>0.0021</b>	<b>0.1492</b>	<b>0.0595</b>

$C_e$  = Concentration of test substance in the supernatant at the end of adsorption

$X/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (adsorption step)

$C_1$  = Concentration of test substance in the supernatant at the end of desorption

$X_1/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (desorption step)

**Table B.8.1.5.1-12 Proportions of [ $^{14}\text{C}$ ] inpyrfluxam adsorbed to and desorbed from Brierlow soil**

	<b>Adsorption</b>		<b>Desorption</b>	
<b>Application rate (<math>\mu\text{g/mL}</math>)</b>	<b>Ads %</b>	<b>Not ads %</b>	<b>Des %</b>	<b>Not des %</b>
1	66.6	33.4	28.2	71.8
1	66.4	33.6	28.7	71.3
<b>Mean</b>	<b>66.5</b>	<b>33.5</b>	<b>28.4</b>	<b>71.6</b>
0.3	68.5	31.5	26.5	73.5
0.3	68.1	31.9	28	72
<b>Mean</b>	<b>68.3</b>	<b>31.7</b>	<b>27.2</b>	<b>72.8</b>
0.1	69.1	30.9	26.8	73.2
0.1	68.2	31.8	27	73
<b>Mean</b>	<b>68.6</b>	<b>31.4</b>	<b>26.9</b>	<b>73.1</b>
0.03	69.6	30.4	27.3	72.7
0.03	68.7	31.3	26.8	73.2
<b>Mean</b>	<b>69.1</b>	<b>30.9</b>	<b>27</b>	<b>73</b>
0.01	71.9	28.1	24.8	75.2
0.01	71.3	28.7	25.1	74.9
<b>Mean</b>	<b>71.6</b>	<b>28.4</b>	<b>24.9</b>	<b>75.1</b>

Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.



**Table B.8.1.5.1-13 Concentration of [<sup>14</sup>C] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with Kenslow soil for adsorption and desorption**

	Adsorption			Desorption		
Nominal application rate (µg/mL)	Adsorption C <sub>e</sub> (µg/mL)	Weight ads in soil (µg)	X/m (µg/g)	Desorption C <sub>1</sub> (µg/mL)	Weight des in soil (µg)	X <sub>1</sub> /m (µg/g)
1	0.3105	17.3862	6.9406	0.1935	12.9602	5.1737
1	0.3767	15.1924	6.0648	0.1678	11.5515	4.6114
<b>Mean</b>	<b>0.3436</b>	<b>16.2893</b>	<b>6.5027</b>	<b>0.1807</b>	<b>12.2559</b>	<b>4.8926</b>
0.3	0.0879	5.2707	2.094	0.0565	3.9641	1.5749
0.3	0.0879	5.2364	2.0996	0.0559	3.9460	1.5822
<b>Mean</b>	<b>0.0879</b>	<b>5.2535</b>	<b>2.0968</b>	<b>0.0562</b>	<b>3.9550</b>	<b>1.5786</b>
0.1	0.027	1.6651	0.6687	0.0175	1.2519	0.5028
0.1	0.0279	1.6969	0.6812	0.0186	1.2583	0.5051
<b>Mean</b>	<b>0.0274</b>	<b>1.6810</b>	<b>0.675</b>	<b>0.018</b>	<b>1.2551</b>	<b>0.5039</b>
0.03	0.0088	0.5451	0.218	0.0057	0.4130	0.1652
0.03	0.009	0.5586	0.2248	0.0057	0.4251	0.1711
<b>Mean</b>	<b>0.0089</b>	<b>0.5518</b>	<b>0.2214</b>	<b>0.0057</b>	<b>0.4190</b>	<b>0.1681</b>
0.01	0.0033	0.2038	0.082	0.0021	0.1552	0.0625
0.01	0.003	0.2025	0.0811	0.002	0.1548	0.062
<b>Mean</b>	<b>0.0031</b>	<b>0.2032</b>	<b>0.0816</b>	<b>0.002</b>	<b>0.1550</b>	<b>0.0623</b>

C<sub>e</sub> = Concentration of test substance in the supernatant at the end of adsorption

X/m = Weight adsorbed (µg)/weight of dry soil (adsorption step)

C<sub>1</sub> = Concentration of test substance in the supernatant at the end of desorption

X<sub>1</sub>/m = Weight adsorbed (µg)/weight of dry soil (desorption step)

**Table B.8.1.5.1-14 Amounts and proportions of [<sup>14</sup>C] inpyrfluxam adsorbed to and desorbed from Kenslow soil**

	Adsorption		Desorption	
Application rate (µg/mL)	Ads %	Not ads %	Des %	Not des %
1	69.4	30.6	25.5	74.5
1	61.9	38.1	24.0	76.0

<b>Mean</b>	<b>65.6</b>	<b>34.4</b>	<b>24.7</b>	<b>75.3</b>
0.3	70.9	29.1	24.8	75.2
0.3	70.8	29.2	24.6	75.4
<b>Mean</b>	<b>70.9</b>	<b>29.1</b>	<b>24.7</b>	<b>75.3</b>
0.1	71.4	28.6	24.8	75.2
0.1	71.2	28.8	25.8	74.2
<b>Mean</b>	<b>71.3</b>	<b>28.7</b>	<b>25.3</b>	<b>74.7</b>
0.03	71.6	28.4	24.2	75.8
0.03	71.6	28.4	23.9	76.1
<b>Mean</b>	<b>71.6</b>	<b>28.4</b>	<b>24.1</b>	<b>75.9</b>
0.01	71.5	28.5	23.8	76.2
0.01	73.2	26.8	23.6	76.4
<b>Mean</b>	<b>72.4</b>	<b>27.6</b>	<b>23.7</b>	<b>76.3</b>

Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.

**Table B.8.1.5.1-15 Concentration of [<sup>14</sup>C] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with Clipstone soil for adsorption and desorption**

	<b>Adsorption</b>			<b>Desorption</b>		
<b>Nominal application rate (µg/mL)</b>	<b>Adsorption C<sub>e</sub> (µg/mL)</b>	<b>Weight ads (µg)</b>	<b>X/m (µg/g)</b>	<b>Desorption C<sub>1</sub> (µg/mL)</b>	<b>Weight des (µg)</b>	<b>X<sub>1</sub>/m (µg/g)</b>
1	0.4781	12.4243	4.9539	0.1878	8.2382	3.2848
1	0.4705	13.6119	5.4666	0.2004	9.1186	3.6621
<b>Mean</b>	<b>0.4743</b>	<b>13.0181</b>	<b>5.2103</b>	<b>0.1941</b>	<b>8.6784</b>	<b>3.4734</b>
0.3	0.1467	3.9675	1.5851	0.0640	2.5296	1.0106
0.3	0.1475	3.9720	1.5907	0.0633	2.5473	1.0201
<b>Mean</b>	<b>0.1471</b>	<b>3.9697</b>	<b>1.5879</b>	<b>0.0636</b>	<b>2.5384</b>	<b>1.0154</b>
0.1	0.0438	1.2302	0.4895	0.0193	0.7993	0.3181
0.1	0.0471	1.2139	0.4852	0.0194	0.7734	0.3091
<b>Mean</b>	<b>0.0454</b>	<b>1.2221</b>	<b>0.4874</b>	<b>0.0194</b>	<b>0.7863</b>	<b>0.3136</b>
0.03	0.0144	0.4091	0.1625	0.0058	0.2796	0.1111
0.03	0.0162	0.3668	0.1460	0.0057	0.2419	0.0963
<b>Mean</b>	<b>0.0153</b>	<b>0.3880</b>	<b>0.1543</b>	<b>0.0057</b>	<b>0.2607</b>	<b>0.1037</b>
0.01	0.0053	0.1393	0.0557	0.0018	0.1006	0.0402

0.01	0.0047	0.1506	0.0600	0.0021	0.1034	0.0412
<b>Mean</b>	<b>0.0050</b>	<b>0.1449</b>	<b>0.0578</b>	<b>0.0019</b>	<b>0.1020</b>	<b>0.0407</b>

$C_e$  = Concentration of test substance in the supernatant at the end of adsorption

$X/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (adsorption step)

$C_1$  = Concentration of test substance in the supernatant at the end of desorption

$X_1/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (desorption step)

**Table B.8.1.5.1-16 Amounts and proportions of [ $^{14}\text{C}$ ] inpyrfluxam adsorbed to and desorbed from Clipstone soil**

	<b>Adsorption</b>		<b>Desorption</b>	
<b>Application rate (<math>\mu\text{g/mL}</math>)</b>	<b>Ads %</b>	<b>Not ads %</b>	<b>Des %</b>	<b>Not des %</b>
1	51.4	48.6	33.7	66.3
1	54.1	45.9	33.0	67.0
<b>Mean</b>	<b>52.8</b>	<b>47.2</b>	<b>33.4</b>	<b>66.6</b>
0.3	52.3	47.7	36.2	63.8
0.3	52.1	47.9	35.9	64.1
<b>Mean</b>	<b>52.2</b>	<b>47.8</b>	<b>36.1</b>	<b>63.9</b>
0.1	53.2	46.8	35.0	65.0
0.1	50.9	49.1	36.3	63.7
<b>Mean</b>	<b>52.0</b>	<b>48.0</b>	<b>35.7</b>	<b>64.3</b>
0.03	53.4	46.6	31.7	68.3
0.03	47.8	52.2	34.0	66.0
<b>Mean</b>	<b>50.6</b>	<b>49.4</b>	<b>32.9</b>	<b>67.1</b>
0.01	51.5	48.5	27.7	72.3
0.01	56.2	43.8	31.3	68.7
<b>Mean</b>	<b>53.9</b>	<b>46.1</b>	<b>29.5</b>	<b>70.5</b>

Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.

**Table B.8.1.5.1-17 Concentration of [<sup>14</sup>C] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with Hareby soil for adsorption and desorption**

	Adsorption			Desorption		
Nominal application rate (µg/mL)	Adsorption C <sub>e</sub> (µg/mL)	Weight ads (µg)	X/m (µg/g)	Desorption C <sub>1</sub> (µg/mL)	Weight des (µg)	X <sub>1</sub> /m (µg/g)
1	0.4738	12.6388	5.0738	0.2201	7.7585	3.1146
1	0.4636	12.6859	5.1091	0.2188	7.7583	3.1246
<b>Mean</b>	<b>0.4687</b>	<b>12.6624</b>	<b>5.0915</b>	<b>0.2195</b>	<b>7.7584</b>	<b>3.1196</b>
0.3	0.1479	3.9249	1.5581	0.0645	2.4712	0.9810
0.3	0.1417	3.9205	1.5669	0.0656	2.4528	0.9803
<b>Mean</b>	<b>0.1448</b>	<b>3.9227</b>	<b>1.5625</b>	<b>0.0650</b>	<b>2.4620</b>	<b>0.9807</b>
0.1	0.0421	1.3125	0.5202	0.0210	0.8404	0.3331
0.1	0.0410	1.2593	0.5063	0.0203	0.7978	0.3208
<b>Mean</b>	<b>0.0415</b>	<b>1.2859</b>	<b>0.5133</b>	<b>0.0206</b>	<b>0.8191</b>	<b>0.3270</b>
0.03	0.0132	0.4284	0.1707	0.0069	0.2744	0.1093
0.03	0.0129	0.4089	0.1640	0.0063	0.2660	0.1067
<b>Mean</b>	<b>0.0130</b>	<b>0.4187</b>	<b>0.1674</b>	<b>0.0066</b>	<b>0.2702</b>	<b>0.1080</b>
0.01	0.0044	0.1614	0.0651	0.0023	0.1093	0.0441
0.01	0.0046	0.1695	0.0674	0.0024	0.1130	0.0449
<b>Mean</b>	<b>0.0045</b>	<b>0.1654</b>	<b>0.0663</b>	<b>0.0024</b>	<b>0.1111</b>	<b>0.0445</b>

C<sub>e</sub> = Concentration of test substance in the supernatant at the end of adsorption

X/m = Weight adsorbed (µg)/weight of dry soil (adsorption step)

C<sub>1</sub> = Concentration of test substance in the supernatant at the end of desorption

X<sub>1</sub>/m = Weight adsorbed (µg)/weight of dry soil (desorption step)

**Table B.8.1.5.1-18 Amounts and proportions of [<sup>14</sup>C] inpyrfluxam adsorbed to and desorbed from Hareby soil**

	Adsorption		Desorption	
Application rate (µg/mL)	Ads %	Not ads %	Des %	Not des %
1	51.9	48.1	38.6	61.4
1	52.6	47.4	38.8	61.2
<b>Mean</b>	<b>52.3</b>	<b>47.7</b>	<b>38.7</b>	<b>61.3</b>

0.3	51.7	48.3	37.0	63.0
0.3	52.8	47.2	37.4	62.6
<b>Mean</b>	<b>52.3</b>	<b>47.7</b>	<b>37.2</b>	<b>62.8</b>
0.1	55.6	44.4	36.0	64.0
0.1	55.3	44.7	36.6	63.4
<b>Mean</b>	<b>55.5</b>	<b>44.5</b>	<b>36.3</b>	<b>63.7</b>
0.03	56.8	43.2	36.0	64.0
0.03	56.3	43.7	35.0	65.0
<b>Mean</b>	<b>56.6</b>	<b>43.4</b>	<b>35.5</b>	<b>64.5</b>
0.01	59.8	40.2	32.3	67.7
0.01	59.9	40.1	33.3	66.7
<b>Mean</b>	<b>59.9</b>	<b>40.1</b>	<b>32.8</b>	<b>67.2</b>

Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.

**Table B.8.1.5.1-19 Concentration of [<sup>14</sup>C] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with LAD-SCL-PF soil for adsorption and desorption**

	<b>Adsorption</b>			<b>Desorption</b>		
<b>Nominal application rate (µg/mL)</b>	<b>Adsorption C<sub>e</sub> (µg/mL)</b>	<b>Weight ads (µg)</b>	<b>X/m (µg/g)</b>	<b>Desorption C<sub>1</sub> (µg/mL)</b>	<b>Weight des (µg)</b>	<b>X<sub>1</sub>/m (µg/g)</b>
1	0.4477	13.5481	2.7080	0.2313	8.6538	1.7297
1	0.4782	13.1087	2.6249	0.2251	8.3862	1.6793
<b>Mean</b>	<b>0.4630</b>	<b>13.3284</b>	<b>2.6664</b>	<b>0.2282</b>	<b>8.5200</b>	<b>1.7045</b>
0.3	0.1402	4.1477	0.8342	0.0719	2.6391	0.5308
0.3	0.1399	4.1137	0.8259	0.0719	2.5888	0.5197
<b>Mean</b>	<b>0.1401</b>	<b>4.1307</b>	<b>0.8300</b>	<b>0.0719</b>	<b>2.6140</b>	<b>0.5253</b>
0.1	0.0413	1.3022	0.2610	0.0224	0.8168	0.1637
0.1	0.0450	1.3000	0.2602	0.0232	0.8058	0.1613
<b>Mean</b>	<b>0.0431</b>	<b>1.3011</b>	<b>0.2606</b>	<b>0.0228</b>	<b>0.8113</b>	<b>0.1625</b>
0.03	0.0139	0.4292	0.0857	0.0074	0.2702	0.0540
0.03	0.0146	0.4078	0.0814	0.0076	0.2423	0.0484
<b>Mean</b>	<b>0.0142</b>	<b>0.4185</b>	<b>0.0836</b>	<b>0.0075</b>	<b>0.2563</b>	<b>0.0512</b>
0.01	0.0051	0.1480	0.0298	0.0026	0.0928	0.0187
0.01	0.0050	0.1543	0.0309	0.0027	0.0974	0.0195

<b>Mean</b>	<b>0.0050</b>	<b>0.1511</b>	<b>0.0303</b>	<b>0.0027</b>	<b>0.0951</b>	<b>0.0191</b>
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$C_e$  = Concentration of test substance in the supernatant at the end of adsorption

$X/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (adsorption step)

$C_1$  = Concentration of test substance in the supernatant at the end of desorption

$X_1/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (desorption step)

**Table B.8.1.5.1-20 Amounts and proportions of [ $^{14}\text{C}$ ] inpyrfluxam adsorbed to and desorbed from LAD-SCL-PF soil**

	<b>Adsorption</b>		<b>Desorption</b>	
<b>Application rate (<math>\mu\text{g/mL}</math>)</b>	<b>Ads %</b>	<b>Not ads %</b>	<b>Des %</b>	<b>Not des %</b>
1	54.6	45.4	36.1	63.9
1	52.2	47.8	36.0	64.0
<b>Mean</b>	<b>53.4</b>	<b>46.6</b>	<b>36.1</b>	<b>63.9</b>
0.3	54.1	45.9	36.4	63.6
0.3	54.2	45.8	37.1	62.9
<b>Mean</b>	<b>54.1</b>	<b>45.9</b>	<b>36.7</b>	<b>63.3</b>
0.1	55.9	44.1	37.3	62.7
0.1	53.6	46.4	38.0	62.0
<b>Mean</b>	<b>54.8</b>	<b>45.2</b>	<b>37.6</b>	<b>62.4</b>
0.03	55.3	44.7	37.0	63.0
0.03	52.9	47.1	40.6	59.4
<b>Mean</b>	<b>54.1</b>	<b>45.9</b>	<b>38.8</b>	<b>61.2</b>
0.01	54.0	46.0	37.3	62.7
0.01	55.4	44.6	36.9	63.1
<b>Mean</b>	<b>54.7</b>	<b>45.3</b>	<b>37.1</b>	<b>62.9</b>

Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.

**Table B.8.1.5.1-21 Concentration of [ $^{14}\text{C}$ ] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with Atwater soil for adsorption and desorption**

	<b>Adsorption</b>			<b>Desorption</b>		
<b>Nominal application rate (<math>\mu\text{g/mL}</math>)</b>	<b>Adsorption <math>C_e</math> (<math>\mu\text{g/mL}</math>)</b>	<b>Weight ads (<math>\mu\text{g}</math>)</b>	<b><math>X/m</math> (<math>\mu\text{g/g}</math>)</b>	<b>Desorption <math>C_1</math> (<math>\mu\text{g/mL}</math>)</b>	<b>Weight des (<math>\mu\text{g}</math>)</b>	<b><math>X_1/m</math> (<math>\mu\text{g/g}</math>)</b>
1	0.5390	11.9841	0.9591	0.2382	7.6421	0.6116
1	0.5621	10.7116	0.8554	0.2108	7.0481	0.5628

<b>Mean</b>	<b>0.5506</b>	<b>11.3479</b>	<b>0.9072</b>	<b>0.2245</b>	<b>7.3451</b>	<b>0.5872</b>
0.3	0.1603	3.6501	0.2920	0.0719	2.3022	0.1842
0.3	0.1526	3.4062	0.2730	0.0691	2.1104	0.1691
<b>Mean</b>	<b>0.1565</b>	<b>3.5282</b>	<b>0.2825</b>	<b>0.0705</b>	<b>2.2063</b>	<b>0.1767</b>
0.1	0.0479	1.1243	0.0900	0.0221	0.7068	0.0566
0.1	0.0496	1.1294	0.0904	0.0207	0.7612	0.0610
<b>Mean</b>	<b>0.0487</b>	<b>1.1268</b>	<b>0.0902</b>	<b>0.0214</b>	<b>0.7340</b>	<b>0.0588</b>
0.03	0.0155	0.3858	0.0308	0.0076	0.2378	0.0190
0.03	0.0149	0.3919	0.0313	0.0073	0.2490	0.0199
<b>Mean</b>	<b>0.0152</b>	<b>0.3889</b>	<b>0.0311</b>	<b>0.0074</b>	<b>0.2434</b>	<b>0.0195</b>
0.01	0.0054	0.1457	0.0116	0.0026	0.0960	0.0077
0.01	0.0054	0.1473	0.0118	0.0027	0.0954	0.0076
<b>Mean</b>	<b>0.0054</b>	<b>0.1465</b>	<b>0.0117</b>	<b>0.0027</b>	<b>0.0957</b>	<b>0.0076</b>

$C_e$  = Concentration of test substance in the supernatant at the end of adsorption

$X/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (adsorption step)

$C_1$  = Concentration of test substance in the supernatant at the end of desorption

$X_1/m$  = Weight adsorbed ( $\mu\text{g}$ )/weight of dry soil (desorption step)

**Table B.8.1.5.1-22 Amounts and proportions of [ $^{14}\text{C}$ ] inpyrfluxam adsorbed to and desorbed from Atwater soil**

<b>Application rate (<math>\mu\text{g/mL}</math>)</b>	<b>Adsorption</b>		<b>Desorption</b>	
	<b>Ads %</b>	<b>Not ads %</b>	<b>Des %</b>	<b>Not des %</b>
1	47.3	52.7	36.2	63.8
1	43.2	56.8	34.2	65.8
<b>Mean</b>	<b>45.3</b>	<b>54.7</b>	<b>35.2</b>	<b>64.8</b>
0.3	47.7	52.3	36.9	63.1
0.3	47.3	52.7	38.0	62.0
<b>Mean</b>	<b>47.5</b>	<b>52.5</b>	<b>37.5</b>	<b>62.5</b>
0.1	48.5	51.5	37.1	62.9
0.1	47.7	52.3	32.6	67.4
<b>Mean</b>	<b>48.1</b>	<b>51.9</b>	<b>34.9</b>	<b>65.1</b>
0.03	50.0	50.0	38.4	61.6
0.03	51.3	48.7	36.5	63.5
<b>Mean</b>	<b>50.7</b>	<b>49.3</b>	<b>37.4</b>	<b>62.6</b>
0.01	52.0	48.0	34.1	65.9
0.01	52.1	47.9	35.2	64.8

<b>Mean</b>	<b>52.1</b>	<b>47.9</b>	<b>34.7</b>	<b>65.3</b>
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Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.

**Table B.8.1.5.1-23 Concentration of [<sup>14</sup>C] inpyrfluxam in the aqueous phase and calculated in the soil phase associated with Ibaraki soil for adsorption and desorption. Presented for information purposes only (results not included in overall mean endpoints).**

	<b>Adsorption</b>			<b>Desorption</b>		
<b>Nominal application rate (µg/mL)</b>	<b>Adsorption C<sub>e</sub> (µg/mL)</b>	<b>Weight ads (µg)</b>	<b>X/m (µg/g)</b>	<b>Desorption C<sub>1</sub> (µg/mL)</b>	<b>Weight des (µg)</b>	<b>X<sub>1</sub>/m (µg/g)</b>
1	0.3457	15.8819	6.3757	0.2013	11.3947	4.5744
1	0.3303	16.0231	6.4170	0.1961	11.7089	4.6892
<b>Mean</b>	<b>0.3380</b>	<b>15.9525</b>	<b>6.3963</b>	<b>0.1987</b>	<b>11.5518</b>	<b>4.6318</b>
0.3	0.1033	5.0011	1.9885	0.0598	3.6608	1.4556
0.3	0.1055	4.8720	1.9566	0.0599	3.4958	1.4039
<b>Mean</b>	<b>0.1044</b>	<b>4.9366</b>	<b>1.9726</b>	<b>0.0598</b>	<b>3.5783</b>	<b>1.4297</b>
0.1	0.0321	1.5673	0.6297	0.0186	1.1469	0.4608
0.1	0.0330	1.5460	0.6145	0.0193	1.1125	0.4422
<b>Mean</b>	<b>0.0326</b>	<b>1.5567</b>	<b>0.6221</b>	<b>0.0190</b>	<b>1.1297</b>	<b>0.4515</b>
0.03	0.0097	0.4782	0.1921	0.0055	0.3546	0.1425
0.03	0.0105	0.5182	0.2076	0.0057	0.3884	0.1556
<b>Mean</b>	<b>0.0101</b>	<b>0.4982</b>	<b>0.1999</b>	<b>0.0056</b>	<b>0.3715</b>	<b>0.1490</b>
0.01	0.0036	0.1958	0.0788	0.0021	0.1482	0.0597
0.01	0.0032	0.1921	0.0765	0.0020	0.1477	0.0588
<b>Mean</b>	<b>0.0034</b>	<b>0.1940</b>	<b>0.0777</b>	<b>0.0020</b>	<b>0.1480</b>	<b>0.0592</b>

C<sub>e</sub> = Concentration of test substance in the supernatant at the end of adsorption

X/m = Weight adsorbed (µg)/weight of dry soil (adsorption step)

C<sub>1</sub> = Concentration of test substance in the supernatant at the end of desorption

X<sub>1</sub>/m = Weight adsorbed (µg)/weight of dry soil (desorption step)



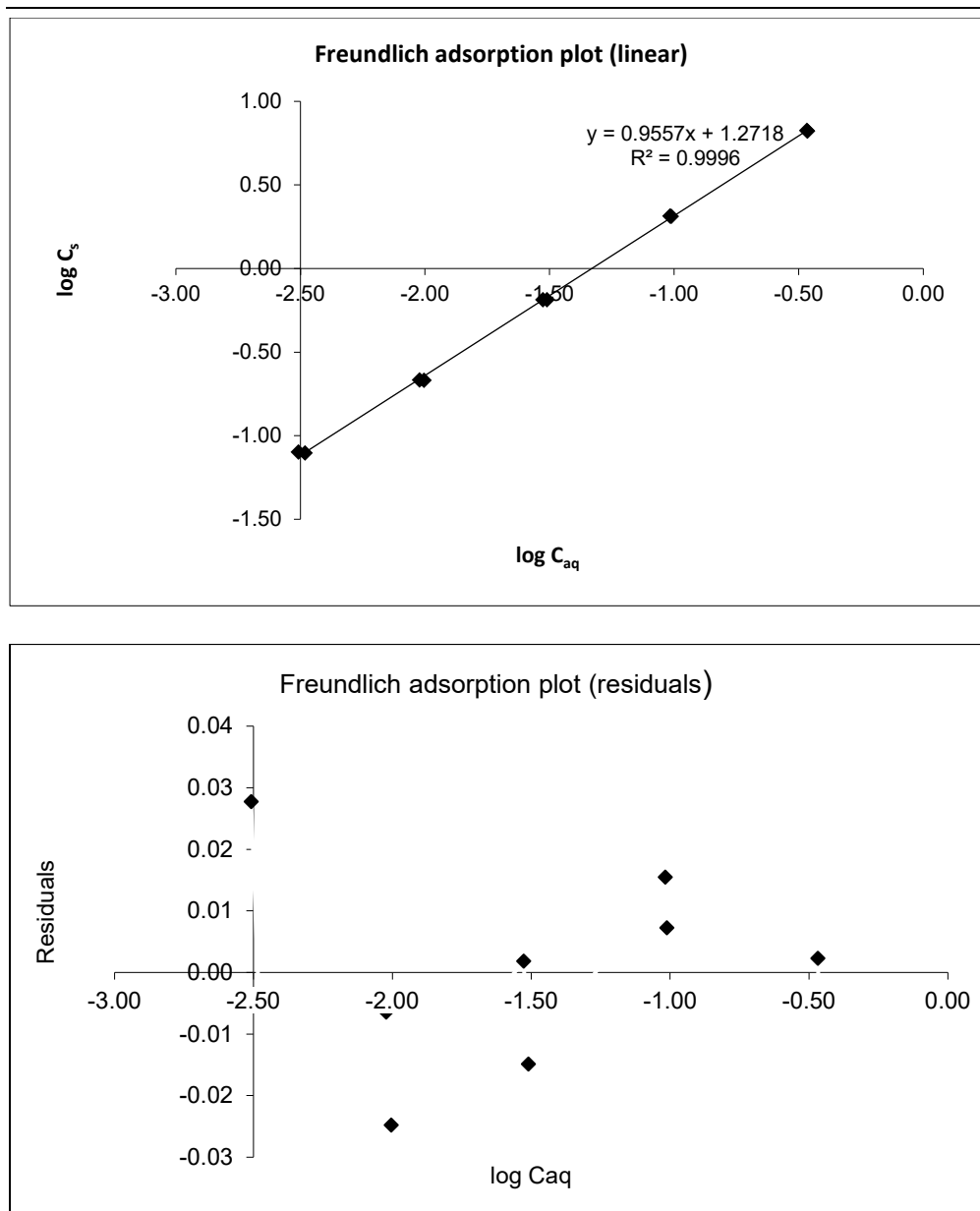
**Table B.8.1.5.1-24 Amounts and proportions of [<sup>14</sup>C] inpyrfluxam adsorbed to and desorbed from Ibaraki soil. Presented for information purposes only (results not included in overall mean endpoints)**

Application rate (µg/mL)	Adsorption		Desorption	
	Ads %	Not ads %	Des %	Not des %
1	64.6	35.4	28.3	71.7
1	65.9	34.1	26.9	73.1
<b>Mean</b>	<b>65.2</b>	<b>34.8</b>	<b>27.6</b>	<b>72.4</b>
0.3	65.6	34.4	26.8	73.2
0.3	65.7	34.3	28.2	71.8
<b>Mean</b>	<b>65.7</b>	<b>34.3</b>	<b>27.5</b>	<b>72.5</b>
0.1	65.9	34.1	26.8	73.2
0.1	65.2	34.8	28.0	72.0
<b>Mean</b>	<b>65.5</b>	<b>34.5</b>	<b>27.4</b>	<b>72.6</b>
0.03	66.4	33.6	25.8	74.2
0.03	66.1	33.9	25.0	75.0
<b>Mean</b>	<b>66.3</b>	<b>33.7</b>	<b>25.4</b>	<b>74.6</b>
0.01	68.3	31.7	24.3	75.7
0.01	70.6	29.4	23.1	76.9
<b>Mean</b>	<b>69.5</b>	<b>30.5</b>	<b>23.7</b>	<b>76.3</b>

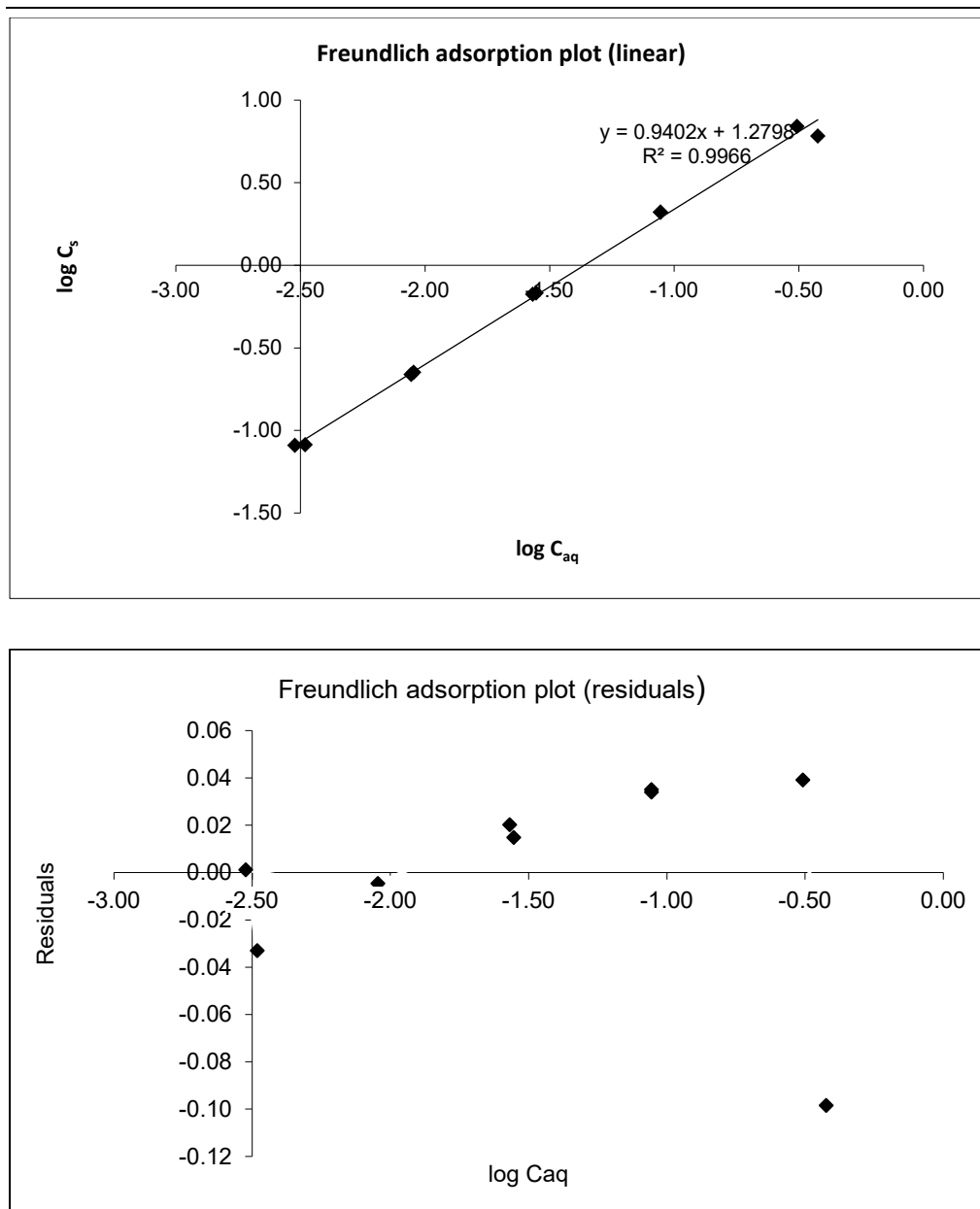
Ads = adsorbed, des = desorbed

Desorption percentages are of the amount remaining in soil after removal of the supernatant.

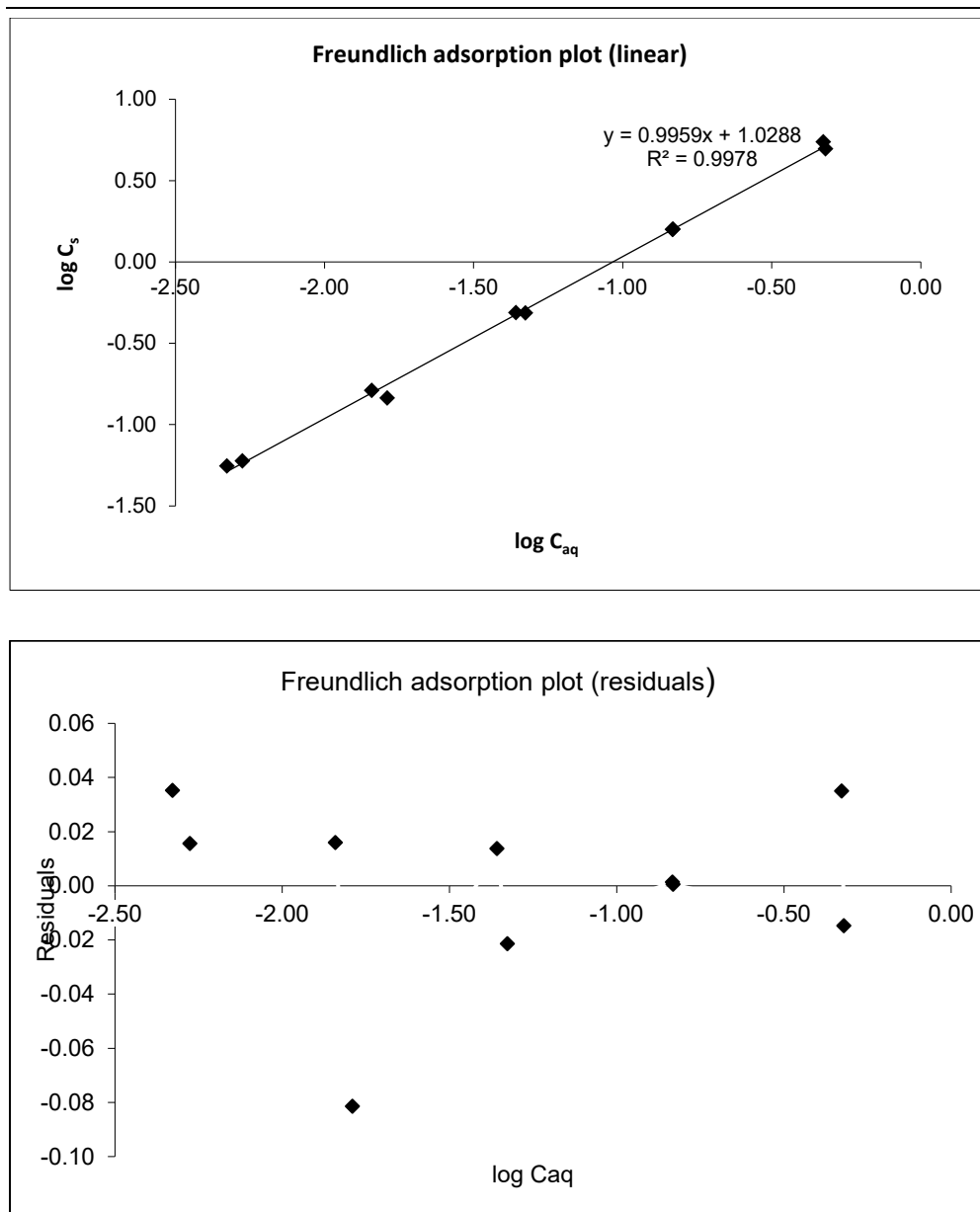
Adsorption and desorption partition coefficients are summarised in Table B.8.1.5.1-25. Isotherm graphs and Freundlich coefficients and exponents are presented in figures B.8.1.5.1-03 to B.8.1.5.1-09.



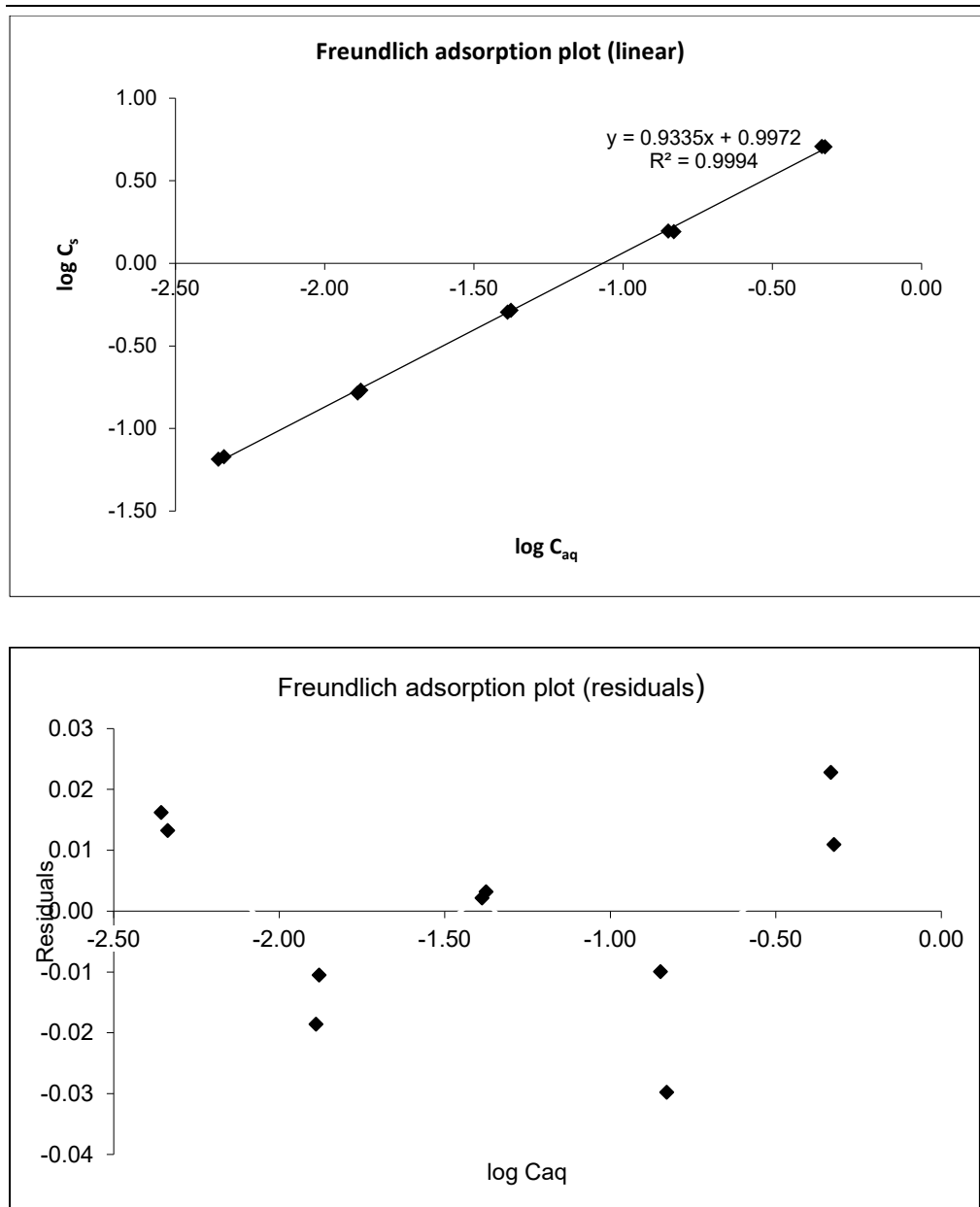
**Figure B.8.1.5.1-03 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in Brierlow soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE.**



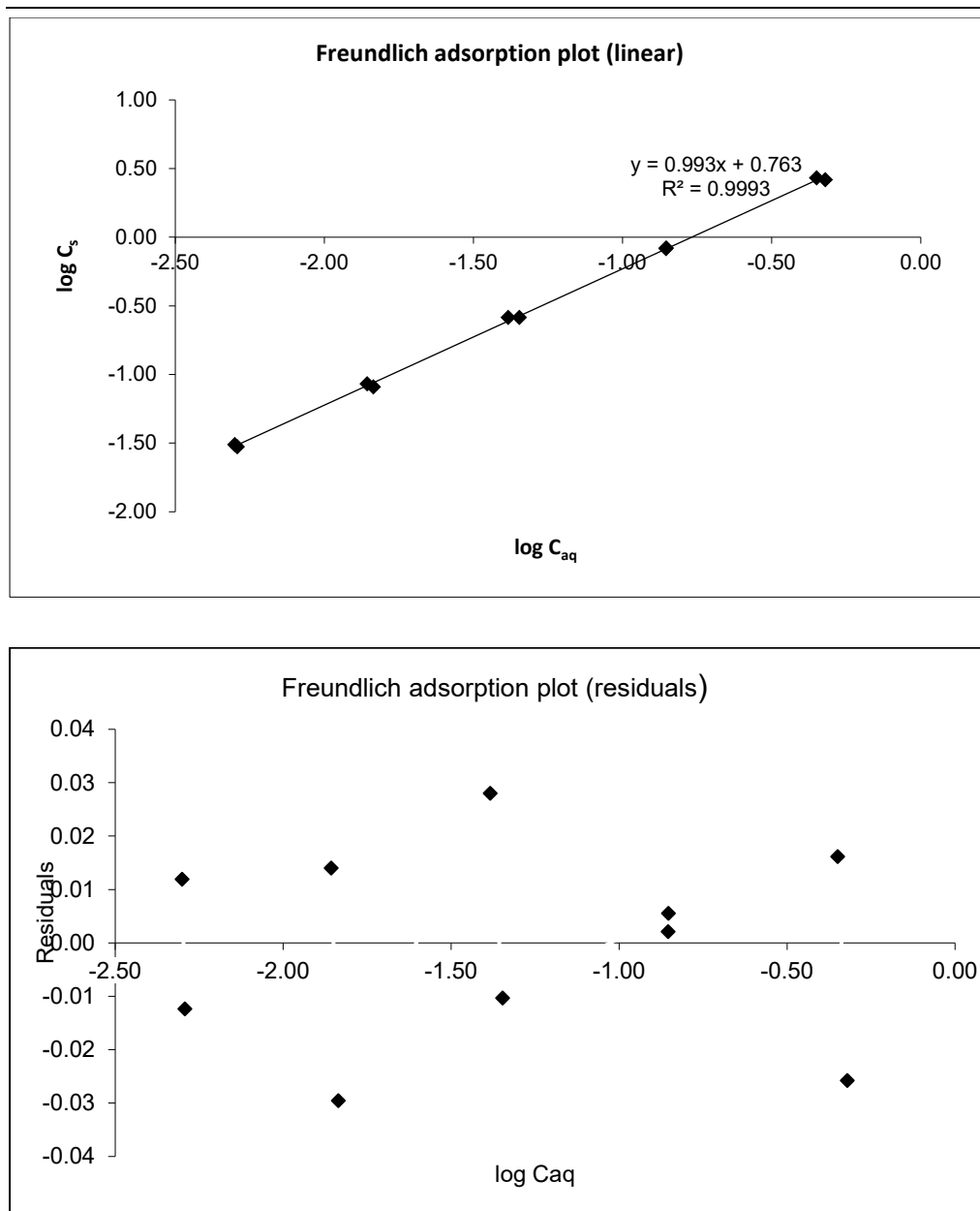
**Figure B.8.1.5.1-04 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in Kenslow soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE.**



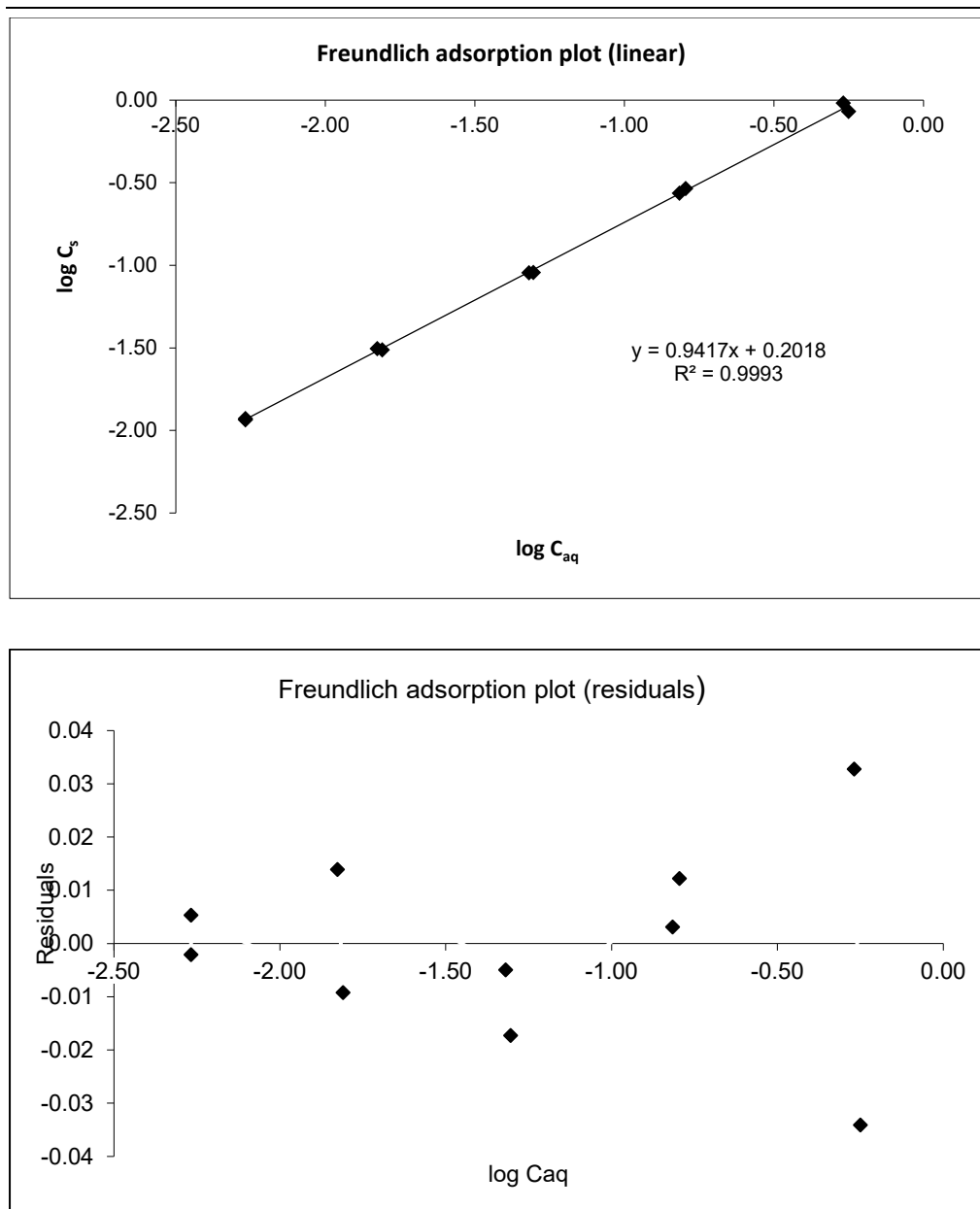
**Figure B.8.1.6.1-05 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in Clipstone soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE.**



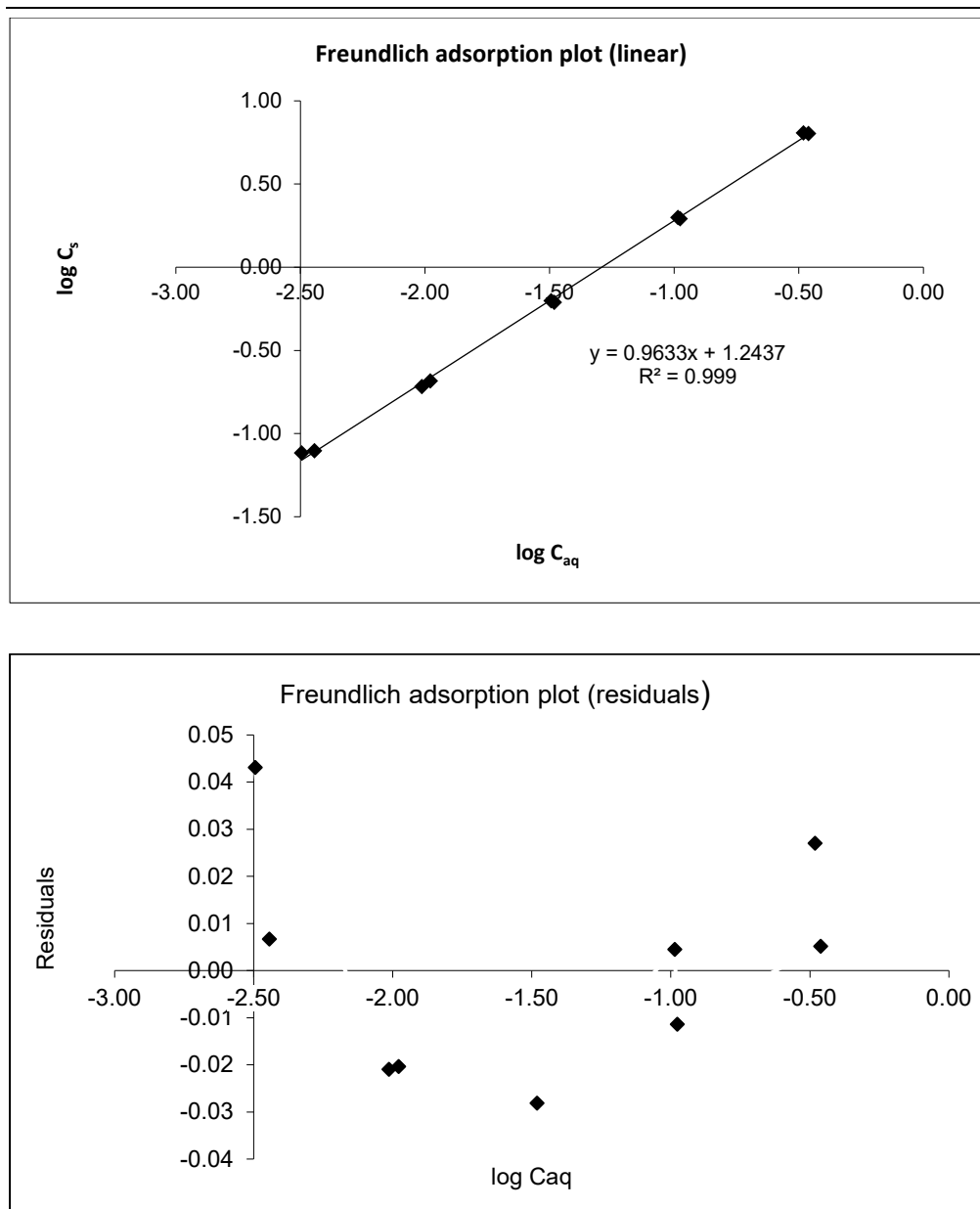
**Figure B.8.1.5.1-06 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in Hareby soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE.**



**Figure B.8.1.5.1-07 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in LAD-SCL-PF soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE.**



**Figure B.8.1.5.1-08 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in Atwater soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE.**



**Figure B.8.1.4.1-09 Top: Adsorption isotherm for [ $^{14}\text{C}$ ] inpyrfluxam in Ibaraki soil. Bottom: Residual plot for the adsorption isotherm. Plotted by HSE. Presented for information purposes only (results not included in overall mean endpoints)**

HSE assessed the applicant's Freundlich isotherms by inputting the aqueous and soil phase concentrations provided by the applicant in Tables B.8.1.4.11- to B.8.1.4.1-23 using the OECD 106 calculation tool. There were small differences between adsorption and desorption  $K_{foc}$  values derived by HSE and those calculated by the applicant, but these were attributed to rounding. Consequently, applicant adsorption values and isotherms were accepted. It is noted that the 95 % confidence intervals of the adsorption  $K_f$  and  $1/n$  values are not reported. HSE has calculated these values and added them to the summary on Table B.8.1.4.1-25.



**Table B.8.1.5.1-25 Applicant derived summary of the key adsorption and desorption values from all soils**

Soil	Organic carbon (%)	pH (CaCl <sub>2</sub> )	Adsorption				Desorption			
			K <sub>f</sub> <sup>ads</sup> (95% C.I.**)	K <sub>foc</sub>	1/n (95% C.I.**)	r <sup>2</sup>	K <sub>f</sub> <sup>des</sup>	K <sub>foc-des</sub>	1/n	r <sup>2</sup>
Brierlow	2.4	6.1	18.71 (17.91 - 22.06)	780	0.9563 (0.955 – 1.010)	0.9997	22.13	922	0.9646	0.9995
Kenslow	3.8	5.5	19.02 (16.56 - 26.72)	500	0.9392 (0.914 – 1.037)	0.9967	25.90	682	0.9753	0.9998
Clipstone	1.2	5.5	10.70 (9.42 - 13.7)	891	0.9963 (0.969 – 1.078)	0.9973	15.17	1264	0.9602	0.9970
Hareby	1.6	7.4	9.91 (9.53 - 12.92)	619	0.9321 (0.926 – 1.012)	0.9994	12.85	803	0.9428	0.9995
LAD-SCL-PF	0.9	8.1	5.79 (5.28 - 7.39)	643	0.9922 (0.983 - 1.079)	0.9993	7.54	838	1.0133	0.9994
Atwater	0.3	7	1.59 (1.50 - 2.07)	531	0.9418 (0.938 - 1.033)	0.9993	2.47	822	0.9788	0.9989
Ibaraki*	2.6	7.3	17.47 (17.28 - 21.73)	672	0.9618 (0.966 - 1.027)	0.9990	20.91	804	0.9523	0.9992

\*Ibaraki soil presented for information purposes only (results not included in overall mean endpoints)

\*\*Calculated by HSE

The pH of the supernatants after the 48 hour equilibrium test were determined and are presented in Table B.8.1.5.1-26 below.

**Table B.8.1.5.1-26 pH measurements of adsorption supernatants**

<b>Nominal application rate (µg/mL)</b>	<b>Brierlow</b>	<b>Kenslow</b>	<b>Clipstone</b>	<b>Hareby</b>	<b>LAD-SCL-PF</b>	<b>Atwater</b>	<b>Ibaraki*</b>
1	6.4	6.3	6.35	7.1	7.43	7.33	7.2
1	6.41	6.3	6.35	7.08	7.42	7.32	7.19
0.3	6.41	6.31	6.36	7.07	7.37	7.33	7.2
0.3	6.42	6.33	6.36	7.06	7.38	7.31	7.2
0.1	6.42	6.33	6.36	7.04	7.35	7.36	7.2
0.1	6.45	6.35	6.36	7.04	7.35	7.38	7.2
0.03	6.44	6.37	6.36	7.04	7.32	7.4	7.2
0.03	6.45	6.37	6.35	7.03	7.37	7.4	7.19
0.01	6.4	6.29	6.35	7.08	7.35	7.38	7.19
0.01	6.43	6.31	6.34	7.07	7.34	7.39	7.19

\*Ibaraki soil presented for information purposes only (results not included in overall mean endpoints)

HSE notes that the pH of the supernatants at equilibrium were measured in the definitive test, rather than measured before contact with the soil. However, this is not considered to be a major deviation for the following reasons:

- It is the pH of the aqueous phase that plays an important role in the adsorption process especially for ionisable substances, and this pH was measured by the applicant;
- the solubility of inpyrfluxam does not change across environmentally relevant pH ranges;
- no hydrolysis of inpyrfluxam was seen at pH 4, 7 and 9 at 50°C;
- there is still enough information (such as pH soils in CaCl<sub>2</sub> and pH of supernatant after sorption step) to investigate pH dependence.

## OECD 106 EVALUATORS CHECKLIST

It is noted that this study was completed in May 2016, which is before the publication of the OECD 106 evaluator checklist and evaluation tool, which was published in November 2017. While guidance should not be applied retrospectively, much of the content of the adsorption checklist was already present in the OECD 106 Guideline, with the checklist providing clarification on how a good study should be conducted. It is therefore considered appropriate to apply the checklist to the current study.

The following are noted regarding the adsorption checklist:

- The recovery of the applied radioactivity was > 90 %, ranging between 91.0 to 97.1 % when NERs are included. However, when NERs are subtracted, the

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parental mass balance is < 90 % for Atwater (mean of 84.05 %) and LAD-SCL-PF (mean of 88.5 %). These checks confirmed that the test item mass balance was > 90 % AR for all but 2 soils, demonstrating that the test item was stable for the adsorption phase for all other soils. The Atwater and LAD-SCL-PF soils are considered further, as they are below the 90% threshold in the definitive test, and attained a >90 % mass balance in the preliminary soil stability test.

- The use of the indirect method was appropriate based on a  $K_d$  \* soil/solution ratio > 0.3 in all soils (see Table B.8.1.5.1-27). This also indicates the chosen soil:solution ratio was acceptable.
- The  $K_{fe}/K_f$  ratio should be < 1.2 for the highest concentration and values > 1.2 should be treated with caution. Values calculated by HSE are shown below (see Table B.8.1.4.1-27). Values were slightly > 1.2 for Atwater soil, possibly due to the unextracted residues accounting for > 5 % of the total recovery. All other soils had ratios < 1.2. HSE notes that while the  $K_{fe}/K_f$  ratio for the Atwater soil is > 1.2, it is a small exceedance, and produced highly linear and statistically well-fitted Freundlich isotherms. This soil also produced low adsorption compared to other soils and exclusion from the data set would result in less conservative, more highly sorbing values being obtained. Therefore the Atwater soil has not been excluded.
- The acceptability of the analytical method was confirmed for the concentration measured, as the LOQ is suitably below the lowest concentration method by OECD 106 guidelines.
- Based on the goodness-of-fit criteria outlined in the EFSA OECD 106 checklist (EFSA, 2017), HSE reviewed the Freundlich adsorption isotherm for each soil through visual analysis of the isotherm and residuals, and through checking the  $r^2$  and  $1/n$  values (Figures B.8.1.4.1-03 to B.8.1.4.1-09). The Freundlich exponents  $1/n$  were in the range of 0.9392 to 0.9963 indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range. Visual fits of the standard regressions were acceptable for all soils and the  $r^2$  values of the standard linear regressions ranged from 0.9967 to 0.9997, all markedly above the recommended value of 0.975.

**Table B.8.1.5.1-27 Summary of results used for quality check (in addition to values reported in Table B.8.1.5.1-25)**

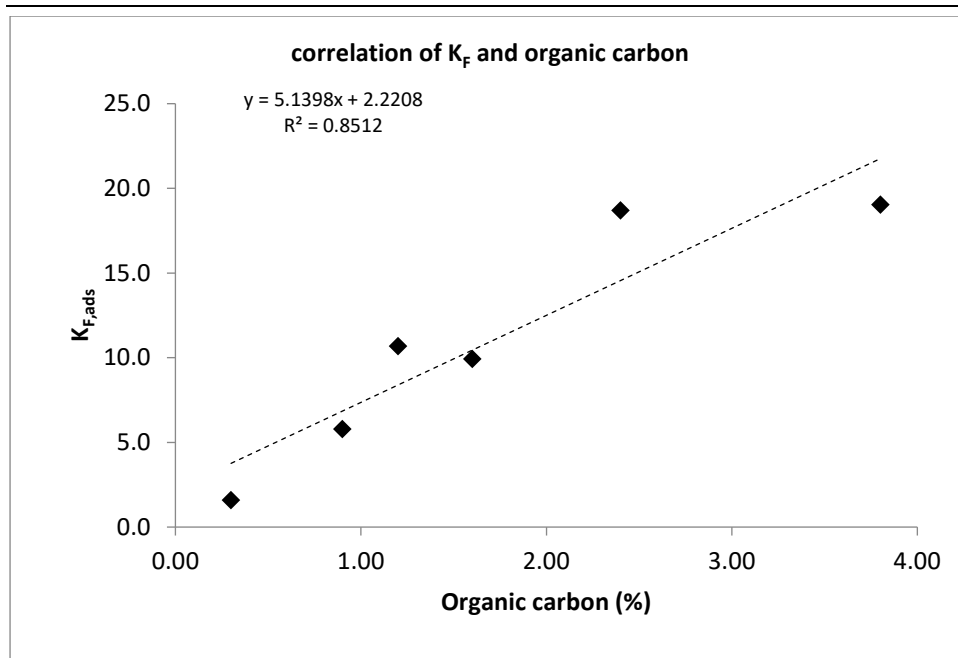
Soil	Units	Brierlow	Kenslow	Clipstone	Hareby	LAD-SCL-PF	Atwater	Ibaraki*
Adsorption method (direct/indirect)	-	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect	Indirect
Soil:solution ratio	(g dw/mL)	1:10	1:10	1:10	1:10	1:05	1:02	1:10
Mass balance (at highest tested conc.)	%	91.5	92.5	91.3	92.9	88.5	84.1	88.9
Adsorbed percentage	%	66.4 - 71.9	61.9 - 73.2	51.4 - 56.2	51.9 - 59.9	52.2 - 55.9	43.2 - 52.1	64.6 - 70.6
$K_d \times$ (soil:solution ratio)	-	2.15 - 2.86	1.79 - 3.00	1.00 - 1.32	1.17 - 1.64	1.22 - 1.40	0.85 - 1.21	2.05 - 2.66
$K_{fE} / K_f$	-	1.068	1.073	1.055	1.054	1.063	1.223	1.010

\*Ibaraki soil presented for information purposes only (results not included in overall mean endpoints)

### Organic carbon dependence

It is noted that the applicant has stated that there was no obvious correlation between the adsorption partition coefficients and organic carbon. HSE disagrees with this statement, and have calculated a Kendall rank correlation coefficient for  $K_f$  of 0.867 using the German support tool (pH Dependence Calculator). This indicates a strong relationship between sorption and percentage organic carbon. The corresponding p-value was 0.012 which is < 0.05 and so this positive relationship is deemed to be statistically significant.

This dependence was also investigated by creating a linear regression plot and conducting statistical analysis using Excel. The linear regression is displayed in Figure B.8.1.5.1-10. This plot consists of only 6 data points (Ibaraki soil was excluded), however it does show an acceptable visual fit to the data (p-value = 0.00875).



**Figure B.8.1.5.1-10 Linear regression investigating the organic carbon dependence of [ $^{14}\text{C}$ ] inpyrfluxam**

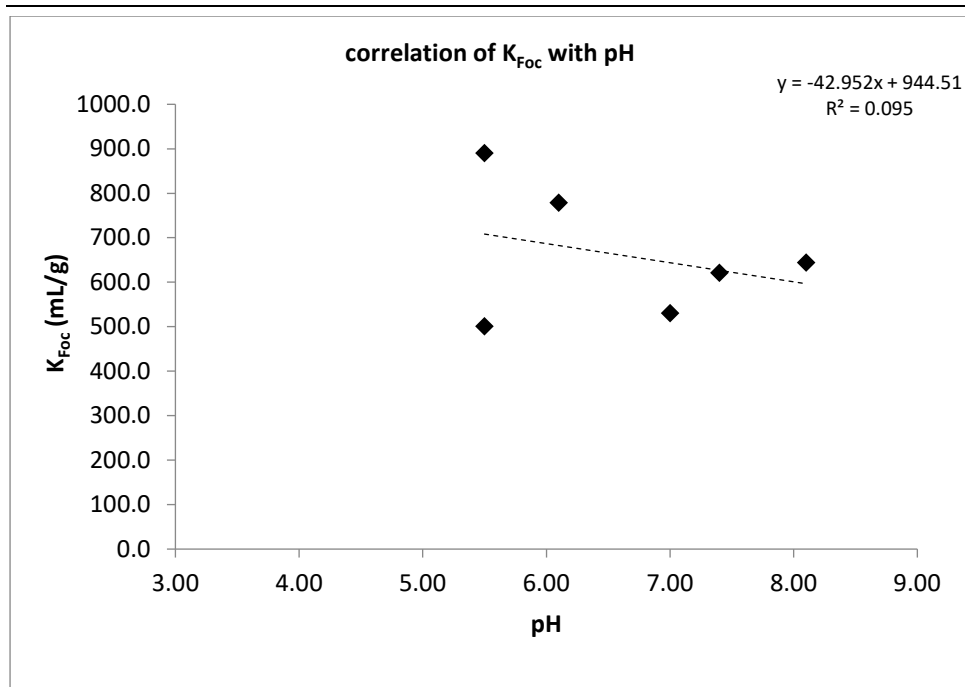
HSE considered the results from both analyses and suggests that, due to the strong relationship demonstrated by the Kendall rank correlation coefficient, and the acceptable visual fit of the linear regression plot, there is a relationship between sorption and organic carbon.

### pH dependence

The applicant has also stated that there is no relationship between the adsorption partition coefficients and pH.

HSE calculated a Kendall rank correlation coefficient for  $K_{oc}$  of 0.00 using the German support tool (pH Dependence Calculator), this indicates that there is no correlation between sorption and pH. The corresponding p-value was 1.00 which is  $> 0.05$  also confirming that the correlation between pH and  $K_{foc}$  is not statistically significant.

This dependence was further investigated by creating a linear regression plot (see Figure B.8.1.5.1-11) and conducting statistical analysis using Excel. The p-value for this regression is 0.5523 which is above the 0.05 guideline for statistical significance. The linear plot indicates that there is no relationship between  $K_{foc}$  and pH. The  $r^2$  value is small (0.095) indicating there is no relationship between sorption and pH. HSE considered the results from both analyses and agrees that the adsorption of inpyrfluxam is not dependent on pH.



**Figure B.8.1.5.1-11 Linear regression investigating the relationship between pH and  $K_{foc}$  in [ $^{14}C$ ] inpyrfluxam on seven soils**

## CONCLUSIONS

The Freundlich adsorption coefficients ( $K_{foc}$ ) for the seven test soils calculated ranged from 500 to 891 mL/g (geomean: 650 mL/g), which is in good agreement to HSE derived values of 501.2 to 890.5 mL/g. Therefore HSE accepts the applicant's values.

Using the Briggs classifications for the estimation of the mobility of active substances in soil, inpyrfluxam can be classified as having a low mobility in soils.

The Freundlich desorption coefficients ( $K_{focdes}$ ) were slightly higher than the corresponding adsorption coefficients indicating a degree of irreversibility to the adsorption.

HSE considers this study acceptable for validation.

**Table B.8.1.5.1-28 Summary of accepted inpyrfluxam adsorption endpoints (HSE values)**

Inpyrfluxam							
Soil Type	OC (%)	Soil pH (CaCl <sub>2</sub> )	$K_d$ (mL/g)	$K_{doc}$ (mL/g)	$K_f$ (mL/g)	$K_{foc}$ (mL/g)	1/n
Brierlow	2.4	6.1	-	-	18.71	780	0.956
Kenslow	3.8	5.5	-	-	19.02	500	0.939
Clipstone	1.2	5.5	-	-	10.70	891	0.996

Hareby	1.6	7.4	-	-	9.91	619	0.932
LAD-SCL-PF	0.9	8.1			5.79	643	0.992
Atwater	0.3	7	-	-	1.59	531	0.942
Ibaraki*	2.6	7.3	-	-	17.47	672	0.962
<b>Geometric mean (if not pH dependent)</b>					<b>8.39</b>	<b>647</b>	
<b>Arithmetic mean (if pH dependent)</b>							<b>0.960</b>
<b>pH dependence</b>					<b>No</b>		

\*Volcanic soil; not considered in calculation of mean values. The impact of excluding this soil was considered. With the Ibaraki soil, the geometric mean  $K_{\text{foc}}$  is 650 mL/g and the arithmetic mean  $1/n$  is 0.962, while with the Ibaraki soil excluded the geometric mean  $K_{\text{foc}}$  is 647 mL/g and the arithmetic mean  $1/n$  is 0.960. The soil is well within the range of values derived and the inclusion or exclusion of the soil has minimal impact on the adsorption parameters. As no information on the amorphous iron and aluminium oxide content of the soil has been provided, it is unclear whether this soil is relevant to GB agricultural conditions. Consequently this soil has been excluded from the data set.

**B.8.1.5.2. Adsorption and desorption in soil of metabolite 3'-OH-S-2840**

<b>Reference:</b>	KCA 7.1.3.1.2/02
<b>Report:</b>	[ <sup>14</sup> C] 3'-OH-S-2840: Adsorption/Desorption in Soil. Smithers Viscient (ESG) Ltd., UK, Study Number: 3201398; Sumitomo Chemical Co., Ltd. Report TPM-0032
<b>Author(s) &amp; Year:</b>	██████████ (2017)
<b>Address:</b>	Test Facility - Smithers Viscient (ESG) Ltd. 108 Woodfield Drive, Harrogate North Yorkshire, HG1 4LS UNITED KINGDOM
<b>Guidelines:</b>	OECD Guideline 106 (January 2000) OCSP Guideline 835.1230 (October 2008)
<b>GLP:</b>	Yes

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Soil:solution ratios were not investigated in the preliminary study.	Minor deviation. As adsorption % was >50% for all soils and resulting K <sub>d</sub> *soil:solution ratios were > 0.3, this is considered acceptable.
No transport information was provided for the soil samples from the sampling site to testing facilities.	Minor omission. Acceptable as soil properties were confirmed to not be significantly altered on arrival.
Single extractions were conducted on soil for mass balance calculations instead of the recommended two extractions.	Minor deviation. Recoveries still resulted in >90% AR.



The LOD/LOQ reported was only 1 order of magnitude above the lowest nominal concentration.	Minor deviation. Acceptable as the lowest concentration in the supernatant was still above the LOD/LOQ.
In the 3 hour preliminary equilibrium test, >1% of the total volume was removed for analysis.	Minor deviation. Acceptable as this occurred in only one test at a small exceedance (1.6%).

### HSE conclusion on deviations

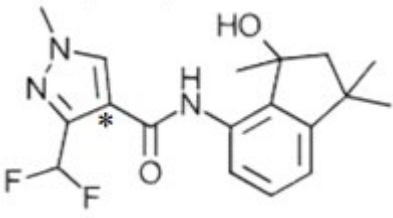
HSE has validated the study and considers it to be acceptable. The deviations noted by HSE have not invalidated the study.

## INTRODUCTION

The adsorption and desorption characteristics of [<sup>14</sup>C]3'-OH-S-2840 was determined in three soils (two from the UK and one from France) at 20 ± 2°C in the dark using the indirect method. The study was carried out in accordance to OECD 106 guidelines. A tier 1 preliminary assessment was conducted to determine the substance stability, solubility and a tier 2 test determined the adsorption equilibrium time. A tier 3 assessments was then carried out to produce the relevant adsorption/desorption isotherms.

## MATERIALS

<b>Test material</b>	[Pyrazolyl-4- <sup>14</sup> C]3'-OH-S-2840
<b>Specific activity:</b>	2.22 GBq/mmol
<b>Lot/Batch:</b>	RIS2015-005
<b>Purity:</b>	Radiochemical Purity ≥99.2% HSE considers the purity suitable for use in the test.
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	Stable (at least for the test conditions)

<b>Structure:</b>	 <p>*: position of radiolabel. HSE notes that the radiolabel is positioned on the pyrazole moiety and is a suitably stable position for the radiolabel.</p>
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The study was conducted with three different soils. None of the soils were obtained from arable land and none had any history of pesticide use for the past 5 years. A summary of the physical and chemical properties of the soils is provided in Table B.8.1.5.2-1. All soils were sieved (2 mm), air-dried, and kept in the dark at room temperature until use.

**Table B.8.1.5.2-1: Soil physiochemical properties**

Soil Characterisation	Quilen (Soil A)	Hareby (Soil B)	Clipstone (Soil C)
Sampling location	France	UK	UK
Sampling depth	10-20 cm	4-10 cm	5-15 cm
Particle size distribution			
Sand (%)	30	33	86
Silt (%)	50	33	8
Clay (%)	20	34	6
Texture <sup>1</sup>	Silt loam	Clay loam	Loamy sand
pH (0.01M CaCl <sub>2</sub> )	7.3	7.4	5.5
% organic matter	5.0	2.8	2.1
% organic carbon	2.9	1.6	1.2
CEC (meq/100g)	26.1	12.2	7.8

<sup>1</sup> USDA classification; CEC = Cation Exchange Capacity

The soils used were stored for no more than 2 years; HSE finds that re-analysis of soil parameters is not required as storage was less than 3 years. No pesticide was applied for at least 5 years from before the date of sampling.

OECD 106 recommends soils with a range of parameters with particular consideration to pH, organic carbon (%OC) and clay content. HSE notes the soils presented by the applicant demonstrate a good range in clay content and a sufficient range in pH (measured in 0.01M CaCl<sub>2</sub>). Ideally, the soils would present a greater range in %OC, but the range of OC is wide enough over the three soils to determine if it has an impact on adsorption of the metabolite and to calculate K<sub>oc</sub> values. Additionally, the soils fall within the range of properties provided as examples by the guidance. HSE finds this demonstrates an acceptable range and all soil parameters are therefore considered suitable for use in the study.

HSE notes that the applicant has not provided information on the transport conditions of the soil samples from the sampling site. HSE considers this a minor deviation as the soil parameters are acceptable and were confirmed upon arrival at the testing facilities which indicates no significant alterations to the soil properties have occurred.

## STUDY DESIGN

### I. Experimental conditions

HSE notes the applicant conducted a preliminary test to assess adsorption to the test vessels. The applicant conducted a tier 2 test to find the equilibrium time for adsorption followed by a test for determining the stability of the substance. Finally, a tier 3 test was conducted at multiple concentrations to determine the adsorption isotherms for adsorption and desorption. Details of each test are given below.

For each test, different application solutions were made up from an initial stock solution of 0.146 mg/mL 3'-OH-S-2840 in acetonitrile. The stock solutions were reduced to dryness and the test substance dissolved in CaCl<sub>2</sub> to create the application solutions. HSE notes that OECD 106 recommends organic solvent remains < 0.1% of the aqueous phase. As the acetonitrile was dissolved before creating the final application solutions for each test, HSE finds this acceptable. Furthermore, HSE notes the applicant should provide information pertaining to storage conditions for the stock solution during the testing procedures. Although this information was not supplied, a stability test confirmed the substance as stable (see findings below) and concentrations of the application solution made from the stock solution were confirmed by LSC.

Stock solutions and application solutions were characterised by HPLC and TLC where the resulting purity was then calculated (99.17%).

The OECD 106 guidance recommends all soils are equilibrated with a minimum volume of 45 cm<sup>3</sup> 0.01 M CaCl<sub>2</sub> and then made up to 50 cm<sup>3</sup> using the stock solution with the stock solution not exceeding 10% of the total volume. The applicant reported soils were equilibrated with 22.5 cm<sup>3</sup> CaCl<sub>2</sub> and made solutions up to 25 cm<sup>3</sup> using 2.5 cm<sup>3</sup> of application solution. HSE notes that the OECD experimental guidance is

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a suggestion and since an appropriate soil:solution ratio was used resulting in sufficient adsorption, the use of half the volume of  $\text{CaCl}_2$  for equilibration is considered acceptable by HSE.

## **II. Preliminary Test**

The control in the preliminary test for adsorption to the test vessel was conducted by the applicant using 0.005  $\mu\text{g/mL}$  test substance in 0.01 M  $\text{CaCl}_2$  solution made up from the stock solution. The control substance was left for 24 hours and radioactivity was measured before and after shaking. Stability was tested following the tier 2 adsorption kinetics test to determine equilibrium time (see tier 2 section below).

HSE notes the applicant did not conduct any investigation into the soil:solution ratio. The OECD 106 guidance and accompanying OECD 106 evaluators checklist both consider investigation of the ratio an important step for determining acceptable ranges and minimising errors. However, the applicant has demonstrated that soil adsorption for all soils is >50% with a maximum mean adsorption of 86.7% (Quilen). Additionally, HSE has verified that the calculated  $K_d \times \text{soil/solution ratio}$  is >0.3 for all results with a reasonable level of sorption. HSE therefore considers this an acceptable deviation.

## **III. Tier 2 Test**

An adsorption equilibrium time test was performed on all soils at 0.05  $\mu\text{g/mL}$  for periods of up to 48 hours. Solutions were shaken for 3, 6, 24 and 48 hours. On each sampling occasion, samples were centrifuged (34 minutes at 5000 g). HSE notes that OECD 106 requirements for centrifugation time are dependent on soil density which has not been provided. If centrifugation times are insufficient for full phase separation, this will result in more test substance being detected in the liquid phase, and results in lower, more conservative sorption values. HSE therefore does not view this as a major deviation. Aliquots were removed for determination of radioactivity and replaced with an equivalent volume of fresh 0.01 M calcium chloride solution. After removal of aliquots, soils were re-mixed with the calcium chloride solution and returned to the shaker. The applicant has noted for the 6, 24 and 48 hour tests 200  $\mu\text{L}$  was removed from a total volume of 25 mL accounting for <1% while for the 3 hour test, 400  $\mu\text{L}$  was removed which is >1% of the total volume. OECD 106 notes the volume removed from the total solution should not exceed 1%; however, this occurred in a single test and did not exceed 1% of the total volume significantly (1.6%). HSE therefore agrees with the applicant this is unlikely to impact study results significantly and notes this as a minor deviation.

Additionally, a desorption equilibrium time test was performed on all soils at 0.05  $\mu\text{g/mL}$  for periods of up to 72 hours. Following the 48 hour adsorption period, the samples were centrifuged (34 minutes, 5000 g), the supernatants removed and replaced with an equivalent volume of fresh 0.01M calcium chloride solution (25 mL). Samples were shaken vigorously to break up the soil packed at the bottom of the

vessel and to re-mix it with the new solution. Solutions were then placed back on the shaker and aliquots were taken for LSC at 6, 24, 48 and 72 hours.

Stability was determined by the applicant following the test for determining equilibrium time. After the supernatant was removed, the remaining soil was extracted by shaking (20 minutes) three times with acetone (20 mL). Quilen soil was extracted a further two times with acetone: 0.05 M HCl (4:1 v/v, 20 mL). Extraction solvent was removed from the soil by centrifuging (10 minutes at 5000 g) and the radioactivity in the extracts was determined by LSC. One replicate from each of the extracts was then concentrated to ca 1 mL by rotary evaporation and reconstituted in acetone (4 x 0.5 mL). Adsorption supernatants and soil extracts were then analysed by HPLC.

#### **IV. Tier 3 Test**

To determine Freundlich adsorption isotherms, duplicate test vessels were prepared for each soil (12.5 or 5 g dry weight soil). The soil was pre-equilibrated with 0.01M  $\text{CaCl}_2$  (22.5 ml) overnight at  $20 \pm 2^\circ\text{C}$  in the dark using Teflon tubes. Soils were then treated with application solutions of [ $^{14}\text{C}$ ]3'-OH-S-2840 (2.5 ml) made up from a stock solution to achieve nominal initial concentrations in the aqueous phase of 0.005, 0.01, 0.05, 0.1 and 0.5  $\mu\text{g/mL}$ . All samples were shaken for 48 hours (the adsorption equilibrium time) then centrifuged for 34 minutes at 5000 g. As much of the adsorption supernatant as possible was removed from each incubation vessel into a new pre-weighed vessel, and weighed aliquots were taken for LSC analysis. The pH of supernatant was determined following the adsorption test. HSE notes OECD 106 recommends that pH of the supernatant is determined both before and after contact with the soil. As the pH was known prior to the test and determined following the adsorption test, HSE finds this acceptable.

The applicant investigated desorption using the serial method outlined in OECD 106 by adding an identical volume of 0.01 M  $\text{CaCl}_2$  to the soil where the solution was removed in the final adsorption step. The mixture was then agitated for 24 hours and subsequently the desorption supernatant was analysed via LSC.

The  $K_d$  value was calculated by the applicant from the soil concentration and adsorption supernatant for both adsorption and desorption. The applicant presented calculations for the Freundlich coefficients ( $K_F$ ) and exponent ( $1/n$ ) for the adsorption and desorption isotherms. An isotherm of the logarithmic concentrations in soil and in solution were plotted and presented for adsorption and desorption.

Mass balance was determined for each soil at the highest concentration. The soil after the desorption phase was rinsed with acetone and radioactivity of the rinse was analysed. See analytical descriptions for more information on mass balance determination.

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## V. Description of analytical procedures

### Analysis of adsorption/desorption supernatants (following tier 2/3 test)

Corresponding test vessels were taken, centrifuged and supernatants analysed by LSC. HSE notes that as the test substance is shown to be stable in the stability test, LSC is an acceptable method for analysis. Additionally, the OECD evaluator checklist states that the volume of water entrained in the soil after centrifugation should be included in the calculation for the aqueous phase (supernatant). Prior to LSC, the applicant determined the weight of the supernatant by measuring the total weight of soil plus solution and subtracting the dry soil weight. The applicant has therefore included entrained water as part of the total volume of supernatant.

### Analysis of soil (following tier 3 test)

Concentration of the test substance in soil were calculated indirectly. OECD guidelines states that the mass of test substance in soil should be calculated as a difference between initial and final concentrations in solution for the indirect method. The applicant has presented detailed examples of soil concentration calculations, and the indirect calculations for both the adsorption and desorption steps have been replicated by HSE. Therefore, as the calculations have been verified by HSE, HSE has relied upon applicant reported data for subsequent calculations and isotherms.

### Analysis for radioactive mass balance

Following desorption in the tier 3 experiment, for the highest dosed concentration of each soil, the radioactive mass balance was determined by an additional acetone extraction of the soil. Residues were air dried, ground and combusted. The radioactivity in the acetone wash was quantified by LSC. Remaining radioactivity in the soil was quantified by combustion with LSC.

HSE notes that OECD 106 and the evaluator tool requires a reliable analytical method over the concentration range conducted in the study. In order to allow for detection and evaluation after partitioning has taken place, the guidance suggests detection limits of the analytical method should be at least two orders of magnitude below the nominal concentration. The applicant has provided additional information that for this study the limit of detection (LOD) is  $\leq 0.6\%$  AR at 0.5 - 0.01  $\mu\text{g/mL}$  and  $\sim 1\%$  AR at 0.01  $\mu\text{g/mL}$  (1.5 x background). Additionally, the applicant has stated that the LOD and LOQ values are regarded as equivalent in the context of radioactivity analysis. At the lower limit, this would provide an LOD (and LOQ) of 0.0001  $\mu\text{g/mL}$  whilst the lowest nominal concentration is 0.005  $\mu\text{g/mL}$ . Therefore, the LOD is only one order of magnitude below the lowest nominal concentration. At this nominal concentration, the lowest concentration reported in the supernatant was 0.0007  $\mu\text{g/mL}$ . This was above to the LOD (and LOQ) and therefore HSE considers that the analytical results were able to be reliably quantified.. HSE therefore notes this as an acceptable minor deviation.

## RESULTS AND DISCUSSION

### I. Mass balance

The recovery of radioactivity at the highest concentration for each soil is shown in Table B.8.1.5.2-2 and ranged from 90 to 95%. To recover the test substance from soil, the applicant carried out a single extraction using an acetone solution. HSE notes that OECD guidelines recommend soil extraction is carried out twice; however, the resultant recoveries were still >90%. Furthermore, HSE notes that a mass balance was also measured and calculated as part of the stability test where in addition to the acetone wash, the test utilised a second extraction via 0.05 M HCl which also resulted in comparable recoveries of >90% shown in Table B.8.1.5.2-3. HSE therefore considers this an acceptable minor deviation and all mass balances are suitable. Table B.8.1.4.2-3 recovery % values are subject to rounding.

**Table B.8.1.5.2-2: Recovery of radioactivity following a 48 hour adsorption phase and a 24 hour desorption phase at 0.5 µg/mL**

Soil	Vessel	Recovery of radioactivity (%)				
		Adsorption Supernatant	Desorption Supernatant	Acetone Wash	Unextracted from soil	Total
Quilen	ISOA1	20.3	11.5	38.6	21.0	91.4
	ISOA2	20.6	12.1	37.3	20.3	90.3
	Mean	20.5	11.8	38	20.7	90.9
Hareby	ISOB1	41.4	19.5	26.9	6.7	94.5
	ISOB2	40.8	20.4	26.0	7.2	94.4
	Mean	41.1	20.0	26.5	7.0	94.5
Clipstone	ISOC1	24.5	14.2	40.1	15.3	94.1
	ISOC2	24.2	13.9	40.5	15.2	93.8
	Mean	24.4	14.1	40.3	15.3	94.0

**Table B.8.1.5.2-3: Recovery of radioactivity and test item from each 48-hour adsorption test sample**

<b>Soil</b>	<b>Radioactivity in adsorption supernatant (%)</b>	<b>Radioactive equivalent of test item in adsorption supernatant (%)</b>	<b>Test item in adsorption supernatant (%)</b>	<b>Radioactivity in soil (%)</b>	<b>Radioactive equivalent of test item in soil (%)</b>	<b>Test item in soil (%)</b>	<b>Total recovery of test item (%)</b>
<b>Quilen</b>	11.7	99.2	11.6	78.9	99.4	78.4	90.1
<b>Hareby</b>	33.5	99.0	33.2	64.2	98.8	63.5	96.6
<b>Clipstone</b>	17.7	99.3	17.6	79.5	99.1	78.8	96.4

## II. Main test findings

### Preliminary Test

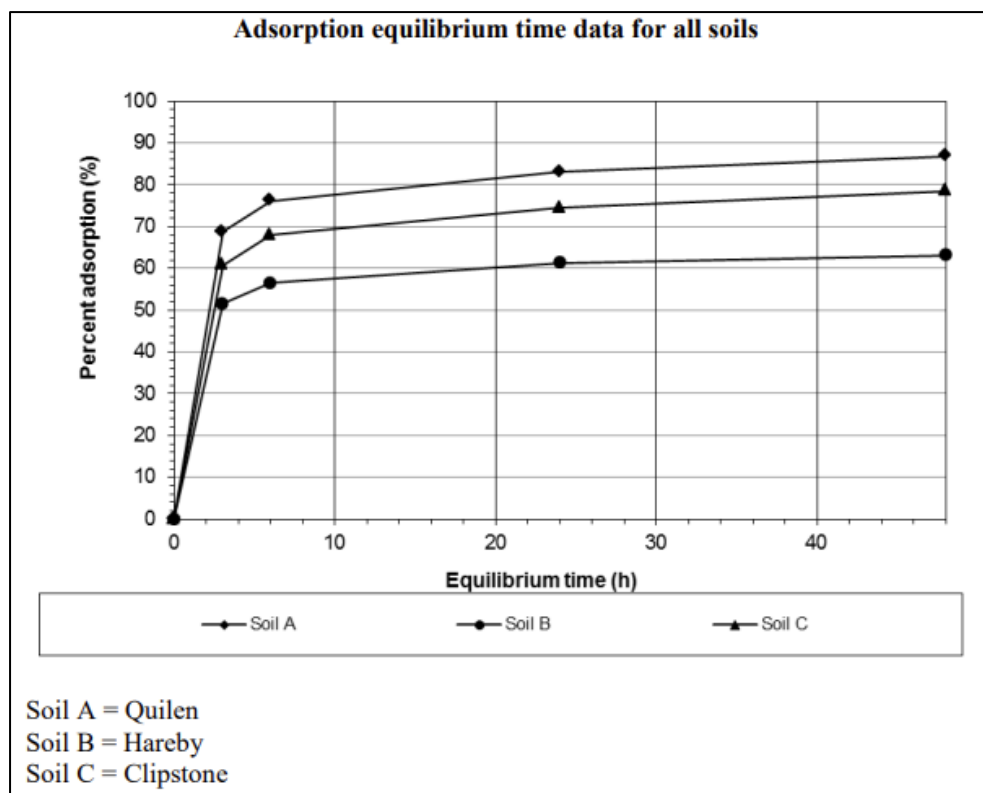
A soil:solution ratio of 1:2 (w/v) for the Clipstone soil and 1:5 w/v for the Quilen and Hareby soils were used by the applicant, as well as the use of Teflon tubes, an adsorption equilibrium time of 48 hours and a desorption equilibrium time of 24 hours. HSE notes sorption was >50% in all soils whilst values were not too high so as to affect the accuracy of measured concentrations in the aqueous phase (maximum mean of 86.7% in Quilen). HSE notes the control resulted in a maximum difference of 1% test substance between 0h and 24h. Therefore, HSE agrees with the applicant that [<sup>14</sup>C]3'-OH-S-2840 does not significantly adsorb to the test vessels.

### Tier 2

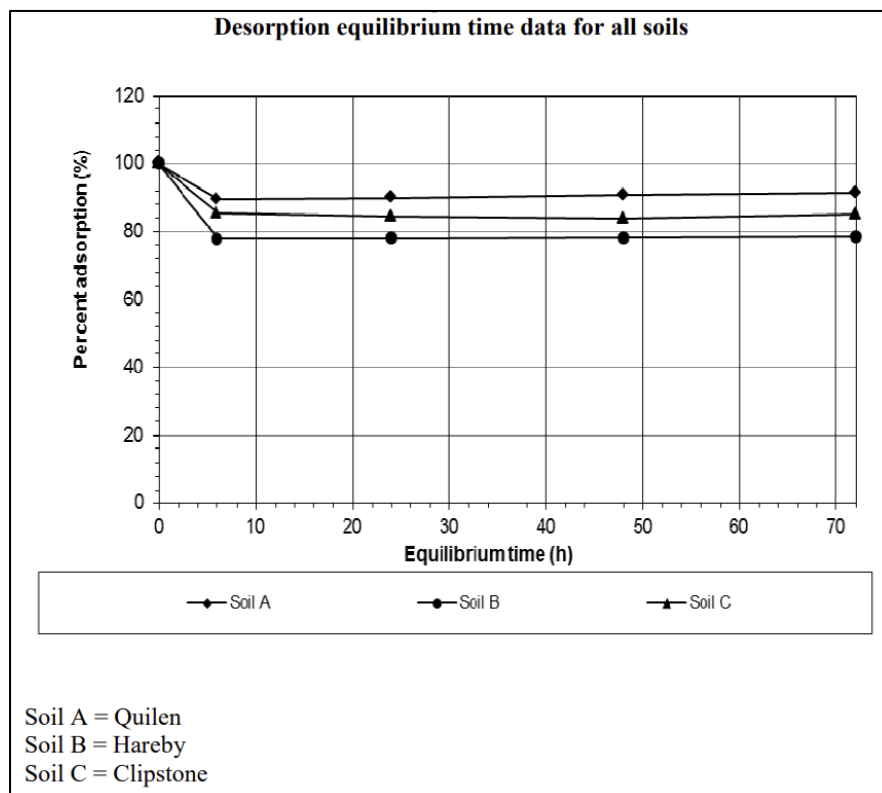
The applicant determined an equilibration time of 48 hours following a test where sampling was carried out at 3, 6, 24 and 48 hours. The graphs showing the percentage of test substance as a function of equilibrium time for both adsorption and desorption are presented in Figure B.8.1.5.2-1 and Figure B.8.1.5.2-2 respectively. Based on the adsorption and desorption equilibrium time graphs, an adsorption equilibrium time of 48 hours and a desorption equilibrium time of 24 hours were chosen for all soils. HSE agrees with these equilibrium times as the % adsorbed increased up to 48 hours. Adsorption of [<sup>14</sup>C]3'-OH-S-2840 to soil at the



1:2 w/v for Clipstone soil and 1:5 w/v soil to aqueous phase for Quilen and Hareby soils at 48 hours was between ca 63 and 87% and the adsorption levels were considered suitable for further study.



**Figure B.8.1.5.2-1: Adsorption equilibrium time data for all soils**



### Figure B.8.1.5.2-2: Desorption equilibrium time data for all soils

Following the 48 hour test, the applicant conducted a stability test on the adsorption supernatant and soil for each soil. The total radioactivity recovered from the supernatant and soil ranged between 90.6-97.7% AR. This was then quantified by HPLC of which a total of 90.1-96.6% was determined as [<sup>14</sup>C]3'-OH-S-2840. The total recovery of the test substance was therefore between 98.8-99.4%. HSE agrees with the applicant that the high recovery of [<sup>14</sup>C]3'-OH-S-2840 (>98.8%) in all soils demonstrate stability of the test substance.

#### Tier 3

Based on the goodness-of-fit criteria outlined in the EFSA OECD 106 checklist, HSE performed all relevant quality checks as part of confirming the acceptability of the study and of the reported endpoints. A table summarising the checklist criteria is given in Table B.8.1.5.2-4 below. These checks confirmed that the mass balance of [<sup>14</sup>C]3'-OH-S-2840 was >90% (90.1-96.6%), and % adsorption was >50% (53.6-86.8%) and therefore were acceptable. HSE calculated the  $K_{FE} / K_F$  ratio as a method for ascertaining the impact of any systematic errors. As the preliminary stability test mass balance was determined using radio-HPLC, HSE has calculated  $K_{FE}/K_F$  using these recovery values. In all results the value of this ratio are <1.2 (maximum 1.147, Quilen), this suggests low potential for systematic errors. Therefore, HSE considers any potential systematic error would not have significant impact on the study results and finds this acceptable. All  $R^2$  values are > 0.99 indicating a strong goodness-of-fit of the data to the linear regression isotherms. Additionally, the  $K_d$  \* soil:solution ratio is >0.3 in all soils (minimum of 1.012) indicating the indirect method used was appropriate as per the OECD checklist.

**Table B.8.1.5.2-4: Summary of OECD checklist criteria for [<sup>14</sup>C]3'-OH-S-2840 in 3 soils**

Soil	Quilen	Hareby	Clipstone
Soil:solution ratio	1:5	1:5	1:2
Radioactive mass balance of test item from preliminary stability test (%)	90.1	96.6	96.4
Mean adsorption (%)	76.4 – 86.7	53.7 – 60.0	70.2 – 74.3

<b>K<sub>d</sub> * soil:solution ratio</b>	3.24 – 6.54	1.16 – 1.5	2.36 – 2.90
<b>K<sub>F</sub></b>	14.26	5.58	4.81
<b>1/n</b>	0.8791	9.9561	0.9729
<b>R<sup>2</sup></b>	0.9942	0.9993	0.9983
<b>K<sub>Foc</sub></b>	492	439	401
<b>K<sub>FE</sub> / K<sub>F</sub> at highest concentration</b>	1.147	1.066	1.053

The % adsorption and % desorption are shown in Table B.8.1.5.2-05 below. The measured concentrations in the adsorption supernatant along with soil concentrations calculated indirectly by the applicant are presented in Table B.8.1.5.2-6 to B.8.1.5.2-8 below.

**Table B.8.1.5.2-5: Proportions of [<sup>14</sup>C]3'-OH-S-2840 adsorbed to and desorbed from three soils**

<b>Soil</b>	<b>Nominal Concentration (µg/mL)</b>				
	<b>0.5</b>	<b>0.1</b>	<b>0.05</b>	<b>0.01</b>	<b>0.005</b>
<b>Mean % adsorption (%)</b>					
Quilen	76.4	84.5	85.7	84.8	86.7
Hareby	53.7	56.5	57.4	60.0	57.9
Clipstone	70.2	73.8	74.3	74.3	72.7
<b>Mean % desorption (%)</b>					
Quilen	13.4	12.2	11.8	10.7	8.7
Hareby	31.6	34.1	34.7	30.1	32.0
Clipstone	15.7	15.6	15.0	15.8	15.0

**Table B.8.1.5.2-6: Concentrations of [<sup>14</sup>C]3'-OH-S-2840 equivalents (based on distribution of radioactivity and indirectly calculated) in Quilen soil**

<b>Nominal Concentration (µg/mL)</b>	<b>Adsorption</b>		<b>Desorption</b>	
	<b>C<sub>e</sub> (µg/mL)</b>	<b>X/m (µg/g)</b>	<b>C<sub>1</sub> (µg/mL)</b>	<b>X<sub>1</sub>/m (µg/g)</b>
0.5	0.1120	1.8284	0.0551	1.5914
	0.1142	1.8353	0.0592	1.5815
0.1	0.0157	0.4242	0.0110	0.3722
	0.0157	0.4288	0.0111	0.3767
0.05	0.0075	0.2207	0.0055	0.1941
	0.0073	0.2193	0.0053	0.1939
0.01	0.0017	0.0465	0.0011	0.0414
	0.0016	0.0466	0.0010	0.0418
0.005	0.0007	0.0223	0.0004	0.0203
	0.0007	0.0223	0.0004	0.0204

**Table B.8.1.5.2-7: Concentrations of [<sup>14</sup>C]3'-OH-S-2840 equivalents (based on distribution of radioactivity and indirectly calculated) in Hareby soil**

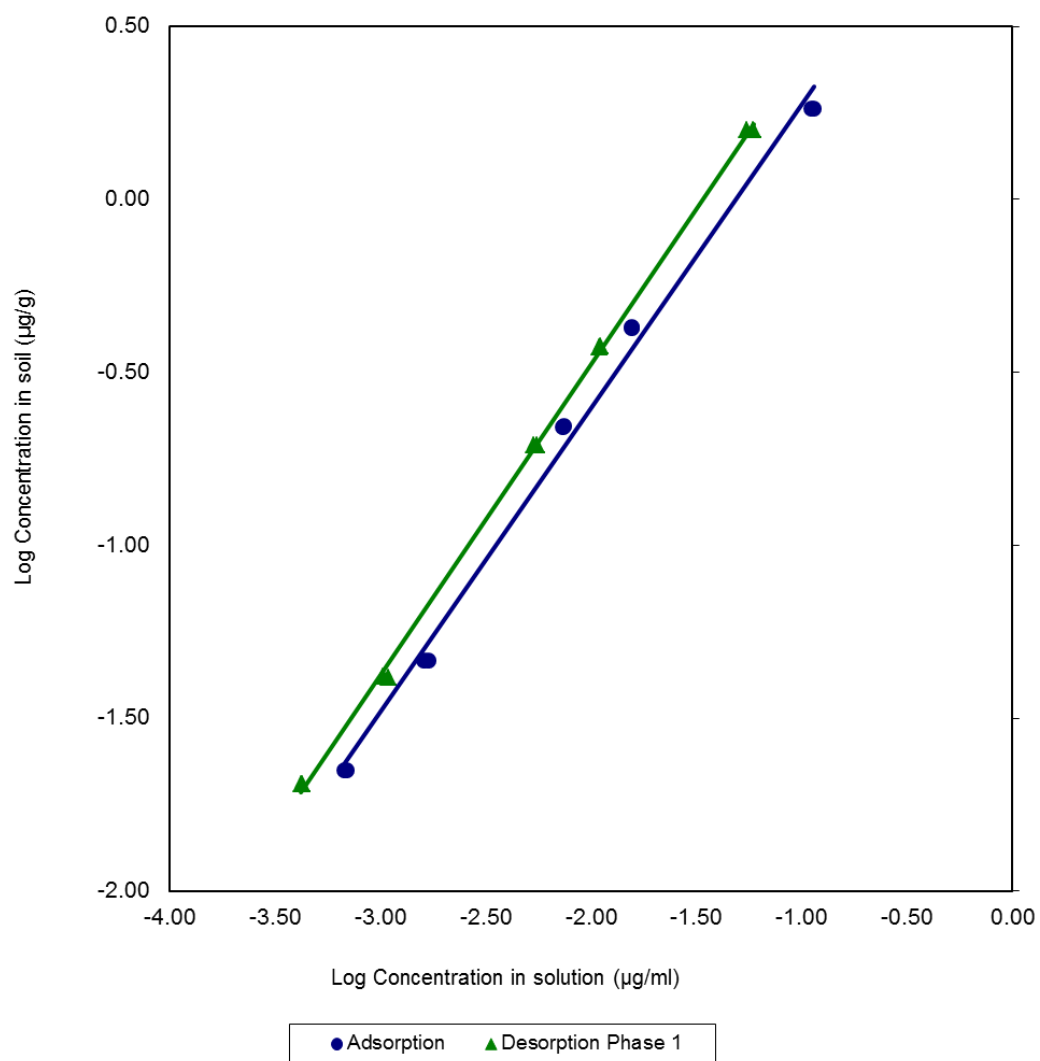
<b>Nominal Concentration (µg/mL)</b>	<b>Adsorption</b>		<b>Desorption</b>	
	<b>C<sub>e</sub> (µg/mL)</b>	<b>X/m (µg/g)</b>	<b>C<sub>1</sub> (µg/mL)</b>	<b>X<sub>1</sub>/m (µg/g)</b>
0.5	0.2228	1.2886	0.0944	0.8888
	0.2240	1.3060	0.0990	0.8847
0.1	0.0443	0.2849	0.0216	0.1888
	0.0436	0.2864	0.0221	0.1876
0.05	0.0219	0.1466	0.0114	0.0951
	0.0220	0.1488	0.0113	0.0980
0.01	0.0043	0.0321	0.0022	0.0225
	0.0043	0.0323	0.0022	0.0226

0.005	0.0022	0.0147	0.0010	0.0101
	0.0022	0.0150	0.0011	0.0101

**Table B.8.1.5.2-8: Concentrations of [<sup>14</sup>C]3'-OH-S-2840 equivalents (based on distribution of radioactivity and indirectly calculated) in Clipstone soil**

<b>Nominal Concentration (µg/mL)</b>	<b>Adsorption</b>		<b>Desorption</b>	
	<b>C<sub>e</sub> (µg/mL)</b>	<b>X/m (µg/g)</b>	<b>C<sub>1</sub> (µg/mL)</b>	<b>X<sub>1</sub>/m (µg/g)</b>
0.5	0.1439	0.6727	0.0681	0.5653
	0.1433	0.6811	0.0666	0.5765
0.1	0.0266	0.1488	0.0140	0.1255
	0.0264	0.1487	0.0139	0.1257
0.05	0.0135	0.0758	0.0069	0.0645
	0.0130	0.0777	0.0070	0.0660
0.01	0.0028	0.0159	0.0014	0.0135
	0.0027	0.0160	0.0015	0.0133
0.005	0.0014	0.0074	0.0007	0.0064
	0.0014	0.0075	0.0007	0.0064

The applicant has calculated the adsorption and desorption isotherms relying on measured supernatants and indirectly calculated soil adsorption data. The calculations for indirect soil adsorption were evaluated by HSE and deemed acceptable. The isotherms and residuals are presented in Figure B.8.1.5.2-3 to Figure B.8.1.5.2-8 below. HSE notes the applicant did not provide residuals for the isotherms, therefore results presented were produced by HSE. The applicant calculated the Freundlich coefficient ( $K_F$ ) and Freundlich exponent ( $1/n$ ) for each adsorption/desorption isotherm of each soil. The overall sorption characteristics of [<sup>14</sup>C]3'-OH-S-2840 are shown in Table B.8.1.5.2-9. HSE used the OECD calculator tool (v2) to repeat and confirm the Freundlich coefficient isotherm calculations and other calculated values provided by the applicant. All repeated calculations resulted in values that were identical or with acceptable similarity. HSE could not identify a reason for minor differences in reproducing the applicant calculations..



**Figure B.8.1.5.2-3: Adsorption and desorption isotherms for [ $^{14}\text{C}$ ]3'-OH-S-2840 on Quilen soil ( $R^2$  of 0.9942)**

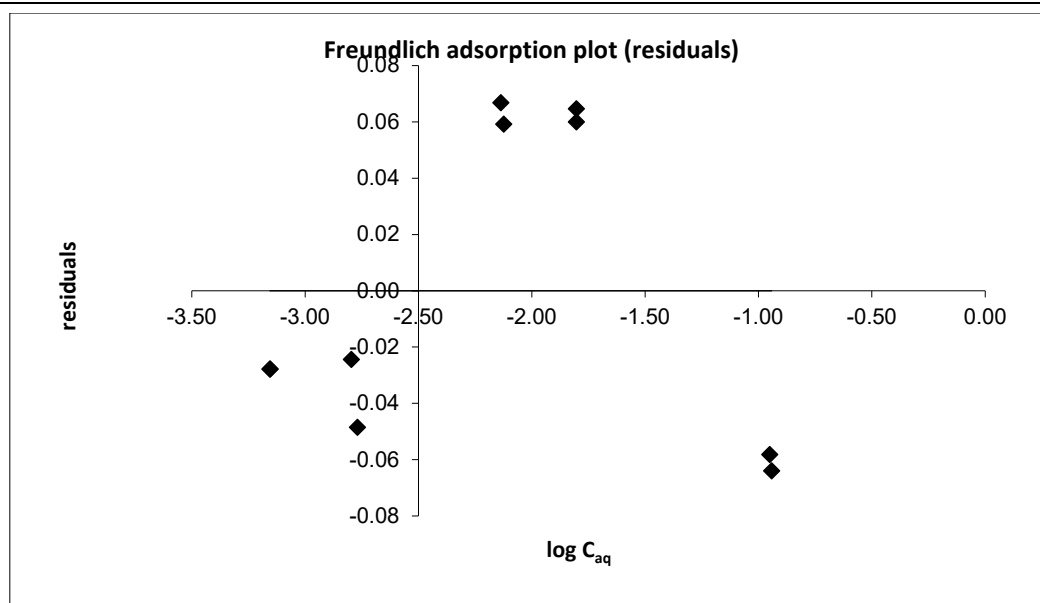
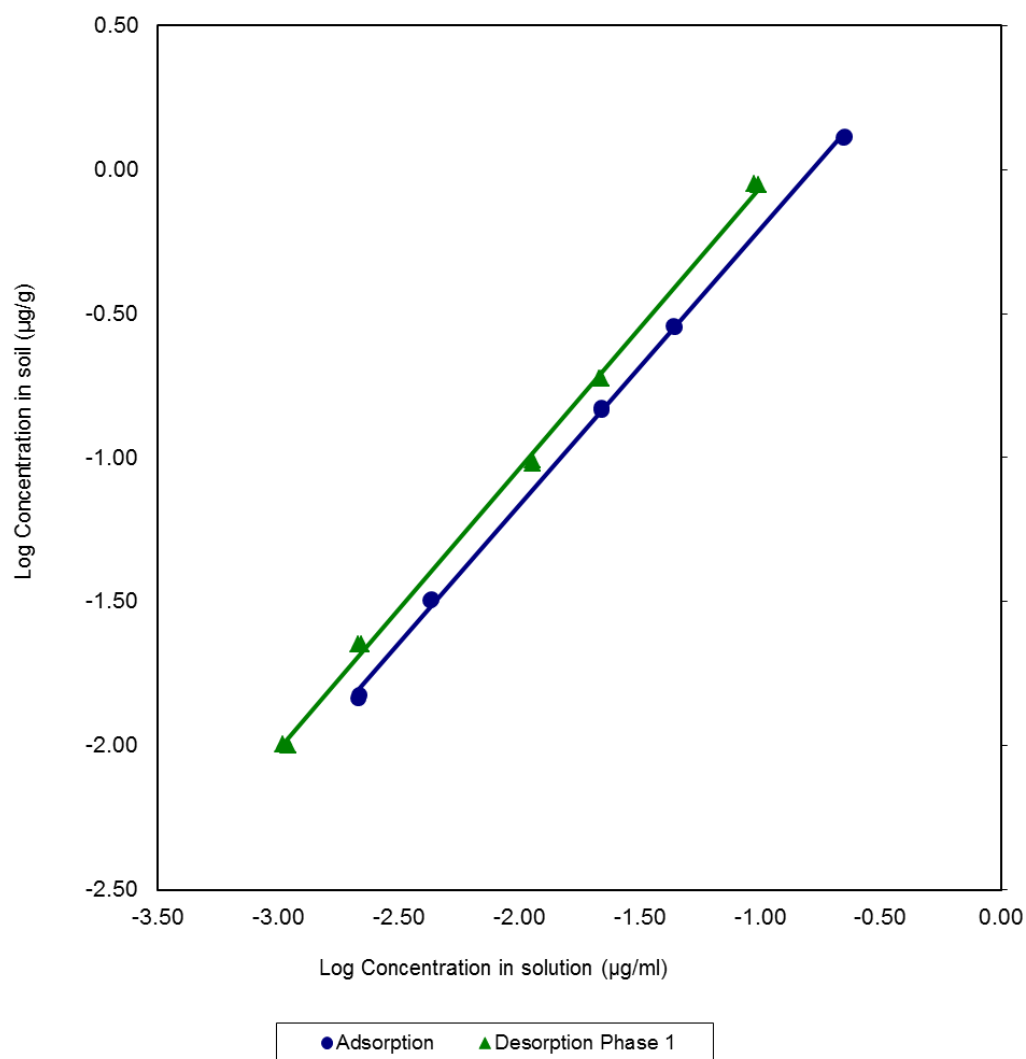
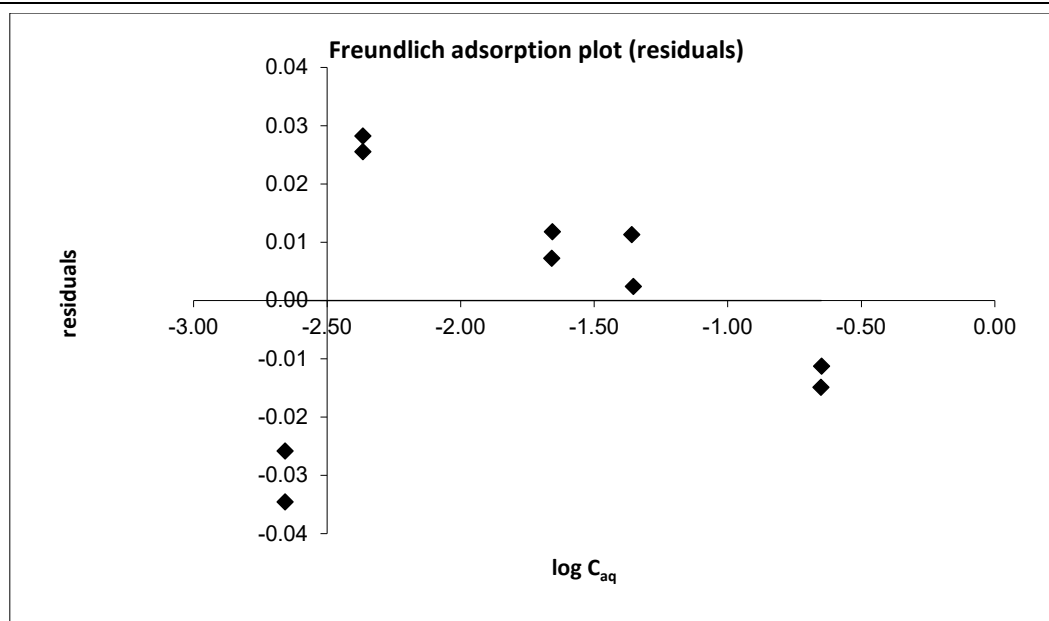


Figure B.8.1.5.2-4: Residuals of adsorption isotherm for [ $^{14}\text{C}$ ]3'-OH-S-2840 on Quilen soil (HSE results)

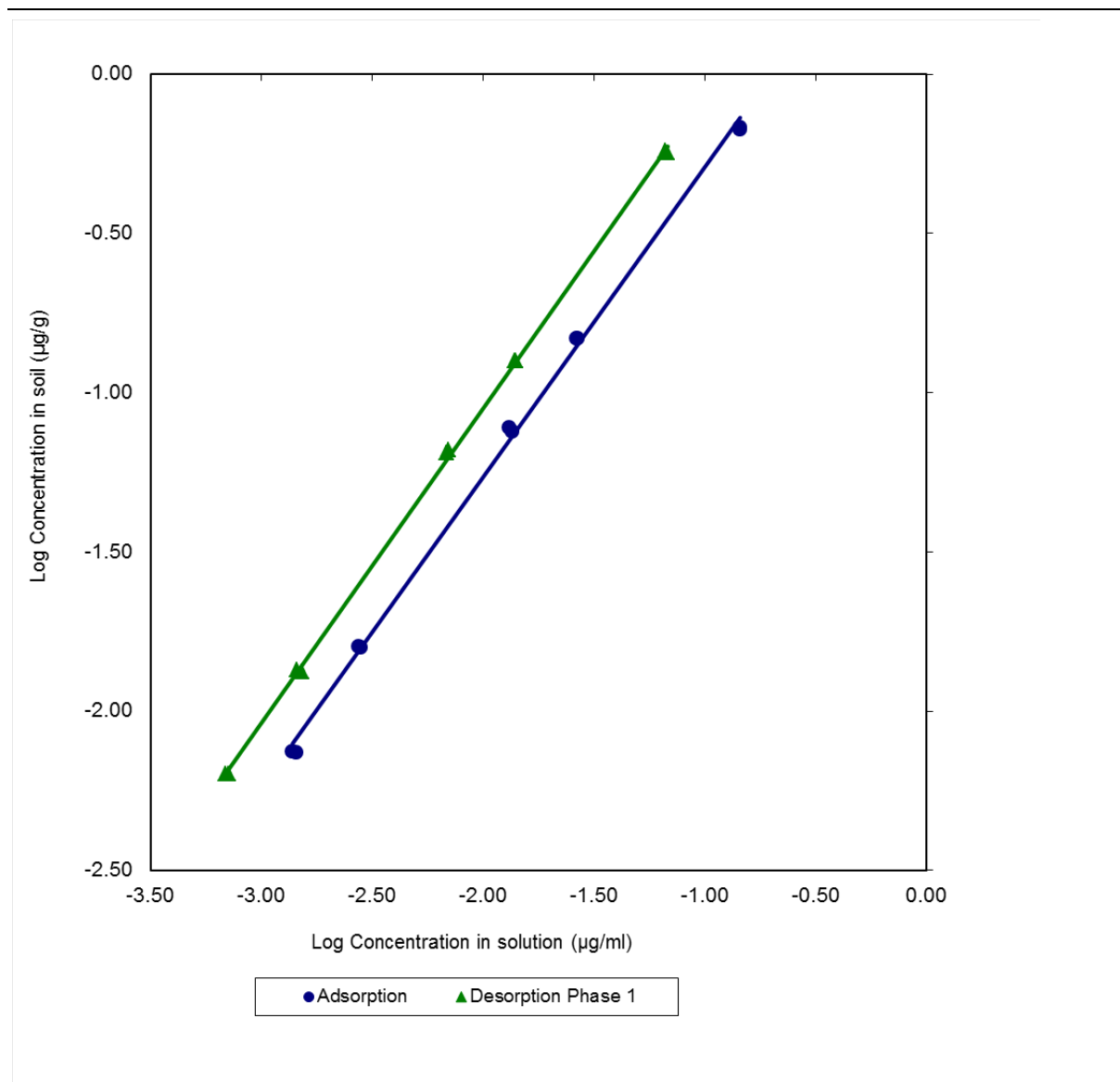


**Figure B.8.1.5.2-5: Adsorption and desorption isotherms for [ $^{14}\text{C}$ ]3'-OH-S-2840 on Hareby soil ( $R^2$  of 0.9993)**

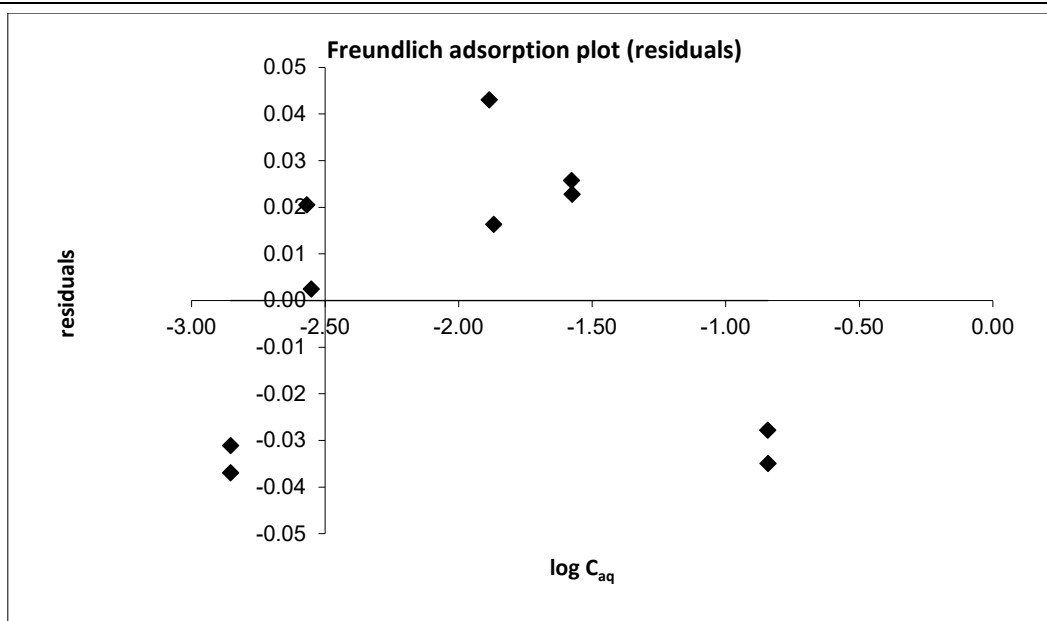




**Figure B.8.1.5.2-6: Residuals of adsorption isotherm for [ $^{14}\text{C}$ ]3'-OH-S-2840 on Hareby soil (HSE results)**



**Figure B.8.1.5.2-7: Adsorption and desorption isotherms for [ $^{14}\text{C}$ ]3'-OH-S-2840 on Clipstone soil ( $R^2$  of 0.9983)**



**Figure B.8.1.5.2-8: Residuals of adsorption isotherm for [ $^{14}\text{C}$ ]3'-OH-S-2840 on Clipstone soil (HSE results)**

**Table B.8.1.5.2-9: Adsorption and desorption characteristics of [<sup>14</sup>C]3'-OH-S-2840 on three soils**

Soil	Organic carbon (%)	pH (CaCl <sub>2</sub> )	K <sub>F</sub> <sup>ads</sup>	K <sub>Foc</sub>	1/n	r <sup>2</sup>
<b>Adsorption</b>						
Quilen	2.9	7.3	14.26	492	0.8791	0.9942
Hareby	1.6	7.4	5.58	349	0.9561	0.9993
Clipstone	1.2	5.5	4.81	401	0.9729	0.9983
Geometric mean				<b>410</b>		
Arithmetic mean					<b>0.936</b>	
<b>Desorption</b>			K <sub>F</sub> <sup>des</sup>	K <sub>Foc-des</sub>	1/n	r <sup>2</sup>
Quilen	2.9	7.3	20.97	723	0.8992	0.9991
Hareby	1.6	7.4	8.22	514	0.9765	0.9984
Clipstone	1.2	5.5	8.44	703	0.9873	0.9996

K<sub>F</sub><sup>ads/des</sup> = Freundlich adsorption/desorption partition coefficient

K<sub>Foc</sub> = Coefficient of adsorption per unit organic carbon

K<sub>Foc-des</sub> = Coefficient of desorption per unit organic carbon

1/n = Slope of the Freundlich adsorption isotherm

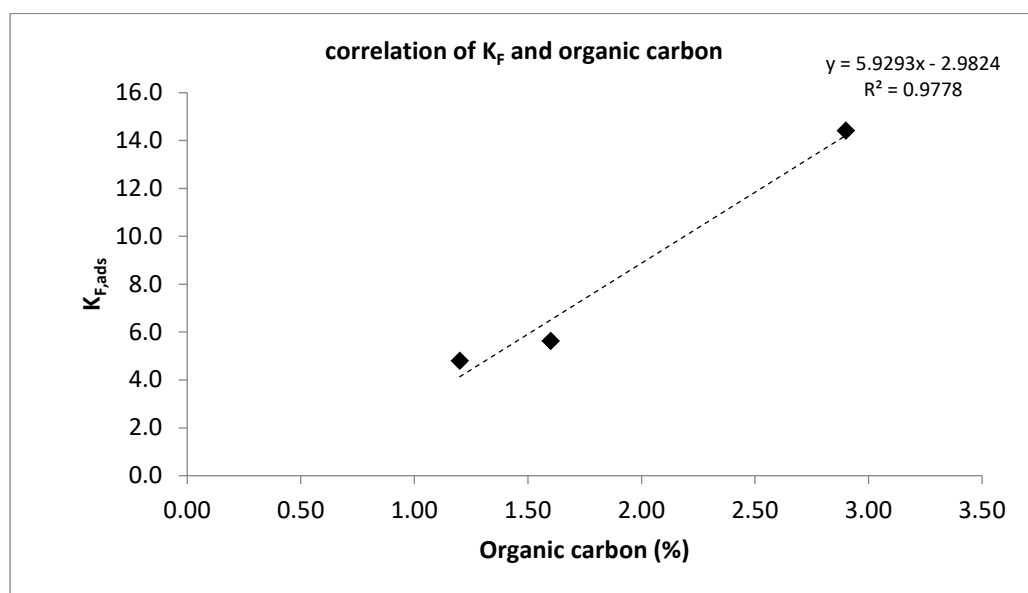
The acceptability of the analytical method was confirmed over the entire range of concentrations measured (reported LOD/LOQ of 1% AR equivalent to 0.0001 mg/L). HSE confirmed the use of the indirect method was appropriate based on a K<sub>d</sub> \* soil/solution ratio >0.3 in all soils. The graphical fits of the Freundlich equation are presented above based on the standard linear regression form using log-log transformed data alongside the associated residual plots. The R<sup>2</sup> of the standard linear regressions ranged from 0.991 to 0.9996 and the visual fit of both the standard regression and the residual plots were acceptable.

HSE notes there were minor differences between the applicant reported K<sub>Foc</sub> and 1/n values to those calculated by HSE using the OECD 106 calculator tool. All differences in 1/n were <0.01 and are therefore insignificant. For K<sub>Foc</sub>, the largest difference in HSE calculated and applicant reported values was 5.2 in a single soil (Quilen). As the K<sub>Foc</sub> is relatively high, a difference of 5.2 is unlikely to impact outcomes in subsequent calculations and modelling at later stages when used as an endpoint. Additionally, the difference in the HSE calculated K<sub>Foc</sub> geomean (412.1 L/kg) and that reported by the applicant (410 L/kg) was smaller (2.1 L/kg difference).

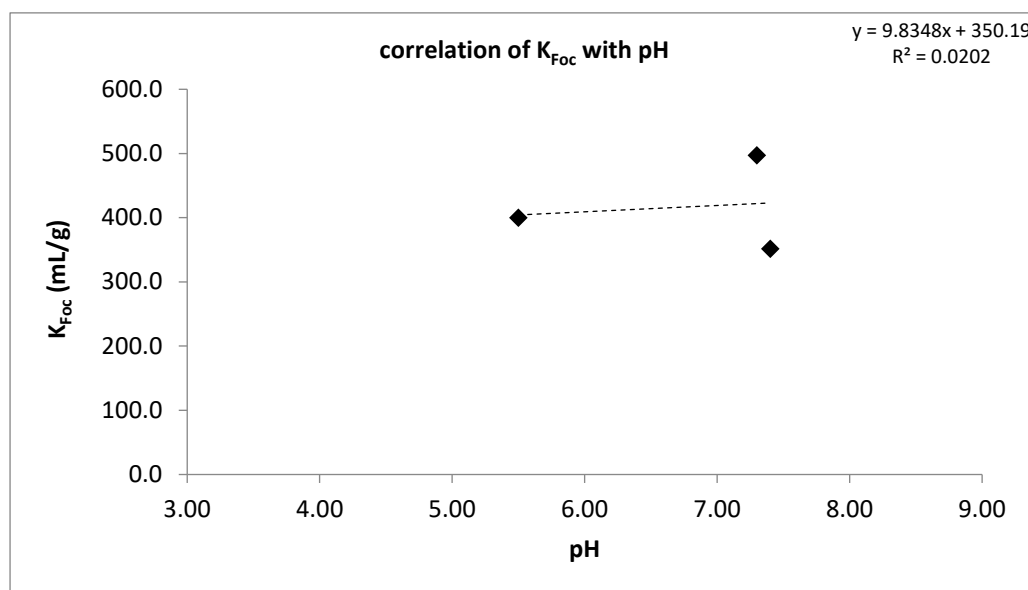
Therefore, HSE concludes these differences are not significant and therefore the reported values are acceptable.

#### Investigation of pH dependence

Correlations between  $K_F$  and organic carbon and  $K_{Foc}$  with pH were assessed below in Figure B.8.1.5.2-9 and B.8.1.5.2-10.



**Figure B.8.1.5.2-9: Correlation of  $K_F$  and organic carbon in 3 soils produced by HSE using the OECD 106 calculator tool**



**Figure B.8.1.5.2-10: Correlation of  $K_{foc}$  with pH in 3 soils produced by HSE using the OECD 106 calculator tool**

All data was subsequently checked with the OECD spreadsheet calculator and the existing results were considered to be valid. Results in the linear regression model of pH and  $K_{\text{foc}}$  indicate that there was no evidence of pH dependence; however, the model showed poor goodness of fit ( $r^2 = 0.0202$ ). Additionally, HSE calculated a Kendall rank correlation coefficient for  $K_{\text{oc}}$  of -0.333 using the German support tool (pH Dependence Calculator). This indicates a moderate negative correlation between the pH and sorption. As the corresponding p-value (1.00) was  $>0.05$ , any correlation cannot be considered as statistically significant. As only 3 soils have been tested, HSE finds that further statistical testing for pH dependence would not produce reliable or meaningful statistical conclusions. Consideration of the mechanistic characteristics of the molecule is presented below.

Values for log  $K_{\text{ow}}$ , for molecules generally, typically range between -3 (very hydrolytic) and +10 (extremely lipophilic/hydrophobic). The data for log  $K_{\text{ow}}$  (see Section B.1, [REDACTED] 2016a) show that there is a slight tendency towards the metabolite being more hydrophilic at a pH value of 6.5; however, no further data is provided at any other pH. Furthermore, no data on the solubility, hydrolytic stability or the pKa of the metabolite have been provided. In terms of molecular structural, the 3'-OH-S-2840 metabolite is structurally identical to the parent substance except for the replacement of a hydrogen by an -OH functional group. The difference in the molecule leads to a reduction in log  $K_{\text{ow}}$  compared to the parent (3.65 at pH 7.1-7.3) indicating a slight increase of hydrophilic properties. There is no data or mechanistic reason to indicate pH dependent sorption.

For the parent substance, which is structurally similar, it is noted however that studies submitted in the Section B.1 demonstrate that the solubility of the parent does not change across the environmentally relevant pH range. Similarly, there is no hydrolysis of inpyrfluxam at 50 °C and pH 4, 7 or 9. While the overall structure of the metabolite is extremely similar to that of the parent, given the complexity of the molecule, it is difficult to confirm how much the properties of the metabolite deviate from that of the parent. However, it is concluded that based on the information available, neither the statistical or mechanistic data indicate that metabolite is readily ionisable.

Therefore, consideration of pH dependence for 3'-OH-S-2840 in the exposure assessment is not required.

## CONCLUSION

Adsorption  $K_{\text{foc}}$  values ranged from 349 to 492 L/kg with a **geomean of 410 L/kg** (Freundlich exponent (1/n) from 0.8791 to 0.9729 with an arithmetic **mean of 0.936**) and desorption  $K_{\text{foc}}$  desorption values ranged from 514 to 723 L/kg (Freundlich exponent (1/n) from 0.8992 to 0.9873). These values were slightly higher than the

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corresponding adsorption coefficients indicating a degree of irreversibility to the adsorption. There was no evidence of any pH dependence.

HSE repeated the Freundlich coefficient isotherm calculations by inputting the applicant raw data into the HSE OECD 106 calculation tool (v2). HSE reproduced the results presented by the applicant with only minor differences. ( $<0.01$  for  $1/n$  values;  $<0.2$  for  $K_F$ ). Factoring in the overall quality criteria on the goodness of fit and parameter reliability, HSE finds the applicant conclusions acceptable.

**B.8.1.5.3. Adsorption and desorption in soil of metabolite 1'-COOH-S-2840**

<b>Data Point:</b>	KCA 7.1.3.1.2/01
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2017a
<b>Report Title:</b>	[ <sup>14</sup> C]1'-COOH-S-2840A and B: Adsorption/Desorption in Soil
<b>Study number</b>	3201397
<b>Guideline(s) followed in study:</b>	OECD Test Guideline No 106 (January 2000)
<b>GLP?</b>	Yes

<b>Deviations from guideline</b>	<b>HSE assessment of deviations</b>
It is not stated if the stock solutions and application solutions were prepared immediately before use or were stored, and if so the storage conditions and duration.	This is considered to be a minor omission as the stability of the test item in the test system was verified and mass balances were >90 %.
<b>HSE conclusion</b>	
The study is accepted as suitable for use in the risk assessment.	



## INTRODUCTION

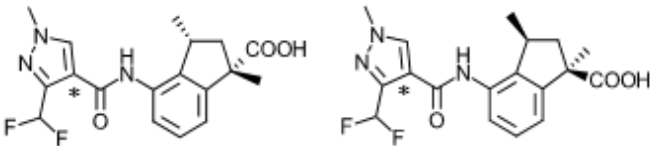
The adsorption and desorption behaviour of [ $^{14}\text{C}$ ]1'-COOH-S-2840A and B, a metabolite of inpyrfluxam, was studied in five soils in batch equilibrium experiments in the dark in the laboratory at  $20 \pm 2$  °C. The study followed the OECD Guideline No. 106, and was conducted to Good Laboratory Practice (GLP) standards. 1'-COOH-S-2840A and B was applied at nominal test concentrations of 1, 0.5, 0.1, 0.05 and 0.01 µg/mL in aqueous 0.01 M  $\text{CaCl}_2$  solution; the test therefore covered a concentration range across two orders of magnitude in accordance with guideline requirements.

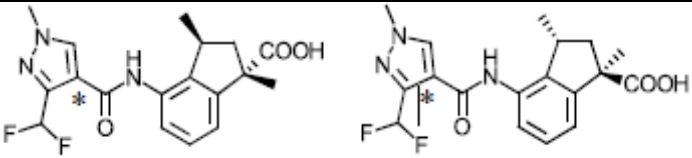
Metabolite 1'-COOH-S-2840 consists of two diastereomers, 1'-COOH-S-2840A and 1'-COOH-S-2840B. As these diastereomers may have different physical and chemical properties, 1'-COOH-S-2840A and 1'-COOH-S-2840B sorption parameters have been calculated for both separately within the study.

## MATERIALS AND METHODS

### I. Test item

**Table B.8.1.5.3-01 Radiolabelled test substance**

<b>[Pyrazolyl-4-<math>^{14}\text{C}</math>]1'-COOH-S-2840A</b>	 <p>Radiolabel position denoted by *</p>
<b>Specific activity</b>	5.81 MBq/mg (2.11 GBq/mmol)
<b>Radiochemical purity</b>	99.5 % (stated)

<b>[Pyrazolyl-4-<math>^{14}\text{C}</math>]1'-COOH-S-2840B</b>	 <p>Radiolabel position denoted by *</p>
<b>Specific activity</b>	5.81 MBq/mg (2.11 GBq/mmol)
<b>Radiochemical purity</b>	99.4 % (stated)

**Table B.8.1.5.3-02 Non-Radiolabelled test substance**

<b>Name</b>	1'-COOH-S-2840A
<b>Chemical purity</b>	100 % w/w

<b>Name</b>	1'-COOH-S-2840B
<b>Chemical purity</b>	99.6 % w/w

**II. Test soils**

Five soils with varying characteristics were included in the test.

**Table B.8.1.5.3-03 Characteristics of test soils used in the adsorption and desorption study of [<sup>14</sup>C] 1'-COOH-S-2840A and B**

	<b>Quilen</b>	<b>Hareby</b>	<b>Clipstone</b>	<b>Speyer 2.3</b>	<b>Atwater</b>
<b>Soil code</b>	A	B	C	D	E
<b>Sampling Location</b>	Pas de Calais, France	Lincolnshire, UK	Nottinghamshire, UK	Rheinland-Pfalz, Germany	California, USA
<b>Land use</b>	Grassland	Grassland	Grassland	Uncultivated	Unknown
<b>Pesticide use history</b>	None in last 5 years	None in last 10 years	None in last 5 years	None in last 5 years	None in last 10 years
<b>Sampling Depth</b>	10-20 cm	4-10 cm	5-15 cm	Ca 20 cm	0-3 inches (ca.7.5 cm)
<b>Particle size(%w/w)<sup>1</sup></b>					

<b>Clay (&lt;2µm) (%)</b>	20	34	6	6	15
<b>Silt (2–50µm) (%)</b>	50	33	8	34	13
<b>Sand (50–2000µm) (%)</b>	30	33	86	60	72
<b>Texture (USDA)</b>	Silt loam or Loam	Clay loam	Loamy sand	Sandy loam	Sandy loam
<b>pH (0.01 M CaCl<sub>2</sub>)</b>	7.3	7.4	5.5	5.8	7.0
<b>Organic Matter (%)</b>	5.0	2.8	2.1	1.2	0.5
<b>Organic Carbon (%)</b>	2.9	1.6	1.2	0.7	0.3
<b>CEC<sup>2</sup> (meq/100 g soil)</b>	26.1	12.2	7.8	7.3	10.3

<sup>1</sup>USDA particle size distribution

<sup>2</sup>Cation Exchange Capacity

The pH (CaCl<sub>2</sub>) ranges between 5.5 and 7.4 with both acidic and basic soils included; this is similar to the range of 4.5 to 7.5 given in the OECD 106 Guideline. The organic carbon content ranges between 0.5 and 5.0 % and the clay content between 6 and 34 %. It is noted that no very high clay soils are included, but overall HSE is satisfied that the soils show a sufficiently wide range of soil properties.

Information regarding the nature of the sites, land use and pesticide use history was supplied. No details of conditions during soil transport were given, but as soil properties were tested after arrival at the test facility, this is not considered to be a major deviation. Samples were air dried, sieved (2 mm), thoroughly mixed and stored in the dark at room temperature in loosely tied plastic bags until analysis. The test item was applied on 22 August 2016 according to the Quality Assurance Statement, implying a soil storage time of up to 2 years. The Guideline states that soils stored for 3 years can be used providing that soil properties are re-determined

prior to testing. HSE accept that the reported soil properties are likely to be representative of the soils at the time the soils were dosed.

Moisture contents of stored soils were determined by drying a portion (ca. 105 °C). The wet : dry weight ratio was calculated to allow the dry weight equivalent of the dispensed soil to be calculated from the measured wet weight. All calculations were based on soil dry weight.

## STUDY DESIGN

### I. Experimental Conditions

Separate stock solutions containing 1'-COOH-S-2840A (0.158 mg/L, SS1A) and 1'-COOH-S-2840B (0.157 mg/L, SS1B) were available in acetonitrile; concentrations were determined by liquid scintillation counting (LSC). These solutions were used directly for the solubility test. Application solutions were used to assess adsorption to the test vessels (application solution 2). Aliquots of SS1A and SS1B (both 6 µL) were reduced to dryness under nitrogen and dissolved in calcium chloride solution (0.01 M; 10 mL). Solution concentrations were determined by LSC to be 0.093 µg 1'-COOH-S-2840A/L and 0.095 µg 1'-COOH-S-2840B/L. Further application solutions and the concentration of the solutions determined by LSC as 10.29 µg 1'-COOH-S-2840A/L and 10.06 µg 1'-COOH-S-2840B/L.

Five concentrations spanning two orders of magnitude were used in the definitive test. For both 1'-COOH-S-2840 A and B, stock solution (1.6 mL) were dissolved in calcium chloride solution (0.01 M) to produce a nominal treatment solution concentration of 10 µg/mL, with further concentrations prepared by serial dilution. No additional solvent was used.

Storage conditions and duration of the stock solutions and application solutions were not stated. The radiochemical purity of selected stock and application solutions was checked prior to use with HPLC and TLC and found to be ≥ 98.0% for 1'-COOH-S-2840A and ≥ 98.2% for 1'-COOH-S-2840B.

Preliminary tests were conducted to determine solubility of the test items at concentrations of 10 µg/mL in 0.01 M calcium chloride solution, adsorption levels to plastic and Teflon® tubes and the adsorption and desorption equilibrium over 48 and 72 h respectively. The stability of the test item in 0.01 M calcium chloride solution over 48 h was also investigated for all 5 soils.

- Solubility of both isomers (10 µg/mL) was assessed in glass vessels containing calcium chloride solution (0.01 M). Stock Solution 1 (A) (630 µL) or Stock Solution 1 (B) (640 µL) was added to the vessel, the solvent evaporated to dryness under nitrogen and the dried residue dissolved in CaCl<sub>2</sub> solution (0.01 M). Recovery in the solution was determined immediately by LSC.

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- Adsorption to both plastic and Teflon® containers was tested at nominal test substance concentrations of 0.01 µg/L in calcium chloride solution (0.01 M) or application solution 2 (1 mL) in calcium chloride solution (0.01 M, 9 mL). Radioactivity in solution was assessed before and after shaking (24 h).
  - The recovery of [<sup>14</sup>C]1'-COOH-S-2840A and [<sup>14</sup>C]1'-COOH-S-2840B was tested in duplicate for all soil types following a contact time of 48 h. Vessels were removed from the shaker and centrifuged (9 minutes, 4750 rpm (ca 5000 g). It has been considered whether the centrifugation time and speed were appropriate for the density of the soil. No soil bulk densities were given in the study report, but a general internet search suggests densities of 1.3 to 1.6 g cm<sup>-3</sup> for the soil textures included in the study. While these soil bulk densities should be regarded with caution and are only indicative, Figure 1a in the OECD 106 Guideline suggests that a centrifugation time of around 1 h would be appropriate for a centrifugation speed of 5000 rpm; consequently the shaking time and speed may not have been sufficient for these soil textures. Some colloids may therefore have remained in the aqueous phase and been subjected to analysis together with the aqueous phase so that their impact is lost. As this will result in a lower K<sub>foc</sub> and therefore be conservative, this has not been considered further. The supernatant was decanted and the remaining soil extracted by shaking (20 minutes, x3, acetone : 0.5 M HCl (4 : 1 v/v, 25 mL)). Overall radioactivity in the extracts was determined. The extracts were also concentrated for HPLC analysis and recovery of radioactivity specifically attributed to 1'-COOH-S-2840A and B specifically determined. The direct method was therefore used in the preliminary test. The weight of the supernatant was determined from the total weight of the soil added and the solution, by subtracting the dry soil weight. Consequently the weight of CaCl<sub>2</sub> quoted as extracted from soil includes the water entrained in the soil pellet. The liquid entrained in the soil pellet has however been accounted for in the desorption calculations. Weights were converted to volume assuming a density of 1.00 g/mL.
  - The optimum equilibration time was determined using a 1 : 1 w/v soil to aqueous phase ratio and initial concentrations of 1 µg/mL. Solutions were shaken for 3, 6, 24 and 48 h after which samples were centrifuged (9 minutes, 4750 rpm (ca 5000 g) and an aliquot removed for radioactivity determination. The volume removed was replaced with an equivalent volume of fresh CaCl<sub>2</sub> solution (0.01 M), the soil and solution re-mixed and the samples returned to the shaker. Desorption equilibration time was determined using duplicate vessels for each soil. After an adsorption period of 48 h, samples were centrifuged and the supernatants decanted and replaced with an equivalent volume of fresh calcium chloride solution (0.01 M; ca 5 mL). Samples were shaken vigorously to break up the soil pellet and re-mix it with the new solution and the samples shaken for 6, 24, 48 and 72 hours. Radioactivity was then determined in the supernatant.

A soil:solution ratio of 1:1 was used in the definitive test. Following the preliminary tests, for the definitive tests, soils (10 g) were dispensed into pre-weighed TEFLON® tubes and equilibrated with 0.01 M calcium chloride solution (9 mL) overnight before treatment. Duplicate soil samples were used for each soil and test concentration. On the day of the test, treatment solutions (1 mL) were added to the equilibrated vessels. Following shaking (48 h), samples were centrifuged (9 minutes; 4750 rpm(ca. 5000 g)) and the supernatant decanted. Weighed aliquots were analysed by LSC and the pH of the supernatant determined.

For the desorption phase, the removed supernatant was replaced with an equal weight of fresh 0.01 M calcium chloride solution. The soil pellet was broken up and re-mixed with the solution by vigorous shaking. Samples were then shaken (48 h), centrifuged (9 minutes; 4750 rpm(ca. 5000 g)). The mass balance was determined on the post-desorption samples for each solution type at the highest concentration, from triplicate sub-samples (ca 0.2 g). The soil samples were rinsed with acetone, air-dried and ground. Soil samples were combusted and the radioactivity in both the soil pellet and the supernatant determined using LSC.

HPLC and TLC were used to determine stability and radiochemical purity. Samples were co-chromatographed with non-radiolabelled 1'-COOH-S-2840A and 1'-COOH-S-2840B. Following a request for additional information, the applicant clarified that the LOD as ~0.1 % AR based on observation of a chromatogram. No LOQ was given. The adsorption checklist, 'Outcome of the pesticides peer review meeting on the OECD 106 evaluators checklist, EFSA 2017' states that the LOQ should be at least two orders of magnitude below the lowest nominal concentration; the lowest test concentration is 0.01 µg/mL so the LOQ should be 0.0001 µg/mL or lower. The LOD is three orders of magnitude below the lowest concentration, (0.00001 µg/mL), but it might be expected that the LOQ would be slightly higher. (Although not explicitly stated, the LOQ is often calculated by 10 x standard deviation of response or slope and therefore may be 0.00003 µg/mL.) While the LOQ is not stated, it is therefore considered that the method is sufficiently sensitive.

The efficiency of the oxidiser and carry-over between samples were checked at regular intervals during a day's testing using <sup>14</sup>C standards. Combustion efficiencies were 99 ± 5% and therefore no correction factors were applied to reported data. The limit of detection (LOD) for the LSC was taken as 1.5 times the background radioactivity, determined by counting samples with no radioactivity in the same batch as the samples. Following a request for additional information, the applicant clarified that the LOD was equal to <0.5 % AR. The LOQ has not been given.

In the definitive test, weights of supernatants were determined from the total weights of soil plus solution by subtracting the weights of the dry soil (indirect method).

## RESULTS

### I. Preliminary tests

Both [ $^{14}\text{C}$ ]1'-COOH-S-2840A and [ $^{14}\text{C}$ ]1'-COOH-S-2840B were soluble at a concentration of 10 µg/L. LSC analysis demonstrated that ≥97 % AR was recovered from the solution.

Over 24 h, there was no adsorption to Teflon® tubes (recovery ≥98.6 % AR), but slight adsorption to plastic tubes (recovery of test item ≥90.9 % AR). The applicant therefore selected Teflon® tubes, which is accepted by HSE.

**Table B. 8.1.5.3-04 Percentage test item in solution in plastic and Teflon® tubes over 24 h**

Tube type	Soluble 1'-COOH-S-2840A (%)		Soluble 1'-COOH-S-2840B (%)	
	0 h	24 h (after shaking)	0 h	24 h (after shaking)
Plastic	100.3, 93.4	96.0, 95.7	97.8, 94.9	93.3, 90.9
Teflon	101.4, 99.7	104.9, 99.3	101.0, 102.5	99.8, 98.6

The applicant selected a 1:1 w/v soil solution ratio and a concentration of 1 µg/L for the test item. No other soil:solution ratios were tested, but as this ratio is generally accepted as being most appropriate for poorly adsorbed substances, this is accepted by HSE.

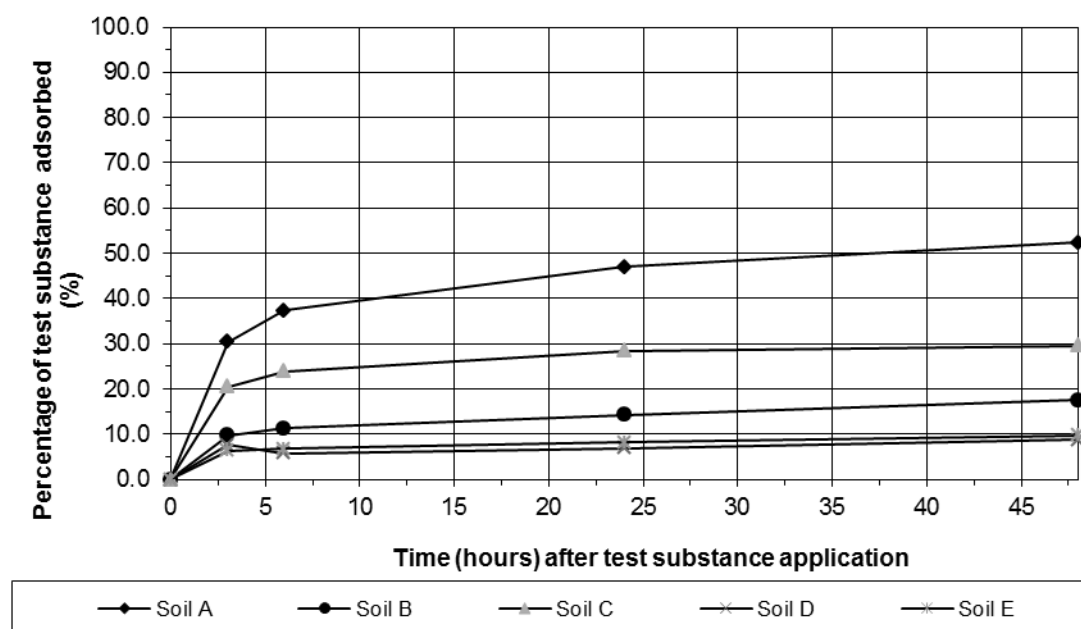
The test item was found to be stable in the test system over 48 hours with a mean recovery of applied radioactivity after extraction (adsorption supernatant plus extract) of between 92.4 % and 97.8 % for [ $^{14}\text{C}$ ]1'-COOH-S-2840A and between 91.7 and 96.9 % for [ $^{14}\text{C}$ ]1'-COOH-S-2840B. Recovery in supernatants and soil extracts was analysed by HPLC and ranged between 92.1 % and 97.4 % for 1'-COOH-S-2840A and 91.0 and 96.9 % for 1'-COOH-S-2840B. HSE has examined the supplied chromatograms and agrees that there is no evidence of degradation products.

The purities of 1'-COOH-S-2840A and B in the extracts were also tested and found to be ≥99 % for supernatants and soil extracts. The applicant therefore judged that the test items were stable in the conditions of the test and that this would be applicable in the definitive test. HSE agrees that stability has been demonstrated in the preliminary test.

## II. Equilibrium test

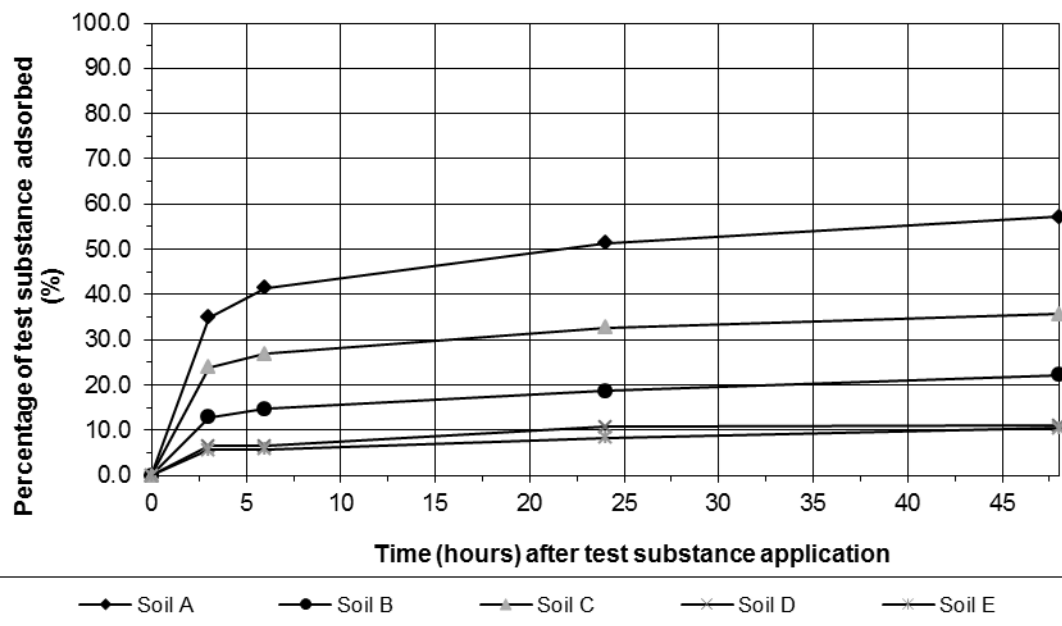
Incubation for 3, 6, 24 and 48 hours was tested. An adsorption equilibrium time of 48 hours was selected for use in the definitive test. For desorption, a 1:1 w/v soil solution ratio and 1 g/mL test substance concentration in the aqueous phase were used to test desorption equilibrium at 6, 24, 48 and 72 h. A desorption equilibrium time of 48 h was selected for use in the definitive test.

Tabulated data for the degree of sorption of the test items have not been supplied. HSE has examined Figure B.8.1.4.3-3 below and notes that the percentage adsorption was still rising between 24 and 48 hours in all soils. Only for 1'-COOH-S-2840B in soil D is the percentage adsorbed similar at both 24 and 48 h. This indicates that equilibrium may not have been reached at 48 hours in the majority of soils. There is no indication of instability of the test item in soil over 48 hours, but it is noted that the total experimental time (adsorption and desorption together) is 96 hours and stability has not been demonstrated over this longer time interval. In addition, the OECD 106 Guideline states that a period of 24 hours is generally sufficient but samples may be collected over a period of 48 hours. As the equilibrium curves have flattened after 24 hours, an equilibrium time of 48 hours is accepted as a compromise between equilibration and potential instability, but the impact of equilibrium potentially not having been reached on the adsorption isotherms is considered further below.



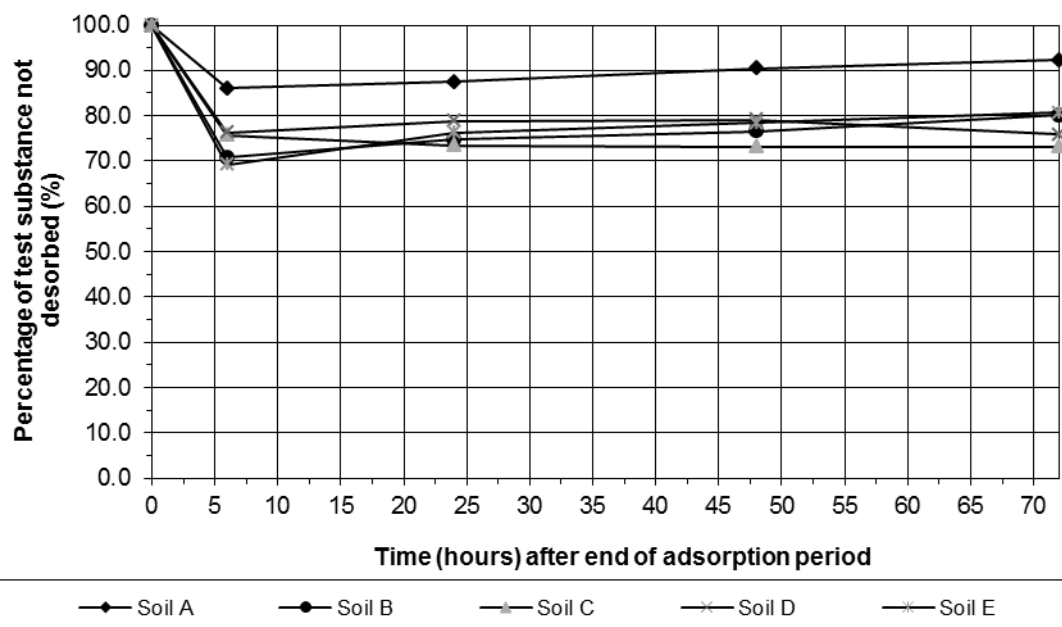
**Figure B.8.1.5.3-01 Adsorption equilibrium time data for all soils 1'-COOH-S-2840A**



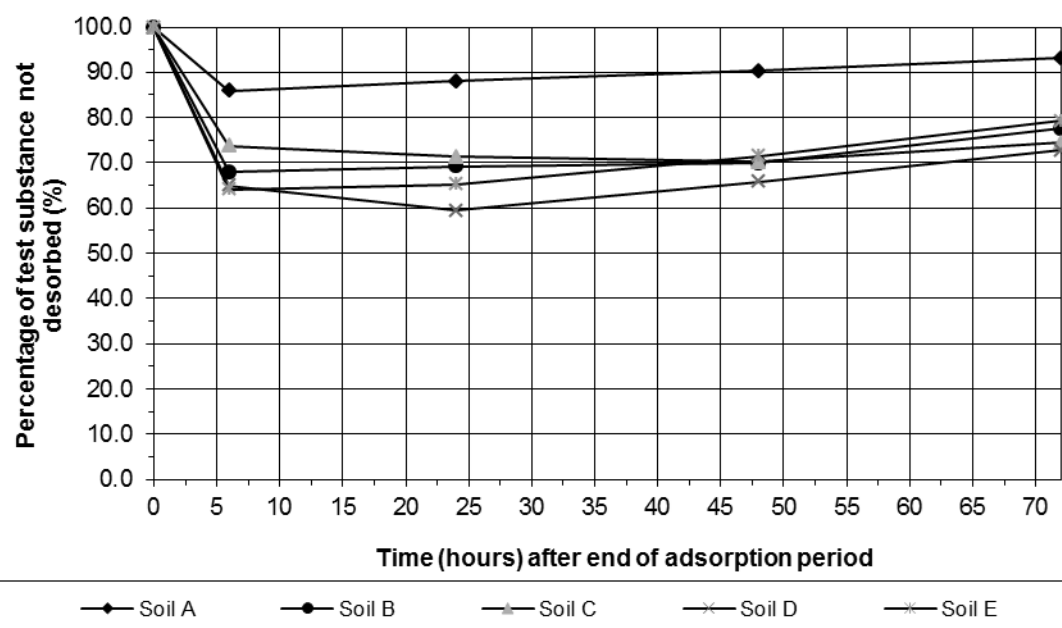


Soil A = Quilen; Soil B = Hareby; Soil C = Clipstone; Soil D = Speyer 2.3; Soil E = Atwater

**Figure B.8.1.5.3-02 Adsorption equilibrium time data for all soils - 1'-COOH-S-2840B**



**Figure B.8.1.5.3-03 Desorption equilibrium time data for all soils - 1'-COOH-S-2840A**



Soil A = Quilen; Soil B = Hareby; Soil C = Clipstone; Soil D = Speyer 2.3; Soil E = Atwater

**Figure B.8.1.5.3-04 Desorption equilibrium time data for all soils - 1'-COOH-S-2840B**

#### Definitive test

In the definitive test, the indirect method was used as stability of the test item was demonstrated in the preliminary test. Conditions used in the definitive test were based on the results outlined in the preliminary tests and the equilibrium test.

The pH of the supernatants was determined. The pH of the 0.01 M calcium chloride mixed with the highest and lowest concentration stock solutions was 6.02 and 6.20 respectively prior to addition of soil. The Guideline states that the pH of the aqueous phase plays an important role in the adsorption process, especially for ionisable substances.

**Table B.8.1.5.3-05 The pH of adsorption supernatants after soil addition - 1'-COOH-S-2840A**

Nominal Dose Level (µg/mL)	Replicate	Adsorption Supernatant pH				
		Quilen	Hareby	Clipstone	Speyer 2.3	Atwater
		(Soil A)	(Soil B)	(Soil C)	(Soil D)	(Soil E)
1	1	7.37	7.09	6.56	6.99	7.09
	2	7.32	7.09	6.48	6.96	7.11
	Mean	7.35	7.09	6.52	6.98	7.10

0.5	1	7.29	7.10	6.44	6.85	6.99
	2	7.24	7.10	6.40	6.86	7.04
	<b>Mean</b>	<b>7.27</b>	<b>7.10</b>	<b>6.42</b>	<b>6.86</b>	<b>7.02</b>
0.1	1	7.19	7.04	6.44	6.76	6.98
	2	7.19	6.99	6.41	6.79	6.99
	<b>Mean</b>	<b>7.19</b>	<b>7.02</b>	<b>6.43</b>	<b>6.78</b>	<b>6.99</b>
0.05	1	7.15	6.97	6.35	6.78	6.99
	2	7.13	6.96	6.32	6.80	7.06
	<b>Mean</b>	<b>7.14</b>	<b>6.97</b>	<b>6.34</b>	<b>6.79</b>	<b>7.03</b>
0.01	1	6.99	6.89	6.32	6.75	6.87
	2	6.98	6.91	6.32	6.76	6.92
	<b>Mean</b>	<b>6.99</b>	<b>6.90</b>	<b>6.32</b>	<b>6.76</b>	<b>6.90</b>
	<b>Mean</b>	<b>7.19</b>	<b>7.02</b>	<b>6.41</b>	<b>6.83</b>	<b>7.01</b>
The pH of 0.01 M calcium chloride mixed with the highest and lowest concentration stock solutions was 6.02 and 6.20 respectively, prior to soil addition.						

**Table B.8.1.5.3-06 The pH of adsorption supernatants after soil addition - 1'-COOH-S-2840B**

Nominal Dose Level (µg/mL)	Replicate	Adsorption Supernatant pH				
		Quilen	Hareby	Clipstone	Speyer 2.3	Atwater
		(Soil A)	(Soil B)	(Soil C)	(Soil D)	(Soil E)
1	1	7.02	6.92	6.23	6.72	7.19
	2	7.08	6.94	6.22	6.74	7.19
	<b>Mean</b>	<b>7.05</b>	<b>6.93</b>	<b>6.23</b>	<b>6.73</b>	<b>7.19</b>
0.5	1	7.05	6.94	6.24	6.76	7.14

	2	7.02	6.93	6.24	6.77	7.22
	<b>Mean</b>	<b>7.04</b>	<b>6.94</b>	<b>6.24</b>	<b>6.77</b>	<b>7.18</b>
0.1	1	7.01	6.91	6.20	6.73	7.11
	2	7.00	6.92	6.28	6.75	7.17
	<b>Mean</b>	<b>7.01</b>	<b>6.92</b>	<b>6.24</b>	<b>6.74</b>	<b>7.14</b>
0.05	1	7.03	6.95	6.26	6.72	7.18
	2	7.01	7.02	6.25	6.71	7.11
	<b>Mean</b>	<b>7.02</b>	<b>6.99</b>	<b>6.26</b>	<b>6.72</b>	<b>7.15</b>
0.01	1	7.11	7.08	6.23	6.80	7.22
	2	7.04	6.98	6.26	6.73	7.24
	<b>Mean</b>	<b>7.08</b>	<b>7.03</b>	<b>6.25</b>	<b>6.77</b>	<b>7.23</b>
	<b>Mean</b>	<b>7.04</b>	<b>6.96</b>	<b>6.24</b>	<b>6.75</b>	<b>7.18</b>
The pH of 0.01 M calcium chloride mixed with the highest and lowest concentration stock solutions was 6.41 and 6.69 respectively, prior to soil addition.						

The actual amounts of test item in comparison to the nominal application rates are shown below.

**Table B.8.1.5.3-07 Nominal and actual application rates used in the test**

<b>Nominal concentration</b>	<b>Actual application rate (µg/mL)</b>
<b>1'-COOH-S-2840A</b>	
0.01	0.009968
0.05	0.05098
0.1	0.1015
0.5	0.4959
1	0.9908
<b>1'-COOH-S-2840B</b>	
0.01	0.009736
0.05	0.05577
0.1	0.1131
0.5	0.507
1	1.032

Adsorption supernatants and concentrated acetone: 0.5 M HCl extracts of soil were analysed by HPLC. The table below shows recovery of [ $^{14}\text{C}$ ]1'-COOH-S-2840A in the supernatants and soil extracts at a concentration of 1 µg/mL. The volume of water entrained in the soil pellet was accounted for by subtracting the weight of dry soil, so that the volume of  $\text{CaCl}_2$  derived includes the entrained water.

**Table B.8.1.5.3-08 Parental mass balance for [<sup>14</sup>C]1'-COOH-S-2840A and [<sup>14</sup>C]1'-COOH-S-2840B at a concentration of 1 µg/mL in the preliminary test (direct method)**

Soil	Recovery of Radioactivity as [ <sup>14</sup> C]1'-COOH-S-2840A (%)			Recovery of Radioactivity as [ <sup>14</sup> C]1'-COOH-S-2840B (%)		
	Adsorption Supernatant	Soil Extract	Total	Soil Extract	Adsorption Supernatant	Total
Quilen	23.9	68.2	92.1	22.1	68.9	91.0
Hareby	47.3	47.3	94.6	41.7	52.9	94.6
Clipstone	41.6	55.8	97.4	38.5	58.4	96.9
Speyer 2.3	59.5	37.5	97.0	56.2	39.7	95.9
Atwater	56.3	40.3	96.5	54.7	41.0	95.6

It is stated in the study report that both [<sup>14</sup>C]1'-COOH-S-2840A and B were ≥99 % in both supernatants and soil extracts, indicating that the test item was stable under the conditions of the test. While the totals in the table above are less than this (91.0 to 97.4 %, this indicate incomplete extraction rather than degradation.

Percentages of [<sup>14</sup>C]1'-COOH-S-2840A and B adsorbed and desorbed and in the supernatants are shown below.

**Table B.8.1.5.3-09 Recovery of radioactivity following 48 hours adsorption and 48 desorption phases: 1'-COOH-S-2840A at a concentration of 1 µg/mL in the definitive test**

Soil	Recovery of Radioactivity (%)					
	Adsorption Supernatant	Desorption Supernatant	Soil Extract	Residue	Total Recovery (%)	Recovery minus unextracted residues (%)
Quilen	24.7	14.9	26.0	34.3	99.9	65.6
	24.6	15.1	26.8	33.8	100.3	66.5

<b>Mean</b>	<b>24.7</b>	<b>15.0</b>	<b>26.4</b>	<b>34.1</b>	<b>100.1</b>	<b>66.1</b>
Hareby	48.8	23.5	16.8	9.5	98.6	89.1
	49.6	24.0	17.5	9.3	100.4	91.1
<b>Mean</b>	<b>49.2</b>	<b>23.8</b>	<b>17.2</b>	<b>9.4</b>	<b>99.5</b>	<b>90.2</b>
Clipstone	42.7	21.4	23.9	11.5	99.5	88.0
	44.4	23.2	21.7	9.9	99.2	89.3
<b>Mean</b>	<b>43.6</b>	<b>22.3</b>	<b>22.8</b>	<b>10.7</b>	<b>99.4</b>	<b>88.7</b>
Speyer 2.3	61.2	21.0	13.8	3.8	99.8	96.0
	60.5	22.9	13.2	3.6	100.2	96.6
<b>Mean</b>	<b>60.9</b>	<b>22.0</b>	<b>13.5</b>	<b>3.7</b>	<b>100.0</b>	<b>96.4</b>
Atwater	57.0	23.5	13.9	5.2	99.4	94.4
	56.9	23.9	14.4	4.9	100.1	95.2
<b>Mean</b>	<b>57.0</b>	<b>23.7</b>	<b>14.2</b>	<b>5.1</b>	<b>99.8</b>	<b>94.9</b>

**Table B.8.1.5.3-10 Recovery of radioactivity following 48 hours adsorption and 48 desorption phases: 1'-COOH-S-2840B at a concentration of 1 µg/mL in the definitive test (direct method)**

Soil	Recovery of Radioactivity (%)					
	Adsorption Supernatant	Desorption Supernatant	Soil Extract	Residue	Total Recovery (%)	Recovery minus unextracted residues (%)
Quilen	23.4	14.0	26.1	32.5	96.0	63.5
	22.5	13.3	23.1	33.2	92.1	58.9
<b>Mean</b>	<b>23.0</b>	<b>13.7</b>	<b>24.6</b>	<b>32.9</b>	<b>94.1</b>	<b>61.3</b>
Hareby	43.9	22.9	21.8	11.1	99.7	88.6

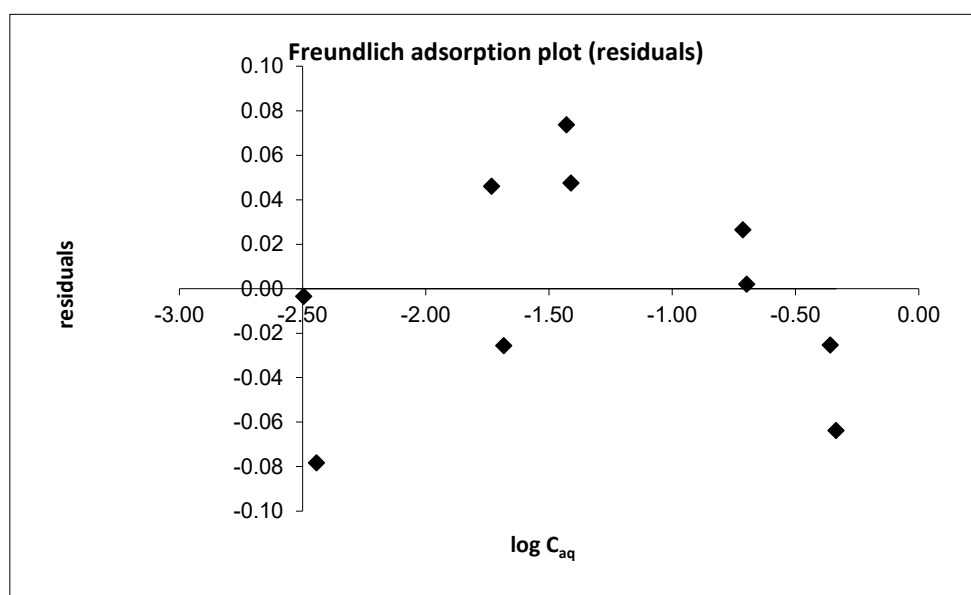
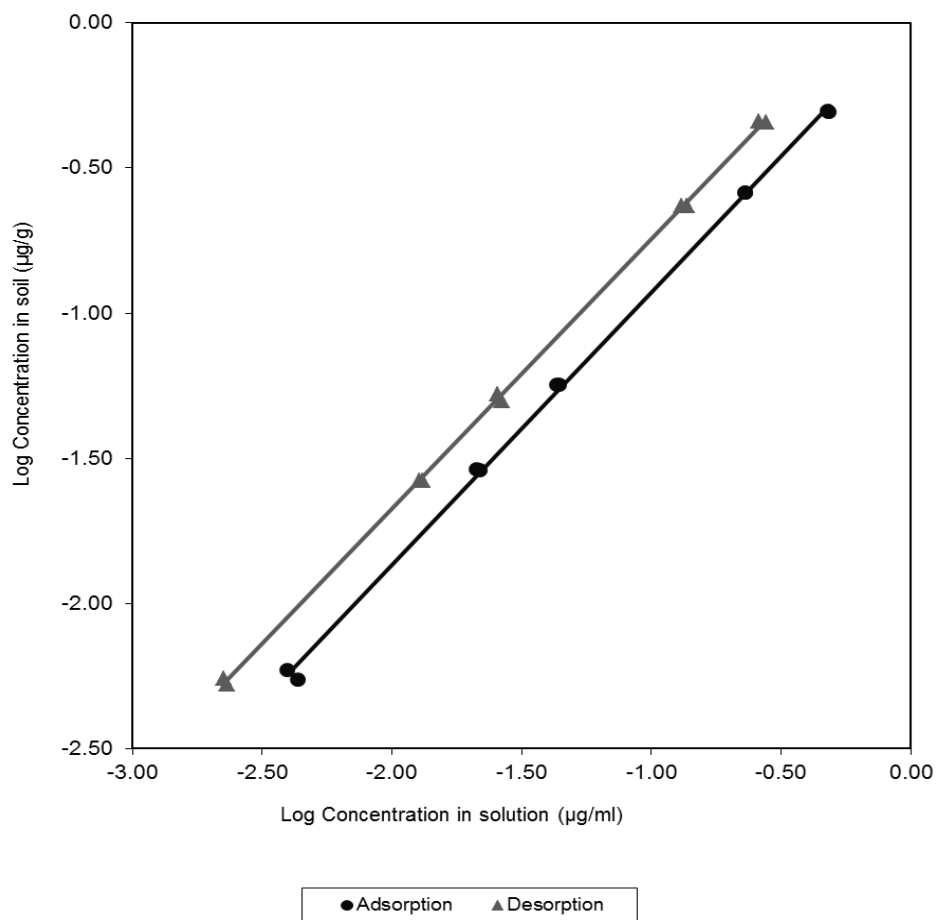
	46.6	22.0	19.9	12.2	100.7	88.5
<b>Mean</b>	<b>45.3</b>	<b>22.5</b>	<b>20.9</b>	<b>11.7</b>	<b>100.2</b>	<b>88.7</b>
Clipstone	39.8	22.1	23.7	11.3	96.9	85.6
	44.2	21.3	22.1	9.8	97.4	87.6
<b>Mean</b>	<b>42.0</b>	<b>21.7</b>	<b>22.9</b>	<b>10.6</b>	<b>97.2</b>	<b>86.6</b>
Speyer 2.3	59.6	22.9	13.6	4.2	100.3	96.1
	58.6	23.7	13.6	3.8	99.7	95.9
<b>Mean</b>	<b>59.1</b>	<b>23.3</b>	<b>13.6</b>	<b>4.0</b>	<b>100.0</b>	<b>96.0</b>
Atwater	56.4	23.5	13.7	5.6	99.2	93.6
	58.0	23.6	12.8	5.3	99.7	94.4
<b>Mean</b>	<b>57.2</b>	<b>23.6</b>	<b>13.3</b>	<b>5.5</b>	<b>99.5</b>	<b>94.1</b>

## Isotherms

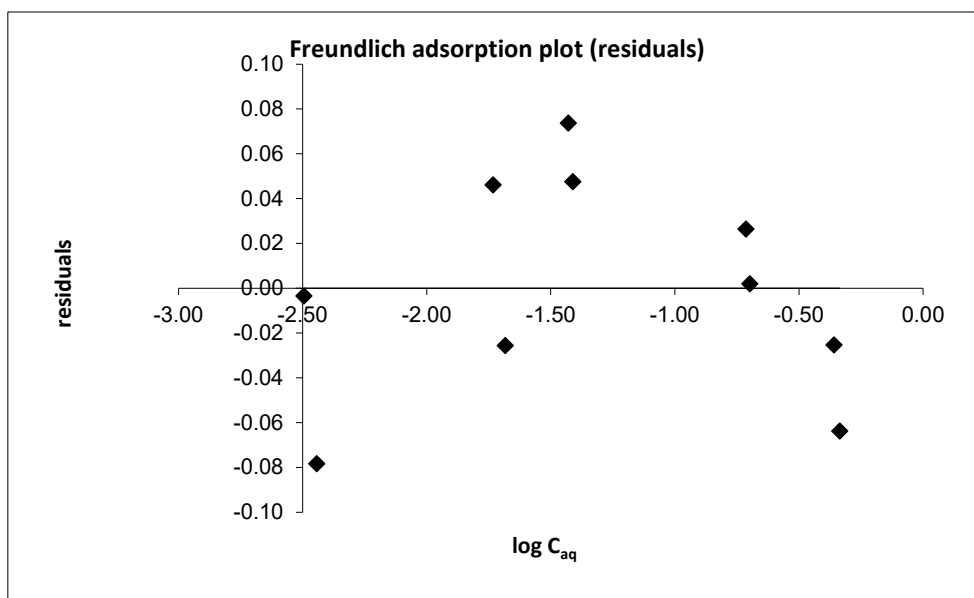
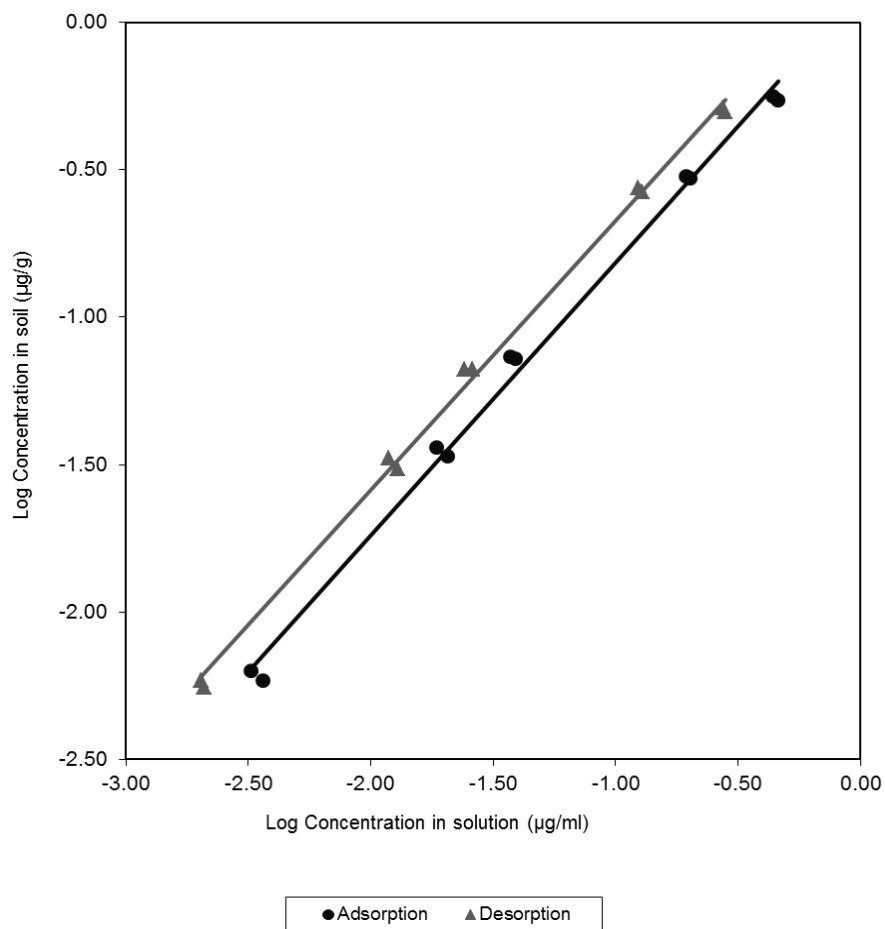
The applicant has calculated both adsorption and desorption isotherms for isomers 1'-COOH-S-2840A and 1'-COOH-S-2840B separately.

The adsorption and desorption parameters derived by the applicant have been verified by HSE. There were small differences between adsorption and desorption  $K_{foc}$  values derived by HSE and those calculated by the applicant, but these were attributed to rounding. Consequently, applicant values were accepted and adsorption values and isotherms presented in this report were derived by the applicant. Plots of the residuals were not supplied by the applicant and have been provided by the HSE for adsorption isotherms. Goodness of fit has not been assessed by HSE for the desorption phase as these values are not used in the exposure assessment.

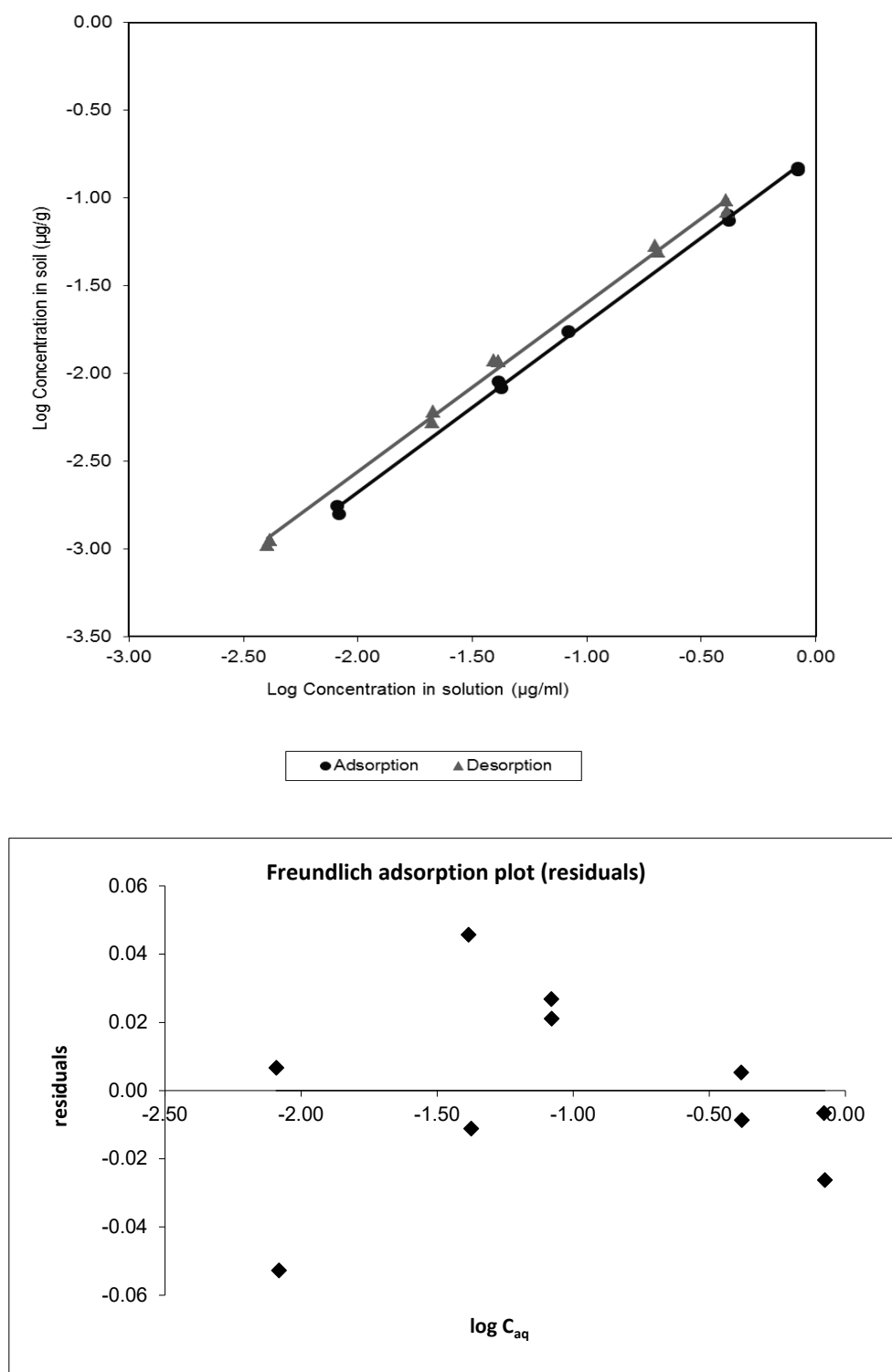


**Quilen**

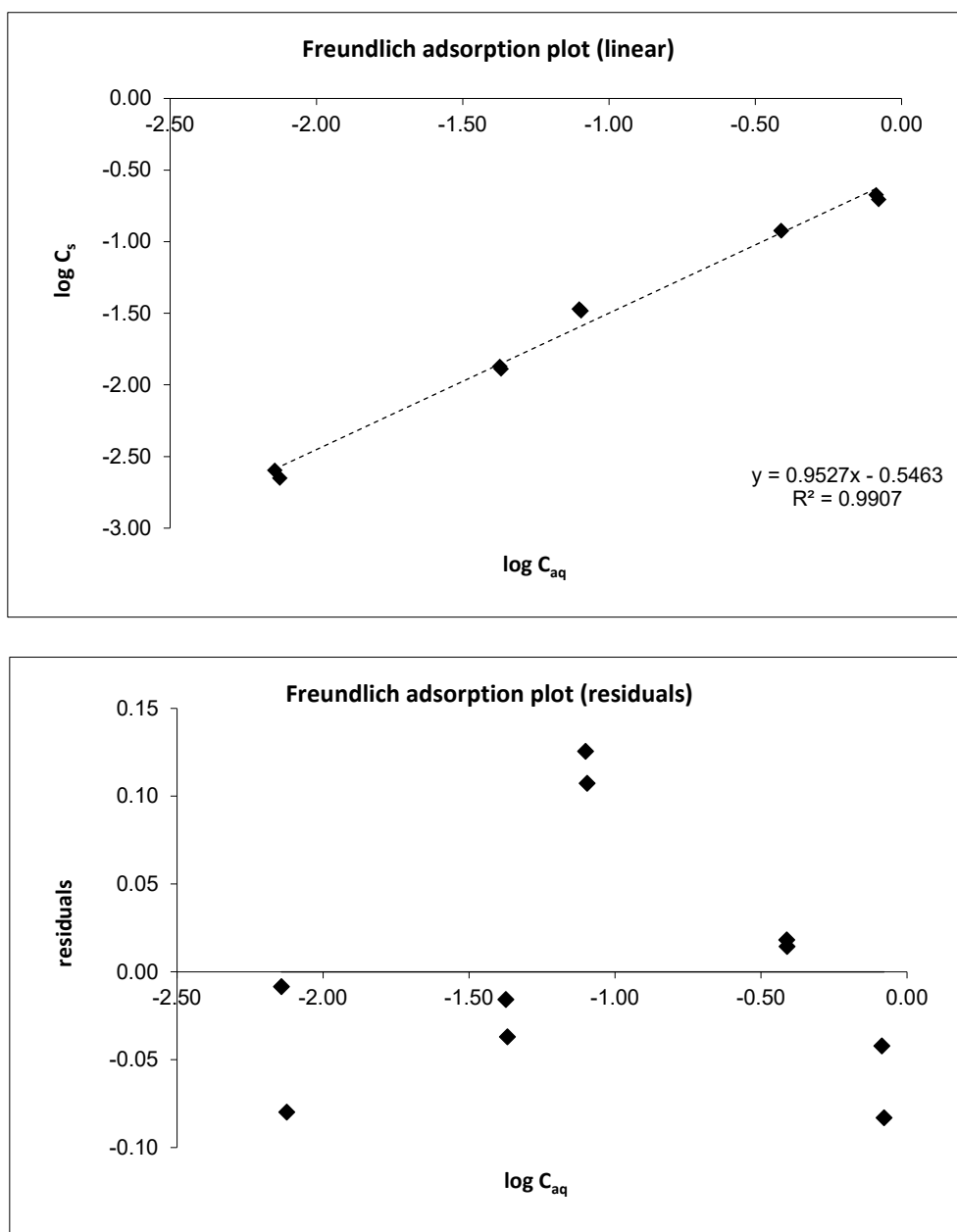
**Figure B.8.1.5.3-05 Adsorption isotherm for [ $^{14}\text{C}$ ]1'-COOH-S-2840A on Quilen soil (no residuals provided by applicant so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9992)**



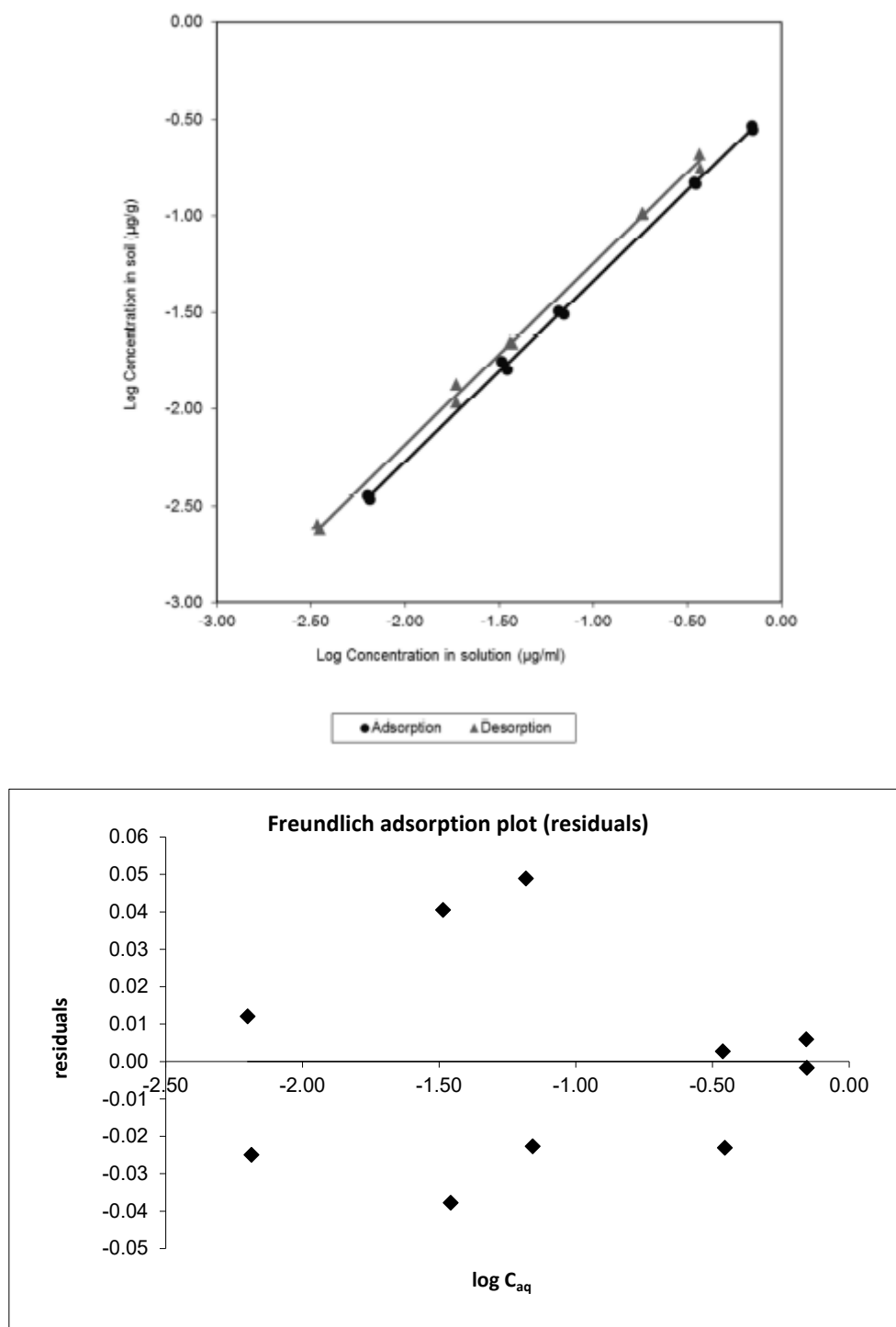
**Figure B.8.1.5.3-06 Adsorption isotherm for [<sup>14</sup>C]1'-COOH-S-2840B on Quilen soil (no residuals provided by applicant so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9955)**

**Hareby Soil**

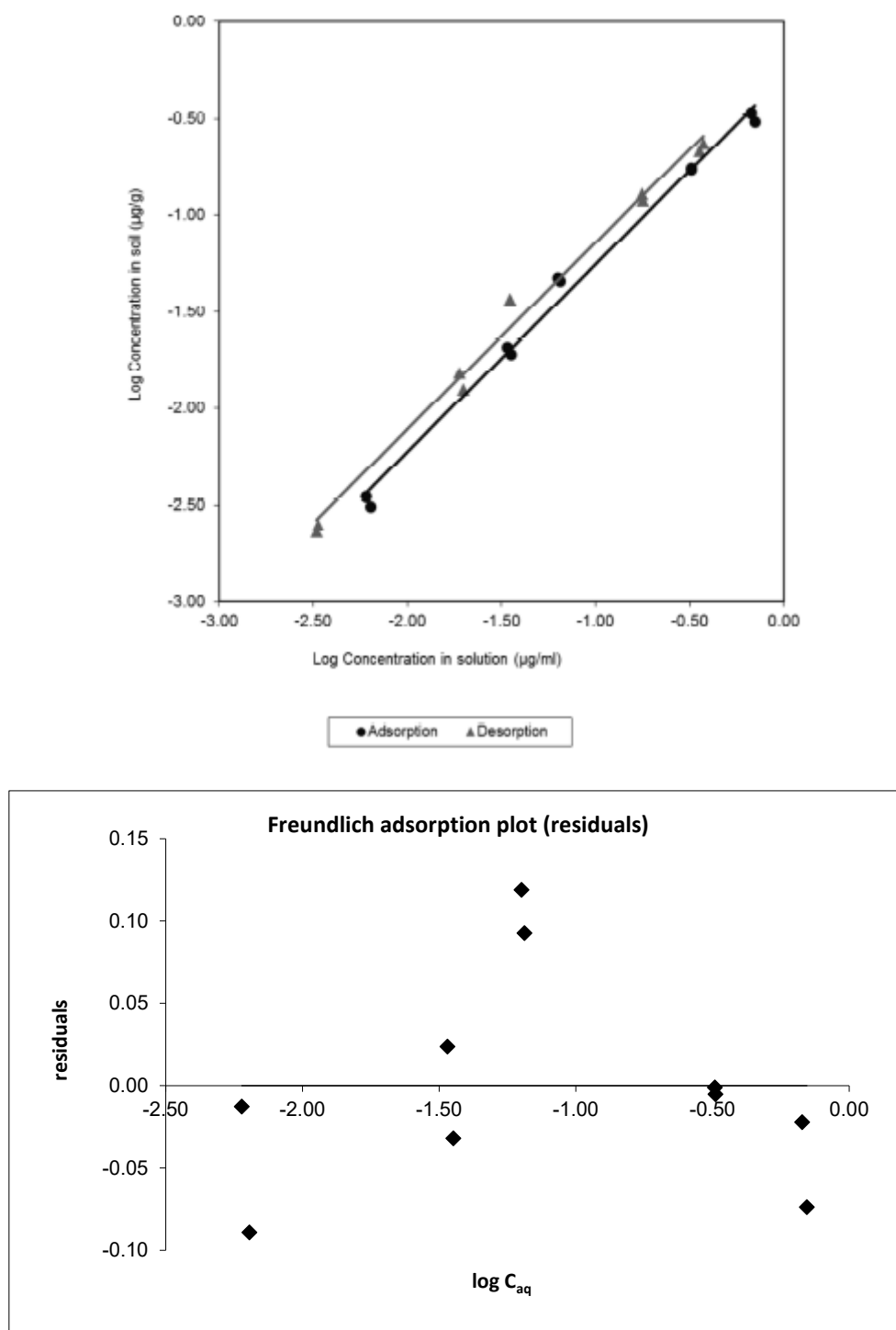
**Figure B.8.1.5.3-07 Adsorption isotherm for [14C]1'-COOH-S-2840A on Hareby soil (no residuals provided by applicant so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9985)**



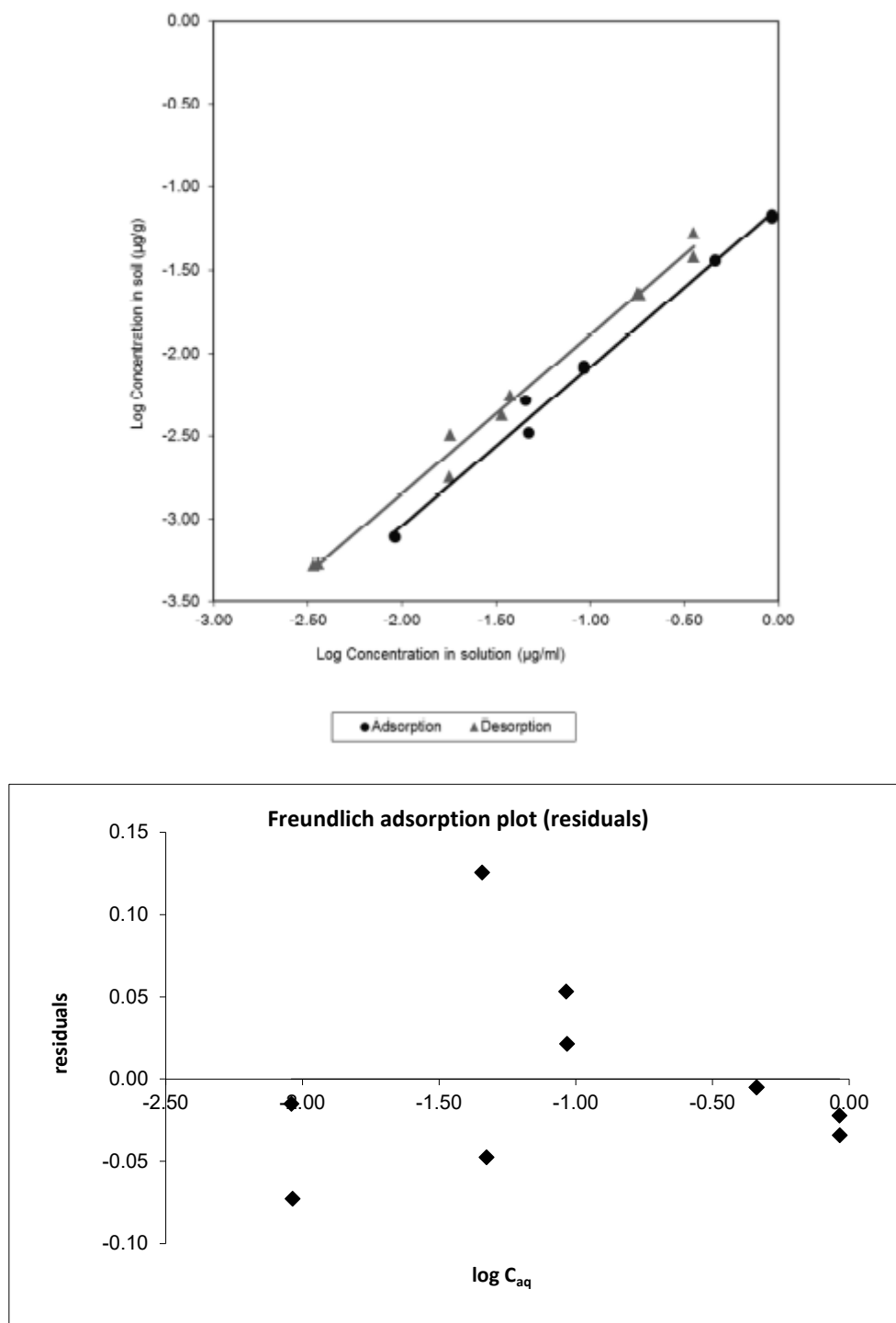
**Figure B.8.1.5.3-08 Adsorption isotherm for [ $^{14}\text{C}$ ]1'-COOH-S-2840B on Hareby soil (error in applicant Freundlich calculations so HSE derived plots are shown for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9907)**

**Clipstone Soil**

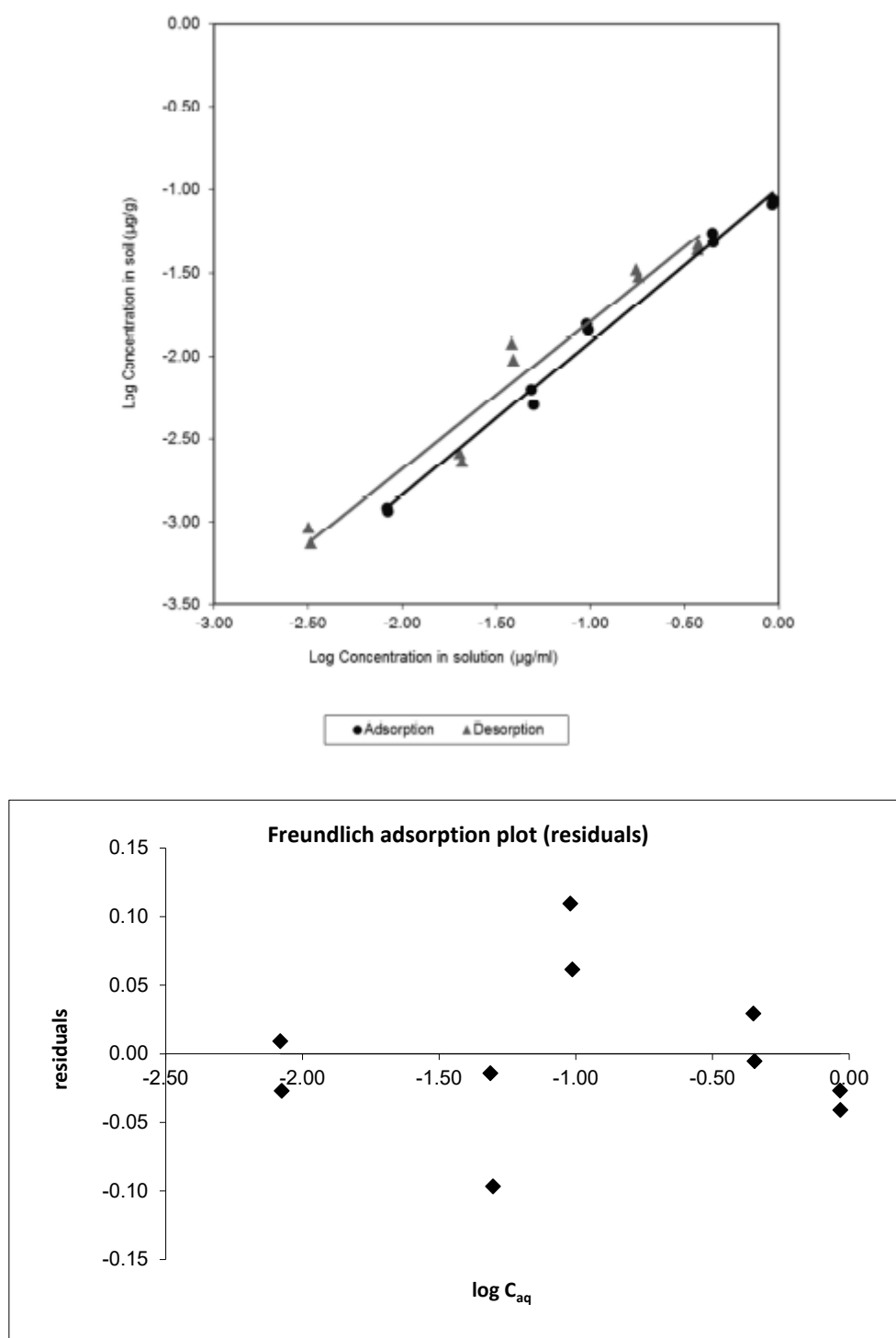
**Figure B.8.1.5.3-09 Adsorption isotherm for [14C]1'-COOH-S-2840A on Clipstone soil (no residuals provided by applicant so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9984)**



**Figure B.8.1.5.3-10 Adsorption isotherm for [<sup>14</sup>C]1'-COOH-S-2840B on Clipstone soil (no residuals provided by applicant so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9984)**

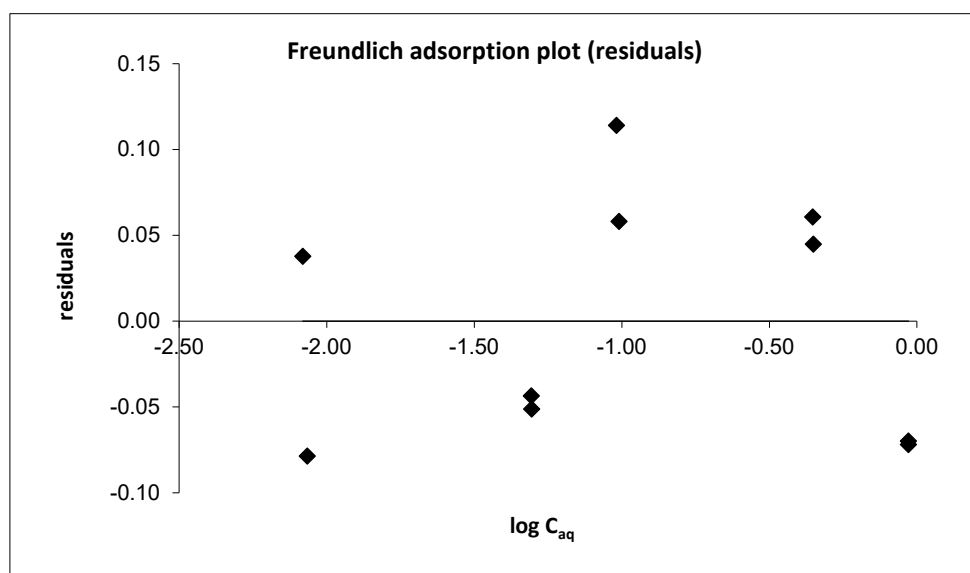
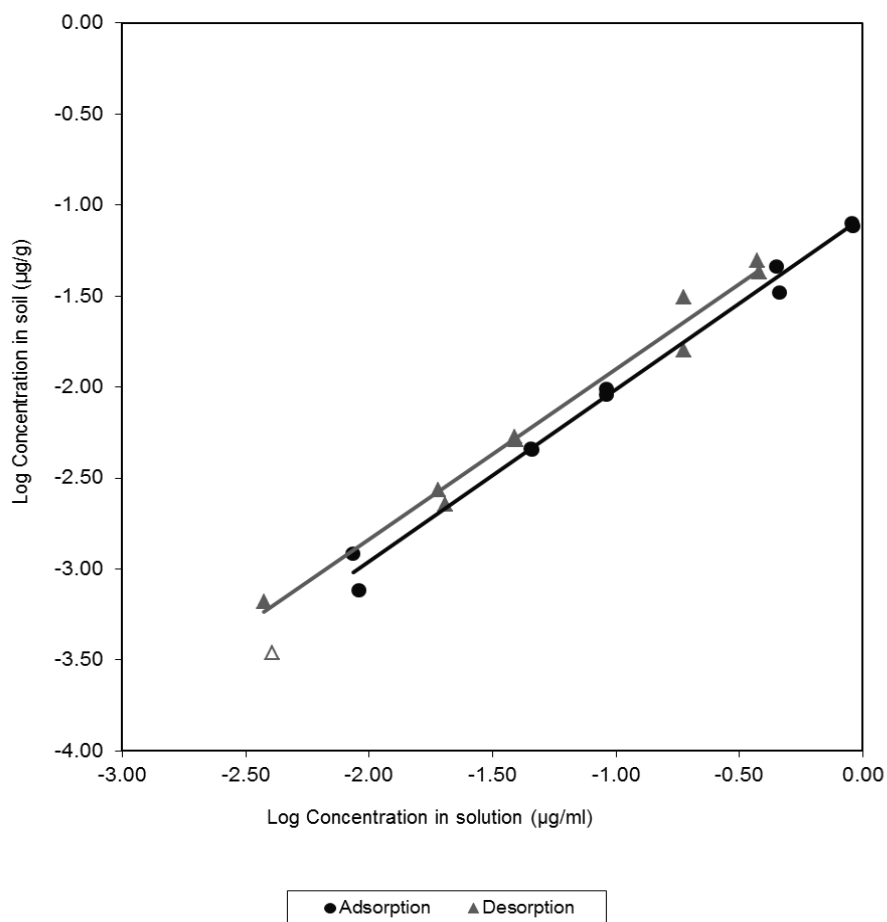
**Speyer 2.3 Soil**

**Figure B.8.1.5.3-11 Adsorption isotherms for [<sup>14</sup>C]1'-COOH-S-2840A on Speyer 2.3 soil (no residuals provided by applicant so derived by HSE so derived by HSE for adsorption phase only; HSE derived r<sup>2</sup> value for adsorption = 0.9939)**



**Figure B.8.1.5.3-12 Adsorption isotherms for [<sup>14</sup>C]1'-COOH-S-2840B on Speyer 2.3 soil (no residuals provided by applicant so derived by HSE so derived by HSE for adsorption phase only; HSE derived r<sup>2</sup> value for adsorption = 0.9935)**



**Atwater Soil**

**Figure B.8.1.5.3-13 Adsorption isotherms for [<sup>14</sup>C]1'-COOH-S-2840A on Atwater soil (no residuals provided by applicant so derived by HSE so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9911)**

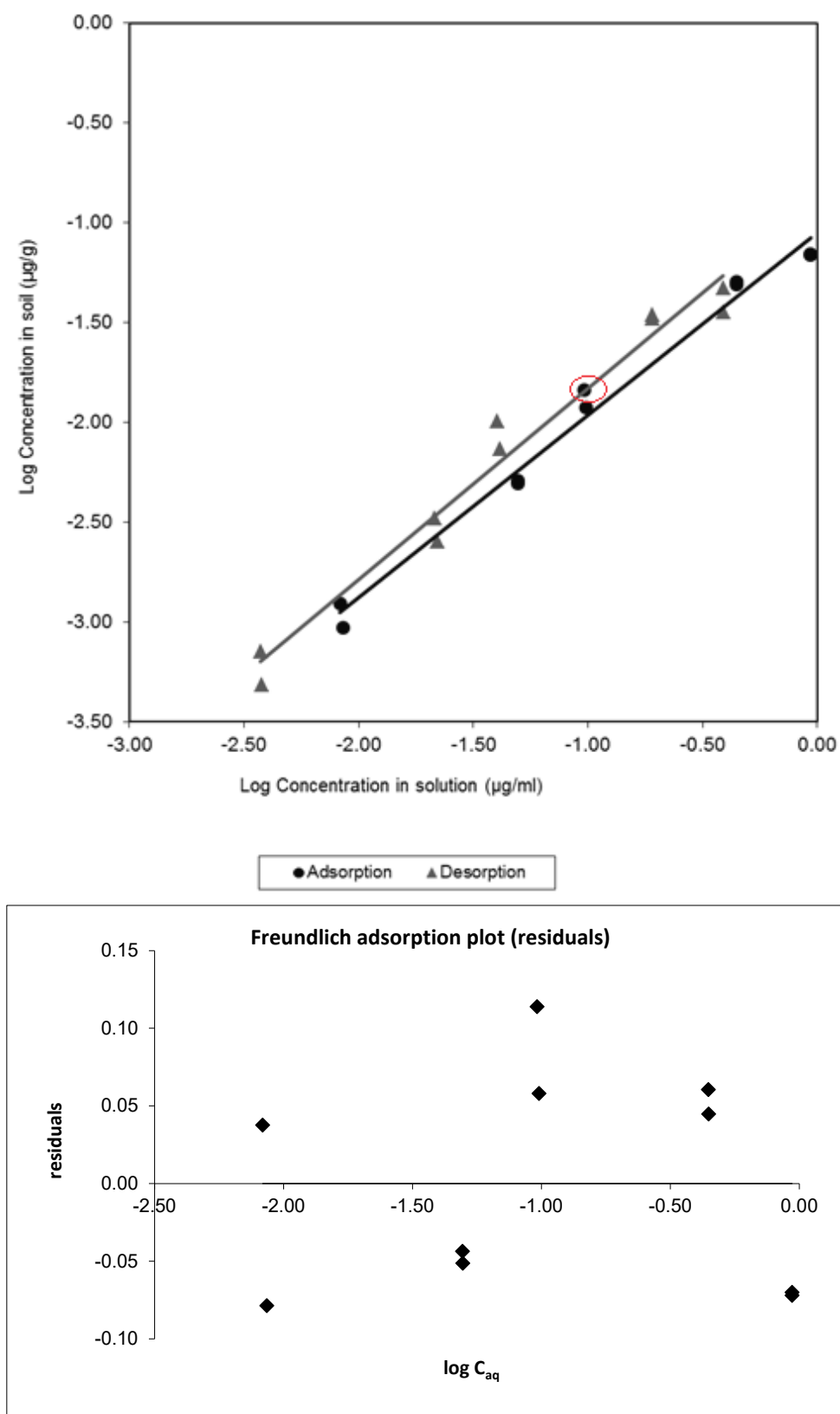
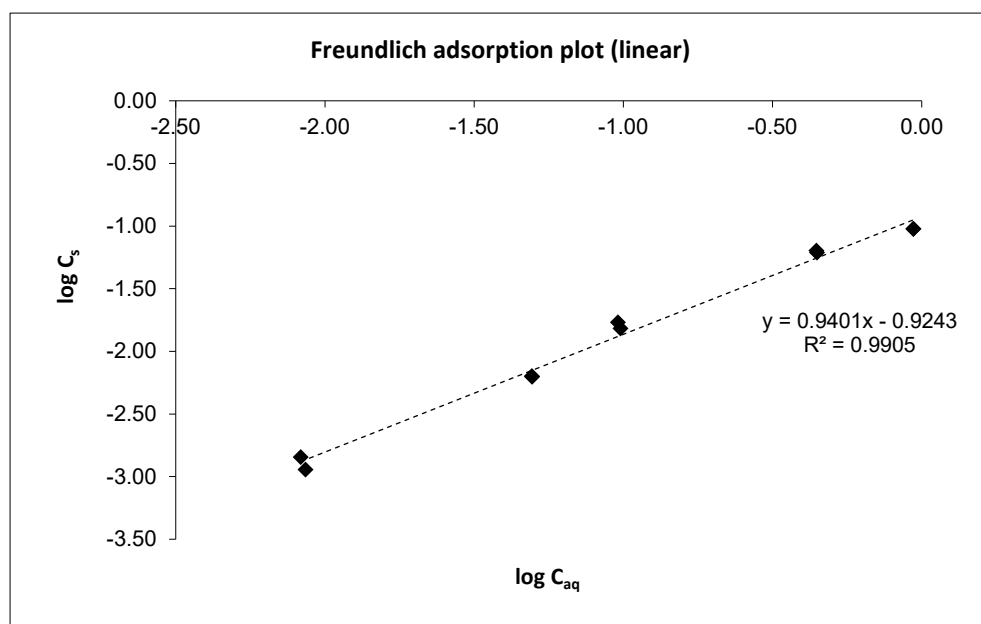


Figure B.8.1.5.3-14 Adsorption isotherms for [<sup>14</sup>C]1'-COOH-S-2840A on Atwater soil (no residuals provided by applicant so derived by HSE so derived by HSE for adsorption phase only; HSE derived  $r^2$  value for adsorption = 0.9911)

HSE was unable to replicate the applicant fit for 1'-COOH-S-2840B in the Atwater soil. This differed from the other soils 1'-COOH-S-2840B and for all soils for 1'-COOH-S-2840A in which a close match was observed between HSE and applicant fits and consequently between the  $1/n$  and  $K_{foc}$  values derived. The  $r^2$  value obtained by HSE was higher at 0.9905, compared to the applicant's value of 0.9859.

Consideration of the data showed that the difference in fit was related to a single data point at a concentration of 0.1  $\mu\text{g/mL}$ . The concentrations measured in solution were similar for the two replicates (0.0960 and 0.0978  $\mu\text{g/mL}$ ) but there is a larger difference between the calculated concentrations in soil for the two replicates (0.0145 and 0.0119  $\mu\text{g/g}$ ). This was not seen in the HSE calculations where the calculated values in soil were very similar. It is considered that, as the concentrations in the aqueous phase were similar, the concentrations adsorbed to soil should also be similar. Replotting the applicant's data after removal of this data point results in a plot very similar to the HSE plot, with an  $r^2$  of 0.9908. As HSE was able to closely replicate the applicant fits and calculated  $K_{foc}$  and  $1/n$  values in all other cases, the decision has been taken to use the HSC fit and associated adsorption parameters for 1'-COOH-S-2840B in the Atwater soil. The HSE fit is shown below.



**Figure B.8.1.5.3-15 HSE derived adsorption isotherm for [ $^{14}\text{C}$ ]1'-COOH-S-2840B on Atwater soil ( $r^2$  value for adsorption = 0.9905)**

The endpoints derived from the HSE and applicant fits are compared below.

**Table B.8.1.5.3-11 Comparison of HSE and applicant sorption parameters for  $^{14}\text{C}$ 1'-COOH-S-2840B on the Atwater soil**

	<b><math>K_{\text{foc}}</math> (mL/g)</b>	<b>1/n</b>	<b><math>r^2</math></b>
<b>HSE</b>	<b>39.7</b>	<b>0.940</b>	<b>0.9905</b>
<b>Applicant</b>	30	0.915	0.9859

Parameters used in bold

## ADSORPTION CHECKLIST

It is noted that this study was completed in February 2017, which is before the publication of the OECD 106 evaluator checklist and evaluation tool, which was published in November 2017. While guidance should not be applied retrospectively, much of the content of the adsorption checklist was already present in the OECD 106 Guideline, with the checklist helping to confirm the acceptability of the fitted sorption parameters and the appropriateness of their use in regulatory models. The checks also help to identify studies where deviations from the OECD guideline could lead to significant and systematic errors in fitted parameters. It is therefore considered appropriate to apply the checklist to the current study.

### Stability and mass balance

The stability of the test item under the conditions of the test has been considered. The applicant tested the stability as part of the preliminary tests and, following extraction of the soil pellet with HCl (0.5 M), the recovery from the supernatant and the soil extract was >90 % (range 92.1 to 97.4 % AR for 1'-COOH-S-2840A and 91.0 to 96.9 % AR for 1'-COOH-S-2840B). The applicant therefore considered that the test item was stable under the conditions of the test.

### $K_d$ x soil:solution ratio is >0.3

The accurate determination of the distribution coefficient,  $K_d$ , is best achieved when the  $K_d$  x soil:solution ratio is >0.3. The values are presented below for all soils and both isomers.

**Table B.8.1.5.3-12  $K_d$  x soil:solution ratios (g/cm<sup>3</sup>) for 1'-COOH-S-2840A and 1'-COOH-S-2840B in all soils calculated by HSE**

<b>Soil</b>	<b>Nominal Concentration (µg/mL)</b>	<b>1'-COOH-S-2840A</b>	<b>1'-COOH-S-2840B</b>
Quilen	0.01	1.49	2.04
	0.01	1.32	1.70
	0.05	1.35	2.01
	0.05	1.40	1.69
	0.1	1.32	1.91
	0.1	1.34	2.04
	0.5	1.16	1.53
	0.5	1.16	1.62
	1	1.08	1.24
	1	1.05	1.36
Hareby	0.01	<b>0.23</b>	0.30
	0.01	<b>0.20</b>	0.35
	0.05	<b>0.21</b>	0.32
	0.05	<b>0.24</b>	0.30
	0.1	<b>0.22</b>	0.41
	0.1	<b>0.22</b>	0.43
	0.5	<b>0.19</b>	0.31
	0.5	<b>0.20</b>	0.31
	1	<b>0.19</b>	<b>0.26</b>
	1	<b>0.18</b>	<b>0.24</b>

Clipstone	0.01	0.58	0.62
	0.01	0.53	0.52
	0.05	0.56	0.57
	0.05	0.46	0.65
	0.1	0.54	0.79
	0.1	0.46	0.74
	0.5	0.41	0.57
	0.5	0.44	0.57
	1	0.42	0.53
	1	0.42	0.47
Speyer 2.3	0.01	<b>0.08</b>	<b>0.17</b>
	0.01	<b>0.10</b>	<b>0.16</b>
	0.05	<b>0.08</b>	<b>0.15</b>
	0.05	<b>0.12</b>	<b>0.12</b>
	0.1	<b>0.10</b>	<b>0.16</b>
	0.1	<b>0.09</b>	<b>0.18</b>
	0.5	<b>0.08</b>	<b>0.13</b>
	0.5	<b>0.08</b>	<b>0.14</b>
	1	<b>0.08</b>	<b>0.12</b>
	1	<b>0.07</b>	<b>0.11</b>
Atwater	0.01	<b>0.16</b>	<b>0.13</b>
	0.01	<b>0.10</b>	<b>0.17</b>
	0.05	<b>0.12</b>	<b>0.13</b>
	0.05	<b>0.13</b>	<b>0.13</b>

	0.1	<b>0.12</b>	<b>0.18</b>
	0.1	<b>0.11</b>	<b>0.16</b>
	0.5	<b>0.08</b>	<b>0.14</b>
	0.5	<b>0.12</b>	<b>0.14</b>
	1	<b>0.10</b>	<b>0.10</b>
	1	<b>0.09</b>	<b>0.10</b>

Values in bold are <0.3

It is possible to calculate accurate values for  $K_d$  for the indirect method when the  $K_d \times$  soil:solution ratio is <0.3 providing that the mass balance is extremely high. The  $K_d \times$  soil:solution ratio is <0.3 for the following soil (parental mass balance shown in brackets):

#### 1'-COOH-S-2840A

- All concentrations for the Hareby soil (94.6 %)
- All concentrations for the Speyer 2.3 soil (97.0 %)
- All concentrations for the Atwater soil (96.5 %)

#### 1'-COOH-S-2840B

- The highest concentration in the Hareby soil (94.6 %)
- All concentrations for the Speyer 2.3 soil (95.9 %)
- All concentrations for the Atwater soil (95.6 %)

As mass balance values were >94.6 % or higher at all concentrations in the preliminary test this is not of concern.

#### Adsorbed percentage

The soil:solution ratios should be selected to ensure that >20 % and preferably >50 % is adsorbed. The percentage adsorption, calculated as the decrease in concentration in the aqueous phase, is shown below.

**Table B.8.1.5.3-13 % adsorbed for 1'-COOH-S-2840A and 1'-COOH-S-2840B in all soils calculated by HSE**

<b>Soil</b>	<b>Nominal Concentration (µg/mL)</b>	<b>1'-COOH-S-2840A</b>	<b>1'-COOH-S-2840B</b>
Quilen	0.01	59.87	67.13
	0.01	56.86	63.02
	0.05	57.43	66.83
	0.05	58.42	62.88
	0.1	56.85	65.69
	0.1	57.24	67.11
	0.5	53.76	60.47
	0.5	53.72	61.87
	1	51.89	55.31
	1	51.21	57.58
Hareby	0.01	<b>18.74</b>	22.97
	0.01	<b>16.73</b>	26.05
	0.05	<b>17.22</b>	24.15
	0.05	<b>19.18</b>	23.26
	0.1	<b>18.13</b>	29.09
	0.1	<b>17.93</b>	29.97
	0.5	<b>16.11</b>	23.67
	0.5	<b>16.56</b>	23.51
	1	<b>15.83</b>	20.66
	1	<b>15.23</b>	<b>19.15</b>



Clipstone	0.01	36.80	38.37
	0.01	34.79	34.26
	0.05	35.86	36.17
	0.05	31.74	39.21
	0.1	35.27	44.12
	0.1	31.53	42.62
	0.5	29.30	36.39
	0.5	30.57	36.17
	1	29.77	34.81
	1	29.40	32.13
Speyer 2.3	0.01	<b>7.70</b>	<b>14.75</b>
	0.01	<b>8.71</b>	<b>13.72</b>
	0.05	<b>7.61</b>	<b>12.68</b>
	0.05	<b>10.95</b>	<b>10.70</b>
	0.1	<b>9.16</b>	<b>14.15</b>
	0.1	<b>8.57</b>	<b>15.56</b>
	0.5	<b>7.60</b>	<b>11.28</b>
	0.5	<b>7.60</b>	<b>12.11</b>
	1	<b>7.13</b>	<b>10.33</b>
	1	<b>6.94</b>	<b>10.03</b>
Atwater	0.01	<b>13.72</b>	<b>11.67</b>
	0.01	<b>8.71</b>	<b>14.75</b>
	0.05	<b>10.36</b>	<b>11.42</b>
	0.05	<b>11.14</b>	<b>11.24</b>

	0.1	<b>10.34</b>	<b>15.12</b>
	0.1	<b>9.66</b>	<b>13.53</b>
	0.5	<b>7.50</b>	<b>12.56</b>
	0.5	<b>10.32</b>	<b>12.17</b>
	1	<b>9.28</b>	<b>9.23</b>
	1	<b>8.17</b>	<b>9.20</b>

Adsorption is >50 % in the Quilen soil for both isomers. Adsorption is >20 % for both isomers in the Clipstone soil and for the 1'-COOH-S-2840B isomer in the Hareby soil (except for one replicate at a concentration of 1 µg/mL which was only slightly <20 % at 19.15 %). Those values not meeting the requirement for 20 % adsorption are highlighted in bold; these are the one data point for 1'-COOH-S-2840B in the Hareby soil, and all data points for the Speyer 2.3 and Atwater soils. The definitive test was conducted at a soil:solution ratio of 1:1, which is usually recommended for poorly adsorbing compounds; it is therefore unlikely that testing further ratios would have improved the adsorption percentage.

The subsequent quality checks including the systematic error check indicate that results from soils with the lowest sorption may be subject to a higher degree of uncertainty. The validity of the endpoints from these soils therefore requires further consideration and the impact of removing some soils from the data set is considered further below.

### Systematic error check

The December 2017 adsorption checklist introduced a new criteria of conducting a systematic error check as systematic errors can arise due to loss of test substance via processes other than sorption when the indirect method is used. The  $K_{fE}/K_f$  ratio should be <1.2 for the highest concentration and values >1.2 should be treated with caution. Values calculated by HSE are shown below.

**Table B.8.1.5.3-14  $K_{fE}/K_f$  ratios calculated by HSE**

Soil	1'-COOH-S-2840A		1'-COOH-S-2840B	
	$K_{fE}/K_f$ ratio	F (% loss)	$K_{fE}/K_f$ ratio	F (% loss)
Quilen	1.180	7.9	1.194	9.0

	1.182		1.185	
Hareby	<b>1.518</b>	5.4	<b>1.354</b>	5.4
	<b>1.549</b>		<b>1.393</b>	
Clipstone	1.096	2.6	1.098	3.1
	1.097		1.107	
Speyer 2.3	<b>1.727</b>	3.0	<b>1.658</b>	4.1
	<b>1.761</b>		<b>1.692</b>	
Atwater	<b>1.606</b>	<b>3.5</b>	<b>1.910</b>	4.4
	<b>1.750</b>		<b>1.917</b>	

Values >1.2 in bold

The  $K_{fe}/K_f$  values are >1.2 for both isomers at the Hareby, Speyer 2.3 and Atwater soils. Adsorption data from these soils should therefore be treated with caution. Values were <1.2 for the Quilen and Clipstone soils.

### Goodness of fit and parameter reliability

The  $r^2$  values for all soils are >0.99 and therefore meet the requirement to be >0.975. No outliers were identified. Plots of residuals above were acceptable with small, random errors.

### CONCLUSION

Adsorption parameters were determined using the indirect method in 5 soils for two isomers, 1'-COOH-S-2840A and 1'-COOH-S-2840B. For adsorption the  $K_{foc}$  values ranged between 11 and 35 L/kg (geometric mean of 21 L/kg) for 1'-COOH-S-2840A, while for 1'-COOH-S-2840B  $K_{foc}$  values ranged between 16 and 45 L/kg (geometric mean of 27 L/kg). The  $1/n$  values ranged between 0.9369 and 0.9660 (arithmetic mean of 0.950) for 1'-COOH-S-2840A. For 1'-COOH-S-2840B, the  $1/n$  values ranged between 0.9266 and 0.9716 (arithmetic mean of 0.937). This showed that adsorption was dependent of concentration over the concentraton range tested.

The applicant measured adsorption over a concentration range spanning two orders of magnitude.

The following are noted regarding the adsorption checklist:

- The parental mass balance is >90 % for all soils.

- The  $K_d \times \text{soil:solution ratio}$  should be  $>0.3$ . The  $K_d \times \text{soil:solution ratio}$  was  $<0.3$  for the Hareby soil (1'-COOH-S-2840A all data points; 1'-COOH-S-2840B 2 data points), Speyer 2.3 and Atwater soils (all data points). This is not necessarily a concern given the high mass balance in all soils and for both isomers, and the fact that an optimal soil: solution ratio of 1:1 was selected.
- The OECD Guideline recommends that a minimum of 20 % should be adsorbed and preferably at least 50 %. In the Hareby soil, approximately 15-19 % was adsorbed for 1'-COOH-S-2840A ( $>20$  % for 1'-COOH-S-2840B in this soil), and similar amounts for both isomers of approximately 7-15 % and 8-15 % AR in the Speyer 2.3 and Atwater soils respectively.
- The systematic errors should be  $<1.2$ . Values  $>1.2$  were obtained for the Hareby soil (1.354-1.549), Speyer 2.3 (1.658-1.761) and Atwater soil (1.606-1.917). The systematic error check provides an estimate of how much the experimental  $K_{oc}$  may be overestimated compared to the actual  $K_{oc}$  when using the indirect method. This is because with the indirect method all loss of concentration in the liquid phase is attributed to sorption to soil, when in reality some additional loss may occur as a result of degradation and/or formation of non-extracted residues (which ideally should not be accounted for in equilibrium sorption measurements). Based on the analytical measurements during the preliminary test, both the A and B isomers were present at  $\geq 99\%$  in the supernatants and soil extracts, suggesting that the test compounds were stable under the conditions used. The loss values (f %) used in the systematic error check were therefore likely due to incomplete solvent extraction rather than test item instability.

**Table B.8.1.5.3-15 Summary of adsorption checklist parameters: 1'-COOH-S-2840A**

Parameter	Quilen (France)	Hareby (UK)	Clipstone (UK)	Speyer 2.3 (Germany)	Atwater (USA)
Adsorption method	Indirect	Indirect	Indirect	Indirect	Indirect
Soil:solution ratio (g dw/mL)	1:1	1:1	1:1	1:1	1:1
Parental recovery at highest concentration (%)	92.1	94.6	97.4	97.0	96.5
% Adsorbed (range)	51.21- 59.87	15.23- 19.18	29.30-36.80	6.94-10.95	7.50- 13.72
$K_{fE}/K_f$ ratios	1.180/	<b>1.518/</b>	1.096/	<b>1.727/</b>	<b>1.606/</b>

	1.182	<b>1.549</b>	1.097	<b>1.761</b>	<b>1.750</b>
<b>K<sub>d</sub> x soil: solution ratio</b>	1.05–1.49	0.18-0.24	0.41-0.56	0.07-0.12	0.10-0.16
<b>Visual fit</b>	Good	Good	Good	Good	Good
<b>K<sub>f,ads</sub> (95 % confidence intervals)</b>	1.057 (0.981-1.139)	0.190 (0.174-0.207)	0.409 (0.373-0.448)	0.080 (0.068-0.095)	0.094 (0.077-0.115)
<b>K<sub>foc</sub> (L/kg)</b>	35	11	33	11	28
<b>1/n (95 % confidence intervals)**</b>	0.940 (0.918-0.962)	0.963 (0.932-0.993)	0.936 (0.905-0.966)	0.956 (0.895-1.017)	0.937 (0.864-1.009)
<b>R<sup>2</sup></b>	0.9989	0.9987	0.9990	0.9941	0.9913

**Soils failing the systematic error check (>1.2) highlighted in bold**

\*\*Confidence intervals for 1/n calculated by HSE; as corresponding 1/n values are presented, these differ slightly from values presented in Table B.8.1.5.3-20 which were calculated by the applicant and are accepted for use in the exposure assessment.

The impact on the adsorption parameters of including and excluding the soils with K<sub>fe</sub>/K<sub>f</sub> >1.2 is considered in the table below.

**Table B.8.1.5.3-16 Comparison of mean adsorption parameters including and excluding soils that fail the systematic error check: 1'-COOH-S-2840A**

<b>Soil</b>	<b>K<sub>foc</sub> (L/kg)</b>	<b>1/n</b>
Quilen (France)	35	0.939
Hareby (UK)	11	0.966
Clipstone (UK)	33	0.937

Speyer 2.3 (Germany)	11	0.962
Atwater (USA)	28	0.945
<b>Mean of all soils (geomean for <math>K_{foc}</math> and arithmetic mean for <math>1/n</math>)</b>	<b>20.8</b>	<b>0.948</b>
<b>Mean of soils with <math>K_{fe}/k_f &lt; 1.2</math> (geomean for <math>K_{foc}</math> and arithmetic mean for <math>1/n</math>)</b>	<b>34.0</b>	<b>0.938</b>

As noted above, <20 % was adsorbed in the Hareby, Speyer 2.3 and Atwater soils for 1'-COOH-S-2840A. The  $K_{fe}/K_f$  values are also >1.2 for these soils. The data from these soils may potentially be unreliable and the implications of excluding these soils has been considered.

As the Hareby, Speyer 2.3 and Atwater soils are not very dissimilar to the values obtained from the other soils and will result in slightly more conservative mean parameters, HSE concludes that all 5 soils should be included in the data set.

**Table B.8.1.5.3-17 Summary of adsorption checklist parameters: 1'-COOH-S-2840B**

<b>Parameter</b>	<b>Quilen (France)</b>	<b>Hareby (UK)</b>	<b>Clipstone (UK)</b>	<b>Speyer 2.3 (Germany)</b>	<b>Atwater (USA)</b>
<b>Adsorption method</b>	Indirect	Indirect	Indirect	Indirect	Indirect
<b>Soil:solution ratio (g dw/mL)</b>	1:1	1:1	1:1	1:1	1:1
<b>Parental recovery at highest concentration (%)</b>	91.0	94.6	96.9	95.9	95.6
<b>% Adsorbed (range)</b>	55.31-67.13	19.15-29.97	32.13-44.12	10.03-14.75	9.20-15.12

<b>K<sub>fe</sub>/K<sub>f</sub> ratios</b>	1.194/ 1.185	<b>1.354/</b> <b>1.393</b>	1.098/ 1.107	<b>1.658/</b> <b>1.692</b>	<b>1.910/</b> <b>1.917</b>
<b>K<sub>d</sub> x soil: solution ratio</b>	1.24-2.04	0.24-0.41	0.47-0.79	0.11-0.18	0.10-0.18
<b>Visual fit</b>	Good	Good	Good	Good	Good
<b>K<sub>f,ads</sub> 95 % confidence intervals)</b>	1.355 (1.132- 1.621)	0.284 (0.229- 0.353)	0.556 (0.449- 0.687)	0.122 (0.103- 0.144)	0.119 (0.097- 0.146)
<b>K<sub>f</sub></b>	1.30	0.25	0.52	0.10	0.119*
<b>K<sub>foc</sub> (L/kg)</b>	45	16	44	15	40*
<b>1/n 95 % confidence intervals)</b>	0.927 (0.876- 0.978)	0.953 (0.877- 1.028)	0.972 (0.902- 1.042)	0.931 (0.870- 0.992)	0.940 (0.865- 1.015)*
<b>R<sup>2</sup></b>	0.9949	0.9881	0.9915	0.9905	0.9905*

\*Values calculated by HSE

Soils failing the systematic error check (>1.2) highlighted in bold

The impact on the adsorption parameters of including and excluding the soils with K<sub>fe</sub>/K<sub>f</sub> >1.2 is considered in the table below.

**Table B.8.1.5.3-18 Comparison of mean adsorption parameters including and excluding soils that fail the systematic error check: 1'-COOH-S-2840B**

<b>Soil</b>	<b>K<sub>foc</sub> (L/kg)</b>	<b>1/n</b>
Quilen (France)	45	0.927
Hareby (UK)	16	0.949
Clipstone (UK)	44	0.972

Speyer 2.3 (Germany)	15	0.923
Atwater (USA)*	40	0.940
<b>Mean of all soils (geomean for <math>K_{foc}</math> and arithmetic mean for <math>1/n</math>)</b>	<b>28.6</b>	<b>0.9422</b>
<b>Mean of soils with <math>K_{fe}/k_f &lt; 1.2</math> (geomean for <math>K_{foc}</math> and arithmetic mean for <math>1/n</math>)</b>	<b>44.5</b>	<b>0.9495</b>

\*HSE values, all other values calculated by applicant

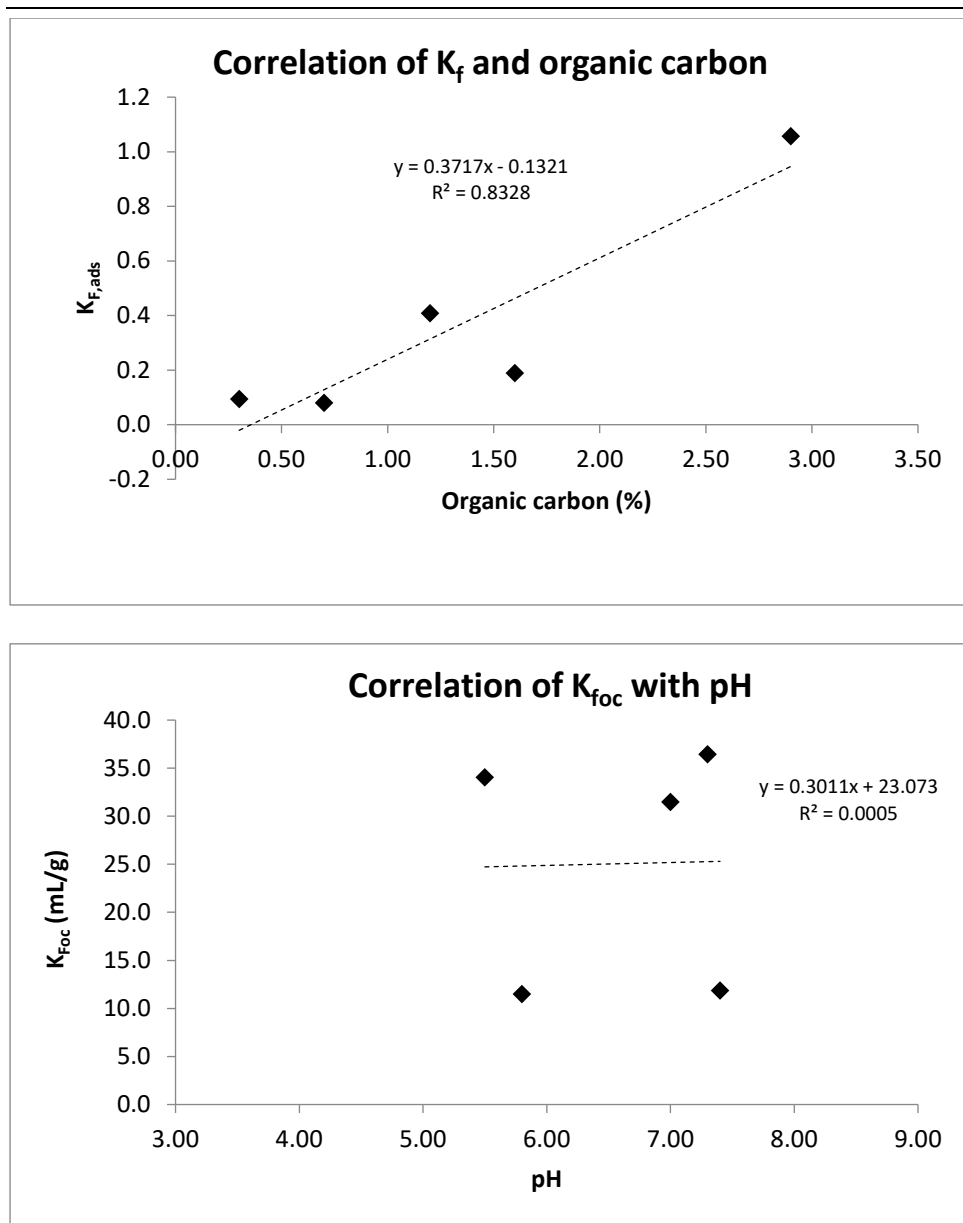
For 1'-COOH-S-2840B, adsorption is <20 % and the  $K_{fe}/K_f$  values are >1.2 in the Speyer 2.3 and Atwater soils. For the Hareby soil, the systematic errors are 1.354-1.393 and so above the 1.2 cutoff, while one percentage sorbed is marginally below 20 % (19.15 %). The Hareby soil therefore fails the criteria. It is noted however that the Hareby soil has the second highest  $1/n$  and therefore it is considered appropriate to retain this in the data set in order to ensure that the geometric mean is a conservative reflection of the data set.

The potential for removing the Speyer 2.3 and Atwater soils from the data set has been considered. In general, HSE considers the systematic error check to be an important criteria in determining the acceptability of results from sorption tests using the indirect method. In this case HSE has put less weight on the outcome of this check and accepted soils where strictly this criterion failed. This was partly to ensure the retention of conservative values and to avoid biasing the selection of soils in favour of those where sorption was strongest (and the checks most likely to pass). In addition, the preliminary test showed good evidence of test item stability ( $\geq 99\%$  present in supernatant and soils extracts as respective test items). The loss value (f %) used in the systematic error check was therefore likely due to incomplete solvent extraction rather than test item instability. HSE considers incomplete solvent extraction to be less important than test item stability when considering the outcome of this check. Overall HSE considered it reasonable to retain all soils in the dataset.

### pH Dependence

Correlations between  $K_f$  and organic carbon and  $K_{foc}$  with soil pH are assessed below.



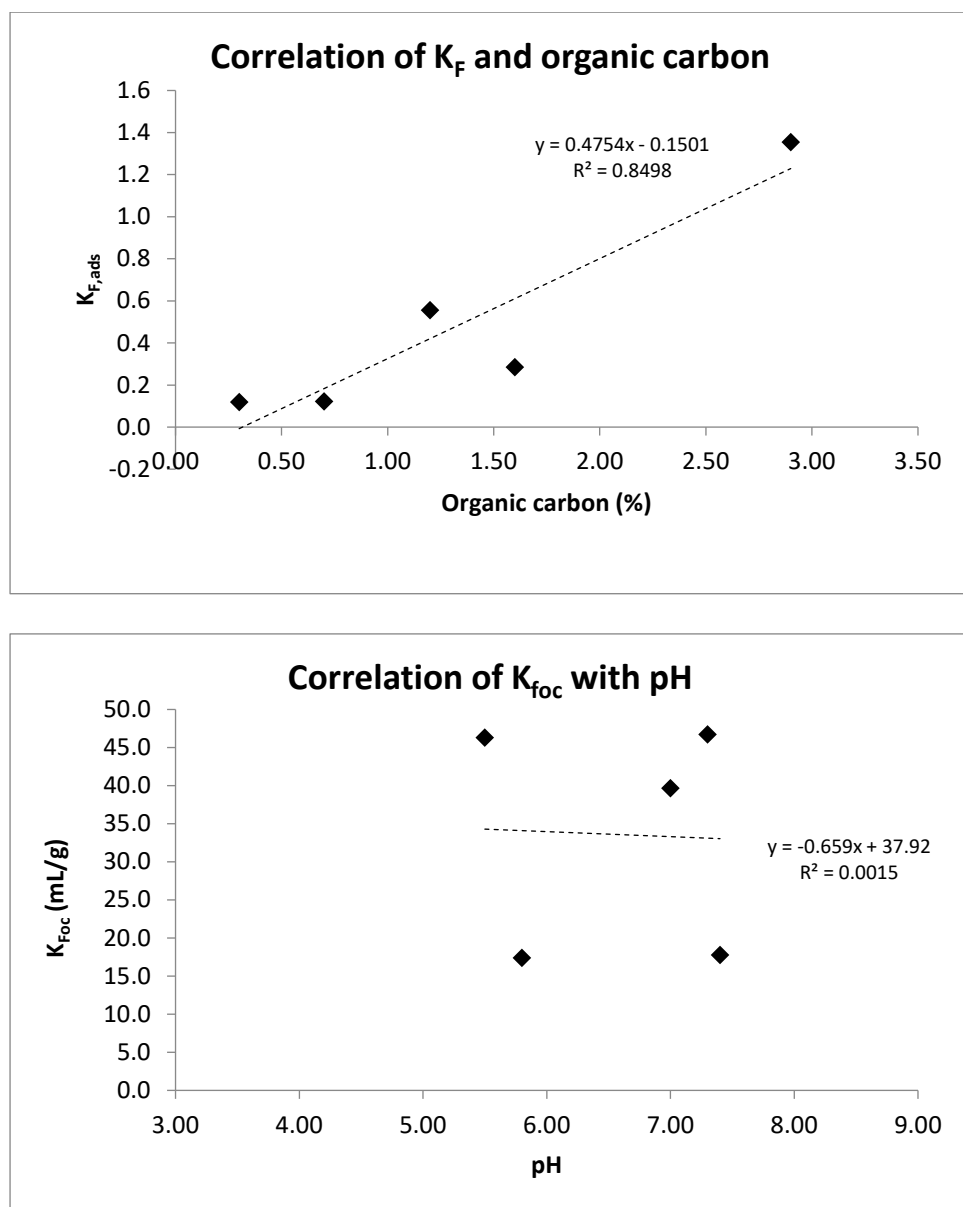


**Figure B.8.1.5.3-16 Correlations between  $K_f$  and organic carbon and  $K_{foc}$  with pH for 1'-COOH-S-2840A**

For 1'-COOH-S-2840A and using linear regression, the data show a correlation between  $K_f$  and organic carbon content of the soils ( $r^2 = 0.8328$ ). HSE calculated a Kendall rank correlation coefficient for the adsorption partition coefficient of 0.800 ( $p$ -value = 0.043) using the German support tool (pH Dependence Calculator). This indicates a strong positive correlation which is statistically significant.

HSE calculated a Kendall rank correlation coefficient for  $K_{foc}$  and pH of -0.200 using the German support tool (pH Dependence Calculator). This indicates a weak negative correlation between sorption and pH. The corresponding  $p$ -value was 0.806; as this is  $>0.05$  this correlation is considered not to be statistically significant.

X



**Figure B.8.1.5.3-17 Correlations between  $K_f$  and organic carbon and  $K_{foc}$  with pH for 1'-COOH-S-2840A**

For 1'-COOH-S-2840B, and using linear regression, the data show a correlation between  $K_f$  and organic carbon content of the soils ( $r^2 = 0.8498$ ). HSE calculated a Kendall rank correlation coefficient for the adsorption partition coefficient of 0.800 ( $p$ -value = 0.043) using the German support tool (pH Dependence Calculator). This indicates a strong positive correlation which is statistically significant.

HSE calculated a Kendall rank correlation coefficient for  $K_{foc}$  and pH of 0.000 with a corresponding  $p$ -value of 1.000 using the German support tool (pH Dependence Calculator). This indicates no correlation between sorption and pH.

The data suggests that there is no correlation between  $K_{\text{foc}}$  and pH with  $r^2$  values of 0.0005 for isomer 1'-COOH-S-2840A and 0.0015 for 1'-COOH-S-2840B. For both diastereomers, the tau value was 0 and the p value was 1.000, showing that there was no correlation between the two parameters. The mechanistic data has been considered to see if it corroborates the statistical data.

The structure has been considered to see if it contains ionisable functional groups. The presence of a carboxylic acid group is noted which suggests that ionisation might occur.

This is corroborated by the mechanistic data, which is as follows for 1'-COOH-S-2840:

**Table B.8.1.5.3-19 Change in the partition coefficient of 1'-COOH-S-2840A and 1'-COOH-S-2840B across a range of pH at 25 °C**

pH	1'-COOH-S-2840A	1'-COOH-S-2840B
5	0.84	0.97
7	<0.3	
9		

Values for log  $P_{\text{ow}}$ , for molecules generally, typically range between -3 (very hydrophilic) and +10 (extremely lipophilic/hydrophobic). The data for log  $P_{\text{ow}}$  (see Section B.1, [REDACTED] and [REDACTED] 2016) show that there is a tendency towards the metabolite being more hydrophilic at higher pH values, but, while there is some difference across the pH range due to the logarithmic scale, there is little difference between the two isomers. This data supports the conclusion that there is no mechanistic evidence for pH dependence of 1'-COOH-S-2840 and also indicates that the two isomers are likely to exhibit similar characteristics.

No data on the solubility or the pKa of the metabolite have been provided. It is noted however that that studies submitted in the Section B.1 demonstrate that the solubility of the parent does not change across the environmentally relevant pH range. The test item was also stable under the conditions of the sorption study. The structure of the metabolite differs to that of the parent in that a methyl group is replaced by a carboxylic acid group. The carboxylic acid functional group and log  $P_{\text{ow}}$  <1 might indicate a tendency towards being hydrophilic and greater ionisation for the metabolite, as confirmed by the higher  $K_{\text{foc}}$  values of the parent compared to the metabolite (geometric mean of 647 mL/g for parent and 23.7 mL/g for the metabolite (see below)). Given the complexity of the molecule however, it is difficult to confirm how much the properties of the metabolite deviate from that of the parent. It is

concluded however that based on the information available, no pH dependence is apparent as the molecule is fully dissociated >pH 5.

Consideration of pH dependence for 1'-COOH-S-2840 (A and B) in the exposure assessment is not required.

**Table B.8.1.5.3-20 Summary of endpoints for use in the risk assessment**

<b>Soil</b>	<b>Soil type (USDA)</b>	<b>C<sub>org</sub> (%)</b>	<b>pH (CaCl<sub>2</sub>)</b>	<b>K<sub>f</sub> (mL/g)</b>	<b>K<sub>foc</sub> (mL/g)</b>	<b>1/n</b>	<b>R<sup>2</sup></b>
<b>1'-COOH-S-2840A</b>							
<b>Quilen (Pas de Calais, France)</b>	Silt loam or Loam	2.9	7.3	1.057	35	0.939	0.9989
<b>Hareby (Lincolnshire, UK)</b>	Clay loam	1.6	7.4	0.190	11	0.966	0.9987
<b>Clipstone (Nottinghamshire, UK)</b>	Loamy sand	1.2	5.5	0.409	33	0.937	0.9990
<b>Speyer (Rheinland-Pfalz, Germany)</b>	Sandy loam	0.7	5.8	0.080	11	0.962	0.9941
<b>Atwater (California, USA)</b>	Sandy loam	0.3	7.0	0.094	28	0.945	0.9913
<b>pH dependence</b>					<b>No</b>		
<b>Geometric mean</b>					20.8		
<b>Arithmetic mean</b>						0.950	

1'-COOH-S-2840B							
<b>Quilen (Pas de Calais, France)</b>	Silt loam or Loam	2.9	7.3	1.30	45	0.927	0.9949
<b>Hareby (Lincolnshire, UK)</b>	Clay loam	1.6	7.4	0.25	16	0.949	0.9881
<b>Clipstone (Nottinghamshire, UK)</b>	Loamy sand	1.2	5.5	0.52	44	0.972	0.9915
<b>Speyer (Rheinland-Pfalz, Germany)</b>	Sandy loam	0.7	5.8	0.10	15	0.923	0.9905
<b>Atwater (California, USA)</b>	Sandy loam	0.3	7.0	0.09	40	0.940	0.9905
<b>pH dependence</b>					No		
<b>Geometric mean</b>					28.6		
<b>Arithmetic mean</b>						0.942	

HSE data for Atwater soil, 1'-COOH-S-2840B, all other data applicant data

For 1'-COOH-S-2840A, the  $K_{\text{foc}}$  values for adsorption ranged between 11 and 35 L/kg (geometric mean of 21 L/kg). The corresponding  $1/n$  values ranged between 0.937 and 0.966 (arithmetic mean of 0.950). For 1'-COOH-S-2840B, the  $K_{\text{foc}}$  values for adsorption ranged between 16 and 45 L/kg (geometric mean of 27 L/kg). The corresponding  $1/n$  values ranged between 0.927 and 0.972 (arithmetic mean of 0.942). The adsorption parameters for the two isomers are therefore similar, with the values for 1'-COOH-S-2840B are overall slightly higher.

For desorption of 1'-COOH-S-2840A, the  $K_{\text{foc}}$  values ranged between 15 and 53 L/kg (geometric mean of 29 L/kg), while the  $1/n$  values were 0.929 to 0.963 (arithmetic mean of 0.945). For desorption of 1'-COOH-S-2840B, the  $K_{\text{foc}}$  values ranged between 22 and 61 L/kg (geometric mean of 36 L/kg), while the  $1/n$  values were 0.916 to 0.985 (arithmetic mean of 0.943). The desorption parameters for the two isomers are therefore similar.

Desorption parameters were slightly higher than adsorption parameters indicating a degree of irreversibility.

### Comparison of sorption parameters for 1'-COOH-S-2840A and 1'-COOH-S-2840B

GB Guidance, 'Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers' provides recommendations for consideration of adsorption studies for diastereomers to be used alongside the EFSA Guidance document, 'Guidance of EFSA on risk assessments for active substance of plant protection products that have stereoisomers as components and impurities and for transformation products of active substances that may have stereoisomers' (2019). The need for including both diastereomers separately or using mean values of the adsorption parameters of 1'-COOH-S-2840A and B in the exposure assessment has been considered in line with this guidance. The guidance recommends focusing on the potential for differences in sorption between diastereomers and to focus on the factors most likely to influence sorption. The GB guidance proposes an approach based on consideration of the individual  $K_{foc}$  values, taking into account supporting information on the substance as a whole. This includes consideration of the susceptibility of the compound to leaching based on the  $K_{foc}$  value and degradation rate and the overall leaching potential. The appropriateness of an exposure assessment based on average sorption behaviour relative to separate exposure assessments based on the sorption behaviour of each diastereomer can then be considered.

The adsorption parameters for the two isomers of 1'-COOH-S-2840 are compared below.

**Table B.8.1.5.3-21 Comparison of 1'-COOH-S-284A , 1'-COOH-S-2840B and mean sorption endpoints (HSE data)**

	<b>1'-COOH-S-2840A</b>	<b>1'-COOH-S-2840B</b>	<b>Mean of both diastereomers*</b>
<b>Adsorption</b>			
<b><math>K_{foc}</math> (L/kg)</b>	20.8	28.6	24.4
<b>1/n</b>	0.950	0.942	0.946

\*Means of the two individual mean values for two isomers;  $K_{foc}$  geometric mean, 1/n arithmetic mean HSE data for Atwater soil, 1'-COOH-S-2840B, all other data applicant data

The sorption parameters are similar for the two diastereomers. In particular, the 1/n for adsorption is very similar for the two diastereomers and this parameter is known to be particularly influential in groundwater modelling. The  $K_{foc}$  values indicate that 1'-COOH-S-2840 is of very high mobility. This means that small differences in the sorption parameters can have a big influence on the behaviour of the substance.

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The modelling endpoint for 1'-COOH-S-2840 derived for use in the exposure assessment is 74.7 days (both stereoisomers combined); 1'-COOH-S-2840 is therefore of medium persistence in the EFSA persistence classification. The 80<sup>th</sup> percentile PEC<sub>gw</sub> value calculated by the applicant exceed 0.1 µg/L in all scenarios (range 0.081 to 0.429 µg/L), based on a  $K_{foc}$  of 24 L/kg and 1/n 0.943. As this metabolite has a relatively long DT<sub>50</sub> value and is of very high mobility, there is some evidence that the differences in sorption parameters will make some difference to groundwater exposure for this metabolite. As the 0.1 µg/L trigger value is exceeded in several scenarios, acceptable levels for this metabolite will be dependent on mammalian toxicology data. Input parameters for modelling will be considered further in the CP Part B.8.

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**B.8.1.5.4. Aged sorption**

No studies were submitted or required.

**B.8.1.6. Mobility in soil**

No studies were submitted or required.

The study of mobility in soil is triggered when it is not possible to obtain reliable adsorption co-efficient values for four soils from laboratory adsorption studies. However, the study of mobility in soils was not triggered as inpyrfluxam consistently demonstrated  $K_{oc}$  values greater than 25 mL/g. Therefore, the potential mobility can be determined from the adsorption/ desorption studies under B.8.1.4 and the data requirements 7.1.4.1.1 (column leaching studies of the active substance), 7.1.4.1.2 (column leaching studies of metabolites) and 7.1.4.2 (lysimeter studies) were not required for this active substance.

**B.8.1.6.1. Column leaching**

No column leaching studies were performed.

**B.8.1.6.2. Lysimeter studies**

No lysimeter studies were performed.

**B.8.1.7. Persistence in soil**

Persistence (P) or very persistent (vP) criteria are defined according to Section 3.7.2.1 and 3.7.3.1, respectively, of Annex II of EC Regulation 1107/2009 as follows:

*An active substance, safener or synergist fulfils the persistence criterion where:*

- *The half-life in soil is higher than 120 days.*

*An active substance, safener or synergist fulfils the 'very persistent' criterion where:*

- *The half-life in soil is higher than 180 days.*

The relevant endpoints for the persistence assessment were identified based on the DG SANCO working document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides" [SANCO 2012. DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides". Brussels: European Commission Health and Consumers Directorate-General. Report 25.09.2012 - rev. 3.]. According to this document, when available, field degradation half-lives are relevant for the P and vP assessment.



The degradation of inpyrfluxam was investigated in four soils under aerobic conditions in the laboratory studies (KCA 7.1.1.1/01 and 7.1.1.1/02). Additionally, the dissipation of inpyrfluxam was investigated under field conditions, with field plots established in representative growing regions of Europe, or in one case, soil representative of European growing regions (Ontario). (see KCA 7.1.2.21.1/05 and KCA 7.1.2.1.1/07). In the Ontario trial, inpyrfluxam was applied to bare soil. Necessary measures to minimise the impact of soil surface processes were not performed, and as such only data points recorded after 10mm of cumulative irrigation and/or rainfall were included in the kinetic evaluation. In the European field trials, inpyrfluxam was incorporated into soil to exclude surface processes and to enable a straightforward generation of modelling DegT<sub>50</sub> as input for calculation of predicted environmental concentrations.

The kinetic evaluation was performed in order to derive best-fit field degradation parameters for inpyrfluxam according to the FOCUS kinetics guidance [*FOCUS. (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Version 1.1, 440 pp*]]. HSE excluded degradation endpoints derived from field studies conducted outside of Europe which were not representative of European climate and soil conditions (see B.8.1.2.1.1).

An additional kinetic evaluation was performed to derive degradation parameters that can be used as inputs for modelling in line with the EFSA Guidance [EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT<sub>50</sub> values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662]. By the guidance, as the geomean laboratory DegT<sub>50</sub> exceeded 240 days, only field DegT<sub>50</sub> values were used to derive an endpoint. The non-normalised DegT<sub>50</sub> of inpyrfluxam from field studies ranged from 10.9 – 383 days. This half-life describes the degradation rate in bulk soil: degradation due to surface processes is not included.

Due to the exclusion of surface processes in the kinetic evaluation, the DegT<sub>50</sub> values derived from data collected in the relevant field studies is appropriate for an initial conservative assessment of persistence of inpyrfluxam in soil. Considering the DegT<sub>50</sub> values of 10.9 to 383 days derived from the suitable field studies, inpyrfluxam constitutes a borderline case in which a weight of evidence approach is required.

While laboratory studies have not been considered for endpoint determination, they are included here as part of the approach for concluding on the persistence of inpyrfluxam. These are summarised in Table B.8.1.6-01 alongside the field studies.

**Table B.8.7.1-01 Studies considered for evaluating the persistence of inpyrfluxam in soil**

Trigger criteria	Value	Study	Comments	Criteria met?
<b>DT<sub>50</sub> soil &gt;180 days</b>	<p>Longest DegT<sub>50</sub> = 383 days</p> <p>Geomean = 221.8 days (using SFO and DT<sub>90</sub>/3.32 of biphasic values)</p>	<p><i>S-2399: Terrestrial Field Soil Dissipation of S-2399 on Bare Ground in Ontario, Canada.</i></p> <p>██████████, 2017, KCA 7.1.2.21.1/05</p> <p><i>Soil dissipation study after application of S-2399 at four different locations in Europe – 2016/2018</i></p> <p>██████████, 2018, KCA 7.1.2.21.1/07</p>	<p><u>Soil aerobic field (non-normalised):</u></p> <p>Longest worst case DT<sub>50</sub> = 383 days, Sandy loam, SFO kinetics.</p> <p>Geometric mean DT<sub>50</sub> = 221.8 days (using DT<sub>90</sub>/3.32 of bi-phasic values, range 164 - 383 days)</p> <p>Three of five soils breach the 180 day trigger.</p>	Yes
<b>DT<sub>50</sub> soil &gt;180 days</b>	<p>Longest DegT<sub>50</sub> = 301 days</p> <p>Geomean = 240 days (using SFO and DT<sub>90</sub>/3.32 of biphasic values)</p>	<p><i>Aerobic Soil Metabolism of [Phenyl-<sup>14</sup>C] S-2399 and [Pyrazolyl 4 <sup>14</sup>C] S-2399; Amended Report</i></p> <p>██████████, 2017, KCA 7.1.1.1/01</p> <p><i>S-2399: Degradation under Aerobic Conditions in Soil Rate</i></p> <p>██████████, 2017, KCA 7.1.1.1/02</p>	<p><u>Soil aerobic laboratory (normalised):</u></p> <p>Longest worst case DT<sub>50</sub> = 301 days, 3 soils: two loam, one silt loam. DFOP kinetics, DT<sub>90</sub>/3.32.</p> <p>Geometric mean DT<sub>50</sub> = 240 days (using DT<sub>90</sub>/3.32 of bi-phasic</p>	Yes

Trigger criteria	Value	Study	Comments	Criteria met?						
		<p><i>Recalculation of the laboratory aerobic degradation rate of S-2399 (inpyrfluxam) in soil according to FOCUS Kinetics Guidance</i></p> <p>██████████ &amp; ██████████, 2023</p>	<p>values, range 121 - 301 days)</p> <p>Three of four soils breach the 180 day trigger.</p>							
Weight of evidence	One soil; DegT <sub>50</sub> >10,000 days	<p><i>S-2399: Anaerobic Soil Metabolism</i></p> <p>██████████ &amp; ██████████, 2017, KCA 7.1.1.2/01</p>	<p><u>Soil anaerobic laboratory (normalised):</u></p> <p>One soil tested, showing no degradation</p>	-						
Weight of evidence	Longest DT <sub>50</sub> = 763	<p><i>Photodegradation of [<sup>14</sup>C] S-2399 in/on Soil by Artificial Sunlight</i></p> <p>██████████, 2014, KCA 7.1.1.3/01</p>	<p><u>Soil photolysis:</u></p> <p>All kinetics SFO.</p> <table><tr><th>Sunlight equivalence</th><th>DT<sub>50</sub> range (d)</th></tr><tr><td>Summer, 30°N</td><td>703-763</td></tr><tr><td>Summer, 40°N</td><td>634-688</td></tr></table>	Sunlight equivalence	DT <sub>50</sub> range (d)	Summer, 30°N	703-763	Summer, 40°N	634-688	-
Sunlight equivalence	DT <sub>50</sub> range (d)									
Summer, 30°N	703-763									
Summer, 40°N	634-688									

Trigger criteria	Value	Study	Comments		Criteria met?
			Summer, 50°N	591-641	

HSE find that three out of five field studies breach the 180 day trigger for classification as ‘very persistent’. The geometric mean of the field studies was also found to sit above the trigger value. Three of four laboratory studies were found to breach the 180 day trigger, as does the geometric mean value of the laboratory studies.

Anaerobic degradation (see B.8.1.1.1.5) and photodegradation (see B.1.1.1.6) were also considered. No degradation was observed under anaerobic conditions, while photodegradation was found to have DT<sub>50</sub>'s of 591-763 days at 30-50°N. Both of these studies therefore support classification of inpyrfluxam as ‘very persistent’ using a weight of evidence approach.

HSE therefore considers that while the range of DegT<sub>50</sub> values for inpyrfluxam does include values significantly below the trigger, inpyrfluxam should be classed as ‘very persistent’.

## B.8.2. Fate and behaviour in Water and Sediment

The applicant investigated the fate and behaviour of inpyrfluxam in the aquatic environment through a series of studies that investigated the chemical and photochemical degradation [see B.8.2.1], and biological degradation [see B.8.2.2]. Additionally, the applicant investigated the potential effects of water treatment procedures on inpyrfluxam and its metabolites [see B.8.2.3]. Furthermore, the persistence of inpyrfluxam in the aquatic environment is assessed.

Two major metabolites, defined as breakdown products reaching 10% or more of the applied radioactivity of 5 % or more at two consecutive time points, were observed in aquatic system studies and these are summarised in Table B.8.2-01.

**Table B.8.2-01 Metabolites identified in aquatic degradation studies**

<b>Metabolite identity</b>	<b>Relevant studies</b>	<b>Peak formation (% AR)</b>
<b>1'-COOH-S-2840 isomers A and B combined</b>	Water-sediment	13.1% whole system 10.0 % water / 4.8 % sediment
<b>3'-OH-S-2840</b>	Indirect photolysis Water-sediment	8.6 % 6.8 % whole system 2.9 % water / 6.0 % sediment

**Route and rate of chemical and photochemical degradation in aquatic systems**

The applicant investigated the route and rate of chemical and photochemical degradation in the aquatic environment in three studies covering aqueous hydrolysis, and direct and indirect aqueous photolysis. Table B.8.2-02 summarises the relevant studies. Kinetic evaluations were performed for the photolysis studies to derive trigger endpoints in both cases. One major metabolite was identified to be a product of indirect photochemical degradation, peaking at 11.5% AR after 15 days of irradiation.

**Table B.8.2-02 Laboratory studies investigating the chemical and photochemical degradation of inpyrfluxam in aquatic systems.**

<b>Laboratory study</b>	<b>Study type</b>	<b>Endpoints calculated?</b>
<div style="background-color: black; width: 100px; height: 15px; margin-bottom: 5px;"></div> 2016 KCA 7.2.1.1/1	Aqueous hydrolysis	None
<div style="background-color: black; width: 100px; height: 15px; margin-bottom: 5px;"></div> 2015a KCA 7.2.1.2/1	Direct aqueous photolysis (sterilised buffer)	Trigger

<div style="display: flex; align-items: center;"> <div style="width: 20px; height: 20px; background-color: black; margin-right: 5px;"></div> <div style="width: 20px; height: 20px; background-color: black; margin-right: 5px;"></div> 2015b KCA 7.2.1.3/1 </div>	Indirect aqueous photolysis (sterilised natural water)	Trigger
--	--	---------

The aqueous hydrolysis of inpyrfluxam was investigated at three pH levels (4, 7 and 9) over 5 days at 50 °C [see KCA 7.2.1.1/1]. The applicant also investigated the enantiomer ratio for any changes through the duration of the study. Inpyrfluxam was hydrolytically stable in aqueous solution at all 3 pH levels, with all samples measuring above 96.2% AR after 5 days. It was not possible to calculate degradation rates, and as a result it is concluded that hydrolysis is not a significant route of degradation for inpyrfluxam. No *R* to *S* isomerisation of the test substance occurred.

The aqueous photolysis of inpyrfluxam was explored in two studies, in both sterile aqueous buffer and sterile natural water. Both studies were radiolabelled with one radiolabel position [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam used for the study in aqueous buffer and two radiolabel positions ie ([pyrazolyl-4-<sup>14</sup>C] inpyrfluxam and [phenyl-U-<sup>14</sup>C] inpyrfluxam) for the sterile natural water. In the study with sterile aqueous buffer solution, the degradation of inpyrfluxam was investigated under 15 days of continuous artificial irradiation (equivalent to 42 days of natural sunlight at 40°N) [see KCA 7.2.1.2/1]. After 15 days, there was little or no decrease in inpyrfluxam levels with inpyrfluxam remaining around 100% AR in the irradiated samples and in the dark control samples. No DT<sub>50</sub> was determined according to FOCUS due to the negligible degradation observed. In the study with sterile aqueous natural water, the degradation of inpyrfluxam was investigated under 16 days of continuous artificial irradiation (equivalent to 34 days of natural sunlight at 40°N) [see KCA 7.2.1.2/2]. After 16 days, there was little or no decrease in ([pyrazolyl-4-<sup>14</sup>C] inpyrfluxam levels with inpyrfluxam remaining close to 100% AR in the photolysis samples and declining to a mean of 92.6 % AR in the [phenyl-U-<sup>14</sup>C] inpyrfluxam. In the dark control samples, a mean of 97.6 to 102.8 % AR inpyrfluxam remained at study end. The DT<sub>50</sub> values were determined to be 92 days ([pyrazolyl-4-<sup>14</sup>C] inpyrfluxam) and 41.2 days ([phenyl-U-<sup>14</sup>C] inpyrfluxam) in artificial light, or 197 days ([pyrazolyl-4-<sup>14</sup>C] inpyrfluxam) and 88 days ([phenyl-U-<sup>14</sup>C] inpyrfluxam) in natural sunlight (all after correction for the dark control). The degradation rates suggest that photolytic degradation in natural waters may contribute somewhat to the overall degradation of inpyrfluxam, depending on the significance of other potential removal processes. Quantum yield was calculated in the direct photolysis test but was not verified by HSE due to the lack of significant degradation.

Overall, the radiolabelling was adequate for following the metabolism of inpyrfluxam in these studies.

**Table B.8.2-03 Summary of trigger endpoints for the indirect photolysis of inpyrfluxam following 16 days of continuous irradiation**

	<b>DT<sub>50</sub> in Suntest (days)</b>	<b>DT<sub>50</sub> in Suntest (hours)<sup>1</sup></b>	<b>US (40 °N summer)<sup>2</sup> (days)</b>	<b>OECD (30-50 °N, summer)<sup>3</sup> (days)</b>	<b>JMAFF (35 °N spring)<sup>4</sup> (days)</b>
PYR label	74.4	1786	159	145	465
PH label	37.6	902	81	73	235
HSE (corrected for dark control)					
PYR label	92	2208	197	179	576
PH label	41.2	989	88	80	258

<sup>1</sup> Continuous Suntest irradiation<sup>2</sup> Average summer irradiation in the 300-800 nm range at 40 °N latitude<sup>3</sup> Average summer irradiation in the 300-400 nm range at 30-50 °N latitude<sup>4</sup> Average spring irradiation in the 300-400 nm range at 35 °N latitude in Tokyo**Route and rate of biological degradation in aquatic systems**

The applicant submitted three laboratory studies to investigate the route and rate of biological degradation of inpyrfluxam in aquatic systems, supplemented by a kinetic modelling study. Studies were performed using [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam and [phenyl-U-<sup>14</sup>C] inpyrfluxam. Table B.8.2-05 summarises the relevant studies.

**Table B.8.2-05 Laboratory studies investigating biological degradation of inpyrfluxam in aquatic systems.**

<b>Laboratory aquatic study</b>	<b>Study type</b>	<b>Endpoints calculated?</b>
██████████ 2016 KCA 7.2.2.1/1	Ready biodegradability (CO <sub>2</sub> evolution)	None
██████████ 2017 KCA 7.2.2.2/1	Aerobic mineralisation	Trigger
██████████ 2017b	Water/sediment	None

<p>██████ and ██████ 2017a</p> <p>KCA 7.2.2.3/1 and 2</p>		
<p>██████ and ██████ (2023)</p> <p>Submitted in response to admissibility check</p>	<p>Kinetic evaluation of water/sediment study</p>	<p>Modelling and trigger</p>

The ready biodegradability of inpyrfluxam was studied by measuring the formed carbon dioxide (OECD 301 B: CO<sub>2</sub> evolution test) [see KCA 7.2.2.1/1]. The study passed all validity criteria. Inpyrfluxam cannot be classified as readily biodegradable by the criteria set forth in OECD Guideline 301 B since it did not achieve 60 % CO<sub>2</sub> evolution within a 10-day window by day 28. The toxicity control demonstrated that inpyrfluxam does not cause the inoculum activity to cross the OECD 301 B inhibitory threshold.

The aerobic mineralisation of inpyrfluxam was investigated in natural water in a pelagic test [See KCA 7.2.2.2/1]. The applicant studied two radiolabels ([pyrazolyl-4-<sup>14</sup>C] inpyrfluxam and [phenyl-U-<sup>14</sup>C] inpyrfluxam) at two concentrations: 10 µg/L and 100 µg/L. No significant degradation of inpyrfluxam was observed, with 96.5 % AR ([pyrazolyl-4-<sup>14</sup>C] inpyrfluxam) and 92.1 % AR ([phenyl-U-<sup>14</sup>C] inpyrfluxam) remaining in water at the low concentration, and 90.4 % AR and 90.6 % AR remaining at the high concentration after 61 days. Volatiles peaked at 0.4 % AR at 30 days for [phenyl-U-<sup>14</sup>C] inpyrfluxam. The metabolite observed at the highest concentration was not identified as it was present at only 3.9 % AR (14 days after application, [phenyl-U-<sup>14</sup>C] inpyrfluxam, high and low concentration) and was not therefore a major metabolite. Chiral analysis demonstrated that for both phenyl and pyrazolyl-labelled inpyrfluxam, only the R-isomer was present in both the application solution and at 61 days after application; it was therefore concluded that isomerisation to the S-isomer did not occur. The kinetic evaluation conducted by the applicant determined that inpyrfluxam follows single first order degradation with an observed DT<sub>50</sub> of 1540 to 23600 days; these values are extrapolated well beyond the duration of the study and so should be treated with caution.

The biotransformation of inpyrfluxam was investigated in five water-sediment systems across two separate studies under aerobic aquatic conditions using [phenyl-<sup>14</sup>C] inpyrfluxam (PH-label) and [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PY-label).

In the first study (██████ 2017b) [KCA 7.2.2.3/01], biotransformation was investigated in the Golden Lake and Taunton River systems. The majority of the dose remained unchanged after 112 days of aerobic aquatic exposure and ultimate mineralization to



bound residues and CO<sub>2</sub> was minor. Two metabolites were observed above 5% AR: 3'-OH-S-2840 (max. 6.8% AR), and 1'-COOH-S-2840, (max. 13.1% AR). N-demethylation of the pyrazolyl ring to produce N-des-Me-S-2840 was minor as well as hydrolysis of the amide bond to produce the pyrazolyl derivatives DFPA and DFPA-CONH<sub>2</sub>. Whole system aerobic aquatic half-lives were estimated at >10,000 d (DFOP) and 758 d (SFO) for Golden Lake and Taunton River, respectively.

In the second study (██████ and ██████ 2017a) [KCA 7.2.2.3/02], biotransformation was investigated in the Goose River, Sharkey and Weweantic River systems. The degradation pattern of inpyrfluxam was similar in all three sediment systems with inpyrfluxam declining to 80.0 to 85.0 % AR by the end of the 111 day study. Aerobic total-system half-lives were estimated at 212, 364 and 395 days (SFO fit, CAKE 3.7, verified with KinGUI). Metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 (as 1'-COOH-S-2840 A + 1'-COOH-S-2840 B) at maximum levels of 2.9 % AR and 8.1 % AR respectively.

*Comparison of behaviour of 1'-COOH-S-2840 isomers in water/sediment studies*

Changes in enantiomeric excess were considered by HSE for sections B.8.2.2.3.1 and B.8.2.2.3.2 in accordance with the principles outlined in the EFSA Guidance document, 'Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers' (2019) and the 'GB Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers'. It was identified that changes in enantiomeric excess did not occur in all five of the water-sediment systems tested. A single exposure assessment for the sum of the isomers is therefore considered appropriate.

Overall across the five systems tested, inpyrfluxam was observed to partition significantly from the water phase into the sediment phase. As such, the sediment compartment is considered to be the relevant compartment against which to assess inpyrfluxam persistence criteria. The very persistent trigger in freshwater sediment has been determined as a DT<sub>50</sub> value > 180 days, according to the criteria in Regulation (EC) No 1107/2009. Following determination of the total system trigger/persistence endpoints in Table B.8.2.2.3.2-28, inpyrfluxam can be observed to exceed this trigger value in all five systems.

### B.8.2.1. Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

#### B.8.2.1.1. Hydrolytic degradation

<b>Report:</b>	KCA 7.2.1.1/01
<b>Title</b>	[ <sup>14</sup> C] S-2399: Hydrolysis at pH 4, 7 and 9
<b>Author</b>	██████████ (2016)
<b>Document No.:</b>	TPM-0030
<b>Guidelines</b>	OECD Guideline 111 – Hydrolysis as a function of pH (Apr 2004) US EPA OCSP Test Guidelines No. 835.2120
<b>GLP?</b>	Yes
<b>Acceptability?</b>	Yes
<b>Study relied upon?</b>	Yes

<b>Deviation</b>	<b>HSE assessment of deviation</b>
One radiolabel only [pyrazolyl-4- <sup>14</sup> C] inpyrfluxam. Phenyl ring not labelled.	Minor deviation as [ <sup>14</sup> C] inpyrfluxam did not degrade over 10 % AR, metabolites based on phenyl ring structure should not be produced in amounts >10 % AR.
Dates of preparation of buffers are not included.	Minor omission as buffers are shown to be sterile and pH-stable in the study conduct section.

<b>Previous evaluations:</b>	None – report submitted as part of a new active substance registration.
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## INTRODUCTION

The rate and route of hydrolysis of [<sup>14</sup>C] inpyrfluxam was studied in three aqueous buffer solutions using [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam. For a Tier 1 preliminary test, [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam was applied at 1.00 µg/mL to 10.0 mL sterile pH 4, 7, and 9 buffer solutions, incubated in the dark at 50 ± 0.5 °C and samples analysed at 0 and 5 days.

The applicant conducted the study to both OECD and EPA guidelines. HSE has used the OECD Test Guideline No. 111 and Commission Regulation (EU) No. 283/2013 in

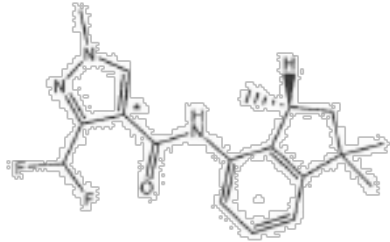
accordance with Retained Regulation (EC) No 1107/2009 to assess the validity of this study.

## MATERIALS AND METHODS

### 1. Test items

One labelled test item was used in this study: [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam, with a radiochemical purity of 99.3 %. This purity is acceptable to HSE. The chemical structure is as follows:

**Table B.8.2.1.1-01 Radiolabelled test substance information**

<b>Specific activity:</b>	2.11 GBq/mmol
<b>Lot/Batch:</b>	CFQ41802
<b>Radiochemical Purity:</b>	99.3 % by HPLC
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	stable
<b>Chemical structure</b>	 <p>*Denotes position of <sup>14</sup>C label</p>

HSE notes that only one radiolabel location is included in this study – [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam. The OECD 111 Guideline states that test items with more than one ring should preferably have the additional rings labelled as well.

The proposed degradation pathway in aquatic systems (Section B.8.2.2.3.1, Figure B.8.2.2.3.1-01) shows four metabolites in the water sediment studies have the pyrazole ring in the structures. However, there is one water sediment metabolite, ATMI, which only includes the phenyl ring and so would not be captured if indeed formed in this hydrolysis study.

The actual study results show that [<sup>14</sup>C] inpyrfluxam did not degrade over 10% of applied radioactivity over the 5 day incubation period at all tested pH conditions at elevated temperature. Mean values of metabolites were all < 5 % AR, due to the high recovery of test item at the study end (96.2 - 98.9 % mean AR). Therefore, HSE concludes that metabolites based on the unlabelled ring structure would not be produced in quantities warranting identification by OECD 111, under these test conditions. Furthermore, HSE considers it unlikely that novel aquatic metabolites of inpyrfluxam would have formed in the hydrolysis study and not be present in the other

abiotic or biotic water studies. The single radiolabel position used in the study was therefore accepted by HSE.

### Non-radiolabelled Test Substance

The following information was provided for the non-radiolabelled test substance, inpyrfluxam AS.

**Table B.8.2.1.1-02 Non-radiolabelled test substance information**

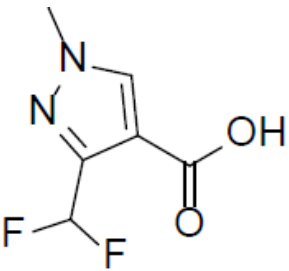
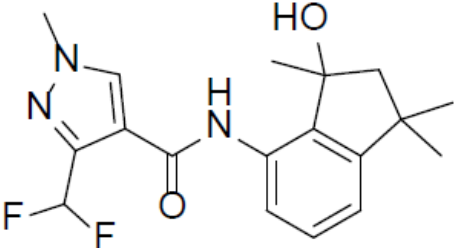
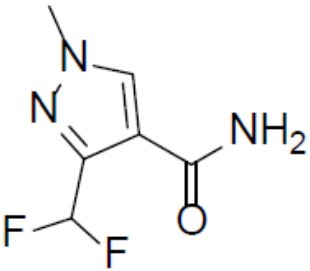
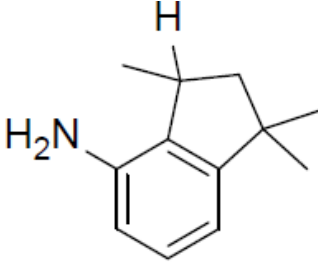
<b>Lot/Batch:</b>	YT3424G
<b>Chemical Purity:</b>	99.9% as inpyrfluxam
<b>Chemical Name (IUPAC):</b>	3-(difluoromethyl)-1-methyl-N-[(3'R)-1',1',3'-trimethyl-2', 3'-dihydro-1'H-inden-4'-yl]-1H-pyrazole-4-carboxamide
<b>Expiration Date:</b>	22 May 2016

### Non-radiolabelled Reference Substances

Six unlabelled reference items were also used for identification purposes: inpyrfluxam (pure *R*-isomer), S-2490 (pure *S*-isomer of inpyrfluxam), DFPA, 3'OH-S-2840, DFPA-CONH<sub>2</sub> and ATMI. Purities were within acceptable range (97.8 - 99.8 %).

**Table B.8.2.1.1-03 Reference substances**

Reference Substance	Lot Number	Expiration Date	Chemical Purity (%)	Chemical Structure
Inpyrfluxam (pure <i>R</i> -isomer)	AS 2375b	05/10/2016	99.8	
S-2940 (pure <i>S</i> -isomer of inpyrfluxam)	AS 2387a	05/10/2016	99.8	

DFPA	AS 2378a	06/01/2017	99.2	
3'OH-S-2840*	AS 2379b	21/09/2016	97.8	
DFPA-CONH <sub>2</sub>	AS 2382b	30/09/2016	97.9	
ATMI	AS 2383a	12/10/2016	99.7	

\*S-2840: Racemic compound of inpyrfluxam (R-isomer) and S-2940 (S-isomer).

All aqueous solutions were prepared using purified reagent water meeting ASTM Type I requirements purified with a Millipore, Milli-Q® Direct 8 system. All chemicals and solvents were at least reagent grade and were obtained from commercial sources.

## 2. Aqueous pH Buffer Test Solutions

Aqueous pH buffers used during this study were prepared as described below. It is noted that the dates of preparation of the buffers were not included in the report. The buffers are, however, shown to be sterile and pH stable in the study conduct section, and as the test facility is GLP certified, HSE does not consider this to be a major

deviation. It is also noted that reagent sources are not stated. However, the applicant records that all chemicals were reagent grade and obtained from commercial sources.

**Table 8.2.1.1-04 Preparation of aqueous pH Buffer Test Solutions**

<b>pH 4</b>	410 mL of 0.01 M acetic acid solution was combined with 90 mL of 0.01 M sodium acetate solution. The resultant buffer had a pH of 4.0 and was not adjusted.
<b>pH 7</b>	98 mL of 0.02 M potassium phosphate monobasic solution was combined with 152 mL of 0.02 M potassium phosphate dibasic solution, and then diluted to 500 mL with purified reagent water. The resultant buffer had a pH of 7.2, which was adjusted to 7.0 with 1.0 M hydrochloric acid.
<b>pH 9</b>	0.3107 g of boric acid was added to a 500-mL volumetric flask and diluted partially with purified reagent water. 2.5 mL of a 1.0 M sodium hydroxide solution was added to the volumetric flask containing the boric acid and the solution was diluted to a final volume of 500 mL with purified reagent water. The resultant buffer had a pH of 9.0 and was not adjusted.

Buffers were sterilized in an autoclave at approximately 121 °C and 15 psi for 90 minutes. Prior to dosing, buffers were purged with nitrogen to exclude oxygen.

### 3. Dosing Solution Preparation

An isotopically-diluted stock solution was prepared by combining 5.0 mL of the purified radiolabelled stock solution with 1.5 mL of the primary non-radiolabelled stock solution, and diluting it to a final volume of 10 mL with acetonitrile. This isotopically-diluted stock solution was then analysed by LSC and based on this analysis and the calculated specific activity (147,240 dpm/μg), the concentration was determined to be 0.247 mg/mL upon preparation. This isotopically-diluted stock solution was used to prepare the test samples.

To create the dosing solutions, three separate 392-μL aliquots of the isotopically diluted stock solution were diluted to 100 mL with the appropriate sterile aqueous buffers (pH 4, 7, or 9) to dose in bulk to obtain a measured concentration of [<sup>14</sup>C] inpyrfluxam of 1.00 μg/mL (1,473,321 dpm per sample). The organic solvent in the test system was <1 % v/v. The test concentration of 1.00 μg/mL selected for this study is less than one-half of the water solubility of [<sup>14</sup>C] inpyrfluxam at 20 °C.

Individual samples were then prepared by aliquoting 10.0 mL of the bulk dosed solution into sterile 10-mL amber vials. These were flushed with nitrogen, and then capped with sterile Teflon®-lined septas and aluminium crimp caps.

Duplicate samples for each aqueous buffer solution were analysed immediately after dosing (Day 0 analysis). An additional 15 samples (5 per pH buffer) were incubated in a thermostatic water bath in the dark at  $50 \pm 0.5$  °C and analysed after 5 days.

#### 4. Analytical Methods

Samples that were not immediately analysed were stored in a freezer. All samples were analysed generally within one day of sampling. HSE notes that an exact temperature of freezer storage has not been given, however no degradation is observed during the test and therefore the storage conditions and duration are deemed suitable and not to have affected the outcome of the test.

All samples were measured for total radioactivity (LSC) and were analysed by HPLC/RAM to determine the distribution of test item and potential metabolites, as well as for determination of isomerization of [ $^{14}\text{C}$ ] inpyrfluxam. Samples were analysed by co-chromatography with reference standards, and direct comparison of retention times between radiochemical and UV detection was used to identify regions in the radiochemical chromatograms.

Conditions and procedures used throughout the analysis of the samples during this study followed the procedures used in the method implementation, with the following exception; 4 mL of acetonitrile was added to samples on Day 5 to compensate for evaporation of sample buffer.

Material balance was calculated for each sample by dividing the amount of recovered radioactivity by the amount of dosed radioactivity.

Using the method of Currie (1968), the limits of detection (LOD) and quantification (LOQ) were calculated. The LSC limit of detection (LOD) and LOQ are summarised below.

**Table 8.2.1.1-05 Limits of Detection and Quantification**

<b>Background Matrix</b>	<b>LOD (dpm)</b>	<b>LOQ (dpm)</b>	<b>LOD (%AR)</b>	<b>LOQ (%AR)</b>
<b>pH 4</b>	21.51	93.17	0.0175	0.0759
<b>pH 7</b>	21.65	93.17	0.0176	0.0759
<b>pH 9</b>	21.28	93.56	0.0173	0.0762

*Note: For HPLC/RAM, LOD was set as 100 dpm integrated area, with LOQ as three times LOD, or 300 dpm.*

HSE notes that the LOQ is much lower than the guidance recommended 10% of the initial applied concentration, therefore this analytical method is suitable for quantification in this study.

## RESULTS

### 1. Temperature, pH and Sample Sterility

Temperatures did not deviate from the mean by more than 0.5 °C during the course of the study, therefore HSE considers these to be acceptable.

**Table 8.2.1.1-06 Temperature Measurements during the Hydrolysis Testing with [<sup>14</sup>C] inpyrfluxam**

Test Day	Minimum temperature °C	Current temperature °C <sup>a</sup>	Maximum temperature °C
0	50	50	50
1	50	50	50.1
2	50	50	50.1
3	50	50.1	50.1
4	50	50	50.1
5	50	50	50

<sup>a</sup>Current temperature reflects the temperature at the time of recording.

The pH values during the study were 3.9, 7.0 and 8.9-9.0 for the test solutions at pH 4, 7 and 9, respectively (Table 8.2.1.1-04). HSE considers these to be acceptable.

**Table 8.2.1.1-07 pH Measurements during the Hydrolysis Testing with [<sup>14</sup>C] inpyrfluxam**

Test Day	pH 4.0	pH 7.0	pH 9.0
0 (after dosing)	3.9	7	9
5	3.9	7	8.9

At sampling, an additional sample from each pH level was evaluated for sterility by aliquoting a portion onto a Petrifilm aerobic count plate and incubating for at least 48 hours. HSE accepts that the results of the sterility checks indicate that all dosed samples were sterile.

**Table 8.2.1.1-08 Sterility evaluation during the Hydrolysis Testing with [<sup>14</sup>C] inpyrfluxam**

Buffer	Samples Day 0	Samples Day 5
4	Negative	Negative
7	Negative	Negative
9	Negative	Negative

*Note: "Positive" indicates the presence of microbes. "Negative" indicates the absence of microbial colony formation.*



## Mass balance

Mean mass balances ranged from 97.7 % to 98.2 % AR at day 0 and from 96.2 % to 98.9 % AR at day 5 for all systems. HSE notes that material balance over the course of the incubation period was within the acceptable range of 90 to 110 % applied radioactivity.

Carbon dioxide and volatile organic compounds were not collected. HSE conclude that it is unlikely that volatile organic compounds and CO<sub>2</sub> would be produced >5 % AR as the material balance for all tests were ≥97 % AR (single replicate).

**Table 8.2.1.1-09**      **Mass balance of [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam – expressed as mean % AR for different pH samples**

<b>Matrix</b>	<b>HPLC/RAM (% AR)</b>	
	<b>Average [<sup>14</sup>C] inpyrfluxam at Incubation Start (% AR)</b>	<b>Average [<sup>14</sup>C] inpyrfluxam at Incubation End (% AR)</b>
pH 4	98.2	98.9
pH 7	97.7	96.2
pH 9	98.1	97.7

### 3. Distribution of [<sup>14</sup>C] inpyrfluxam and Degradants

The data for the distribution of [<sup>14</sup>C] inpyrfluxam and degradants are summarised in the table below.

**Table 8.2.1.1-10**      **Mass balance of [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam – expressed as % AR**

	<b>pH 4 %TAR*</b>			<b>pH 7 %TAR*</b>			<b>pH 9 %TAR*</b>		
<b>Sampling Day</b>			<b>Mean</b>			<b>Mean</b>			<b>Mean</b>
0	97.7	98.7	<b>98.2</b>	97.2	98.3	<b>97.7</b>	97.5	98.7	<b>98.1</b>
5	101	97.0	<b>98.9</b>	98.2	94.1	<b>96.2</b>	97.1	99.6	<b>98.4</b>

\*TAR = Total Applied Radioactivity

The amount of [<sup>14</sup>C] inpyrfluxam at day 5 ranged between 101 and 97.0 % mean AR for pH 4, between 94.1 and 98.2 % mean AR for pH 7, and 97.1 and 99.6 % mean AR for pH 9 at 50°C, showing it is stable at these pH levels. Observed degradation did not exceed 10 % AR.

HSE notes that the applicant did not submit any further hydrolytic results. Given that hydrolytic degradation was minor (< 5 %) at all studied pH levels, HSE concludes that this study is sufficient and no further hydrolysis studies are necessary, as [<sup>14</sup>C] inpyrfluxam has been demonstrated to be hydrolytically stable at 50°C.

#### 4. Chiral analysis of [<sup>14</sup>C] inpyrfluxam

Samples were analysed by chiral analysis as described above. The data for distribution of [<sup>14</sup>C] inpyrfluxam are summarised in the table below.

**Table 8.2.1.1-11 Isomeric distribution of [Pyrazolyl-4-<sup>14</sup>C] inpyrfluxam – expressed as % AR**

Matrix	Average Chiral HPLC/RAM Distribution (% AR)					
	Day 0 % [ <sup>14</sup> C] inpyrfluxam ( <i>R</i> -Isomer)	Day 5 % [ <sup>14</sup> C] inpyrfluxam ( <i>R</i> -Isomer)	Day 0 % [ <sup>14</sup> C] inpyrfluxam ( <i>S</i> -Isomer)	Day 5 % [ <sup>14</sup> C] inpyrfluxam ( <i>S</i> -Isomer)	Day 0 % Others	Day 5 % Others
pH 4	98.2	98.9	-	-	-	-
pH 7	97.7	96.2	-	-	-	-
pH 9	98.1	98.4	-	-	-	-

Chiral analysis determined that no *R* to *S* isomerization of the test substance occurred in any of the three test systems. HSE has validated the representative chromatograms provided by the applicant in their study report and confirmed that the compound identities match given retention times or *r<sub>f</sub>* values ascribed to reference standards.

## CONCLUSION

Inpyrfluxam in sterile aqueous solution at pH 4, 7, and 9 for 5 days at 50°C did not produce degradation products that were detected at levels ≥ 10% AR that would trigger further study. Additionally, there was no *R* to *S* isomerisation during the study.

No extended study at 25 °C is considered necessary. Hydrolysis is not considered to be a significant route of degradation and inpyrfluxam is considered to be hydrolytically stable. HSE considers this study acceptable overall.

**B.8.2.1.2. Direct photochemical degradation**

<b>Data Point:</b>	KCA 7.2.1.2/01
<b>Report Author:</b>	
<b>Report Year:</b>	2015a
<b>Report Title:</b>	Photodegradation of [ <sup>14</sup> C] S-2399 in Sterilized pH 7 Buffer by Artificial Sunlight
<b>Study number</b>	2642W and 2642W-1
<b>Guideline(s) followed in study:</b>	US EPA OPPTS Guideline 835.2240 OECD Guideline 316 for the Testing of Chemicals: Phototransformation of Chemicals in Water – Direct Photolysis
<b>GLP?</b>	Yes

<b>Deviations from guideline</b>	<b>HSE assessment of deviations</b>
For the irradiated samples, the temperature dropped to 21.5 to 22 °C from day 12 to day 13 before returning to >24 °C. The temperature in the dark controls rose to approximately 26.5 °C on day 7.	The deviations in temperature were of short duration and not expected to affect the outcome of the test.
<b>HSE assessment of deviations</b>  The study is acceptable to derive endpoints for use in the exposure assessment.	

**INTRODUCTION**

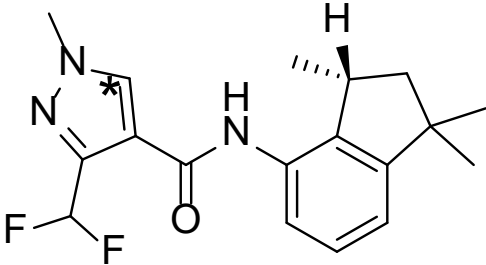
The photolytic degradation of the new active substance inpyrfluxam was studied in sterilised phosphate buffer at pH 7. The study followed the OECD 316 Guideline on

photo transformation of chemicals and the US EPA OPPTS Guideline 835.2240, and was conducted to Good Laboratory Practice (GLP) standards.

## MATERIALS AND METHODS

### I. Test item

**Table B.8.2.1.2-01 Radiolabelled test substance**

<p><b>[pyrazolyl-4-<sup>14</sup>C]</b> <b>inpyrfluxam</b></p>	 <p>Radiolabel position denoted by *</p>
<p><b>Specific activity</b></p>	<p>2.11 GBq/mmol</p>
<p><b>Radiochemical purity</b></p>	<p>96.4 % (analysed)</p>

**Table B.8.2.1.2-02 Non-Radiolabelled test substance**

<p><b>Name</b></p>	<p>Inpyrfluxam</p>
<p><b>Chemical purity</b></p>	<p>99.9 %</p>

<p><b>Name</b></p>	<p>Inpyrfluxam TG</p>
<p><b>Chemical purity</b></p>	<p>99.8 %</p>

<p><b>Name</b></p>	<p>3'OH-S-2840</p>
<p><b>Chemical purity</b></p>	<p>99.9 %</p>

<b>Name</b>	DFPA-CONH <sub>2</sub>
<b>Chemical purity</b>	99.5 %

<b>Name</b>	<i>p</i> -nitroacetophenone
<b>Chemical purity</b>	99.9 %

<b>Name</b>	Pyridine, anhydrous
<b>Chemical purity</b>	99.9 %

<b>Name</b>	DFPA
<b>Chemical purity</b>	99.3 %

Reference standard solutions (5 mg/mL) were prepared in acetonitrile.

## II. Test system

Buffer solutions were sterilised by passing through a 0.2 µm filter into an attached sterile container. All glassware was autoclaved (121 °C; 0.1 MPa; 30 min) prior to use. The pH meter was calibrated prior to measuring the pH before addition of the test item. Dosing procedures were conducted in a biological safety cabinet under aseptic conditions.

It is known that inpyrfluxam is stable to aqueous hydrolysis at pH 7 and therefore this pH was chosen for the aqueous photolysis study. All solutions were prepared using HPLC grade water. The 0.01 M phosphate buffer was prepared by weighing sodium phosphate monobasic monohydrate ((NaH<sub>2</sub>PO<sub>4</sub>•H<sub>2</sub>O); 1.38 g) and diluting to volume (1 L) with deionised water. The pH was adjusted with aqueous NaOH solution (10 %).

Stock solutions of [<sup>14</sup>C] inpyrfluxam were prepared by diluting stock solution with sterile pH 7 buffer.

**Table B.8.2.1.2-03 Preparation of dose solutions**

<b>Dose Solution</b>	<b>Volume of Stock Solution (μL)</b>	<b>Volume of Buffer (mL)</b>	<b>Final Concentration of dose solution (DPM/mL)</b>
Preliminary	63	65	$3.966 \times 10^5$
Definitive	165	170	$4.016 \times 10^5$

Aliquots (5 mL) of dose solutions were transferred into sample tubes. The concentration of acetonitrile in aqueous samples was <1 % by volume. Triplicate aliquots of each dosing solution were taken at least before and after application to determine dose concentration and homogeneity of solutions during dosing. Time 0 samples were analysed by HPLC to confirm stability of the analyte. Sterility of the test solutions and samples at the final sampling point were also assessed by placing aliquots on trypticase soy agar, with plates incubated (35 °C; at least 2 days) and examined for growth of aerobic bacteria.

The test item was exposed to light via a Heraeus Suntest CPS+ unit equipped with an Xenon arc lamp fitted with a quartz glass filter with IR-reflective coating and a special UV glass filter to block radiation <290 nm to simulate outdoor sunlight exposure. The Suntest CPS+ was set at a light intensity of 600 W/m<sup>2</sup>, which gave an average intensity of 61.3 W/m<sup>2</sup> in the 290-400 nm range and 497 W/m<sup>2</sup> in the 290-800 nm range, at the level of the samples. A spectrometer was used to measure the light intensity and spectral distribution prior to the experimental start, between the two experimental sets and after the study. The spectral distribution at the level of the samples was recorded in the 290 nm and 290 to 800 nm ranges. The light intensity for the light source was determined by integrating the total light intensity over the 290 to 800 nm range.

Irradiated samples were placed in quartz tubes with screw caps with Teflon-lined silicon septums, while Pyrex sample tubes were used for the dark controls.

Irradiated samples were placed in a deionised water bath at a temperature of  $25 \pm 1$  °C with continuous circulation using a temperature-controlled circulation bath, with the temperature monitored continuously in a surrogate tube in the water bath. Dark control samples were placed in an incubator maintained at  $25 \pm 1$  °C, with the temperature monitored continuously.

Light exposed traps were connected to ethylene glycol (EG) and NaOH traps to collect volatiles.

### III. Chemical actinometry

The quantum yield in aqueous solution was determined from concurrently run tubes containing nitroacetophenone (PNAP) and pyridine (PYR), the photolytic behaviour of which is known. The quantum yield and consequently the half-life of the mixture is dependent on the concentration of PYR at a fixed molar concentration of PNAP, which was based on analytical experience and the literature and the ratio of PNAP to PYR selected based on the expected half-life of inpyrfluxam. Both irradiated and dark control samples were incubated alongside inpyrfluxam samples. Aliquots were analysed by HPLC allowing a standard curve to be generated across 5 concentrations ranging between  $0.1 \times 10^{-5}$  M to  $1.5 \times 10^{-5}$  M.

### IV. Preliminary study

Approximate degradation rates of [ $^{14}\text{C}$ ] inpyrfluxam in pH 7 phosphate buffer were calculated via a preliminary study. Duplicate tubes were prepared and sampled after 3 and 7 days irradiation, while dark control samples were prepared and sampled in duplicate at time 0 and after 3 and 7 days incubation. Time 0 samples were sacrificed immediately after dosing, while the remaining samples were connected to the trapping systems. After the specified incubation period, the pH of the samples was measured, the contents decanted, vials rinsed with acetonitrile (0.5 mL) and the rinsing added to the aqueous sample. Samples were analysed by LSC for mass balance and also by HPLC with a Beta-RAM radioisotope detector. The contents of the traps was also analysed by LSC.

### V. Definitive study

Twelve quartz sample tubes were prepared for duplicate sampling at 6 time points for light exposed samples. Fourteen Pyrex sample tubes were prepared for sampling at 6 time points (plus time 0) for the dark controls. The nominal concentration of inpyrfluxam was 1.06  $\mu\text{g/mL}$ .

At each sampling time, duplicate samples were collected. The pH of samples was measured and the contents of the vials decanted, vials rinsed with acetonitrile (0.5 mL) and the rinsing added to the aqueous sample. Aliquots ( $3 \times 0.1$  mL) were radioassayed by LSC. Quantitation was conducted with HPLC. Samples were analysed directly by reverse phase HPLC with a Beta-Ram radioisotope detector. The contents of the traps (EG and NaOH) were analysed by LSC in triplicate (0.5 mL). Aliquots (mL) of the light and dark actinometer samples were also taken at each sampling event and analysed by HPLC.

### VI. Sample storage

Samples were analysed by HPLC and LSC on the same day they were collected. All samples were stored in a refrigerator when not in use. Traps for volatiles were stored

at ambient temperature. Actinometer samples were stored in a refrigerator until the final sampling event prior to HPLC analysis. The applicant states all samples were analysed within one day of collection.

Reference standards and reference standard solutions were stored frozen when not in use.

## **VII. Confirmatory Chromatography by Thin Layer Chromatography (TLC)**

Two-dimensional TLC analysis was conducted on selected samples to confirm the HPLC peak assignment for inpyrfluxam. Reference standards were visualised using short-wave UV (254 nm) light and the plates scanned for  $^{14}\text{C}$  detection.

## **VIII. Chiral HPLC Analysis of Selected Samples**

The isomer composition of inpyrfluxam in irradiated and dark control samples was assessed by analysing selected extracts by normal phase HPLC. Aliquots (1 mL) of the aqueous samples were partitioned into hexane (2 x 1 mL) and the organic layers separated and concentrated under nitrogen for normal phase HPLC chiral analysis.

## **IX. Sensitivity and Detection Limit**

For Beta-RAM chromatograms, the LOQ observed in HPLC chromatograms was determined by an experiment in which varying volumes of  $^{14}\text{C}$  labelled material were injected and the DPM detected compared to the actual DPM injected. This showed that the smallest injection for which acceptable recoveries were obtained was 258 dpm.

# **RESULTS AND DISCUSSION**

## **I. Radiochemical Purity and Stability of [ $^{14}\text{C}$ ] inpyrfluxam Under Conditions of Administration**

Reverse phase HPLC was used to determine that the radiochemical purity of inpyrfluxam was 96.4 % before test start. Reverse phase HPLC analysis of T0 samples also demonstrated that the test substance was stable under the conditions of administration. The dose solutions were found to be homogenous during the application processes, with a relative standard deviation of 0.9 % for the definitive study.

## **II. UV/Vis Spectrum of inpyrfluxam and Buffer Solutions**

The UV/Vis Spectrum of inpyrfluxam in acetonitrile and the pH 7 phosphate buffer were used to determine the maximum half-life of photolysis assuming a conservative default quantum yield of 1 as a worst case. The maximum upper limit photolysis rate



constant was determined as  $0.028 \text{ days}^{-1}$ , and the maximum half-life was determined as 24.9 days. The exposure was conducted in pH 7 phosphate buffer solutions since inpyrfluxam is stable to hydrolysis at pH 7.

### III. Preliminary Experiment

Samples were continuously irradiated for up to 7 days. Inpyrfluxam degraded slowly in pH 7 buffer under the conditions of the test. The preliminary test was used to determine the dose rate of the definitive test. A dose rate of 10 g/mL and <1 % acetonitrile were used in the definitive test during 15 days of continuous irradiation.

### IV. Definitive Experiment

The test was conducted over 15 days, with the continuous irradiation for irradiated samples equivalent to 42 summer days at 40 °N. Integrated light intensity averaged  $61.3 \text{ W/m}^2$  for the 290-400 nm range and  $497 \text{ W/m}^2$  for the 290-800 nm range.

The applicant reported that a deviation from the expected temperature range was recorded for both the irradiated and dark control samples. For the irradiated samples, the temperature was generally between approximately 24 and 26.2 °C, but dropped to 21.5 to 22 °C from day 12 to day 13 before returning to >24 °C. The temperature in the dark controls rose to approximately 26.5 °C on day 7. As the deviations in temperature are of short duration it is not expected that they affected the outcome of the test.

The pH of aqueous samples was measured at sampling and was a mean of 6.91 (range 6.69 to 7.13) in irradiated samples and 6.92 (range 6.65 to 7.08) in dark controls.

Samples were confirmed as sterile throughout the test.

### V. Material balance

Total recoveries (sum of radioactivity in aqueous samples and traps) averaged  $103.1 \pm 1.7\%$  for irradiated samples and  $104.4 \pm 2.2\%$  AR in dark controls.

**Table B.8.2.1.2-04 Material balance for irradiated samples**

Sample	% Applied dose			
	Aqueous phase	EG trap	NaOH	Total recovery
Replicate 1 (T0)	102.4	NA	NA	102.4
Replicate 2 (T0)	101.9	NA	NA	101.9

<b>Mean (T0)</b>	<b>102.2</b>	<b>NA</b>	<b>NA</b>	<b>102.2</b>
Replicate 1 (T1)	103.5	0.0	0.0	103.5
Replicate 2 (T1)	103.9	0.0	0.0	103.9
<b>Mean (T1)</b>	<b>103.7</b>	<b>0.0</b>	<b>0.0</b>	<b>103.7</b>
Replicate 1 (T3)	105.3	0.0	0.0	105.3
Replicate 2 (T3)	104.4	0.0	0.0	104.4
<b>Mean (T3)</b>	<b>104.9</b>	<b>0.0</b>	<b>0.0</b>	<b>104.9</b>
Replicate 1 (T6)	101.5	0.0	0.1	101.6
Replicate 2 (T6)	104.0	0.0	0.0	104.0
<b>Mean (T6)</b>	<b>102.8</b>	<b>0.0</b>	<b>0.1</b>	<b>102.8</b>
Replicate 1 (T9)	99.1	0.0	0.1	99.2
Replicate 2 (T9)	104.9	0.0	0.0	104.9
<b>Mean (T9)</b>	<b>102.0</b>	<b>0.0</b>	<b>0.1</b>	<b>102.1</b>
Replicate 1 (T13)	102.1	0.0	0.0	102.1
Replicate 2 (T13)	103.0	0.0	0.1	103.1
<b>Mean (T13)</b>	<b>102.6</b>	<b>0.0</b>	<b>0.1</b>	<b>102.6</b>
Replicate 1 (T15)	102.1	0.0	0.0	102.1
Replicate 2 (T15)	105.0	0.0	0.1	105.1
<b>Mean (T15)</b>	<b>103.6</b>	<b>0.0</b>	<b>0.1</b>	<b>103.6</b>
<b>Mean</b>				<b>103.1</b>
<b>STD</b>				<b>1.7</b>

**Table B.8.2.1.2-05 Material balance for dark control samples**

<b>Sample</b>	<b>% Applied dose</b>			
	<b>Aqueous phase</b>	<b>EG trap</b>	<b>NaOH</b>	<b>Total recovery</b>
Replicate 1 (T1)	103.2	0.0	0.0	103.2
Replicate 2 (T1)	108.1	0.0	0.0	108.1
<b>Mean (T1)</b>	<b>105.7</b>	<b>0.0</b>	<b>0.0</b>	<b>105.7</b>
Replicate 1 (T3)	103.4	0.0	0.0	103.4
Replicate 2 (T3)	102.1	0.0	0.0	102.1
<b>Mean (T3)</b>	<b>102.8</b>	<b>0.0</b>	<b>0.0</b>	<b>102.8</b>
Replicate 1 (T6)	106.0	0.0	0.0	106.0
Replicate 2 (T6)	103.2	0.0	0.0	103.2
<b>Mean (T6)</b>	<b>104.6</b>	<b>0.0</b>	<b>0.0</b>	<b>104.6</b>
Replicate 1 (T9)	106.2	0.0	0.0	106.2
Replicate 2 (T9)	102.7	0.0	0.0	102.7
<b>Mean (T9)</b>	<b>104.5</b>	<b>0.0</b>	<b>0.0</b>	<b>104.5</b>
Replicate 1 (T13)	104.9	0.0	0.0	104.9
Replicate 2 (T13)	107.2	0.0	0.0	107.2
<b>Mean (T13)</b>	<b>106.1</b>	<b>0.0</b>	<b>0.0</b>	<b>106.1</b>
Replicate 1 (T15)	104.6	0.0	0.0	104.6
Replicate 2 (T15)	100.8	0.0	0.0	100.8
<b>Mean (T15)</b>	<b>102.7</b>	<b>0.0</b>	<b>0.0</b>	<b>102.7</b>
<b>Mean</b>				<b>104.4</b>
<b>STD</b>				<b>2.2</b>

Radiocarbon recovered in the aqueous samples were >99 % AR throughout the study for all samples tested. NaOH traps represented up to an average of 0.1 % AR in Day 15 light exposed samples while organic volatiles trapped in the ethylene glycol traps were below limits of quantitation throughout the study. Traps for volatiles in dark controls samples represented <0.1 % AR throughout the study period.

HPLC analysis showed that inpyrfluxam was stable in both irradiated and dark control samples during the test. In the irradiated samples, inpyrfluxam ranged from 94.3 to 97.1 % AR. Metabolite 3'-OH-S-2840 was detected at up to 5.9 % AR, but was present in T0 samples and the post-dose purity check as a minor impurity. Results for the dark control samples were similar with inpyrfluxam ranging from 95.0 to 97.5 % AR and with 3'-OH-S-2840 again a minor component ranging between 2.7 and 5.2 % AR.

## VI. Chiral analysis

The isomeric ratio of inpyrfluxam was analysed by normal phase HPLC, which showed that *R*-inpyrfluxam was the only isomer present in both irradiated and dark control samples and that changes in the isomeric ratio of inpyrfluxam were unlikely to occur in water as a result of photolytic irradiation.

## VII. Degradation of [<sup>14</sup>C] inpyrfluxam in sterile pH 7 buffer

**Table B.8.2.1.2-06 Components detected in irradiated samples**

Sample	% Applied dose			
	Inpyrfluxam	3'-OH-S-2840	Organic Volatiles	CO <sub>2</sub>
Replicate 1 (T0)	99.2	3.2	NA	NA
Replicate 2 (T0)	98.9	3.0	NA	NA
<b>Mean (T0)</b>	<b>99.1</b>	<b>3.1</b>	<b>NA</b>	<b>NA</b>
Replicate 1 (T1)	97.6	5.9	<LOD	<LOD
Replicate 2 (T1)	98.2	5.7	<LOD	<LOD
<b>Mean (T1)</b>	<b>97.9</b>	<b>5.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T3)	102.1	3.2	<LOD	<LOD
Replicate 2 (T3)	101.4	3.0	<LOD	<LOD

<b>Mean (T3)</b>	<b>101.8</b>	<b>3.1</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T6)	95.8	5.7	<LOD	0.1
Replicate 2 (T6)	98.3	5.7	<LOD	<LOD
<b>Mean (T6)</b>	<b>97.1</b>	<b>5.7</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T9)	95.7	3.4	<LOD	0.1
Replicate 2 (T9)	101.0	3.9	<LOD	<LOD
<b>Mean (T9)</b>	<b>98.4</b>	<b>3.7</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T13)	98.6	3.5	<LOD	<LOD
Replicate 2 (T13)	99.3	3.7	<LOD	0.1
<b>Mean (T13)</b>	<b>99.0</b>	<b>3.6</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T15)	97.4	4.7	<LOD	<LOD
Replicate 2 (T15)	101.0	4.0	<LOD	0.1
<b>Mean (T15)</b>	<b>99.2</b>	<b>4.4</b>	<b>&lt;LOD</b>	<b>0.1</b>

NA – not applicable, not measured

**Table B.8.2.1.2-07 Components detected in dark control samples**

<b>Sample</b>	<b>% Applied dose</b>			
	<b>Inpyrfluxam</b>	<b>3'-OH-S-2840</b>	<b>Organic Volatiles</b>	<b>CO<sub>2</sub></b>
Replicate 1 (T1)	100.0	3.2	<LOD	<LOD
Replicate 2 (T1)	103.6	4.5	<LOD	<LOD
<b>Mean (T1)</b>	<b>101.8</b>	<b>3.9</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T3)	100.3	3.1	<LOD	<LOD
Replicate 2 (T3)	99.1	3.0	<LOD	<LOD
<b>Mean (T3)</b>	<b>99.7</b>	<b>3.1</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>

Replicate 1 (T6)	101.4	4.6	<LOD	<LOD
Replicate 2 (T6)	100.3	2.9	<LOD	<LOD
<b>Mean (T6)</b>	<b>100.9</b>	<b>3.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T9)	102.7	3.5	<LOD	<LOD
Replicate 2 (T9)	99.5	3.2	<LOD	<LOD
<b>Mean (T9)</b>	<b>101.1</b>	<b>3.4</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T13)	102.1	<LOD *	<LOD	<LOD
Replicate 2 (T13)	104.5	2.7	<LOD	<LOD
<b>Mean (T13)</b>	<b>103.3</b>	<b>1.4</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T15)	99.4	5.2	<LOD	<LOD
Replicate 2 (T15)	97.9	2.9	<LOD	<LOD
<b>Mean (T15)</b>	<b>98.7</b>	<b>4.1</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>

\*Given as 0.0 in study report; replaced by <LOD by HSE

In the irradiated samples and dark control samples, inpyrfluxam remained relatively constant at approximately 100 % AR allowing for analytical variation. Metabolite 3'-OH-S-2840 was also detected at generally 3 to 5 % AR; it is noted that this metabolite was present as an impurity at a level of 3.14 % in the post-dose purity check at T0 and so was not necessarily formed as a photodegradation product. Organic volatiles and CO<sub>2</sub> were present at maximums of <LOD and 0.1 % AR respectively in both the irradiated and dark controls. The data does not therefore indicate that inpyrfluxam will photodegrade in sterile buffer at pH 7.

The applicant has calculated degradation rates using linear regression. These are not acceptable as FOCUS Kinetics principles should have been used. Nevertheless, the degradation rates have not been recalculated as no degradation was observed in the samples. It is noted that, while not calculated using the appropriate methodology, the applicant's degradation rates are 3465 days in irradiated samples and 2310 days in dark controls; these values can be viewed as indicative of the long degradation rates in the sterile buffer. Degradation is therefore considered to be negligible. It is also observed that the applicant stated that the degradation rates could not be correctly calculated as inpyrfluxam was stable under the conditions of the test.

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## VIII. Quantum yield

The applicant has calculated the quantum yield of inpyrfluxam; due to the lack of degradation in the sterile buffer, this has not been verified.

## CONCLUSIONS

The photolysis of [<sup>14</sup>C] inpyrfluxam was studied in a sterile pH7 phosphate buffered solution during 15 days continuous irradiation, alongside dark control samples. The irradiation was approximately equivalent to 42 days at 40 °N. Volatiles were present at very low levels (≤0.1 % AR), while the only metabolite detected was 3'-OH-S-2840; this was also present in post-dose purity checks and therefore is not considered to be a degradation product produced during photolysis.

No degradation rates have been calculated by HSE, but direct aqueous photolysis in sterile buffer is expected to be negligible.

**B.8.2.1.3. Indirect photochemical degradation**

<b>Data Point:</b>	KCA 7.2.1.2/02
<b>Report Author:</b>	
<b>Report Year:</b>	2015b
<b>Report Title:</b>	Photodegradation of [ <sup>14</sup> C] S-2399 in Sterilized Natural Water by Artificial Sunlight
<b>Study number</b>	2644W and 2644W-1
<b>Guideline(s) followed in study:</b>	US EPA OPPTS Guideline 835.2240  OECD Guideline 316 for the Testing of Chemicals: Phototransformation of Chemicals in Water – Direct Photolysis  J-MAFF No.12-Nousan-8147 Studies of Photolytic Fate in Water (2-6-2)
<b>GLP?</b>	Yes

<b>Deviations from guideline</b>	<b>HSE assessment of deviations</b>
The applicant reported that a deviation from the expected temperature range was recorded for both the irradiated and dark control samples. For the irradiated samples, the temperature was generally between approximately 24 and 26.2 °C, but dropped to 21.5 to 22 °C from day 12 to day 13 before returning to >24 °C. The temperature in the dark controls rose to approximately 26.5 °C on day 7.	Minor deviation. As the deviations in temperature were of short duration it is not expected that they affected the outcome of the test.
<b>HSE conclusion</b>	



The study is acceptable to derive endpoints for use in the exposure assessment.

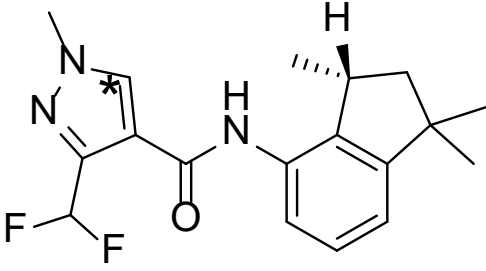
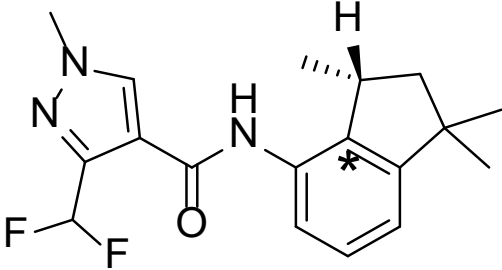
## INTRODUCTION

The photolytic degradation of the new active substance inpyrfluxam was studied in sterilised natural water. The study followed the OECD draft Guideline on photo transformation of chemicals in soil and the US EPA OPPTS Guideline 835.2410, and was conducted to Good Laboratory Practice (GLP) standards.

## MATERIALS AND METHODS

### I. Test item

Table B.8.2.1.3-01 Radiolabelled test substance

<p><b>[pyrazolyl-4-<sup>14</sup>C]</b> <b>inpyrfluxam</b></p>	 <p>Radiolabel position denoted by *</p>
<p><b>Specific activity</b></p>	<p>2.11 GBq/mmol</p>
<p><b>Radiochemical purity</b></p>	<p>95.6 % (analysed)</p>
<p><b>[phenyl-U-<sup>14</sup>C]</b> <b>inpyrfluxam</b></p>	 <p>Radiolabel position denoted by *</p>

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<b>Specific activity</b>	4.51 GBq/mmol
<b>Radiochemical purity</b>	99.0 % (analysed)

**Table B.8.2.1.3-02 Non-Radiolabelled test substance**

<b>Name</b>	Inpyrfluxam
<b>Chemical purity</b>	99.9 %

<b>Name</b>	Inpyrfluxam TG
<b>Chemical purity</b>	99.8 %

<b>Name</b>	3'OH-S-2840
<b>Chemical purity</b>	99.9 %

<b>Name</b>	DFPA-CONH <sub>2</sub>
<b>Chemical purity</b>	99.5 %

<b>Name</b>	DFPA-CONH <sub>2</sub>
<b>Chemical purity</b>	99.5 %

<b>Name</b>	ATMI
<b>Chemical purity</b>	99.9 %

<b>Name</b>	DFPA
<b>Chemical purity</b>	99.3 %

Reference standard solutions (5 mg/mL) were prepared in acetonitrile.

## II. Test system

The natural water used in the study was collected from Tuckahoe State Park, Crouse Mill, Queen Anne, MD on 01 July 2024. After being shipped to the laboratory, it was stored in a refrigerator (temperature not stated) when not in use. The properties of the water are shown below:

**Table B.8.2.1.3-03 Summary of Characterisation of the Natural Water from Lake Tuckahoe, MD**

<b>Name</b>	<b>Lake Tuckahoe Water 2440W-066</b>
<b>Sampling location</b>	Tuckahoe State Park, Queen Anne, MD (US)
<b>Date of collection</b>	July 1, 2014
<b>Time of collection</b>	9:16 am
<b>pH at collection</b>	6.96
<b>Dissolved Oxygen (DO) at collection</b>	7.2 mg/L
<b>Temperatures</b>	27.2 °C (air), 26.6 °C (water)
<b>Depth of collection</b>	5-10 cm
<b>Storage conditions</b>	Typically <4°C
<b>Received at PTRL</b>	July 3, 2014
<b>Water Characterization from Agvise Laboratories, Inc.</b>	
<b>pH</b>	7.5
<b>Calcium (ppm)</b>	16
<b>Magnesium (ppm)</b>	6.4
<b>Sodium (ppm)</b>	7.5
<b>Hardness (mg equivalent CaCO<sub>3</sub>/L)</b>	68
<b>Conductivity (mmhos/cm)</b>	0.21
<b>Sodium Adsorption Ratio (SAR)</b>	0.40
<b>Total Dissolved Solids (ppm)</b>	56
<b>Turbidity (NTU)</b>	1.06
<b>Total Organic Carbon (ppm)*</b>	0.269

\*Total organic carbon was measured prior to use in this study

Solutions were sterilised by passing through a 0.2 µm filter into an attached sterile container. All glassware was autoclaved (121 °C; 0.1 MPa; 30 min) prior to use. The pH meter was calibrated prior to measuring the pH before addition of the test item. Dosing procedures were conducted in a biological safety cabinet under aseptic conditions.

Stock solutions of [ $^{14}\text{C}$ ] inpyrfluxam in acetonitrile were prepared by diluting stock solution with sterile lake water.

**Table B.8.2.1.3-04 Preparation of dose solutions**

<b>Dose Solution</b>	<b>Stock Solution Used</b>	<b>Volume of Stock Solution (<math>\mu\text{L}</math>)</b>	<b>Volume of Natural Water (mL)</b>	<b>Final Concentration of dose solution (DPM/mL)</b>
Preliminary	PYR Label	91	100	$3.94 \times 10^5$
Definitive-PYR	PYR Label	156	200	$3.71 \times 10^5$
Definitive-PH	PH Label	185.2	185	$7.99 \times 10^5$

Aliquots (5 mL) of dose solutions were transferred into sample tubes. The concentration of acetonitrile in aqueous samples was  $<1\%$  by volume. Triplicate aliquots of each dosing solution were taken at least before and after application to determine dose concentration and homogeneity of solutions during dosing. Time 0 samples were analysed by HPLC to confirm stability of the analyte. Sterility of the test solutions and samples at the final sampling point were also assessed by placing aliquots on trypticase soy agar, with plates incubated ( $35\text{ }^{\circ}\text{C}$ ; at least 2 days) and examined for growth of aerobic bacteria.

The test item was exposed to light via a Heraeus Suntest CPS+ unit equipped with an Xenon arc lamp fitted with a quartz glass filter with IR-reflective coating and a special UV glass filter to block radiation  $<290\text{ nm}$  to simulate outdoor sunlight exposure. The Suntest CPS+ was set at a light intensity of  $600\text{ W/m}^2$ , which gave an average intensity of  $48.9\text{ W/m}^2$  in the 290-400 nm range and  $402\text{ W/m}^2$  in the 290-800 nm range, at the level of the samples. A spectrometer was used to measure the light intensity and spectral distribution prior to the experimental start, between the two experimental sets and after the study. The spectral distribution at the level of the samples was recorded in the 290 to 400 nm and 290 to 800 nm ranges. The light intensity for the light source was determined by integrating the total light intensity over the 290 to 800 nm range.

Irradiated samples were placed in quartz tubes with screw caps with Teflon-lined silicon septums, while Pyrex sample tubes were used for the dark controls.

Irradiated samples were placed in a deionised water bath at a temperature of  $25 \pm 1\text{ }^{\circ}\text{C}$  with continuous circulation using a temperature-controlled circulation bath, with the temperature monitored continuously in a surrogate tube in the water bath. Dark

control samples were placed in an incubator maintained at  $25 \pm 1$  °C, with the temperature monitored continuously.

Light exposed traps were connected to ethylene glycol (EG) and NaOH traps to collect volatiles and carbon dioxide.

### **III. Preliminary study**

Approximate degradation rates of [ $^{14}\text{C}$ ] inpyrfluxam in natural water were calculated via a preliminary study. Duplicate tubes were prepared and sampled after 3 and 7 days irradiation, while dark control samples were prepared and sampled in duplicate at time 0 and after 3 and 7 days incubation. Time 0 samples were sacrificed immediately after dosing, while the remaining samples were connected to the trapping systems. After the specified incubation period, the pH of the samples was measured, the contents decanted, vials rinsed with acetonitrile (0.5 mL) and the rinsing added to the aqueous sample. Samples were analysed by LSC for mass balance and also by HPLC with a Beta-RAM radioisotope detector. The contents of the traps was also analysed by LSC.

### **IV. Definitive study**

Twelve quartz sample tubes were prepared for duplicate sampling at 6 time points for light exposed samples. Fourteen Pyrex sample tubes were prepared for sampling at 6 time points (plus time 0) for the dark controls. The nominal concentrations of inpyrfluxam were 0.98 and 1.00  $\mu\text{g/mL}$  in PYR and PH labelled samples, respectively.

At each sampling time, duplicate samples were collected. The pH of samples was measured and the contents of the vials decanted, vials rinsed with acetonitrile (0.5 mL) and the rinsing added to the aqueous sample. Aliquots (3 x 0.1 mL) were radioassayed by LSC. Quantitation was conducted with HPLC. Samples were analysed directly by reverse phase HPLC with a Beta-Ram radioisotope detector. The contents of the traps (EG and NaOH) were analysed by LSC in triplicate (0.5 mL). Aliquots ( mL) of the light and dark actinometer samples were also taken at each sampling event and analysed by HPLC.

### **V. Sample storage**

Samples were analysed by HPLC and LSC on the same day they were collected. All samples were stored in a refrigerator when not in use. Traps for volatiles were stored at ambient temperature. The applicant states all samples were analysed within one day of collection.

Reference standards and reference standard solutions were stored frozen when not in use.

## **VI. Confirmatory Chromatography by Thin Layer Chromatography (TLC)**

Two-dimensional TLC analysis was conducted on selected samples to confirm the HPLC peak assignment for inpyrfluxam and known degradates. Reference standards were visualised using short-wave UV (254 nm) light and the plates scanned for  $^{14}\text{C}$  detection.

## **VII. Chiral HPLC Analysis of Selected Samples**

The isomer composition of inpyrfluxam in irradiated and dark control samples was assessed by analysing selected extracts by normal phase HPLC. Aliquots (1 mL) of the aqueous samples were partitioned into hexane (2 x 1 mL) and the organic layers separated and concentrated under nitrogen for normal phase HPLC chiral analysis.

## **VIII. Sensitivity and Detection Limit**

For Beta-RAM chromatograms, the LOQ observed in HPLC chromatograms was determined by an experiment in which varying volumes of  $^{14}\text{C}$  labelled material were injected and the DPM detected compared to the actual DPM injected. This showed that the smallest injection for which acceptable recoveries were obtained was 258 dpm.

# **RESULTS AND DISCUSSION**

## **I. Radiochemical Purity and Stability of [ $^{14}\text{C}$ ] inpyrfluxam Under Conditions of Administration**

Reverse phase HPLC was used to determine that the radiochemical purities of inpyrfluxam were 95.6 % for the PYR label and 99.0 % for the PH label before test start. Reverse phase HPLC analysis of T0 samples was conducted to demonstrate the stability of the test substance under the conditions of administration. The dose solutions were found to be homogenous during the application processes, with a relative standard deviation of 1.0 to 2.5 % for the definitive study.

## **II. Preliminary Experiment**

Samples were continuously irradiated for up to 7 days. Inpyrfluxam degraded slowly in natural water under the conditions of the test. The preliminary test was used to determine the sampling schedule of the definitive test.

## **III. Definitive Experiment**

The test was conducted over 16 days, with the continuous irradiation for irradiated samples equivalent to 34 summer days at 40 °N. Integrated light intensity averaged 48.9 W/m<sup>2</sup> for the 290-400 nm range and 402 W/m<sup>2</sup> for the 290-800 nm range.

The applicant reported that a deviation from the expected temperature range was recorded for both the irradiated and dark control samples. For the irradiated samples, the temperature was generally between approximately 24 and 26.2 °C, but dropped to 21.5 to 22 °C from day 12 to day 13 before returning to >24 °C. The temperature in the dark controls rose to approximately 26.5 °C on day 7. As the deviations in temperature are of short duration it is not expected that they affected the outcome of the test.

The pH of aqueous samples was measured at sampling. For the PYR label the pH was a mean of 6.33 (range 5.40 to 7.08) in irradiated samples and 6.24 (range 5.75 to 6.87) in dark controls. For the PH label the pH was a mean of 7.11 (range 6.34 to 7.85) in irradiated samples and 6.93 (range 6.31 to 7.78) in dark controls.

Samples were confirmed as sterile throughout the test.

#### IV. Material balance

For the PYR label total recoveries (sum of radioactivity in aqueous samples and traps) averaged  $102.6 \pm 2.3$  % for irradiated samples and  $104.3 \pm 2.1$  % AR in dark controls. For the PH label total averaged  $98.7 \pm 2.5$  % for irradiated samples and  $98.6 \pm 1.3$  % AR in dark controls.

##### *PYR label*

**Table B.8.2.1.3-05 Material balance for irradiated samples for the PYR label**

Sample	% Applied dose			
	Aqueous phase	EG trap	NaOH	Total recovery
Replicate 1 (T0)	103.6	NA	NA	103.6
Replicate 2 (T0)	103.6	NA	NA	103.6
<b>Mean (T0)</b>	<b>103.6</b>	<b>NA</b>	<b>NA</b>	<b>103.6</b>
Replicate 1 (T1)	103.6	0.0	0.0	103.6
Replicate 2 (T1)	103.3	0.0	0.0	103.3
<b>Mean (T1)</b>	<b>103.5</b>	<b>0.0</b>	<b>0.0</b>	<b>103.5</b>
Replicate 1 (T4)	98.7	0.0	0.0	98.7
Replicate 2 (T4)	100.0	0.0	0.0	100.0



<b>Mean (T4)</b>	<b>99.4</b>	<b>0.0</b>	<b>0.0</b>	<b>99.4</b>
Replicate 1 (T7)	105.6	0.0	0.0	105.6
Replicate 2 (T7)	101.3	0.0	0.0	101.3
<b>Mean (T7)</b>	<b>103.5</b>	<b>0.0</b>	<b>0.0</b>	<b>103.5</b>
Replicate 1 (T10)	102.1	0.0	0.1	102.2
Replicate 2 (T10)	104.8	0.0	0.1	104.9
<b>Mean (T10)</b>	<b>103.5</b>	<b>0.0</b>	<b>0.1</b>	<b>103.6</b>
Replicate 1 (T14)	104.9	0.0	0.2	105.1
Replicate 2 (T14)	104.7	0.0	0.1	104.8
<b>Mean (T14)</b>	<b>104.8</b>	<b>0.0</b>	<b>0.2</b>	<b>105.0</b>
Replicate 1 (T16)	98.7	0.0	0.1	98.8
Replicate 2 (T16)	11.4	0.0	0.1	101.5
<b>Mean (T16)</b>	<b>99.9</b>	<b>0.0</b>	<b>0.1</b>	<b>100.0</b>
<b>Mean</b>				<b>102.6</b>
<b>STD</b>				<b>2.3</b>

Table B.8.2.1.3-06 Material balance for dark control samples for the PYR label

<b>Sample</b>	<b>% Applied dose</b>			
	<b>Aqueous phase</b>	<b>EG trap</b>	<b>NaOH</b>	<b>Total recovery</b>
Replicate 1 (T1)	105.2	0.0	0.0	105.2
Replicate 2 (T1)	106.6	0.0	0.0	106.6
<b>Mean (T1)</b>	<b>105.9</b>	<b>0.0</b>	<b>0.0</b>	<b>105.9</b>
Replicate 1 (T4)	99.4	0.0	0.0	99.4
Replicate 2 (T4)	106.1	0.0	0.0	106.1

<b>Mean (T4)</b>	<b>102.8</b>	<b>0.0</b>	<b>0.0</b>	<b>102.8</b>
Replicate 1 (T7)	105.2	0.0	0.0	105.2
Replicate 2 (T7)	104.7	0.0	0.0	104.7
<b>Mean (T7)</b>	<b>105.0</b>	<b>0.0</b>	<b>0.0</b>	<b>105.0</b>
Replicate 1 (T10)	102.9	0.0	0.0	102.9
Replicate 2 (T10)	104.0	0.0	0.0	104.0
<b>Mean (T10)</b>	<b>103.5</b>	<b>0.0</b>	<b>0.0</b>	<b>103.5</b>
Replicate 1 (T14)	105.3	0.0	0.0	105.3
Replicate 2 (T14)	106.5	0.0	0.0	106.5
<b>Mean (T14)</b>	<b>105.9</b>	<b>0.0</b>	<b>0.0</b>	<b>105.9</b>
Replicate 1 (T16)	102.0	0.0	0.0	102.1
Replicate 2 (T16)	103.5	0.0	0.0	103.5
<b>Mean (T16)</b>	<b>102.8</b>	<b>0.0</b>	<b>0.0</b>	<b>102.8</b>
<b>Mean</b>				<b>104.3</b>
<b>STD</b>				<b>2.1</b>

Radiocarbon recovered in the aqueous samples were >98 % AR throughout the study for all samples tested. NaOH traps represented an average of 0.1 % AR in day 16 light exposed samples having peaked at 0.2 % AR on day 14 while organic volatiles trapped in the ethylene glycol traps were below limits of quantitation throughout the study. Traps for volatiles in dark controls samples represented <0.1 % AR throughout the study period.

HPLC analysis showed that PYR-label inpyrfluxam was stable in both irradiated and dark control samples during the test. In the irradiated samples, inpyrfluxam ranged from 94.3 to 104.8 % AR. Minimal radioactivity was detected in either trap (maximum 0.2 % AR). Results for the dark control samples were similar with inpyrfluxam ranging from 102.8 to 105.9 % AR and with minimal radioactivity detected in either trap (maximum <0.1 % AR).

*PH label***Table B.8.2.1.3-07 Material balance for irradiated samples for the PH label**

<b>Sample</b>	<b>% Applied dose</b>			
	<b>Aqueous phase</b>	<b>EG trap</b>	<b>NaOH</b>	<b>Total recovery</b>
Replicate 1 (T0)	101.4	NA	NA	101.4
Replicate 2 (T0)	100.1	NA	NA	100.1
<b>Mean (T0)</b>	<b>100.8</b>	<b>NA</b>	<b>NA</b>	<b>100.8</b>
Replicate 1 (T1)	100.5	0.0	0.0	100.5
Replicate 2 (T1)	101.0	0.0	0.0	101.0
<b>Mean (T1)</b>	<b>100.8</b>	<b>0.0</b>	<b>0.0</b>	<b>100.8</b>
Replicate 1 (T3)	100.5	0.0	0.0	100.5
Replicate 2 (T3)	102.6	0.0	0.0	102.6
<b>Mean (T3)</b>	<b>101.6</b>	<b>0.0</b>	<b>0.0</b>	<b>101.6</b>
Replicate 1 (T7)	97.0	0.0	0.3	97.3
Replicate 2 (T7)	99.1	0.0	0.5	99.6
<b>Mean (T7)</b>	<b>98.1</b>	<b>0.0</b>	<b>0.4</b>	<b>98.5</b>
Replicate 1 (T10)	96.9	0.1	0.7	97.7
Replicate 2 (T10)	98.3	0.1	0.7	99.1
<b>Mean (T10)</b>	<b>97.6</b>	<b>0.1</b>	<b>0.7</b>	<b>98.4</b>
Replicate 1 (T14)	93.4	0.1	0.6	94.1
Replicate 2 (T14)	94.6	0.1	0.7	95.4
<b>Mean (T14)</b>	<b>94.0</b>	<b>0.1</b>	<b>0.7</b>	<b>94.8</b>
Replicate 1 (T16)	91.3	0.2	5.0	96.5

Replicate 2 (T16)	93.8	0.2	2.3	96.3
<b>Mean (T16)</b>	<b>92.6</b>	<b>0.2</b>	<b>3.7</b>	<b>96.4</b>
<b>Mean</b>				<b>98.7</b>
<b>STD</b>				<b>2.5</b>

Table B.8.2.1.3-08 Material balance for dark control samples for the PH label

Sample	% Applied dose			
	Aqueous phase	EG trap	NaOH	Total recovery
Replicate 1 (T1)	97.7	0.0	0.0	97.7
Replicate 2 (T1)	100.5	0.0	0.0	100.5
<b>Mean (T1)</b>	<b>99.1</b>	<b>0.0</b>	<b>0.0</b>	<b>99.1</b>
Replicate 1 (T3)	100.9	0.0	0.1	101.0
Replicate 2 (T3)	100.4	0.0	0.1	100.5
<b>Mean (T3)</b>	<b>100.7</b>	<b>0.0</b>	<b>0.1</b>	<b>100.8</b>
Replicate 1 (T6)	99.2	0.0	0.0	99.2
Replicate 2 (T6)	97.8	0.0	0.0	97.8
<b>Mean (T6)</b>	<b>98.5</b>	<b>0.0</b>	<b>0.0</b>	<b>98.5</b>
Replicate 1 (T9)	98.2	0.0	0.0	98.2
Replicate 2 (T9)	97.7	0.0	0.0	97.7
<b>Mean (T9)</b>	<b>98.0</b>	<b>0.0</b>	<b>0.0</b>	<b>98.0</b>
Replicate 1 (T13)	98.3	0.0	0.0	98.3
Replicate 2 (T13)	97.6	0.0	0.0	97.6
<b>Mean (T13)</b>	<b>98.0</b>	<b>0.0</b>	<b>0.0</b>	<b>98.0</b>
Replicate 1 (T15)	97.2	0.0	0.0	97.2

Replicate 2 (T15)	97.9	0.0	0.0	97.9
<b>Mean (T15)</b>	<b>97.6</b>	<b>0.0</b>	<b>0.0</b>	<b>97.6</b>
<b>Mean</b>				<b>98.6</b>
<b>STD</b>				<b>1.3</b>

Total recovery was >96 % in all samples and within the range of 90-110 % AR. In irradiated samples, radiocarbon recovered in the aqueous samples declined from a mean of 100.8 % AR to a mean of 92.6 % AR at study end. Radioactivity present in NaOH traps increased from <0.1 % AR at day 0 to an average of 3.7 % AR in Day 16 in light exposed samples while organic volatiles trapped in the ethylene glycol traps increased very slightly during the course of the study to a mean of 0.2 % AR. In dark controls, radiocarbon recovered in the aqueous samples were >97 % AR throughout the study for all samples tested. Traps for volatiles in dark control samples represented <0.1 % AR throughout the study period.

HPLC analysis showed that inpyrfluxam declined during the test, with a faster decline in the irradiated than the dark control samples. In the irradiated samples, inpyrfluxam ranged from 100.8 to 92.6 % AR. Radioactivity detected in the EG trap was minimal throughout the study, but 3.7 % AR was detected in the NaOH trap at study end. In the dark control samples inpyrfluxam ranged from 100.7 to 97.6 % AR with minimal radioactivity detected in either trap (maximum 0.1 % AR).

## V. Chiral analysis

The isomeric ratio of inpyrfluxam was analysed by normal phase HPLC, which showed that *R*-inpyrfluxam was the main isomer present in both irradiated and dark control samples and that changes in the isomeric ratio of inpyrfluxam did not occur.

**Table B.8.2.1.3-09 Change in stereoisomer ratios over the course of the test for irradiated and dark control samples**

	% AR					
	0 Days		Irradiated		Dark control	
			16 Days			
	R-isomer	S-isomer	R-isomer	S-isomer	R-isomer	S-isomer
PYR label	96.4	3.6	96.5	3.5	96.5	3.5
PH label	100	0	96.6	3.4	96.5	3.5

**VI. Degradation of [<sup>14</sup>C] inpyrfluxam in sterile natural water***PYR label***Table B.8.2.1.3-10 Components detected in irradiated samples**

Sample	% Applied dose						
	Inpyrfluxam	3'-OH-S-2840	DFPA-CONH <sub>2</sub>	DFPA	Others <sup>1</sup>	Organic Volatiles	CO <sub>2</sub>
Replicate 1 (T0)	99.0	3.3	<LOD	<LOD	1.2	NA	NA
Replicate 2 (T0)	99.5	4.1	<LOD	<LOD	<LOD	NA	NA
<b>Mean (T0)</b>	<b>99.3</b>	<b>3.7</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>0.6</b>	<b>NA</b>	<b>NA</b>
Replicate 1 (T1)	99.0	3.5	<LOD	1.0	<LOD	<LOD	<LOD
Replicate 2 (T1)	99.6	3.7	<LOD	<LOD	<LOD	<LOD	<LOD

<b>Mean (T1)</b>	<b>99.3</b>	<b>3.6</b>	<b>&lt;LOD</b>	<b>0.5</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T4)	92.8	3.8	2.2	<LOD	<LOD	<LOD	<LOD
Replicate 2 (T4)	94.4	4.3	1.3	<LOD	<LOD	<LOD	<LOD
<b>Mean (T4)</b>	<b>93.6</b>	<b>4.1</b>	<b>1.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T7)	96.7	4.1	1.9	2.1	0.7	<LOD	0.1
Replicate 2 (T7)	94.3	4.0	1.0	1.3	0.7	<LOD	<LOD
<b>Mean (T7)</b>	<b>95.5</b>	<b>4.1</b>	<b>1.5</b>	<b>1.7</b>	<b>0.7</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T10)	90.8	5.4	2.6	3.5	<LOD	<LOD	0.1
Replicate 2 (T10)	93.7	6.0	2.5	2.6	<LOD	<LOD	<LOD
<b>Mean (T10)</b>	<b>92.3</b>	<b>5.7</b>	<b>2.6</b>	<b>3.1</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T14)	87.6	7.8	3.6	6.1	<LOD	<LOD	<LOD
Replicate 2 (T14)	93.1	5.7	2.7	3.2	<LOD	<LOD	0.1
<b>Mean (T14)</b>	<b>90.4</b>	<b>6.8</b>	<b>3.2</b>	<b>4.7</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T16)	83.2	6.8	3.5	4.8	<LOD	<LOD	<LOD
Replicate 2 (T16)	88.7	5.7	3.2	3.8	<LOD	<LOD	0.1
<b>Mean (T16)</b>	<b>86.0</b>	<b>6.3</b>	<b>3.4</b>	<b>4.3</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>0.1</b>

NA – not applicable, not measured

<sup>1</sup> Column comprised of peaks that did not coelute with standards, none representing > 0.7% AR

**Table B.8.2.1.3-11 Components detected in dark control samples**

<b>Sample</b>	<b>% Applied dose</b>						
	<b>inpyr fluxa m</b>	<b>3'-OH- S-2840</b>	<b>DFPA- CONH<sub>2</sub></b>	<b>DFPA</b>	<b>Other s<sup>1</sup></b>	<b>Organic Volatile s</b>	<b>CO<sub>2</sub></b>
Replicate 1 (T1)	98.2	4.3	1.5	<LOD	1.2	NA	NA
Replicate 2 (T1)	102.3	3.0	<LOD	<LOD	1.3	NA	NA
<b>Mean (T1)</b>	<b>100.3</b>	<b>3.7</b>	<b>0.8</b>	<b>&lt;LOD</b>	<b>1.3</b>	<b>NA</b>	<b>NA</b>
Replicate 1 (T4)	94.3	3.6	<LOD	<LOD	1.5	<LOD	<LOD
Replicate 2 (T4)	101.2	4.9	<LOD	<LOD	<LOD	<LOD	<LOD
<b>Mean (T4)</b>	<b>97.8</b>	<b>4.3</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>0.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T7)	96.9	6.2	0.9	1.2	<LOD	<LOD	<LOD
Replicate 2 (T7)	98.9	4.4	0.7	<LOD	0.6	<LOD	<LOD
<b>Mean (T7)</b>	<b>97.9</b>	<b>5.3</b>	<b>0.8</b>	<b>0.6</b>	<b>0.3</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T10)	97.3	3.8	0.9	<LOD	0.8	<LOD	<LOD
Replicate 2 (T10)	98.7	3.8	0.7	<LOD	0.8	<LOD	<LOD
<b>Mean (T10)</b>	<b>98.0</b>	<b>3.8</b>	<b>0.8</b>	<b>&lt;LOD</b>	<b>0.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T14)	101.0	3.4	<LOD	<LOD	0.9	<LOD	<LOD
Replicate 2 (T14)	102.0	3.2	1.3	<LOD	<LOD	<LOD	<LOD



<b>Mean (T14)</b>	<b>101.5</b>	<b>3.3</b>	<b>0.7</b>	<b>&lt;LOD</b>	<b>0.5</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T16)	98.8	3.2	<LOD	<LOD	<LOD	<LOD	<LOD
Replicate 2 (T16)	100.3	3.2	<LOD	<LOD	<LOD	<LOD	<LOD
<b>Mean (T16)</b>	<b>99.6</b>	<b>3.2</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>

NA – not applicable, not measured

<sup>1</sup> Column comprised of peaks that did not coelute with standards, none representing >1.3% AR

HPLC analysis showed that inpyrfluxam degraded slowly in irradiated samples during the test. Inpyrfluxam decreased from a mean of 99.0 to 86.0 % AR. Metabolite 3'-OH-S-2840 was detected at 3.7 % AR in T0 samples and increased to a mean of 6.8 % AR at T14 before decreasing slightly to 6.3 % AR. At no time point did this metabolite exceed or approach the threshold of 10 % AR to be considered a major metabolite. This metabolite was reported in the sterile buffer solution study to have been present in the post-dose purity check as a minor impurity, although no similar claims are made in the study report for the current study; the metabolite could therefore be a degradation product formed due to photolysis or an impurity. Metabolites DFPA-CONH<sub>2</sub> and DFPA were also detected in low amounts. DFPA-CONH<sub>2</sub> increased to a mean of 3.4 % AR and DFPA increased to a mean of 4.7 % AR at T14 and decreased slightly to 4.3 % AR at T16. The applicant has also included 'others' which are stated to be peaks that did not coelute with the standards with none representing >0.7 % AR. Organic volatiles and CO<sub>2</sub> were not detected at levels >0.1 % AR.

HPLC analysis showed that inpyrfluxam was stable in the dark control during the test. inpyrfluxam ranged from mean values of 97.8 to 101.5 % AR. Metabolite 3'-OH-S-2840 was detected at up to 5.3 % AR, but was present in T0 samples and the post-dose purity check as a minor impurity. Levels of this metabolite were therefore similar to those of the irradiated samples. The levels present in the dark control have been subtracted from the levels in the irradiated samples to determine whether the levels observed may be as a result of photolytic degradation and whether any trends are observed.

**Table B.8.2.1.3-12 Levels of 3'-OH-S-2840 in irradiated PYR samples compared to dark controls**

<b>Sample</b>	<b>3'-OH-S-2840</b>		
	<b>Irradiated samples</b>	<b>Dark control samples</b>	<b>Difference relative to irradiated samples</b>
Replicate 1 (T0)	3.3	Not measured	Not measured
Replicate 2 (T0)	4.1	Not measured	Not measured
<b>Mean (T0)</b>	<b>3.7</b>	<b>Not measured</b>	<b>Not measured</b>
Replicate 1 (T1)	3.5	4.3	-0.8
Replicate 2 (T1)	3.7	3.0	0.7
<b>Mean (T1)</b>	<b>3.6</b>	<b>3.7</b>	<b>-0.1</b>
Replicate 1 (T4)	3.8	3.6	0.2
Replicate 2 (T4)	4.3	4.9	-0.6
<b>Mean (T4)</b>	<b>4.1</b>	<b>4.3</b>	<b>-0.2</b>
Replicate 1 (T7)	4.1	6.2	-2.1
Replicate 2 (T7)	4.0	4.4	-0.4
<b>Mean (T7)</b>	<b>4.1</b>	<b>5.3</b>	<b>-1.2</b>
Replicate 1 (T10)	5.4	3.8	1.6
Replicate 2 (T10)	6.0	3.8	2.2
<b>Mean (T10)</b>	<b>5.7</b>	<b>3.8</b>	<b>1.9</b>
Replicate 1 (T14)	7.8	3.4	4.4

Replicate 2 (T14)	5.7	3.2	2.5
<b>Mean (T14)</b>	<b>6.8</b>	<b>3.3</b>	<b>3.5</b>
Replicate 1 (T16)	6.8	3.2	3.6
Replicate 2 (T16)	5.7	3.2	2.5
<b>Mean (T16)</b>	<b>6.3</b>	<b>3.2</b>	<b>3.1</b>

This indicates that, up until and including T7, 3'-OH-S-2840 is present at higher amounts in the dark control. The metabolite may be an impurity in the dosing solution as it is present in irradiated samples at T0. From T10 onwards, small amounts of the metabolite are observed in irradiated samples that exceed the levels measured in the dark control, which may indicate some formation through photolytic degradation of S2399. By this metric, levels peak at T14 at 3.5 % AR, before declining to 3.1 % AR at study end. This indicates that this metabolite is formed at very low levels and does not approach the level to 10 % AR needed for this to be considered as a major metabolite. No FOCUS Kinetics analysis of this metabolite has therefore been conducted for the PYR labelled 3'-OH-S-2840.

Metabolites DFPA-CONH<sub>2</sub> and DFPA were also detected in low amounts. DFPA-CONH<sub>2</sub> was detected at a mean of 0.8 % AR and DFPA at a mean of 0.6 % AR. The applicant has also included 'others' which are stated to be peaks that did not coelute with the standards with none representing >0.8 % AR. Organic volatiles and CO<sub>2</sub> were not detected.

#### *PH label*

**Table B.8.2.1.3-13 Components detected in irradiated samples**

<b>Sample</b>	<b>% Applied dose</b>				
	<b>Inpyrfluxam</b>	<b>3'-OH-S-2840</b>	<b>Others<sup>1</sup></b>	<b>Volatiles</b>	<b>CO<sub>2</sub></b>
Replicate 1 (T0)	101.4	<LOD	<LOD	NA	NA
Replicate 2 (T0)	100.1	<LOD	<LOD	NA	NA

<b>Mean (T0)</b>	<b>100.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>NA</b>	<b>NA</b>
Replicate 1 (T1)	100.5	<LOD	<LOD	<LOD	<LOD
Replicate 2 (T1)	101.0	<LOD	<LOD	<LOD	<LOD
<b>Mean (T1)</b>	<b>100.8</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T3)	99.3	1.2	<LOD	<LOD	<LOD
Replicate 2 (T3)	102.6	<LOD	<LOD	<LOD	<LOD
<b>Mean (T3)</b>	<b>101.0</b>	<b>0.6</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T7)	91.8	3.0	1.1	<LOD	0.1
Replicate 2 (T7)	94.2	3.2	<LOD	<LOD	<LOD
<b>Mean (T7)</b>	<b>93.0</b>	<b>3.1</b>	<b>0.6</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T10)	86.7	5.0	<LOD	<LOD	0.1
Replicate 2 (T10)	89.6	5.3	2.9	<LOD	<LOD
<b>Mean (T10)</b>	<b>88.2</b>	<b>5.2</b>	<b>1.5</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T14)	84.1	6.4	<LOD	<LOD	<LOD
Replicate 2 (T14)	84.3	4.9	3.6	<LOD	0.1
<b>Mean (T14)</b>	<b>84.2</b>	<b>5.7</b>	<b>1.8</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T16)	66.8	9.8	5.7	<LOD	<LOD

Replicate 2 (T16)	74.6	7.3	8.6	<LOD	0.1
<b>Mean (T16)</b>	<b>70.7</b>	<b>8.6</b>	<b>7.2</b>	<b>&lt;LOD</b>	<b>0.1</b>

NA – not applicable, not measured

<sup>1</sup> Column comprised of peaks that did not coelute with standards, none representing > 0.8 % AR

**Table B.8.2.1.3-14 Components detected in dark control samples**

Sample	% Applied dose			
	Inpyrfluxam	Others <sup>1</sup>	Organic Volatiles	CO <sub>2</sub>
Replicate 1 (T1)	97.7	<LOD	<LOD	<LOD
Replicate 2 (T1)	99.7	0.8	<LOD	<LOD
<b>Mean (T1)</b>	<b>98.7</b>	<b>0.4</b>	<b>&lt;LOD</b>	<b>0.1</b>
Replicate 1 (T3)	100.9	<LOD	<LOD	0.1
Replicate 2 (T3)	100.4	<LOD	<LOD	0.1
<b>Mean (T3)</b>	<b>100.7</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T6)	99.2	<LOD	<LOD	<LOD
Replicate 2 (T6)	97.8	<LOD	<LOD	<LOD
<b>Mean (T6)</b>	<b>98.5</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T9)	98.2	<LOD	<LOD	<LOD
Replicate 2 (T9)	97.7	<LOD	<LOD	<LOD
<b>Mean (T9)</b>	<b>98.0</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
Replicate 1 (T13)	98.3	<LOD	<LOD	<LOD
Replicate 2 (T13)	97.6	<LOD	<LOD	<LOD
<b>Mean (T13)</b>	<b>98.0</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>

Replicate 1 (T15)	97.2	<LOD	<LOD	<LOD
Replicate 2 (T15)	97.9	<LOD	<LOD	<LOD
<b>Mean (T15)</b>	<b>97.6</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>

<sup>1</sup> Column comprised of peaks that did not coelute with standards, none representing > 0.8% AR

HPLC analysis showed that inpyrfluxam degraded slowly in irradiated samples during the test. Inpyrfluxam decreased from a mean of 100.8 to 70.7 % AR. Metabolite 3'-OH-S-2840 was not detected in T0 samples but increased to a mean of 8.6 % AR at T16 (and 9.8 % AR in one replicate) and was still increasing at study end. The applicant has also included 'others' which are stated to be peaks that did not coelute with the standards with none representing >0.8 % AR, although collectively they reached a mean of 7.2 % AR by study end; HSE has verified from submitted chromatograms that none of these metabolites exceeded 1 % AR. Organic volatiles and CO<sub>2</sub> were not detected at levels >0.1 % AR.

HPLC analysis showed that inpyrfluxam was stable in the dark control during the test. inpyrfluxam ranged from mean values of 98.0 to 100.7 % AR. The applicant has also included 'others' which are stated to be peaks that did not coelute with the standards with none representing >0.4 % AR. Organic volatiles and CO<sub>2</sub> were not detected at levels >0.1 % AR. Metabolite 3'-OH-S-2840 was not detected in the dark control at any time point.

## VII. Photodegradation Rate of [<sup>14</sup>C] inpyrfluxam in soil

The applicant has calculated degradation rates for the parent using linear regression. These are not acceptable as FOCUS Kinetics principles should have been used. Fits displayed below are therefore those generated by HSE. The fitting used FOCUS Kinetics Guidance was conducted using CAKE 3.7. For time 0 samples, the total recovery was used with any metabolites and volatiles added to the parent % AR (although it is acknowledged that metabolite was present in the dosing solution which will result in a small error; as the DT<sub>50</sub> value derived is not used in the exposure assessment this is not considered to be an important issue). As no values approached the LOD or LOQ no consideration of these values according to FOCUS Kinetics was required. In accordance with the FOCUS Kinetics procedure for selecting best fit endpoints, both SFO and FOMC models were run initially in order to determine whether biphasic kinetics gave a better fit, with other biphasic models then run if required.

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*PYR label, irradiated*

**Table B.8.2.1.3-15 Data used in the FOCUS Kinetics evaluation (PYR label, irradiated)**

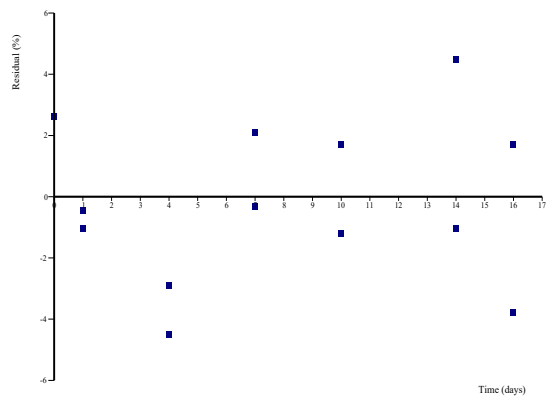
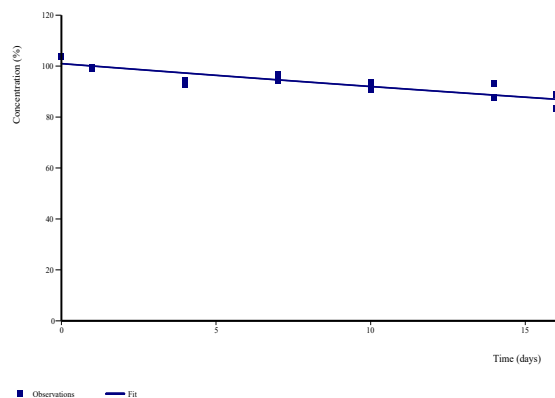
<b>Time Point (days)</b>	<b>% AR</b>
0	103.6
0	103.6
1	99.0
1	99.6
3	92.8
3	94.4
7	96.7
7	94.3
10	90.8
10	93.7
14	87.6
14	93.1
16	83.2
16	88.7

**Table B.8.2.1.3-16 Rate of degradation for parent only PYR label in irradiated samples - HSE kinetic fitting. Selected best fit in bold.**

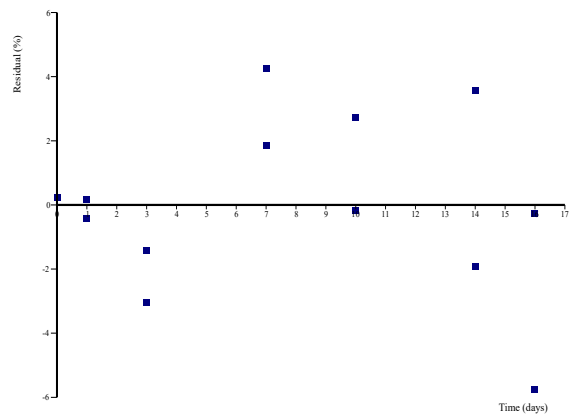
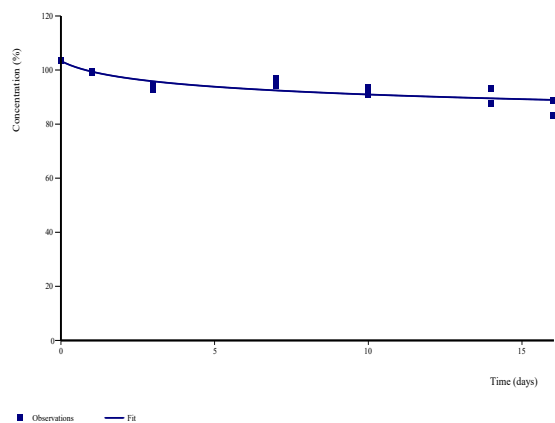
<b>PYR label, irradiated</b>						
Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
<b>SFO</b>	<b>Good</b>	<b>1.62</b>	<b>M<sub>0</sub>: 101 k: 0.009323</b>	<b>k: &lt;0.05</b>	<b>74.4</b>	<b>247</b>
FOMC	Good	1.75	M <sub>0</sub> : alpha: beta:	not applicable	>10,000	>10,000
<p><b>SFO:</b> The visual fit of the SFO model to the data is good; starting concentrations are slightly underestimated, but the decline phase and final time point are well represented. The <math>\chi^2</math> value is low at 1.62 %. The fit is considered to be acceptable.</p> <p>FOMC: The FOMC model shows a good visual fit to the data, representing the starting concentrations, decline curve and final concentrations well. There are no systematic errors in the plot of the residuals. The <math>\chi^2</math> value is 1.75, which is low.</p> <p><b>Conclusion: The FOMC fit is not an improvement on SFO and SFO is accepted (DT<sub>50</sub> = 74.4, DT<sub>90</sub> = 247).</b></p>						



SFO



FOMC



**PYR label, dark control****Table B.8.2.1.3-17 Data used in the FOCUS Kinetics evaluation (PYR label, dark control)**

<b>Time Point (days)</b>	<b>% AR</b>
1	105.2
1	106.6
4	94.3
4	101.2
7	96.9
7	98.9
10	97.3
10	98.7
14	101.0
14	102.0
16	98.8
16	100.3

**Table B.8.2.1.3- 18 Rate of degradation for parent-only PYR label in dark control samples -HSE kinetic fitting. Selected best fit in bold.**

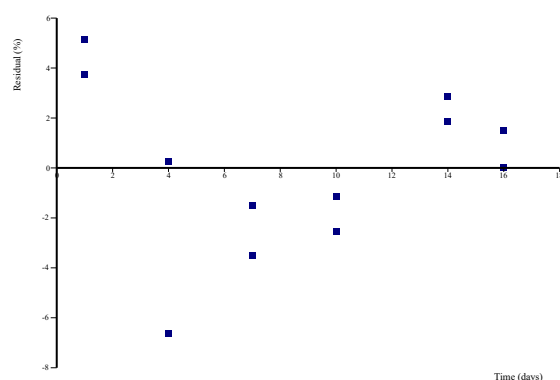
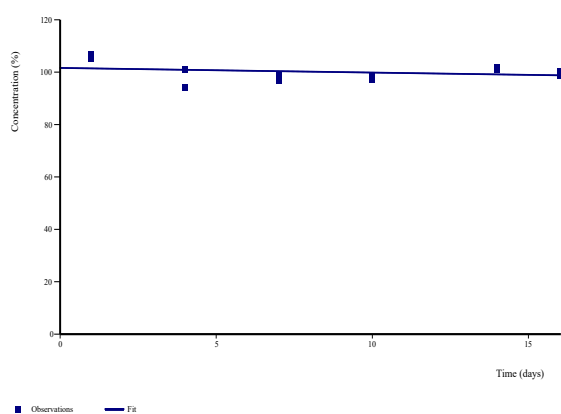
<b>PYR label, dark control</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>Good</b>	<b>2.19</b>	<b>M<sub>0</sub>: 101.6 k: 0.001774</b>	<b>k: &lt;0.05</b>	<b>391</b>	<b>1300</b>
FOMC	Good	1.98	M <sub>0</sub> : 121.1 alpha: 0.01936 beta: 0.000331	not applicab le	>10,000	>10,000

**SFO:** The visual fit of the SFO model to the data is good; the decline phase and final time point are well represented. The  $\chi^2$  value is low at 2.19 %. The fit is considered to be acceptable.

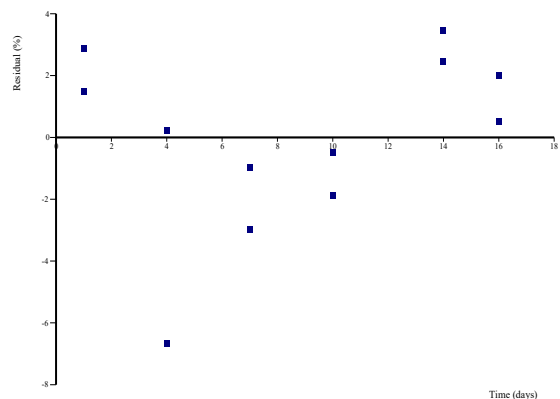
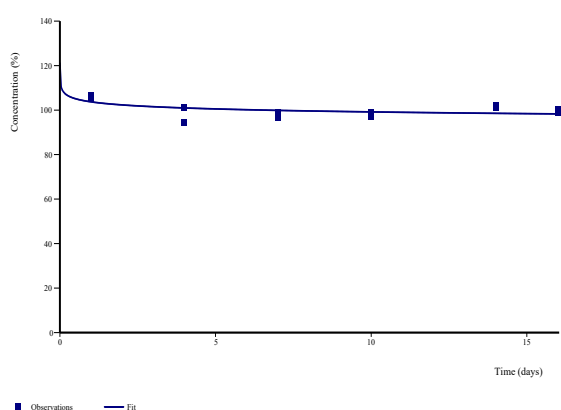
**FOMC:** The visual fit of the SFO and FOMC models is similar, although the FOMC model represents the first data point slightly better. The plots of the residuals are also similar, but with the FOMC model representing earlier data points slightly better and the SFO model representing later data points slightly better. The  $\chi^2$  value for the FOMC model is slightly lower at 1.98 % compared to the value of 2.19 % for the SFO model.

**Conclusion: The FOMC model offers little improvement over SFO. SFO is chosen as the best fit model ( $DT_{50} = 391$ ,  $DT_{90} = 1300$ ).**

### SFO



### FOMC



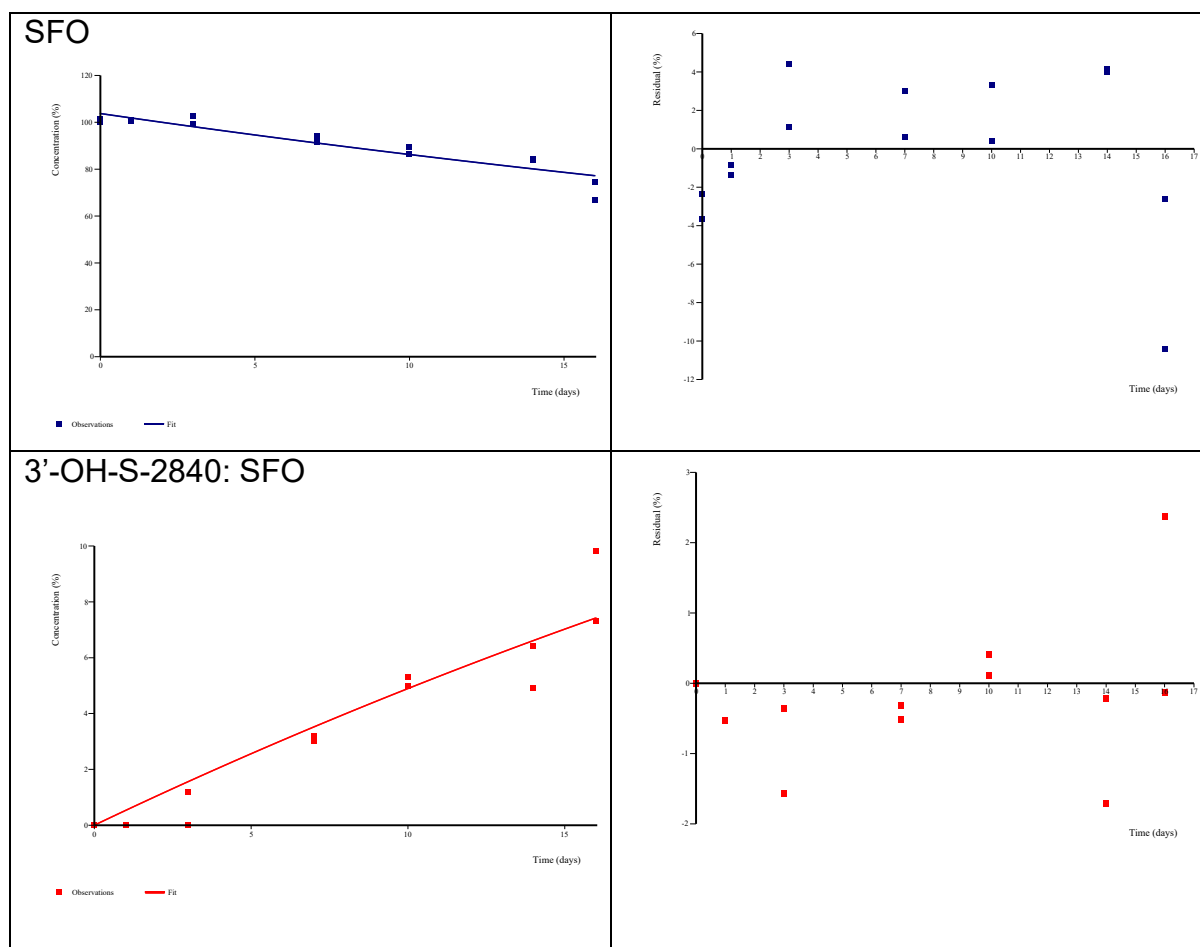
*PH label, irradiated*

**Table B.8.2.1.3-19 Data used in the FOCUS Kinetics evaluation (PH label, irradiated)**

<b>Time Point (days)</b>	<b>% AR</b>	
	<b>Inpyrfluxam</b>	<b>3'-OH-S-2840</b>
0	101.4	0
0	100.1	0
1	100.5	0
1	101.0	0
3	99.3	1.2
3	102.6	0.0
7	91.8	3.0
7	94.2	3.2
10	86.7	5.0
10	89.6	5.3
14	84.1	6.4
14	84.3	4.9
16	66.8	9.8
16	74.6	7.3

**Table B.8.2.1.3- 20 PH label in irradiated samples. SFO fit for parent and metabolite - HSE kinetic fitting. Final parent fit selection in bold.**

<b>PH label, irradiated</b>						
<b>Parent and metabolite (3'-OH-S-2840) SFO</b>						
Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
<b>Parent: SFO</b>	<b>Fair</b>	<b>3.02</b>	<b>M<sub>0</sub>: 103.8</b> <b>k: 0.01846</b>	<b>k: &lt;0.05</b>	<b>37.6</b>	<b>125</b>
3'-OH-S-2840: SFO from parent	Good	16.1	k: 0.0000000115 ffm: 0.28	0.5	>10,000	>10,000
<p><b>Parent SFO:</b> The visual fit of the SFO model to the data is good; the decline phase is well represented although starting concentrations and the final timepoint are slightly overestimated. The <math>\chi^2</math> value is low at 3.02 %. The fit is considered to be acceptable. The metabolite was then added to the fit.</p> <p><b>3'-OH-S-2840 SFO from parent:</b> The SFO model shows a good fit to the parent and the metabolite data, with starting concentrations and the decline phase for parent and the starting concentration and the increase in concentration well represented for the metabolite, although the final time point is overestimated for parent. Closer inspection of the plot of the residuals however shows systematic errors for parent, with the rate of decline consistently overestimated. The <math>\chi^2</math> value was low at 3.02 % for parent, but fairly high for the metabolite at 16.1 %.</p> <p><b>Conclusion:</b> After further consideration of FOMC (see below) model selection is SFO-SFO kinetics as best fit for 3'-OH-S-2840 (parent DT<sub>50</sub> = 37.6, parent DT<sub>90</sub> = 125. Metabolite DT<sub>50</sub> = &gt;10,000, metabolite DT<sub>90</sub> = &gt;10,000).</p>						



**Table B.8.2.1.3-21 PH label in irradiated samples. FOMC fit for parent and SFO fit for metabolite - HSE kinetic fitting.**

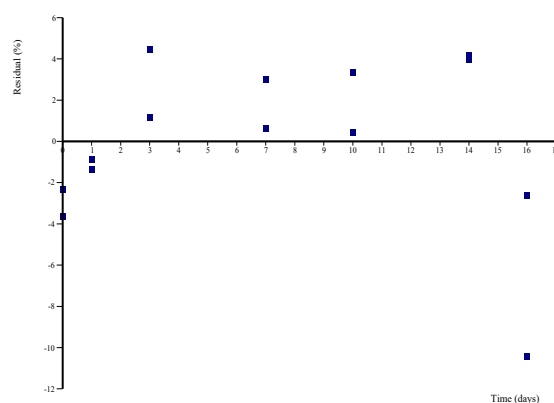
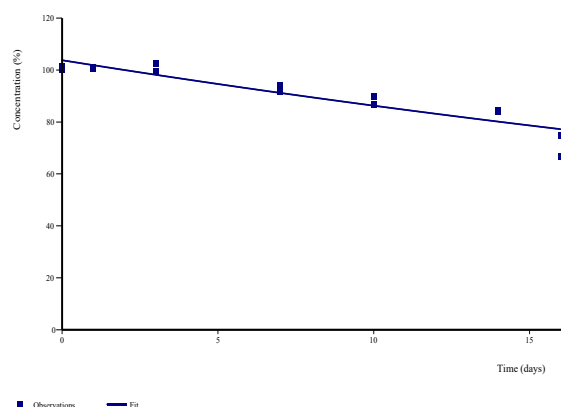
PH label, irradiated						
Parent FOMC and metabolite SFO (3'-OH-S-2840)						
Model	Visual Assesment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
Parent: FOMC	Fair	3.26	M <sub>0</sub> : 103.8 alpha: 2180 beta: 118,000	not applicable	37.6	125 <b>37.6*</b>
3'-OH-S-2840: SFO from parent	Good	16.1	k: 0.0000000115 ffm: 0.2799	0.5	>10,000	>10,000
<b>*DT<sub>90</sub>/3.32</b>						

The FOMC fit is very similar to the SFO fit. The model shows a good fit to the parent and the metabolite data, with starting concentrations and the decline phase for parent and the starting concentration and the increase in concentration well represented for the metabolite, although the final time point is overestimated for parent. Closer inspection of the plot of the residuals however shows systematic errors for parent, with the rate of decline consistently overestimated. The  $\chi^2$  value was low at 3.26 % for parent, but fairly high for the metabolite at 16.1 %; these values are very similar to the  $\chi^2$  values obtained for SFO

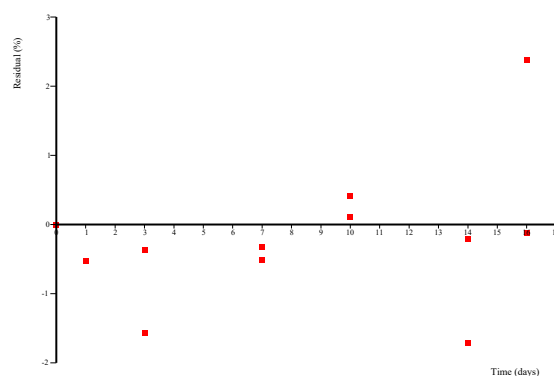
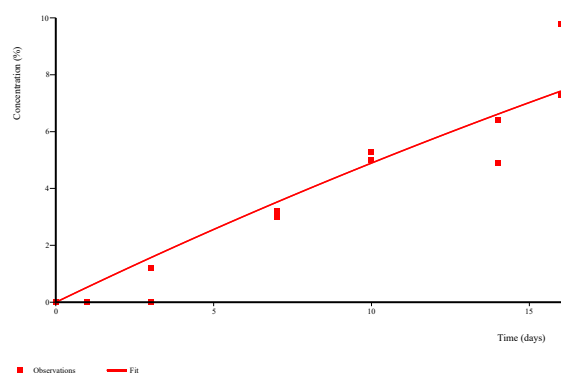
It is noted that for FOMC, the  $DT_{90}/3.32$  is equal to the  $DT_{50}$ , indicating that the fit is SFO.

**Conclusion:** Overall it is considered that the FOMC model does not offer an improvement on the SFO fit and the SFO model is chosen as the best fit model.

### FOMC



### 3'-OH-S-2840: FOMC



*PH label, dark control*

**Table B.8.2.1.3-22 Data used in the FOCUS Kinetics evaluation (PH label, dark control)**

<b>Time Point (days)</b>	<b>% AR</b>
1	97.7
1	100.5
3	100.9
3	100.4
6	99.2
6	97.8
9	98.2
9	97.7
13	98.3
13	97.6
15	97.2
15	97.9

**Table B.8.2.1.3-23 Rate of degradation for parent-only PH label in dark control samples -HSE kinetic fitting. Selected best fit in bold.**

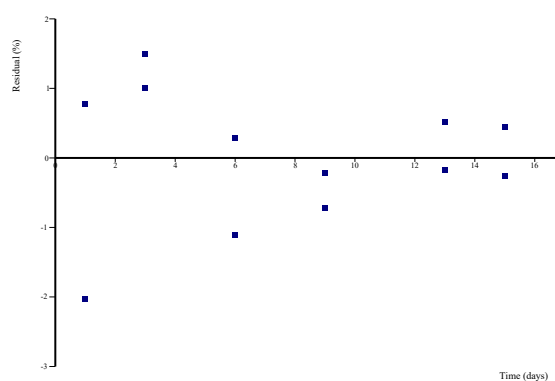
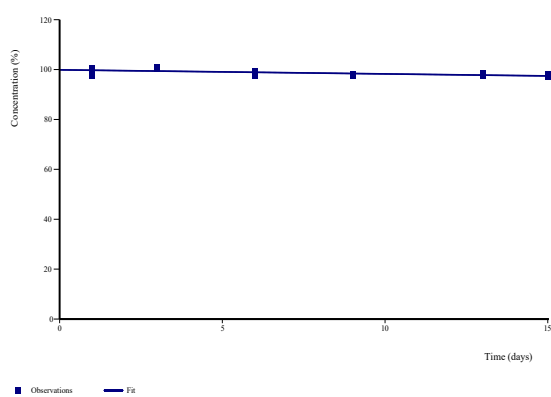
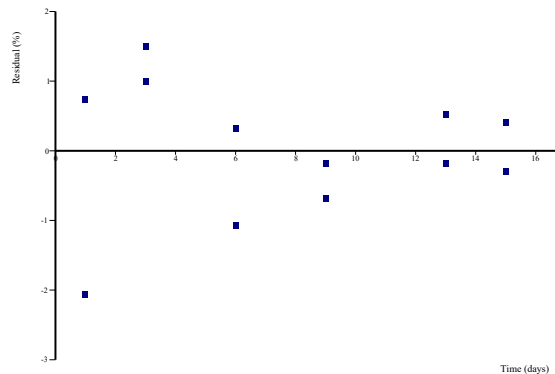
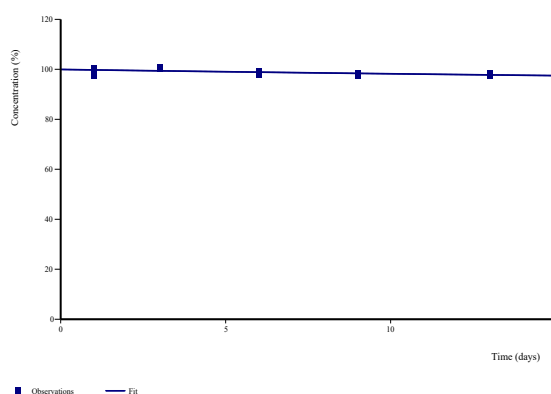
<b>PH label, dark control</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
<b>SFO</b>	<b>Good</b>	<b>0.509</b>	<b>M<sub>0</sub>: 99.89 k: 0.001646</b>	<b>k: &lt;0.05</b>	<b>421</b>	<b>1400</b>
FOMC	Good	0.559	M <sub>0</sub> : 99.96 alpha: 0.08464 beta: 43.62	not applicab le	>10,000	>10,000



**SFO:** The visual fit of the SFO model to the data is good; the decline phase and final time point are well represented. The  $\chi^2$  value is low at 0.509 %. The fit is considered to be acceptable and biphasic models have not been run.

**FOMC:** The FOMC model gives a good visual fit to the model, representing the decline phase and the final timepoint well. The plot of the residuals shows random errors. The  $\chi^2$  value is 0.559, which is low.

**Conclusion:** The  $\chi^2$  values for the SFO and FOMC models are almost identical, with the SFO model having the marginally lower value. The FOMC model offers no improvement on the SFO model and so the SFO model is selected ( $DT_{50} = 421$ ,  $DT_{90} = 1400$ ).

**SFO****FOMC**

**Table B.8.2.1.3-24 Summary of FOCUS Kinetics for PYR and PH labels in the irradiated samples and dark controls, calculated by HSE using CAKE 3.7**

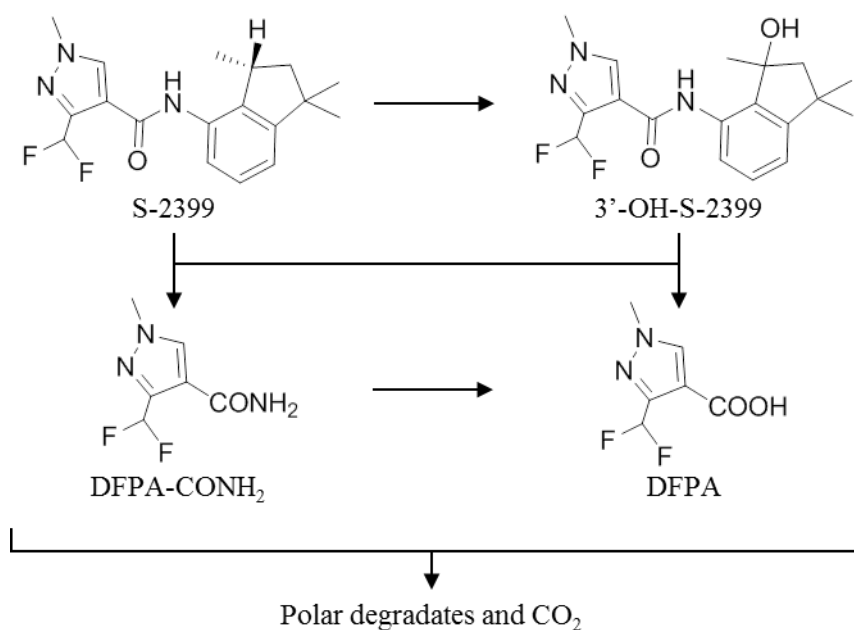
	Visual fit	Model	X <sup>2</sup> (%)	Rate constant	DT <sub>50</sub> (days)	DT <sub>90</sub> (days)
PYR label, irradiated	Good	SFO	1.62	0.009323	74.4	247
PYR label dark control	Good	SFO	2.19	0.001774	391	1300
PYR label, photolysis				0.007549	92	305
PH label, irradiated	Good	SFO	3.02	0.01846	37.6	125
PH label, dark control	Good	SFO	0.509	0.001646	421	1400
PH label, photolysis				0.016814	41.2	137

The degradation rates obtained by HSE are slightly different to those calculated by the applicant, although the broad trends are the same. For the PYR label, degradation was observed in the irradiated samples, with a DT<sub>50</sub> of 74.4 days; slower degradation was observed in the dark controls. For the PH label, degradation was faster in the irradiated samples with a DT<sub>50</sub> of 37.6 days, but degradation in the dark controls was slower than for the PYR label.

The OECD 316 Guideline states that in cases where data for the parent follow first-order kinetics and the parent test chemical rapidly transforms, the same SFO methods should be used to estimate first-order direct photolysis rate constants and half-lives for primary transformation products formed from the parent which are still at substantial concentrations (e.g. 10 % AR). For the PHY label, 3'-OH-S-2840 reached a mean maximum of 6.8 % AR, while for the PH label it reached a mean maximum of 8.6 % AR of which one replicate was 9.8 % AR. For both radiolabels, the metabolite remained below 10 % AR, but levels were still increasing at study end and were close to 10 % AR in the PH label. In addition, a mean of 86.0 % AR for the PYR label and 70.7 % AR for the PH label remains as parent, meaning that there is potential for this metabolite to rise further as the remaining parent material is photolysed. For the PYR label, it was considered appropriate to calculate a DT<sub>50</sub>

value for 3'-OH-S-2840, after accounting for the presence of the metabolite in the dosing solution as an impurity.

The applicant has proposed the following degradation scheme for inpyrfluxam in natural waters:



**Figure B.8.2.1.3-01 Proposed Degradation Pathway of inpyrfluxam in Natural Water When Exposed to Artificial Sunlight**

The intensity of the Xenon lamp was used to convert the DT<sub>50</sub> and DT<sub>90</sub> values into equivalent solar days. This was done using the following equation:

$$T_s = T_a \times I_a / I_s$$

Average Light intensity =	402	W/m <sup>2</sup> (300-800 nm)	I <sub>a</sub>
Average Light intensity =	48.9	W/m <sup>2</sup> (300-400 nm)	I <sub>a</sub>
1 US solar day <sup>A</sup> =	4502	W*h/m <sup>2</sup> /day	I <sub>s</sub>
1 OECD day <sup>B</sup> =	603	W*h/m <sup>2</sup> /day	I <sub>s</sub>
1 JMAFF day <sup>C</sup> =	187.6	W*h/m <sup>2</sup> /day	I <sub>s</sub>
t <sub>1/2</sub> =	857	exposure hours	T <sub>a</sub>
t <sub>1/2</sub> =	77	US solar days	T <sub>s</sub>
t <sub>1/2</sub> =	69	OECD solar days	T <sub>s</sub>

$t_{1/2}$ =	223	JMAFF solar days	Ts
DT <sub>90</sub> =	2845	exposure hours	Ta
DT <sub>90</sub> =	254	US solar days	Ts
DT <sub>90</sub> =	231	OECD solar days	Ts
DT <sub>90</sub> =	742	JMAFF solar days	Ts

<sup>A</sup> Based on Intensity of 1 U.S. Summer Day at 40° N Latitude <sup>1</sup> (300-800 nm)

<sup>B</sup> 67 W/m<sup>2</sup> maximum intensity in 300-400 nm region x 0.75 (factor for varying light intensity throughout the day)

x 12 hours of average sunlight per day

<sup>C</sup> JMAFF Guideline 2-6-2, Notification 13 Seisan No. 3986, October 10, 2001 and Japanese Industrial

<sup>1</sup> Solar Radiation Resource Information, "The solar radiation data manual for buildings, 30 year average monthly solar radiation,

1961-1990, Boulder, CO (40°N Latitude)", National Renewable Energy Laboratory (NREL), Golden Colorado, 1994.

**Table B.8.2.1.3-25 Conversion of DT<sub>50</sub> values observed under the Xenon lamp into equivalent DT<sub>50</sub> values at 30°N, 40°N and 50°N in days: comparison of applicant calculated, HSE calculated and HSE calculated values corrected for dark control**

	<b>DT<sub>50</sub> in Suntest (days)</b>	<b>DT<sub>50</sub> in Suntest (hours)<sup>1</sup></b>	<b>US (40 °N summer)<sup>2</sup> (days)</b>	<b>OECD (30-50 °N, summer)<sup>3</sup> (days)</b>	<b>JMAFF (35 °N spring)<sup>4</sup> (days)</b>
<b>Applicant</b>					
PYR label	87.7	2105	188	171	549
PH label	35.7	857	77	69	223
<b>HSE</b>					
PYR label	74.4	1786	159	145	465
PH label	37.6	902	81	73	235
<b>HSE (corrected for dark control)</b>					
PYR label	92	2208	197	179	576

PH label	41.2	989	88	80	258
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<sup>1</sup> Continuous Suntest irradiation

<sup>2</sup> Average summer irradiation in the 300-800 nm range at 40 °N latitude

<sup>3</sup> Average summer irradiation in the 300-400 nm range at 30-50 °N latitude

<sup>4</sup> Average spring irradiation in the 300-400 nm range at 35 °N latitude in Tokyo

The applicant calculated DT<sub>50</sub> values using linear regression which is not accepted. HSE have recalculated the values using FOCUS Kinetics and have also corrected the photolysis rates for the degradation rate observed in the dark controls and these are the endpoints for use in the exposure assessment.

### VIII. Quantum yield

According to OECD 316, calculation of the quantum yield is optional. This has not been calculated by the applicant.

## CONCLUSIONS

The photolysis of [<sup>14</sup>C] inpyrfluxam was studied in a sterile natural water during 15 or 16 days continuous irradiation, alongside dark control samples. The irradiation was approximately equivalent to 34 days at 40 °N.

Little or no degradation was observed in the dark controls for either label, with volatiles present at ≤0.1 % AR. For the PYR label, the metabolite 3'-OH-S-2840 was present at a mean of 3.7 % AR at the first sampling point and remained relatively constant throughout the study. This may be an impurity in the dosing solution rather than a degradation product. Additional metabolites observed were DFPA-CONH<sub>2</sub> and DFPA which reached maximum levels of 1.5 and 1.2 % AR respectively. Other peaks in chromatograms were a mean maximum of 1.3 % AR. Degradation was slow, amounting to 391 days for the PYR label and 421 days for the PH label under the conditions of the test.

In the irradiated samples, the PYR label degraded to form 3'-OH-S-2840 (mean maximum of 3.5 % AR after correction for the dark control), DFPA-CONH<sub>2</sub> (mean maximum 0.8 % AR, DFPA (mean maximum 0.6 % AR), others (none representing more than 1.3 % AR) and volatiles (maximum 0.2 % AR). The degradation rate was calculated to be 74 days under the conditions of the test or 192 days after correction for the dark control and at 40 °N. For the PH label, the main metabolite was 3'-OH-S-2840 which reached a mean maximum of 8.6 % AR at study end and 9.8 % in one replicate. Metabolite 3'-OH-S-2840 is therefore very close to the trigger of 10 % AR and is still increasing at study end with a mean of 70.7 % AR remaining as unchanged parent, meaning that there is the potential for further formation of this metabolite over time. It is acknowledged however that this study represents a worst case, as this metabolite was not observed in the direct photolysis study and was also continued for the equivalent of 34 days summer sunlight at 40 N and so slightly

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longer than the 30 days specified by the draft OECD 316 Guideline. Other metabolites present did not represent more than 0.8 % individually but collectively represented a mean maximum of 7.2 % AR at study end. Volatiles reached a mean maximum of 3.9 % AR. Degradation was faster than for the PYR label, being 37.6 days under the conditions of the test and 88 days following correction for the dark control and at 40 °N.

The degradation rates suggest that photolytic degradation in natural waters may contribute somewhat to the overall degradation of inpyrfluxam.

**B.8.2.2. Route and rate of biological degradation in aquatic systems****B.8.2.2.1. Ready biodegradability**

<b>Data Point:</b>	KCA 7.2.2.1/01
<b>Report Author:</b>	
<b>Report Year:</b>	2016
<b>Report Title:</b>	S-2399 TGAI – Determination of the Biodegradability of a Test Substance Based on OECD Method 301B (CO <sub>2</sub> Evolution Test)
<b>Guideline(s) followed in study:</b>	OECD Method 301B (CO <sub>2</sub> evolution test)
<b>GLP:</b>	Yes

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Temperature deviation from 22 ± 2 °C, up to 28.5 °C	Minor deviation. Temperature deviation occurred for a period less than 24 hours. Not expected to have effected conclusions of the study.
The applicant does not report the exact nature of the materials used in making stock solutions for the test (e.g purity, supplier).	Not considered as a major deviation as GLP is assured, and they are stated to come from commercial sources and to be of reagent grade.

**HSE conclusion on deviations**

HSE considers that the study was conducted satisfactorily and that the deviation in temperature is not significant as to jeopardise the validity of the study. HSE has evaluated this study, and accepts its conclusions.

## INTRODUCTION

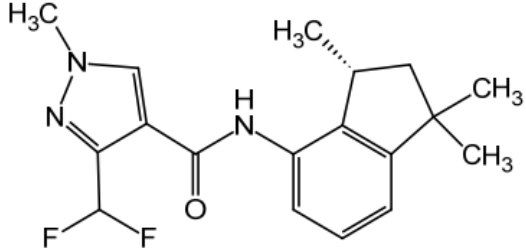
This study was performed to determine the potential for ultimate biodegradation of inpyrfluxam in water by the carbon dioxide evolution method following OECD Test Guideline 301 B. The amount of carbon dioxide (CO<sub>2</sub>) released upon biodegradation of the test substance and a reference substance, sodium benzoate, was measured to assess the potential for ultimate biodegradation.

HSE has validated this study against the OECD 301 section A and B guidelines, which the applicant has also used.

## MATERIALS AND METHODS

The test item inpyrfluxam TG was supplied to the test facility by the applicant. Its properties are summarised in the table below.

**Table B.8.2.2.1-01 Physiochemical properties of inpyrfluxam TG (Inpyrfluxam)**

Parameter	Value
Structural formula	
Empirical formula	C <sub>18</sub> H <sub>21</sub> F <sub>2</sub> N <sub>3</sub> O
Molecular mass	333.38 g/mol
Chemical Purity	95.0 % (sponsor's data)
Water solubility*	1.64 x 10 <sup>-2</sup> (g/L) at 20°C
Vapour Pressure*	3.8 x 10 <sup>-8</sup> Pa at 20 °C
Bacterial Toxicity	Not reported; applicant performed toxicity control

The reference item used was sodium benzoate (99.9 % purity, Sigma Aldrich). Sodium benzoate is known to be readily biodegrade on the timescale of this experiment, so HSE finds this choice of reference acceptable.



## I. Mineral medium

The ingredients of the mineral medium are detailed in Table B.8.2.2.1-02 below. High purity reagent grade water was used for the preparation of the mineral medium and all dosing stock solutions. Only one batch of water, which had been checked by Dissolved Organic Carbon (DOC) analysis, was used. The water contained no more than 10 % of the total carbon content introduced by the test substance.

**Table B.8.2.2.1-02 Composition of stock solutions for mineral salts medium**

	<b>Solution<sup>a</sup></b>	<b>Chemical Formula of Components</b>	<b>Amount (g) per L of Stock</b>	<b>mL of Stock per L of Medium</b>
1	Phosphate buffer	KH <sub>2</sub> PO <sub>4</sub>	8.5	10
		K <sub>2</sub> HPO <sub>4</sub>	21.75	
		Na <sub>2</sub> HPO <sub>4</sub>	26.64	
		NH <sub>4</sub> Cl	0.5	
2	Calcium chloride	CaCl <sub>2</sub>	27.5	1
3	Magnesium sulfate	MgSO <sub>4</sub> · 7H <sub>2</sub> O	22.5	1
4	Ferric chloride <sup>b</sup>	FeCl <sub>3</sub> · 6H <sub>2</sub> O	0.25	1

Based on OECD Document 301B (1992).

<sup>a</sup> Solutions were prepared using purified reagent grade water. The prepared mineral salts medium solutions 1 through 4 were prepared separately and kept refrigerated (approximately 2 to 8 °C) until use on day -1.

<sup>b</sup> In order to avoid having to prepare this solution immediately prior to use, this stock solution was acidified with one drop of concentrated HCl per litre of mineral media stock solution.

HSE notes that the applicant does not report the exact nature of the materials used in making stock solutions for the test (e.g purity, supplier). HSE does not consider this a major deviation as GLP is assured, and they are stated to come from commercial sources and to be of reagent grade.

## II. Inoculum

The activated sludge used for this study was obtained from the Wareham Wastewater Treatment Plant, Wareham, Massachusetts, which receives primarily domestic waste. Approximately 4 L of activated sludge was collected on 9 February 2016 (Same day as testing start date) and transported to Smithers Viscient. HSE accepts this inoculum as suitable for the testing of biodegradation.

Upon arrival at Smithers Viscient, the sludge was passed through a 2-mm sieve and centrifuged at 1000 rpm for ten minutes. The supernatant was discarded, the sludge was washed with mineral medium (SMV No. 07Feb16L37-E) and the contents were centrifuged again and the supernatant was discarded again. The moisture content of the activated sludge was determined to be 95.99 %.

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An inoculum solution with 15 mg suspended solids/mL was prepared (74.81 g wet weight sludge brought to 200 mL with mineral medium), stirred with a Teflon® magnetic stir bar at  $20 \pm 2$  °C and aerated until used. The test substance flasks, the blank flasks, the procedural control flask, and the toxicity control flasks all received 6.0 mL of the inoculum to produce an activated sludge concentration of 30 mg solids/L.

In addition, a 50-g aliquot of fresh soil (Station Street, Wareham, MA), a 25-g aliquot of Taunton River sediment, and a 25-g aliquot of Weweantic River sediment were collected near Smithers Viscient. The soil and sediments were suspended in 1 L of Weweantic River water. The suspension was filtered through glass wool and stored, refrigerated, prior to use. This soil/sediment inoculum was determined to contain 0 % solids. A 3.0 mL aliquot of the soil/sediment filtrate was added to each test vessel containing 2991 mL of mineral medium and 6.0 mL of activated sludge. The preparation is summarised in Table 8.2.2.1-03 below.

**Table B.8.2.2.1-03 Test solution composition after dosing on day 0 for the inpyrfluxam biodegradation study**

Test Vessel	Mineral Medium	Sludge Inoculum <sup>a</sup>	Soil / Sediment Filtrate	Inpyrfluxam <sup>b</sup>	Sodium Benzoate <sup>c</sup>	ThTOC <sup>d</sup>	ThCO <sub>2</sub>
Units	(mL)	(mL)	(mL)	(g)	(mL)	(mg C/L)	Mg CO <sub>2</sub> / L
Inoculum Blank Replicate A	2991	6.0	3.0	NA <sup>e</sup>	NA	0	0
Inoculum Blank Replicate B	2991	6.0	3.0	NA	NA	0	0
Test Suspension Replicate A	2991	6.0	3.0	0.0457	NA	10	36.67
Test Suspension Replicate B	2991	6.0	3.0	0.0457	NA	10	36.67
Procedural Control	2991	6.0	3.0	NA	3.0	10	36.67
Toxicity Control	2991	6.0	3.0	0.0457	3.0	20	73.34
pH Test Vessel	2991	6.0	3.0	0.0457	NA	10	36.67

<sup>a</sup> On day -1, 6.0 mL sludge inoculum (15 mg suspended solids/L solution) were added to mineral medium. Test vessels were attached to a CO<sub>2</sub>-free compressed air gas cylinder and aerated under positive pressure for 24 hours to remove any residual inorganic carbon in the test system prior to test initiation.

<sup>b</sup> Inpyrfluxam carbon content equals 65.71%, determined by TOC analysis

<sup>c</sup> Sodium benzoate stock was prepared at a concentration of 10 mg C/mL.

<sup>d</sup> Theoretical total organic carbon (ThTOC) from test or reference substance

<sup>e</sup> NA = Not Applicable

### III. Test system and conditions

Each test unit of a 4-L glass bottle with a rubber stopper into which one stainless steel needle with a Luer-Lok connection and two pieces of glass tubing were inserted. Prior to test initiation, the test vessels were acid washed and rinsed repeatedly with reagent grade water. The stainless steel needle was extended through the stopper into the test solution serving as a sampling port for solution samples. A rubber cap was used to cover the top of the sample port. The glass tubing provided the inlet and outlet ports for air exchange. CO<sub>2</sub>-free air was pumped

under positive pressure through a hydration flask before entering the test system. The outlet port of each system was connected to two CO<sub>2</sub> effluent gas traps, the first consisting of 200 mL of 0.2 M potassium hydroxide (KOH) and the second trap containing 100 mL of 0.2 M KOH. Each test vessel was placed on a magnetic stir plate located in a dark environmental chamber set to maintain a temperature of  $22 \pm 2$  °C.

On day -1, 2991 mL mineral medium (Table B.8.2.2.1-02). A 6.0-mL aliquot of the activated sludge inoculum and a 3.0-mL aliquot of the soil/sediment filtrate were added to each vessel for a total volume of 3000 mL per vessel. The pH of the test medium was adjusted to 7.40 on day -1 by the addition of 1.0 M sodium hydroxide (NaOH). The six test vessels were attached to a CO<sub>2</sub>-free compressed air gas tank and aerated under positive pressure. The vessels were mixed and purged with CO<sub>2</sub>-free air until day 0 to remove any residual inorganic carbon in the test system prior to test initiation. At test initiation, the test substance (replicates A and B), toxicity control vessel and pH test vessels were each dosed with 0.0457 g of inpyrfluxam. The total nominal fortification was 10 mg C/L in the test substance vessels. The inoculum blank control vessels only received inoculum and mineral medium. The toxicity control vessel also received 3.0 mL of the 10 mg C/mL sodium benzoate stock solution for a total fortification of 20 mg C/L (test and reference substance). The sodium benzoate procedural control was fortified with 3.0 mL of the sodium benzoate stock solution for a final concentration of 10 mg C/L. The pH test vessel was prepared in a similar manner to the test substance vessels. A summary of the dosing procedure is presented in Table B.8.2.2.1-03.

Due to the low solubility of the test substance, DOC was not measured for those samples containing test substance during this study with the exception of the pH Check/TIC sample.

The temperature of the environmental chamber was recorded daily throughout the exposure period using a digital minimum-maximum thermometer (VWR). On test days 1, 3, 5, 7, 10, 14, 17, 21, and 24 a 7-mL sample was removed from the first KOH carbon dioxide trap on each test system and analysed for CO<sub>2</sub> evolution. On day 28, 1 mL of concentrated hydrochloric acid (HCl) was added to each test vessel following pH measurements to terminate biological activity. Aeration was continued overnight to drive any residual inorganic carbon from the test vessels. After overnight aeration, 7-mL samples for analysis of CO<sub>2</sub> evolution were removed from the first and second traps. The amount of evolved CO<sub>2</sub> in each trap was determined using a Shimadzu TOC V-CPH Carbon Analyzer.

#### **IV. Analytical methods**

The amount of carbon dioxide produced by each test system was adjusted by subtracting the CO<sub>2</sub> produced by the blank control. The percent biodegradability for

each test system was calculated using the following equation and is expressed as cumulative percent biodegradation (or percent of theoretical CO<sub>2</sub> production).

$$\% \text{ Ultimate Biodegradability} = \frac{\text{mg CO}_2 \text{ produced}}{\text{mg TOC added} \times 3.667} \times 100$$

where: 3.667 is the molecular weight conversion factor for carbon to carbon dioxide.

#### **Figure B.8.2.2.1-01 Equation showing percentage biodegradation calculation**

HSE is satisfied with the equation used, as it is provided in the OECD 301B guidelines.

## **RESULTS**

### **I. Test conditions**

The applicant states temperature was maintained 22 ± 2 °C, with the exception of a period of less than 24 hours during which the temperature reached a maximum of 28.5 °C on the 19<sup>th</sup> of February 2016 (day 10). The applicant has not provided a temperature log.

HSE notes that this increase in temperature is a deviation from OECD 301 guidelines. An increase of temperature to 28°C would not be expected to kill the microbes in the inoculum and decrease their activity and therefore decrease the degradation rates of the compounds. It is more likely that the temperature increase has increased the microbial activity and therefore increased the degradation rate of the compounds. This assessment is supported by a peak visible in the net cumulative percent CO<sub>2</sub>, for the Test Suspension Mean at day 10, in Figure B.8.2.2.1-02.

As Inpyrfluxam has been found to not readily biodegrade, even with this increase in temperature, repeating this study without the brief increase in temperature would be expected again to yield the same conclusion that inpyrfluxam is not readily biodegradable. This deviation from OECD 301 guidelines is therefore not considered to have impacted study validity.

The pH values measured between 7.20-7.35 in the different vessels at the end of incubation are within the range of pH 6.0-8.5 recommended by the OECD Guideline 301.

**Table B.8.2.2.1-04 pH measurements taken at termination (day 28) of the biodegradation study with inpyrfluxam**

Test Vessel	Replicate	pH at day 28
Test Substance	A	7.30
	B	7.32
Inoculum Blank	A	7.20
	B	7.20
Procedural Control	A	7.34
Toxicity Control	A	7.35

**II. CO<sub>2</sub> evolution**

The total inorganic carbon measured in the KOH traps (Table B.8.2.2.1-05) was used to calculate the cumulative CO<sub>2</sub> evolved from the test vessels (Table B.8.2.2.1-06). The cumulative net percent CO<sub>2</sub> production (blank control values subtracted) are presented in Table B.8.2.2.1-07, and as a graph in figure B.8.2.2.1-02.

**Table B.8.2.2.1-05 Total inorganic carbon (mg C/L) measured in KOH traps during the 28-day biodegradation study with inpyrfluxam**

	Replicate	A	B	A	B		
	Vessel	Inpyrfluxam	Inpyrfluxam	Inoculum Blank	Inoculum Blank	Procedural Control	Toxicity Control
Day		Total inorganic carbon (mg C / L)					
1		21.07	18.59	15.03	13.28	19.33	27.56
3		37.99	35.37	30.07	31.59	107.2	108.1
5		46.17	40.82	40.75	39	133.6	134.4
7		53.61	50.8	51.86	47.1	155.4	157.8
10		64.7	62.83	65.81	51.65	176.4	178.5
14		73.11	72.64	73.16	75.33	202.5	196.8
17		79.85	80.14	84.42	85.63	217.8	210.8
21		89.5	89.39	96.96	94.07	232.9	223.9
24		93.93	94.32	102.9	98.89	239.8	231.8
28	Trap 1	108.3	107.5	118.8	110.4	242.0	245.5
28	Trap 2	26.89	25.12	25.03	24.09	40.35	32.26

**Table B.8.2.2.1-06 Cumulative CO<sub>2</sub> (mg/L) evolved from the inpyrfluxam test vessels during the biodegradation study**

	Replicate	A	B			A	B				
	Vessel	Inpyrfluxam	Inpyrfluxam	Mean	Std. Dev.	Inoculum Blank	Inoculum Blank	Mean	Std. Dev.	Procedural Control	Toxicity Control
day <sup>a,b</sup>		CO <sub>2</sub> (mg / L)									
1		5.15	4.54	4.85	0.43	3.67	3.25	3.46	0.3	4.73	6.74
3		8.96	8.34	8.65	0.44	7.09	7.45	7.27	0.25	25.29	25.5
5		10.5	9.28	9.89	0.86	9.26	8.87	9.07	0.28	30.37	30.56
7		11.73	11.11	11.42	0.43	11.35	10.31	10.83	0.74	34	34.53
10		13.6	13.21	13.41	0.28	13.84	10.86	12.35	2.11	37.09	37.53
14		14.75	14.65	14.7	0.07	14.76	15.19	14.97	0.31	40.84	39.69
17		15.42	15.48	15.45	0.04	16.3	16.54	16.42	0.17	42.06	40.71
21		16.52	16.5	16.51	0.01	17.9	17.36	17.63	0.38	42.99	41.33
24		16.53	16.6	16.57	0.05	18.11	17.41	17.76	0.5	42.21	40.8
28		21.42	21.07	21.25	0.25	22.95	21.43	22.19	1.08	45.46	45.05

<sup>a</sup> Corrected for removal of 7-mL KOH sample from each trap at each sampling interval.

<sup>b</sup> The rounded values presented in this table were calculated based on unrounded experimental results.

Note:

CO<sub>2</sub> mg/L = (TIC value 1st trap \* 3.667) \* ((Vol. remaining in trap 1 / Vol. originally in trap 1) / (Vol. of Test Medium / Vol. KOH trap originally)) + (TIC value 2nd trap \* 3.667) \* ((Vol. remaining in trap 2 / Vol. originally in trap 2) / (Vol. of Test Medium / Vol. KOH trap originally))

Example Calculation for inpyrfluxam, Replicate A (Day 28):

$((108.3 \text{ mg/L} * 3.667) * ((137 \text{ mL} / 200 \text{ mL}) / (3000 \text{ mL} / 200 \text{ mL}))) + ((26.89 \text{ mg/L} * 3.667) * ((100 \text{ mL} / 100 \text{ mL}) / (3000 \text{ mL} / 100 \text{ mL}))) = 21.42 \text{ mg/L}$

Note: 3.667 is the molecular weight conversion factor for carbon to carbon dioxide.

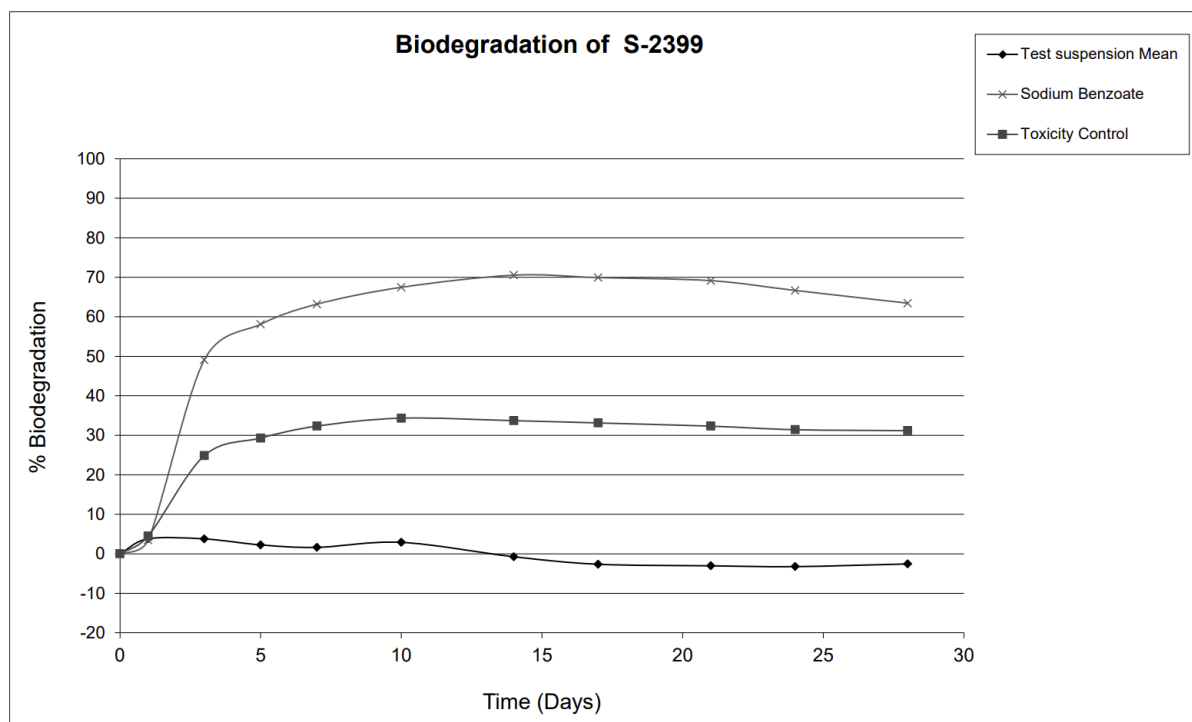
HSE is satisfied that the applicant has correctly accounted for reduced sample volumes.

**Table B.8.2.2.1-07 Cumulative net percent CO<sub>2</sub> evolved (ultimate biodegradation) during the 28-day biodegradation study with inpyrfluxam**

	Replicate	A	B				
	Test Substance	Inpyrfluxam	Inpyrfluxam	Mean	Std. Dev.	Procedural Control	Toxicity Control
Day <sup>a</sup>		Net % CO <sub>2</sub> of theoretical					
1		4.61	2.96	3.78	1.17	3.45	4.47
3		4.61	2.92	3.76	1.19	49.13	24.86
5		3.9	0.59	2.24	2.35	58.11	29.3
7		2.46	0.79	1.63	1.19	63.2	32.32
10		3.42	2.35	2.89	0.76	67.46	34.33
14		-0.62	-0.88	-0.75	0.18	70.54	33.7
17		-2.73	-2.57	-2.65	0.11	69.93	33.12
21		-3.03	-3.08	-3.06	0.04	69.15	32.31
24		-3.34	-3.16	-3.25	0.13	66.67	31.42
28		-2.1	-3.06	-2.58	0.68	63.44	31.17

<sup>a</sup> The rounded values presented in this table were calculated based on unrounded experimental results. All values were corrected for mean inoculum blank CO<sub>2</sub> production.





**Figure B.8.2.2.1-02 Cumulative net percent CO<sub>2</sub> evolved from the test vessels during the 28-day study with inpyrfluxam**

### III. Controls and validity criteria

The reference compound reached the level of  $\geq 60$  % degradation within 14 days, with 70.54 % degraded at 14 d. The OECD 301 B pass threshold of  $>60$  % degradation was met by day 7 of incubation, with 63.20 % degraded.

The toxicity control exhibited degradation rates above the OECD 301 B lower limit of 25 % within 14 days, indicating that the test item is not inhibitory to inoculum activity. At 14 days the toxicity control showed 33.70 % degradation. The threshold was met between 3 d (24.86 % degradation) and 5 d (29.30 %) of incubation.

At the end of the test, the replicates of the test item did not differ by more than the OECD 301 B limit of 20 %. The replicates showed 21.42 and 21.07 mg/L CO<sub>2</sub> produced at 28 days. This is a difference of 0.35 mg CO<sub>2</sub> /L; which HSE calculates to be 1.66 % of 21.07 mg CO<sub>2</sub> /L, therefore  $<20$  % difference.

HSE therefore accepts that all validity criteria of the test method were met.

## CONCLUSION

Based on the extent of CO<sub>2</sub> evolution during this study, inpyrfluxam cannot be classified as readily biodegradable by the criteria set forth in OECD Guideline 301 B since it did not achieve 60 % CO<sub>2</sub> evolution within a 10-day window by day 28.

The reference compound degradation demonstrated the activity of the inoculum and therefore confirmed the validity of the study, and the toxicity control demonstrated that inpyrfluxam does not cause the inoculum activity to cross the OECD 301 B inhibitory threshold.

#### B.8.2.2.2. Aerobic mineralisation in surface water

<b>Data Point:</b>	KCA 7.2.2.2/01
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2017
<b>Report Title:</b>	[ <sup>14</sup> C] S-2399: Aerobic Mineralisation in Surface Water
<b>Document No, Authority registration No:</b>	TPM-0048, 3201629
<b>Substance used:</b>	[Pyrazolyl-4- <sup>14</sup> C] inpyrfluxam (Batch No. CFQ41802; Radiochemical Purity: ≥ 98%); [phenyl- <sup>14</sup> C] inpyrfluxam (Batch No. CFQ41803; Radiochemical Purity: ≥ 98%)
<b>Guideline(s):</b>	OECD Guideline 309 (Aerobic Mineralisation in Surface Water, April 2004)
<b>GLP or GEP:</b>	Yes
<b>Deviations</b>	Yes (see below)
<b>Acceptability:</b>	<p>HSE deemed the study <b>acceptable</b>, providing the applicant will provide the following additional information:</p> <ul style="list-style-type: none"> <li>• Further information on how LOD was detected for HPLC</li> <li>• Limit of Quantification (LOQ) for HPLC</li> <li>• Limit of detection (LOD) and LOQ for chiral HPLC</li> </ul> <p>This information has been provided by the applicant in an RAI.</p>
<b>Study relied upon:</b>	Yes

<b>Deviations</b>	<b>HSE assessment of deviations</b>
Sterile controls only treated with [phenyl- <sup>14</sup> C] inpyrfluxam and not [pyrazolyl- <sup>14</sup> C] inpyrfluxam.	Minor deviation. Position of radiolabel is not thought to affect the extent of degradation.
Three reference items for potential metabolites were not used by the testing facility in co-chromatography.	Minor deviation. No metabolites ≥ 5% AR were detected in the test, therefore HSE accepts there is no requirement to characterise and identify these.
Transport containers are not noted as having been “thoroughly cleansed” prior to use.	Minor deviation. Thorough cleansing prior to use assumed by HSE
Applicant has not stated that the history of agricultural, industrial, and domestic inputs into water body were considered when selecting the sampling site, as is required by OECD 309	Minor deviation. HSE considers that the choice of sampling site, a rural area, is suitable, and that while not explicitly stating that they have considered the input history, the applicant has likely done so.
Test not stated clearly as pelagic or suspended sediment.	Enough information provided in order to proceed with study validation as “pelagic”
Highest used concentration slightly exceeds guideline recommendation, by 0.003 mg/L (3 % above guideline).	Minor deviation, which does not affect study validity
Test vessels were treated six days after the surface water was collected as opposed to guideline of one day.	Minor deviation. The system was shown to still be microbially active during the study.
Each vessel was filled over the one third of the flask volume recommended by OECD guidelines.	Minor deviation. Study was performed with a flow through apparatus adding new air.
The applicant did not extend the study past 61 days despite minimum 20% degradation not being met.	Minor deviation. Minimum degradation was seen in microbially active treated flasks compared to sterile flasks by study end (<10%).

	Therefore, further degradation to 20% would have been unlikely.
Kinetic evaluation data is presented even though prerequisite >20 % active substance degradation not reached.	Minor deviation, with no effect on study validity.

## INTRODUCTION

The applicant determined the mineralisation and degradation rate of inpyrfluxam in natural surface water under dark conditions in a laboratory according to OECD 309 guidelines. The pelagic test system was chosen for the present study and was performed with two concentrations: 10 µg/L (“low” concentration) and 100 µg/L (“high” concentration). The test vessels were attached to a flow-through system for continuous aeration.

Natural water viability was monitored by oxygen content and pH readings, as well as a labelled degradable reference substance, sodium [<sup>14</sup>C]benzoate.

Two radiolabel positions were studied: [phenyl-<sup>14</sup>C] inpyrfluxam and [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam. The applicant also investigated the enantiomeric ratio during the study.

The radioactivity in the water was determined by LSC and HPLC. Parent substance was identified by co-chromatography with the corresponding reference substance using HPLC and TLC.

Kinetic evaluation was conducted using SFO, however the data was not fitted to the biphasic models (FOMC, DFOP or HS) as there was insufficient degradation for kinetic model comparisons to be made.

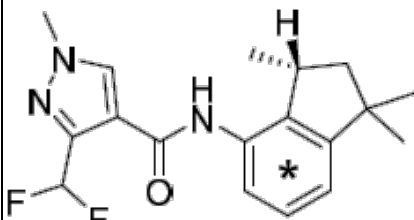
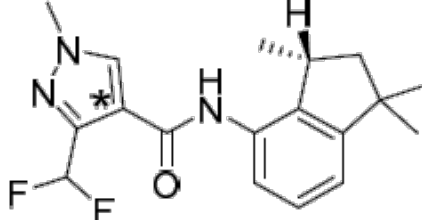
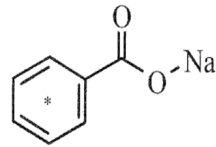
## MATERIAL AND METHODS

### V. Test materials and reference items

The applicant studied the mineralisation of inpyrfluxam in natural surface water using two labelled forms; [phenyl-<sup>14</sup>C] inpyrfluxam (PH-label) and [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PY-label). HSE notes that both ring structures on either side of the likely cleavage point, the bridging amide, have been labelled. Therefore the study represents a suitable source of information on degradation of inpyrfluxam and formation of potential transformation products and is deemed acceptable by HSE.

The details for the test materials are summarised below. In addition, the control item, radiolabelled benzoic acid [ring-<sup>14</sup>C(U)] was used as a reference item to establish that the microbial population was sufficient for the test item to degrade in the surface water.

**Table B.8.2.2-01  $^{14}\text{C}$ -labelled test materials for the study of the aerobic mineralisation of inpyrfluxam**

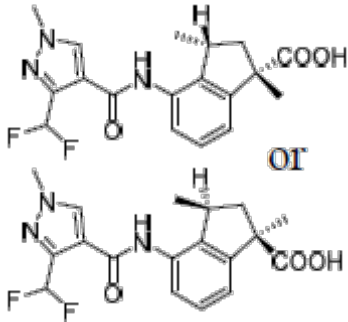
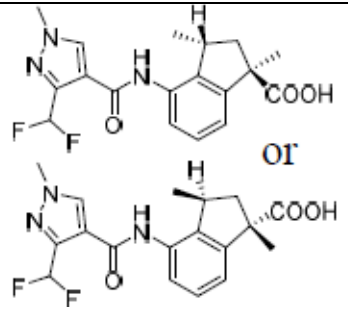
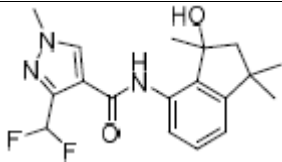
<b>Test Material:</b>	[phenyl- $^{14}\text{C}$ ] inpyrfluxam (PH-label)	[pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam (PY-label)
<b>Lot/Batch:</b>	CFQ41803	CFQ41802
<b>Purity:</b>	Radiochemical purity $\geq 98\%$	Radiochemical purity $\geq 98\%$
<b>CAS number:</b>	Not assigned	Not assigned
<b>Stability of compound:</b>	Stable	Stable
<b>Molecular weight</b>	337.3 (radiolabelled)	335.2 (radiolabelled)
<b>Specific radioactivity of a.s. (MBq mg<math>^{-1}</math>)</b>	13.37 MBq/mg	6.29 MBq/mg
<b>Position of label</b> *denotes position of radiolabel		
<b>Reference substance:</b>	Benzoic Acid, [ring- $^{14}\text{C}$ (U)] sodium salt (sodium [ $^{14}\text{C}$ ] benzoate)	
<b>Lot/Batch:</b>	160811	
<b>Specific activity:</b>	2.22 GBq/mmol	
<b>Purity:</b>	99 %	
<b>CAS#:</b>	Not assigned	
<b>Stability of compound:</b>	Not provided	
<b>Position of label</b> *denotes position of radiolabel		

While chemical and radiochemical purities for [Phenyl- $^{14}\text{C}$ ] inpyrfluxam and [Pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam were stated to be > 98% by the applicant, the radiochemical purities were reassessed by the test facility and found to be 94% and 91% respectively. Both test substances were repurified by HPLC method 1, after which their purities were found to be  $\geq 98\%$  in both labels, by HPLC. This is acceptable to HSE and in line with the OECD 309 guidelines, which state that the radiochemical purity of the test substance should be >95%.

The specific activity values of the compounds were not re-determined at the testing facility, but the values provided by the applicant (see table B.8.2.2.2-01 above) were used.

The applicant supplied an unlabelled inpyrfluxam reference item and five unlabelled metabolites as reference items with sufficient chemical purities (97.7 – 100%).

**Table B.8.2.2.2-02 Unlabelled metabolite reference item details for characterisation of any degradation products (if  $\geq 5\%\text{AR}$ )**

Chemical name	Purity	Date received	Expiration date a	Structure
1'-COOH-S-2840A	100%	8 Dec 2015	3 Feb 2018	
1'-COOH-S-2840B	99.6%	8 Dec 2015	4 Feb 2018	
3'-OH-S-2840	97.8%	8 Dec 2015	13 Sep 2017	

DFPA	99.2%	1 Apr 2016	6 Jan 2017	
ATMI	99.7%	5 Aug 2016	5 Oct 2018	

HSE notes that the applicant also supplied three further reference items for potential metabolites, but these were not used by the testing facility in the co-chromatography. However, since no metabolites of significance ( $\geq 5\%$  applied radioactivity [AR]) were detected in the test, HSE accepts that there was no requirement to characterise and identify these, nor any degradation products against the reference standards.

## VI. Test system

Natural water was collected from The Lake at Studley Royal, Ripon UK and the test system was named 'Fountains Abbey' on 17<sup>th</sup> August, 2016. The water was transported in a car boot at ambient temperatures. Transport duration was less than 1 hour. Prior to use the water was stored in the dark in an environmental chamber routinely maintained at  $4 \pm 2^\circ\text{C}$ , with free access to air. Water was 100  $\mu\text{m}$  sieved prior to use and subsequent characterisation. The water characteristics are summarised in Table B.8.2.2.2-03. The water was dispensed into test vessels on the same day as it was collected, 17<sup>th</sup> August 2016, apart from the sterile group which was dispensed on the 18<sup>th</sup> of August 2016. HSE considers the natural water tested to be appropriate for this study.

HSE notes that the "thoroughly cleansed" condition of the transport container is not noted, as is required in OECD 309 guidelines. HSE views this as a minor deviation, and HSE assumes the containers to be thoroughly cleansed.

**Table B.8.2.2.2-03 Characteristics of the tested natural water**

Water characteristic	Fountains Abbey water
Sampling water temperature	19.1°C
Sampling water oxygen content	10.14 mg/L
Sampling water pH	8.6
Water depth sampled	0-10 cm
Water depth above sediment	30 cm
Water appearance	Clear with slight yellow tinge
Total Organic Carbon (TOC) (mg/L)	0.13
Dissolved Organic Carbon (DOC) (mg/L)	0.00

pH	8.0
Suspended Solids (mg/L)	3
Electrical Conductivity (µS/cm)	285

HSE notes that the applicant has not demonstrated a consideration of the possible historical agricultural, domestic, or industrial inputs to the site, as is desired by OECD 309. However, HSE considers that the choice of sampling site, a rural area, is suitable, and that while not explicitly stating that they have considered the input history, the applicant has likely done so.

HSE notes that the type of study as a 'pelagic' or 'suspended sediment' test is not clearly stated in the report, as is recommended by OECD 309 guidelines. The concentration of suspended solids is below the OECD 309 suspended sediment test level ( $\leq 10$  mg/L) and has therefore been reviewed as pelagic.

## STUDY DESIGN AND METHODS

### I. Experimental Conditions

Water (100 mL) was dispensed into test vessels (250 mL, ca 6 cm diameter amber glass reagent bottles). HSE notes that "up to about a third of the flask volume" is recommended by OECD 309 guidelines to provide sufficient headspace of air to maintain aerobic conditions in a closed system. As this is a flow through system, providing fresh air, 100 mL of water in 250 mL vessels is not considered by HSE as a deviation from OECD 309 guidelines.

The sodium [ $^{14}\text{C}$ ] benzoate reference control sample was prepared by dropwise addition of an application solution into water. This application solution was prepared by dissolving 250 µL of 0.24 mg/mL sodium [ $^{14}\text{C}$ ] benzoate in reverse osmosis (RO) water (6 mL). This was designated as incubation group F.

The sodium [ $^{14}\text{C}$ ] benzoate solvent control sample was prepared in the same way, followed by addition of acetonitrile (29 µL) to assess any impact of the acetonitrile application solvent on the test system. This was designated as incubation group G.

This preparation is summarised in Table B.8.2.2.2-04 below.



**Table B.8.2.2.2-04 Application of test substance**

<b>Incubation Group</b>	<b>Application Solution</b>	<b>Coefficient of variation for concentration</b>	<b>Volume of solution applied (μL)</b>	<b>Mass of test substance applied (μg)</b>	<b>Achieved test substance concentration (mg/L)</b>
A	5(phenyl, low)	0.65	14	1.01	0.0101
B	4(phenyl, high)	1.52	29	10.3	0.103
C	2(pyrazolyl, low)	1.07	20	1.02	0.0102
D	1(pyrazolyl, high)	1.11	11	10.3	0.103
E(sterile)	4(phenyl, high)	1.52	29	10.3	0.103
F	3(benzoate)	0.93	950	10.3	0.103
G	3(benzoate)	0.93	950	10.3	0.103

The mineralisation and degradation rate of inpyrfluxam in a natural surface water were performed using two radiolabels, [phenyl-<sup>14</sup>C] inpyrfluxam (PH-label) and [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PY-label), at 20 ± 2 °C and in the dark for a maximum of 61 days. The test vessels were attached to a flow-through system for continuous aeration and agitated by continuous stirring with a PTFE coated magnetic stir bar. Two concentrations were used for each label. The concentrations of the PY-label were 0.103 mg/L and 0.0102 mg/L, and the concentrations for the PH-label were 0.103 mg/L and 0.0101 mg/L.

OECD 309 guidelines state that the concentration of the highest dosing test substance should not exceed 0.100 mg/L, and the concentration of the lowest dosing test substance should not exceed 0.010 mg/L. HSE notes that the low-concentration and high-concentration values of both labelled substances exceeds those recommended in the OECD 309 guidelines. This can lead to deviations from first-order kinetics. However, the concentrations used barely exceed the recommendations. Furthermore, no significant degradation occurred for either of the concentrations, suggesting that degradation rates are not highly concentration dependant within the timeframe of this study. HSE therefore does not consider this deviation to impact study validity.

The test systems were equipped with 2 M NaOH traps for the collection of evolved <sup>14</sup>CO<sub>2</sub> and ethanediol (ethylene glycol) traps for volatile organic <sup>14</sup>C capture. Sterile samples were tested at the higher concentration (0.103 mg/L).

A subsample of the water for testing was autoclaved twice (121°C, 15 minutes) for use in the sterile vessels. Test vessels including tubing and magnetic stirring bars were similarly sterilised.

Untreated blank controls were used to measure oxygen content and pH. Acetonitrile solvent controls and reference controls using sodium [<sup>14</sup>C] benzoate (0.103 mg/mL) were used to demonstrate that the microbial population was viable in the test system and not affected by acetonitrile at the applied concentration.

It is noted that the sterile controls were only treated with [phenyl-<sup>14</sup>C] inpyrfluxam. However, given that the position of the radiolabel is not thought to affect the extent of degradation, and no degradation of inpyrfluxam could be demonstrated (DT-50 values  $\geq 1.54\text{E}+03$  days) under both sterile and non-sterile conditions, HSE does not deem this to be significant when estimating the extent of biodegradation.

All vessels were treated with their respective substance on 23<sup>rd</sup> August 2016. HSE notes that the vessels were treated six days after the surface water was collected (on 17 August 2016), as opposed to the OECD 309 recommended guideline of one day. However, given that the system was shown to still be microbially active (see “Results” section below), HSE is of the opinion that this did not affect the viability of the study.

## **II. Sampling intervals & procedure**

Duplicate samples were taken at 0, 3, 7, 14, 21, 30 and 61 days after treatment (DAT), as were oxygen and pH measurements of blank samples. Duplicate sterilised water samples were taken at 0, 14, 30 and 61 days after treatment (DAT). The trap solutions were analysed at the same points.

HSE notes that the recommended minimum 20% degradation stated in the OECD guidelines was not met. Despite this, the applicant did not extend the study past 61 days. However, as degradation of the test substance in sterile flasks compared degradation to in microbially active treated flasks by the end of the study was <10%, it can be concluded that biological degradation is not a major pathway of degradation for inpyrfluxam. HSE therefore considers the deviation acceptable and not to impact the outcomes of the study as it is unlikely the test substance would have degraded to 20% before the die off of the microbial population.

At 78 DAT spare test vessels from test and sterile groups had all the trap solutions removed and the 2M NaOH traps replenished. The remaining test vessels were acidified (0.1 mL of concentrated HCl) and returned to the incubation system.

Radioactivity was determined in vessels from reference and solvent control samples (groups F and G) at 78 DAT.

Traps were collected from the vessels treated with [ $^{14}\text{C}$ ] inpyrfluxam at the time of their removal from the incubation system. For vessels incubated for the duration of the test, traps were also collected and replenished at 30 and 61 DAT. At 78 DAT, traps were collected and replenished (NaOH only) prior to acidification of the samples and traps were sampled again at 79 DAT.

Traps associated with vessels from groups F and G (reference and solvent control samples) were collected and replenished at 3, 7, 14, 21, 30 and 61 DAT and were collected only at 78 DAT.

All vessels were removed from the incubation system at 79 DAT.

### III. Description of analytical procedures

Dissolved oxygen and pH were measured in the blank controls at each sampling point.

The water from each vessel and the water used for rinsing the vessel were analysed for radioactive content by LSC and were analysed for test substance and metabolites by HPLC. Then, the vessel was rinsed with acetonitrile, sonicated and the solution analysed by LSC.

The  $^{14}\text{CO}_2$  collected in the NaOH trapping solution and organic volatiles collected in ethanediol traps were counted by LSC.

The potential isomerisation from [ $^{14}\text{C}$ ] inpyrfluxam was evaluated using chiral HPLC.

LOD and LOQ for LSC were set at 1.5 times the background radioactivity.

Background radioactivity was determined by performing LSC on non-radioactive material at the start and end of each sample batch. This has been clarified as typically  $\leq 0.6\%$  AR by the applicant in an RAI.

An LOD of ca.  $0.5\%$  AR was determined for HPLC by identifying the smallest peaks present in the chromatogram

HSE notes that an LOQ for HPLC-RAM had not been provided by the applicant, nor LOD or LOQ for the chiral chromatogram technique (Annex B). This was of concern as they appeared to have a high signal-to-noise ratio. HSE requested clarification these values, and notes that this deviation has been addressed by the applicant in an RAI.

An LOD and LOQ of  $0.5 - 1.0\%$  AR was reported for HPLC-RAM, and  $< 3\%$  LOD and LOQ for chiral HPLC. The applicant has regarded the LOD and LOQ values as equivalent in the context of radioactivity analysis. HSE accepts that the limits for HPLC-RAM are acceptable and in line with OECD 309. For chiral HPLC, the values exceed the acceptable limits of  $1\%$  of the initial amount applied. However this is a minor deviation not expected to affect the acceptability of the study, as chiral analysis showed that inpyrfluxam was the only isomer present throughout the study and the LOD AR % lies below minimum inpyrfluxam AR %.

LOD and LOQ values for TLC are not provided as it was used for qualitative analysis only. HSE considers this reasonable.

## RESULTS

### I. Test system parameters

During incubation, oxygen levels in blank controls were > 8 mg/L at all sampling times, showing that the water samples maintained aerobic conditions. Recorded pH values were in the range 8.1 to 8.5, close to the value of 8.6 recorded at the sampling of the water source. These values are shown in 8.2.2.2/1-05 below.

**Table B.8.2.2.2-05 pH values and oxygen concentrations in lake water obtained from blank control units**

Date(Timepoint)	Blank control 1		Blank control 2	
	Oxygen (mg/L)	pH	Oxygen (mg/L)	pH
23 August 2016 (0DAT)	8.9	8.3	8.8	8.3
26 August 2016 (3DAT)	8.9	8.5	9	8.5
30 August 2016 (7DAT)	9.4	8.4	9.4	8.5
6 September 2016 (14DAT)	9.4	8.5	9.4	8.5
13 September 2016 (21DAT)	9.2	8.1	9.4	8.3
22 September 2016 (30DAT)	9.2	8.4	9.3	8.4
23 October 2016 (61DAT)	9.7	8.4	9.6	8.4

### II. Mass balance

The actual applied amounts of test item [ $^{14}\text{C}$ ] inpyrfluxam were 0.0101 mg/L (phenyl label) or 0.0102 mg/L (pyrazolyl label) for the low application rate and 0.103 mg/L for both labels at the high application rate. The treatment rate for the sodium [ $^{14}\text{C}$ ] benzoate controls was 0.103 mg/L.

Mass balances are presented in Table B.8.2.2.2-06 to 10. In summary, these ranged 90.1 – 100.4% AR (mean values) in the viable test vessels, and 92.8 – 97.0% AR in the sterile vessels (mean values), with the exception of one sample at 61 DAT for the low concentration [pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam. This was found to have a mass balance <90% AR (78.2% AR) and was not included in any further calculations. HSE accepts the applicant's removal of this sample from calculation of the mean mass balance and degradation kinetics, and agrees that the single exception C-61 DAT is anomalous and not statistically significant. This is supported in Table 8.2.2.2-08

which shows that the low mean average is due to a single vessel being outside the 90-110 % range. HSE therefore classifies the mass balances for inpyrfluxam as acceptable by OECD 309 guidelines.

### III. Data

Most of the radioactivity ( $\geq 88$  % AR) was recovered in solution; minimal amounts ( $\leq 2$  % AR) were recovered from vessel walls, and the total volatile radioactivity was  $\leq 0.4$  % AR.

At the lower application rate (0.01 mg/L), inpyrfluxam accounted for 91.2 % - 94.1 % applied radioactivity (AR) immediately after application and 92.1 % - 96.5 % AR at 61 days after treatment (DAT). At the higher application rate (0.1 mg/L), inpyrfluxam accounted for 89.7 % - 92.4 % AR immediately after treatment and 90.4 % - 90.6 % AR at 61 DAT. In sterile samples, mean inpyrfluxam levels at 0 and 61 DAT were 91.3 % AR and 94.2 % AR, respectively.

The applicant provided data for the sterile control. There was detectable mineralisation ( $\leq 0.4$  % AR) in some of the non-sterile vessels but no detectable mineralisation from the sterilised incubation group. Ethanediol traps did not contain any detectable radioactivity. No additional recovery of radioactivity in traps was obtained after acidification at 79 DAT and therefore there was no detectable dissolved carbon dioxide present in the natural water at this time.

**Table B.8.2.2-06 Percent recovery of applied radioactivity recovered from natural water treated with [phenyl- $^{14}\text{C}$ ] inpyrfluxam low concentration (0.01 mg/L)**

Vessel Code	Sampling Interval (days)	Surface Water (Extract 1)	Vessel wash (Extract 2)	Lid wash from Extract 1	Volatiles	Mass Balance
A1	0	90.3	0.4	1.1	NA	93.7
A2		92.3	0.4	NA	NA	92.7
<b>Mean</b>		<b>91.2</b>	<b>0.6</b>	<b>0.6</b>	<b>NA</b>	<b>93.2</b>
A3	3	95.8	0.3	NA	ND	96.1
A4		95.9	0.4	NA	ND	96.3
<b>Mean</b>		<b>95.9</b>	<b>0.4</b>	<b>NA</b>	<b>ND</b>	<b>96.2</b>
A5	7	93	0.3	NA	ND	93.3
A6		93.1	0.6	NA	ND	93.7
<b>Mean</b>		<b>93.1</b>	<b>0.5</b>	<b>NA</b>	<b>ND</b>	<b>93.5</b>
A7	14	94.2	1.2	NA	ND	95.4
A8		91.8	2.3	NA	ND	94.1
<b>Mean</b>		<b>93</b>	<b>1.8</b>	<b>NA</b>	<b>ND</b>	<b>94.8</b>

A9	21	90.1	1.8	NA	0.1	92
A10		89.9	2.1	NA	0.1	92.1
<b>Mean</b>		<b>90</b>	<b>2</b>	<b>NA</b>	<b>0.1</b>	<b>92.1</b>
A11	30	90.3	2.8	NA	0.1	93.2
A12		93.8	0.8	NA	0.1	94.7
<b>Mean</b>		<b>92.1</b>	<b>1.8</b>	<b>NA</b>	<b>0.1</b>	<b>94</b>
A13	61	92.4	0.4	NA	0.2	93
A14		93	0.4	NA	0.2	93.6
<b>Mean</b>		<b>92.7</b>	<b>0.4</b>	<b>NA</b>	<b>0.2</b>	<b>93.3</b>

NA = Not applicable, ND = Not detected (or <0.1% AR)

**Table B.8.2.2.2-07 Percent recovery of applied radioactivity recovered from natural water treated with [phenyl-<sup>14</sup>C] inpyrfluxam, high concentration (0.1 mg/L)**

<b>Vessel Code</b>	<b>Sampling Interval (days)</b>	<b>Surface Water (Extract 1)</b>	<b>Vessel wash (Extract 2)</b>	<b>Volatiles</b>	<b>Mass Balance</b>
B1	0	92.6	0.6	NA	93.2
B2		92.8	0.4	NA	93.2
<b>Mean</b>		<b>92.7</b>	<b>0.5</b>	<b>ND</b>	<b>93.2</b>
B3	3	94.4	0.6	ND	95
B4		95.3	0.8	ND	96.1
<b>Mean</b>		<b>94.9</b>	<b>0.7</b>	<b>ND</b>	<b>95.6</b>
B5	7	92.9	0.3	ND	93.2
B6		93.3	0.4	ND	93.7
<b>Mean</b>		<b>93.1</b>	<b>0.4</b>	<b>ND</b>	<b>93.5</b>
B7	14	92.8	1	ND	93.8
B8		93.5	1	ND	94.5
<b>Mean</b>		<b>93.2</b>	<b>1</b>	<b>ND</b>	<b>94.2</b>
B9	21	90.8	1.5	0.1	92.4
B10		91.6	2.5	0.1	94.2
<b>Mean</b>		<b>91.2</b>	<b>2</b>	<b>0.1</b>	<b>93.3</b>
B11	30	94.6	0.7	0.1	95.4
B12		95.4	1.7	0.6	97.7
<b>Mean</b>		<b>95</b>	<b>1.2</b>	<b>0.4</b>	<b>96.6</b>
B13	61	93.7	0.6	0.2	94.5
B14		93.3	0.5	0.2	94
<b>Mean</b>		<b>93.5</b>	<b>0.6</b>	<b>0.2</b>	<b>94.3</b>

NA = Not applicable, ND = Not detected (or <0.1% AR)

**Table B.8.2.2-08 Percent recovery of applied radioactivity recovered from natural water treated with [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam low concentration (0.01 mg/L)**

<b>Vessel Code</b>	<b>Sampling Interval (days)</b>	<b>Surface Water (Extract 1)</b>	<b>Vessel wash (Extract 2)</b>	<b>Lid wash from Extract 1</b>	<b>Volatiles</b>	<b>Mass Balance</b>
C1	0	94.1	0.6	0.9	NA	95.6
C2		94.3	0.6	NA	NA	94.9
<b>Mean</b>		<b>94.2</b>	<b>0.6</b>	<b>0.5</b>	<b>NA</b>	<b>95.3</b>
C3	3	101.2	0.8	NA	ND	102.0
C4		97.9	0.8	NA	ND	98.7
<b>Mean</b>		<b>99.6</b>	<b>0.8</b>	<b>NA</b>	<b>ND</b>	<b>100.4</b>
C5	7	93.7	ND	NA	ND	93.7
C6		94.8	ND	NA	ND	94.8
<b>Mean</b>		<b>94.3</b>	<b>ND</b>	<b>NA</b>	<b>ND</b>	<b>94.3</b>
C7	14	96.6	1.6	NA	ND	98.2
C8		97.3	1.3	NA	ND	98.6
<b>Mean</b>		<b>97.0</b>	<b>1.5</b>	<b>NA</b>	<b>ND</b>	<b>98.4</b>
C9	21	92.9	1.9	NA	ND	94.8
C10		93.5	1.4	NA	ND	94.9
<b>Mean</b>		<b>93.2</b>	<b>1.7</b>	<b>NA</b>	<b>ND</b>	<b>94.9</b>
C11	30	95.1	1.1	NA	ND	96.2
C12		96.1	0.7	NA	ND	96.8
<b>Mean</b>		<b>95.6</b>	<b>0.9</b>	<b>NA</b>	<b>ND</b>	<b>96.5</b>
C13	61	97.2	0.7	NA	ND	97.9
C14		76.9*	1.3	NA	ND	78.2*
<b>Mean</b>		<b>87.1*</b>	<b>1.0</b>	<b>NA</b>	<b>ND</b>	<b>88.1*</b>

NA = Not applicable, ND = Not detected (or <0.1% AR)

\*C14 values not included in subsequent mean values quoted or any statistical data



**Table B.8.2.2-09 Percent recovery of applied radioactivity recovered from natural water treated with [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam high concentration (0.1 mg/L)**

<b>Vessel Code</b>	<b>Sampling Interval (days)</b>	<b>Surface Water (Extract 1)</b>	<b>Vessel wash (Extract 2)</b>	<b>Lid wash from Extract 1</b>	<b>Volatiles</b>	<b>Mass Balance</b>
D1	0	87.3	0.3	0.2	NA	87.8
D2		92.5	0.8	NA	NA	93.3
<b>Mean</b>		<b>89.9</b>	<b>0.6</b>	<b>0.1</b>	<b>ND</b>	<b>90.6</b>
D3	3	92.8	0.5	NA	ND	93.3
D4		91	0.5	NA	ND	91.5
<b>Mean</b>		<b>91.9</b>	<b>0.5</b>	<b>NA</b>	<b>ND</b>	<b>92.4</b>
D5	7	94.2	0.4	NA	ND	94.6
D6		92.6	0.7	NA	ND	93.3
<b>Mean</b>		<b>93.4</b>	<b>0.6</b>	<b>NA</b>	<b>ND</b>	<b>94</b>
D7	14	90	0.9	NA	ND	90.9
D8		91.2	0.7	NA	ND	91.9
<b>Mean</b>		<b>90.6</b>	<b>0.8</b>	<b>NA</b>	<b>ND</b>	<b>91.4</b>
D9	21	89.6	1.2	NA	ND	90.8
D10		87.5	1.8	NA	ND	89.3
<b>Mean</b>		<b>88.6</b>	<b>1.5</b>	<b>NA</b>	<b>ND</b>	<b>90.1</b>
D11	30	92	0.8	NA	ND	92.8
D12		90.5	1.2	NA	ND	91.7
<b>Mean</b>		<b>91.3</b>	<b>1.0</b>	<b>NA</b>	<b>ND</b>	<b>92.3</b>
D13	61	91.1	0.3	NA	ND	91.4
D14		90.6	0.5	NA	ND	91.1
<b>Mean</b>		<b>90.9</b>	<b>0.4</b>	<b>NA</b>	<b>ND</b>	<b>91.3</b>

NA = Not applicable, ND = Not detected (or <0.1% AR)

**Table B.8.2.2.2-10 Percent recovery of applied radioactivity recovered from sterilised natural water treated with [phenyl-<sup>14</sup>C] inpyrfluxam at the high application rate (0.1 mg/L)**

<b>Vessel Code</b>	<b>Sampling Interval (days)</b>	<b>Surface Water (Extract 1)</b>	<b>Vessel wash (Extract 2)</b>	<b>Volatiles</b>	<b>Mass Balance</b>
E1	0	92.1	0.3	NA	92.4
E2		91.7	0.2	NA	91.9
<b>Mean</b>		<b>91.9</b>	<b>0.3</b>	<b>ND</b>	<b>92.8</b>
E3	14	97.3	0.5	ND	97.8
E4		94.8	0.5	ND	95.3
<b>Mean</b>		<b>96.1</b>	<b>0.5</b>	<b>ND</b>	<b>96.6</b>
E5	30	96.2	0.5	ND	96.7
E6		96.8	0.5	ND	97.3
<b>Mean</b>		<b>96.5</b>	<b>0.5</b>	<b>ND</b>	<b>97.0</b>
E7	61	93.6	0.4	ND	94.0
E8		96.3	0.4	ND	96.7
<b>Mean</b>		<b>95.0</b>	<b>0.4</b>	<b>ND</b>	<b>95.4</b>

NA = Not applicable, ND = Not detected (or <0.1% AR)

The sterile samples were used to distinguish between biotic and abiotic degradation. Mean % AR detected in the water samples at 1 and 61 DAT were 91.3% AR and 94.2% AR in respectively.

Data for sodium [<sup>14</sup>C] benzoate in the reference and solvent controls as below.

The samples demonstrated that the test system was microbially active, with 89.5 – 92.1 % of the applied substance evolved as <sup>14</sup>CO<sub>2</sub> after 78 days, and 89.5 – 86.7% evolved in the sodium [<sup>14</sup>C] benzoate + 29 µL acetonitrile samples. Profiles were similar suggesting that the acetonitrile did not inhibit the microbial degradation of sodium [<sup>14</sup>C] benzoate in the natural water.

**Table B.8.2.2.2-11 Cumulative recovery of radioactivity from reference control vessels treated with sodium [<sup>14</sup>C] benzoate**

<b>Sampling Interval</b>	<b>3 DAT</b>		<b>7 DAT</b>		<b>14 DAT</b>		<b>21 DAT</b>		<b>30 DAT</b>		<b>61 DAT</b>	
<b>Vessel Code</b>	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>
NaOH Trap 1	39.8	40.7	23.5	25.2	14.9	14.9	4.4	5	2.7	0.1	2.8	2.6
NaOH Trap 2	0.2	ND	ND	0.3	0.1	ND	0.1	ND	2.7	ND	0.1	ND
Total in Traps	40.0	40.7	23.5	25.5	15.0	14.9	4.5	5.0	5.4	0.1	2.9	2.6
Mean Total	<b>40.4</b>		<b>24.5</b>		<b>15.0</b>		<b>4.8</b>		<b>2.8</b>		<b>2.8</b>	
Cumulative total	40.0	40.7	63.5	66.2	78.5	81.1	83	86.1	85.8	88.8	88.7	91.4
Cumulative Mean	<b>40.4</b>		<b>64.9</b>		<b>79.8</b>		<b>84.6</b>		<b>87.3</b>		<b>90.1</b>	

ND = Not detected (or &lt;0.1% AR)

<b>Sampling Interval</b>	<b>78 DAT</b>	
<b>Vessel Code</b>	<b>F1</b>	<b>F2</b>
NaOH Trap 1	0.8	0.7
NaOH Trap 2	ND	ND
Total in Traps	0.8	0.7
Mean Total	<b>0.8</b>	
Cumulative total	89.5	92.1
Cumulative Mean	<b>90.8</b>	
Surface Water	2.4	1.8
Mass Balance	91.9	93.9
Mean Mass Balance	<b>92.9</b>	

ND = Not detected (or &lt;0.1% AR)

**Table B.8.2.2-12 Cumulative recovery of radioactivity from solvent control vessels treated with sodium [<sup>14</sup>C] benzoate**

<b>Sampling Interval</b>	<b>3 DAT</b>		<b>7 DAT</b>		<b>14 DAT</b>		<b>21 DAT</b>		<b>30 DAT</b>		<b>61 DAT</b>	
<b>Vessel Code</b>	<b>G1</b>	<b>G2</b>	<b>G1</b>	<b>G2</b>	<b>G1</b>	<b>G2</b>	<b>G1</b>	<b>G2</b>	<b>G1</b>	<b>G2</b>	<b>G1</b>	<b>G2</b>
NaOH Trap 1	40.1	35.2	23.6	24.8	13.1	13.5	4.1	4.4	2.7	2.7	4.4	4
NaOH Trap 2	ND	ND	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total in Traps	40.1	35.4	23.7	24.8	13.1	13.5	4.1	4.4	2.7	2.7	4.4	4
Mean Total	<b>37.8</b>		<b>24.3</b>		<b>13.3</b>		<b>4.3</b>		<b>2.7</b>		<b>4.2</b>	
Cumulative total	40.1	35.4	63.8	60.2	76.9	73.1	81	78.1	83.7	80.8	88.1	84.8
Cumulative Mean	<b>37.8</b>		<b>62.0</b>		<b>75.3</b>		<b>79.6</b>		<b>82.3</b>		<b>86.5</b>	

ND = Not detected (or &lt;0.1% AR)

<b>Sampling Interval</b>	<b>78 DAT</b>	
<b>Vessel Code</b>	<b>G1</b>	<b>G2</b>
NaOH Trap 1	1.4	1.9
NaOH Trap 2	ND	ND
Total in Traps	1.4	1.9
Mean Total	<b>1.7</b>	
Cumulative Total	89.5	86.7
Cumulative Mean	<b>88.1</b>	
Surface Water	3.1	5.2
Mass Balance	92.6	91.9
Mean Mass Balance	<b>92.3</b>	

ND = Not detected (or &lt;0.1% AR)

#### IV. Volatilisation

Ethanediol traps did not contain any detectable radioactivity.  $^{14}\text{CO}_2$  recovered from NaOH traps was insignificant reaching a maximum of 0.4 % AR for the PH-label at high concentration (30 DAT). All other sample contained too low levels of radioactivity to be able to confirm  $\text{CO}_2$ . Supporting data is provided in Tables 8.2.2.2/1-6 to 8.2.2.2/1-10 above.

#### V. Characterisation and identification of residues in water

The quantification of inpyrfluxam and the degradants in Tables B.8.2.2.20-06 to B.8.2.2.2-10 are summarised in Table B.8.2.2.2-13. Results show that no significant degradation of inpyrfluxam was observed. After 61 days, 92.1% AR (phenyl label) and 96.5% AR (pyrazolyl label) remained as unchanged parent for the low concentration; for the high concentration, inpyrfluxam amounted to 90.6% AR (phenyl label) and 90.4% AR (pyrazolyl label). The maximum degradation seen for any incubation group was 1.8 % AR.

**Table B.8.2.2-13 Average percent recovery of [ $^{14}\text{C}$ ] inpyrfluxam and metabolites expressed as applied radioactivity from natural water samples**

Incubation group	% AR present as		
	Inpyrfluxam at 0 DAT	Inpyrfluxam at 61 DAT	Unknowns (max)
PH-label, low rate	91.2	92.1	1.3
PH-label, high rate	92.4	90.6	3.3
PY-label, low rate	94.1	96.5*	3.9
PY-label, high rate	89.7	90.4	3.9
Sterile (PH-label, high rate)	91.3	94.2	0.6

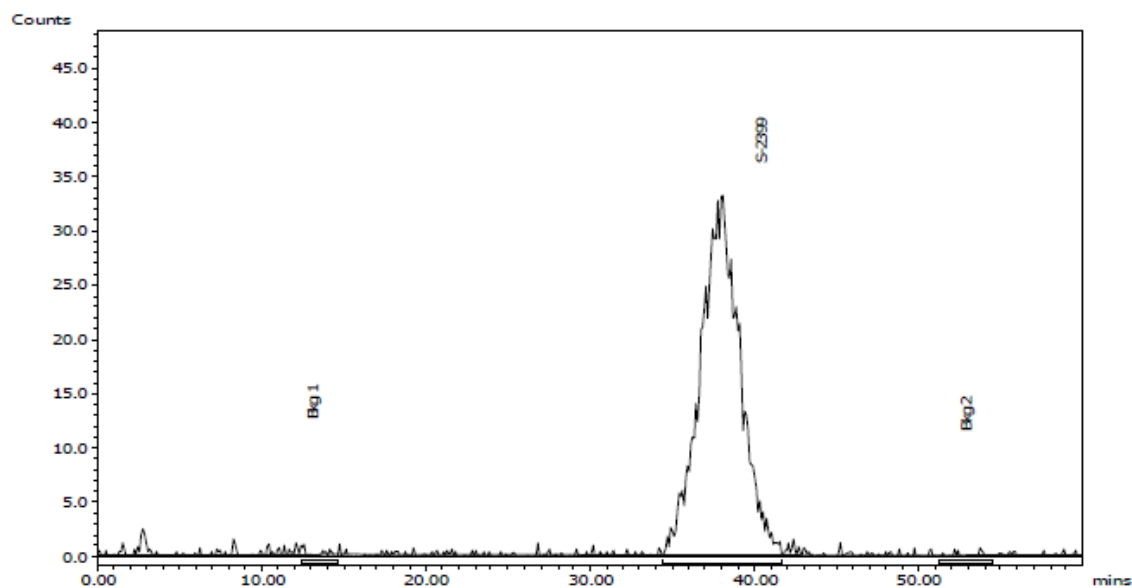
Values are the mean of two replicates

\*One replicate excluded due to low mass balance

The metabolite observed at the highest amount was present at  $\leq 4$  % AR (maximum 3.9 % AR, 14 DAT PY-label at high and low concentration) and it was not identified as it was not a major metabolite.

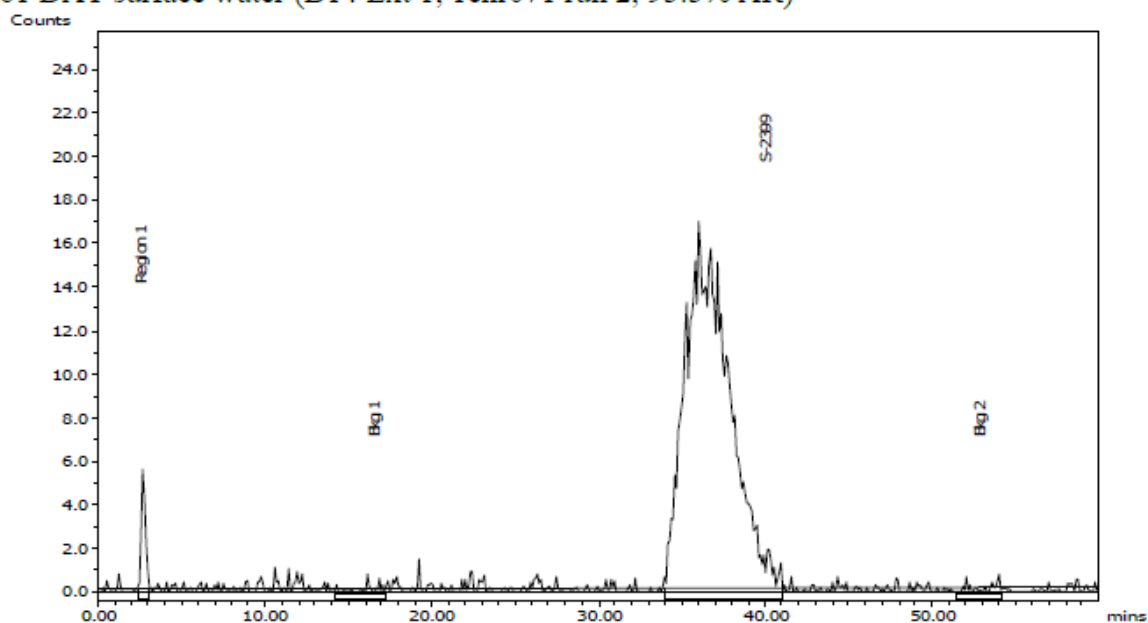
Chiral analysis of the application solution showed that no S-2940 (S-isomer) was present in the starting substance. Chiral analysis was performed on phenyl and pyrazolyl-labelled samples at 61 DAT. All incubation groups were found to only contain inpyrfluxam (R-isomer), and isomerisation to S-2940 (S-isomer) did not occur, as seen in the chromatograms below.

61 DAT surface water (A14 Ext 3, 1chr071 run 1, 93.0% AR)



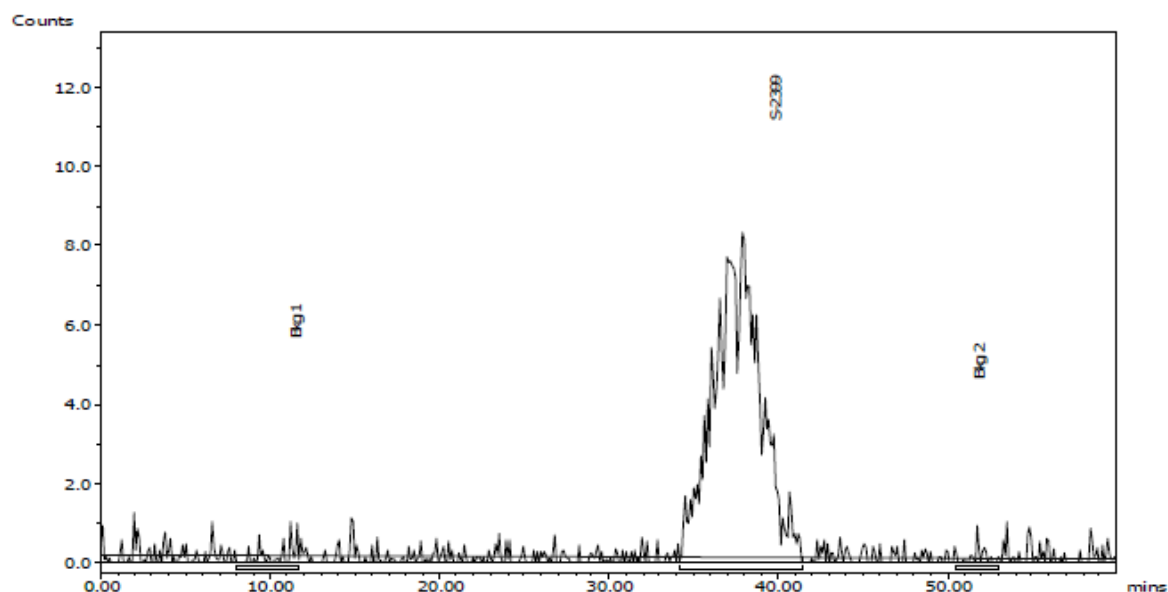
**Figure B.8.2.2.2-01 Chiral HPLC analysis of samples after treatment with [phenyl-<sup>14</sup>C] inpyrfluxam at the low application rate**

61 DAT surface water (B14 Ext 1, 1chr071 run 2, 93.3% AR)



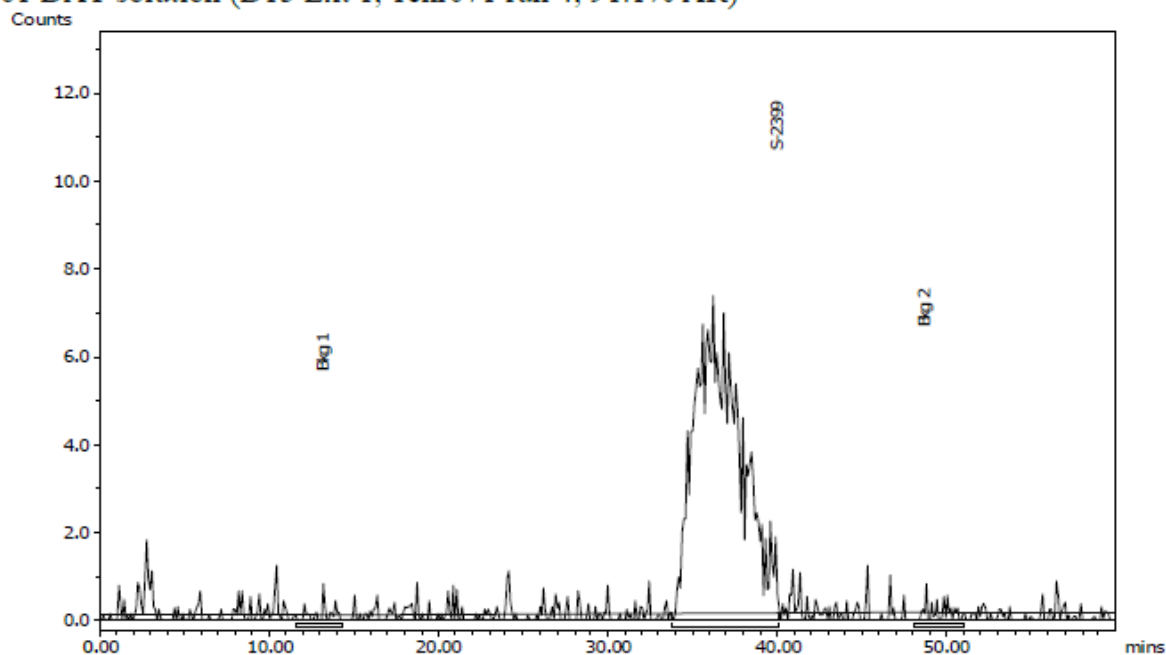
**Figure B.8.2.2.2-02 Chiral HPLC analysis of samples after treatment with [phenyl-<sup>14</sup>C] inpyrfluxam at the high application rate**

61 DAT surface water (C14 Ext 3, 1chr071 run 3, 76.9% AR)



**Figure B.8.2.2.2-03 Chiral HPLC analysis of samples after treatment with [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam at the low application rate**

61 DAT solution (D13 Ext 1, 1chr071 run 4, 91.1% AR)



**Figure B.8.2.2.2-04 Chiral HPLC analysis of samples after treatment with [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam at the high application rate**

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## KINETIC EVALUATION

Degradation rates of [ $^{14}\text{C}$ ] inpyrfluxam were obtained by CAKE software (Version 2.0) in line with FOCUS guidelines) by the applicant. The calculated Single First Order  $\text{DT}_{50}$  values are summarised in Table B.8.2.2.2-14, showing that no degradation of inpyrfluxam could be demonstrated under sterile and non-sterile conditions.

HSE notes that while a kinetic evaluation of the test substance has been conducted, and endpoints provided, the >20 % substance degradation required by OECD 309 for a reliable rate constant to be determined has not occurred. The calculation of these values does not however detract from the validity of the study as the kinetic evaluation is a separate process.

Nevertheless, HSE has checked the data sets used by the applicant for the kinetic evaluation according to the recommendations of the FOCUS workgroup on degradation kinetics [FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration]. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.1 (2014).

### I. Data handling

The values of inpyrfluxam in samples that were used were the individual total values from the two replicates per sampling interval, except for the 61 DAT pyrazolyl low concentration (10  $\mu\text{g/L}$ ) value, where only one replicate was used as the second replicate had a low mass balance.

HSE notes that the two different  $^{14}\text{C}$ -labels were considered in the evaluation in separate fits as opposed to true replicates recommended in the FOCUS guidance, although the two different test concentrations (10  $\mu\text{g/L}$  and 100  $\mu\text{g/L}$ ) were separately modelled using the individual replicates. However, this is considered to be a minor deviation that does not impact study outcomes, given that endpoints for 4 of the 5 fits are not statistically supported.

### II. Results

The calculated Single First Order values are summarised in the table below. HSE deems the visual fit of the five SFO models provided by the applicant to be acceptable, with no systematic deviations in the residuals.  $\chi^2$  error % values determined by the applicant are all below the 15 % FOCUS guideline for laboratory studies and are therefore deemed acceptable by HSE.



**Table B.8.2.2.2-14 Applicant supplied statistical assessment and degradation rate of [<sup>14</sup>C] inpyrfluxam using CAKE 2.0**

Group	SFO				
	DT <sub>50</sub> (days)	DT <sub>90</sub> (days)	k	X <sup>2</sup> error %	Prob. > t
PH-label, low rate	3190	10600	2.17 x 10 <sup>-4</sup>	1.41	0.2418
PH-label, high rate	1540	5120	4.50 x 10 <sup>-4</sup>	1.1	0.0378
PY-label, low rate*	5850	19400	1.18 x 10 <sup>-4</sup>	2.1	0.4125
PY-label, high rate	23600	78500	2.93 x 10 <sup>-5</sup>	1.42	0.4667
Sterile (PH-label, high rate)	3.45 x 10 <sup>12</sup>	1.15 x 10 <sup>13</sup>	2.01 x 10 <sup>-13</sup>	1.6	0.5

\*One replicate excluded due to low mass balance

HSE has assessed these fits and have provided a summary of their own kinetic evaluation using CAKE 3.7 in Table B.8.2.2.2-15 below.

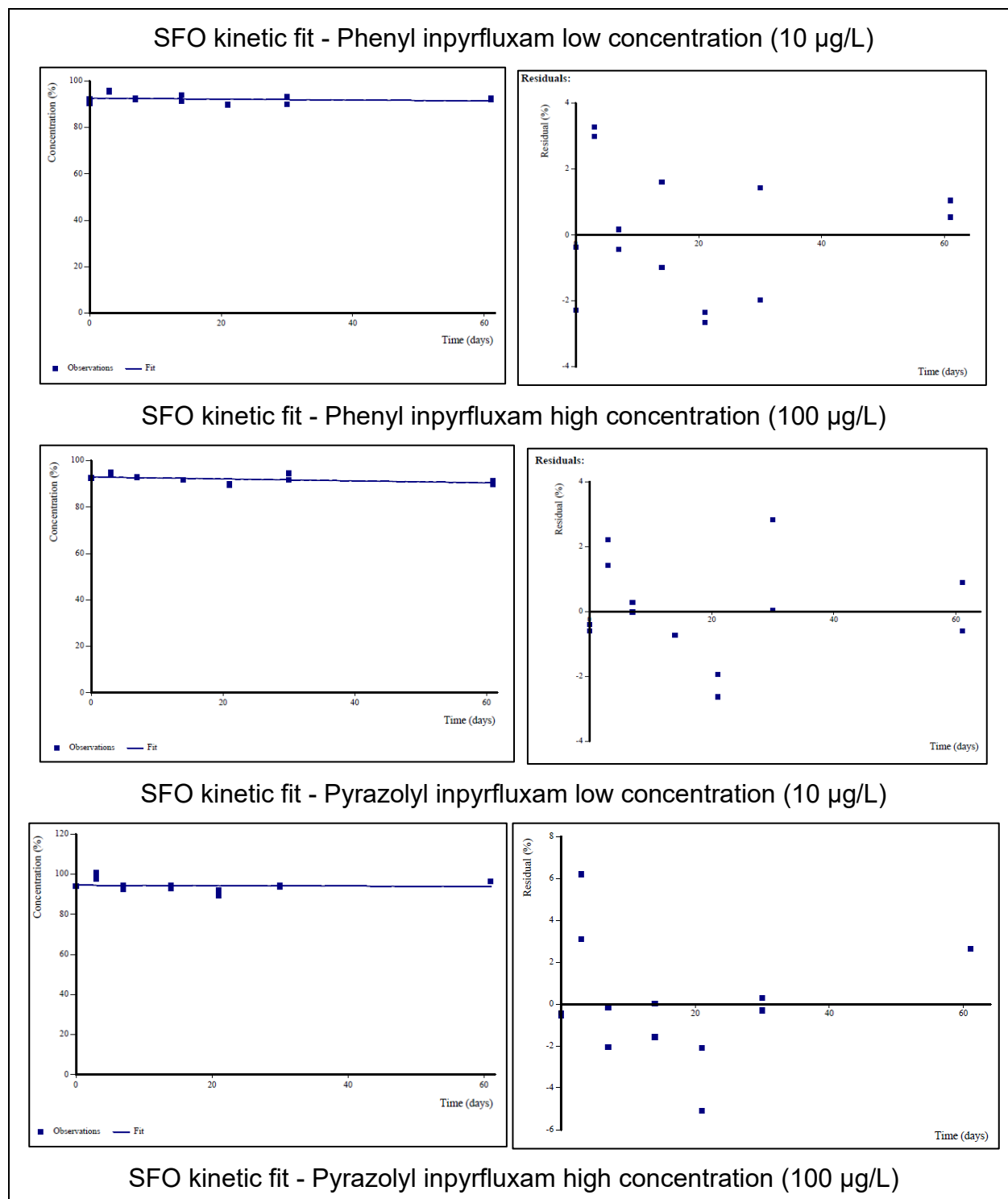
**Table B.8.2.2.2-15 HSE supplied statistical assessment and degradation rate of [<sup>14</sup>C] inpyrfluxam using CAKE 3.7**

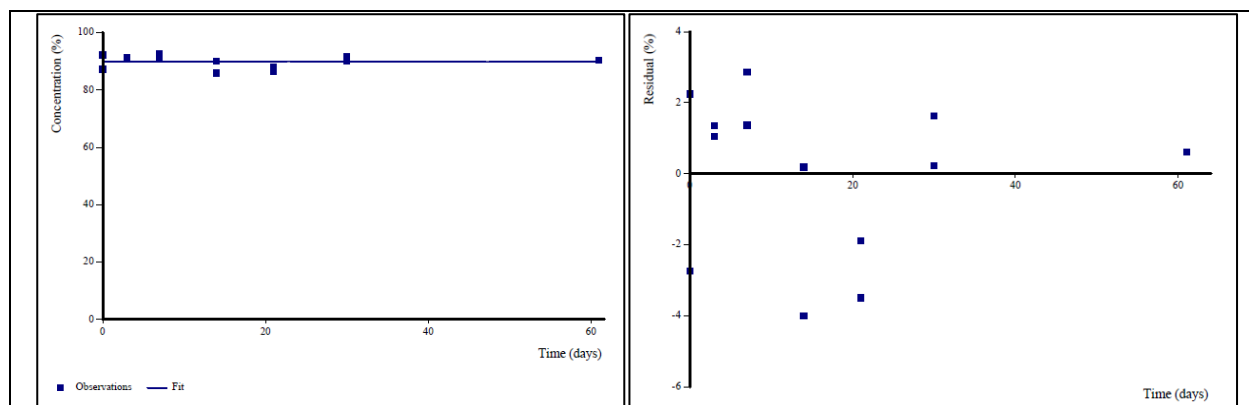
Group	SFO				
	DT <sub>50</sub> (days)	DT <sub>90</sub> (Days)	k	χ <sup>2</sup> error %	Prob. > t
PH-label, low rate	3190	>10000	2.17 x 10 <sup>-4</sup>	1.41	0.2418
PH-label, high rate	1540	5120	4.50 x 10 <sup>-4</sup>	1.1	0.0378
PY-label, low rate*	5850	>10000	1.18 x 10 <sup>-4</sup>	2.1	0.4125
PY-label, high rate	>10000	>10000	2.93 x 10 <sup>-5</sup>	1.43	0.4667
Sterile (PH-label, high rate)	>10000	>10000	2.01 x 10 <sup>-13</sup>	1.6	0.5

As the applicant's kinetic fits are comparable to HSE's kinetic fits, their results are presented and commented upon below.

HSE notes that  $DT_{50}$  and  $DT_{90}$  values are extrapolated well beyond the duration of the study and so should therefore be treated with caution.

**Table B.8.2.2-16 Applicant's kinetic fits and HSE's assessment and conclusion**





SFO: HSE deems the visual fit of the five SFO models provided by the applicant to be acceptable, with no systematic deviations in the residuals.  $\chi^2$  error % values determined by the applicant are all below the 15 % FOCUS guideline for laboratory studies and are therefore deemed acceptable by HSE.

t-test values for 4 of the 5 fits (all but PH-label, high rate) exceed the 0.1 limit for a 'weight of evidence' approach recommended by FOCUS guidelines. This means that the degradation rates provided by the applicant for these 4 fits are not statistically differentiable from 0. This is expected, due to the very small amount of substance degradation measured during the study.

'PH-label, high rate' is an exception. In this case the t-test is passed and the fit and endpoints are deemed acceptable by the HSE.

HSE therefore recommends that the DT<sub>50</sub> and DT<sub>90</sub> endpoints for all fits apart from 'PH-label, high rate' should not be presented, as they infer an accuracy which is not statistically supported. This assessment is supported by the OECD 309 guideline that endpoints should not be provided if <20 % substance degradation has occurred within the study.

Conclusion: As there is no decline phase present in all but one fit, the endpoints cannot be reliably determined. Therefore, whilst the kinetic fits provide a useful summary of degradation in natural surface water, HSE suggests that end points for all but one kinetic fit, PH-label at high concentration, should not be used for persistence assessments.

For the PH-label at high concentration, HSE notes that the difference between the applicant (1540) and HSE derived (1542) DT<sub>50</sub> is 2 days. The difference between the applicant (5120) and HSE derived (5123) DT<sub>90</sub> is 3 days. As such, due to the minimal difference between both values and the clear exceedance of the 60 day 'very persistent' half-life trigger, HSE accepts the applicant supplied DT<sub>50</sub> and DT<sub>90</sub> values for use in the risk assessment.

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## CONCLUSION

No individual metabolites were detected at > 5 % AR at any sampling interval (maximum 3.9 % AR, 14 DAT PY-label at high and low concentration) and so peaks were not identified. No significant degradation of inpyrfluxam was observed to take place under non-sterile or sterile conditions during the study. Therefore aerobic mineralisation is not expected to be a major route of degradation for Inpyrfluxam.

Chiral analysis showed that the R-isomer of inpyrfluxam was the only isomer present throughout the study and that isomerisation to S-2940 (S-isomer) did not occur.

The kinetic evaluation conducted by the applicant determined that inpyrfluxam follows single first order degradation.  $DT_{50}$  values ranged from 1540 to >10,000 days.  $DT_{90}$  values ranged from 5120 days to >10,000 days.

Due to this modelled  $DT_{50}$ , inpyrfluxam is observed to clearly exceed the 40 day very persistent trigger for degradation in fresh water according to the criteria in Regulation (EC) No 1107/2009. However, HSE notes that despite the exceedance of the very persistent trigger value in this study, it does not provide conclusive evidence for the classification of inpyrfluxam as a very persistent compound. Instead, it will be used in weight of evidence approach, with results from the water sediment kinetic analysis evaluation (B.8.2.2.3.1 and B.8.2.2.3.2) considered the primary driver in the determination of the persistence classification of inpyrfluxam.

Overall, HSE accepts this study as suitable for use in the environmental risk assessment.

**B.8.2.2.3. Water / sediment studies****B.8.2.2.3.1. Water / sediment – aerobic study 1**

<b>KCA index:</b>	KCA 7.2.2.3/01 and KCA 7.2.2.3/04
<b>Report Author:</b>	██████████
<b>Report Year:</b>	2017b
<b>Report Title:</b>	S-2399: Degradation under Aerobic Aquatic Conditions; Amended Report 1  Kinetic analysis: Recalculation of the degradation rate of S-2399 (inpyrfluxam) in aquatic systems according to FOCUS Kinetics Guidance
<b>Document number:</b>	TPM-0041
<b>Guidelines:</b>	OECD 308  Kinetic analysis: FOCUS kinetic guidance
<b>GLP:</b>	Underlying study: Yes  Kinetic analysis: N/A
<b>Acceptability:</b>	Yes
<b>Study relied upon:</b>	Yes

<b>Deviation</b>	<b>HSE assessment of deviation</b>
Taunton River sediment collection tool not specified	Minor deviation. Method of collection not expected to affect system properties.
Taunton River sediment collected from top 0-2.5 cm only, whereas OECD 308 specifies upper most 5-10 cm	Minor deviation. Bacterial activity and biomass are generally highest near the sediment surface and decrease with depth. Should not affect outcome of study.
The storage period of the Golden Lake system exceeded the maximum recommended period of 4 weeks by 6 days	Minor deviation. Sediments were demonstrated to be biologically active post-storage (at 0 DAT).

Oxygen saturation, pH and temperature of the water were not measured directly at the collection sites	Minor deviation. Unlikely the sediment characteristics are affected during transport. Furthermore, biomass confirms they were biologically active post collection and handling.
Two coarse sediments used, rather than one fine, one coarse required by OECD 308 guidelines	Minor deviation, however two aerobic studies (7.2.2.3/01 & 02) provided, meeting the OECD 308 guideline between them. The deviation does not void the validity of the study.
Sampling site pesticide histories not provided	Minor deviation. Sites are not in areas where previous pesticide application is to be expected
Taunton River water-sediment ratio is below OECD 308 3:1 minimum guideline ratio	Minor deviation. Not expected to influence the outcome of the test.
Two different values given for acclimation period duration of Taunton river samples (MCA & KCA section 3.1 text; 14-15 days, KCA table 7-8 days)	Minor deviation, as both durations given fall within OECD 308 acceptable durations.
Redox, pH, and O <sub>2</sub> concentration not provided for the acclimation period	Major deviation. These values are required to determine that the systems have approached equilibrium prior to test substance dosing. These have since been provided by the applicant in response to an RAI.
Limit of Detection (LOD) exceeds 1 % of the initial applied dose in some cases, to a maximum of ~1.9 % AR	Minor deviation, attributed by the applicant to matrix effects. Does not effect data handling procedures for inpyrfluxam as all values exceed LOD.

Instances in which LOD exceeds 1% of the initial applied dose not specified.	Minor deviation, as maximum LOD of ~1.9 % AR lies below minimum inpyrfluxam AR %.
LOQ value for HPLC analysis not provided	Major deviation from OECD 308 guidelines. Will be required in order to evaluate degradation rates of inpyrfluxam in water-sediment systems. These have since been provided by the applicant in an RAI.
Golden Lake PH-label AR % recovery falls below 90-110 % OECD 308 guideline	Minor deviation. Measurement is marginally below guideline (minimum 89.5 % AR), for two sampling DAT's only. Not considered to void the validity of the study
<p style="text-align: center;"><b>HSE conclusion on deviations</b></p> <p>HPLC LOQ value to be provided. Redox, pH, and O<sub>2</sub> concentration should be provided for the acclimation period. Following a response by the applicant to an RAI, these omissions have been rectified and the study is permissible.</p>	

## INTRODUCTION

The biotransformation of inpyrfluxam under aerobic aquatic conditions was investigated in two studies, B.8.2.2.3.1 and B.8.2.2.3.2. The former investigated the biotransformation of inpyrfluxam in two water/sediment systems (Golden Lake and Taunton River) using [phenyl-<sup>14</sup>C] inpyrfluxam (PH-label) and [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PY-label).

The aerobic aquatic test systems (50 g of dry weight sediment with approximately 165 mL of water) were dosed with a final water concentrations of 0.018 µg/mL for both radiolabels in the Golden Lake systems, and a final water concentration of 0.015 µg/mL and 0.014 µg/mL for the PH-label and PY-label in the Taunton River systems, respectively.

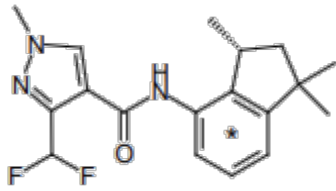
The test systems were incubated at 20±2°C in the dark for a maximum of 112 days and were periodically collected and extracted. The test systems were equipped with NaOH traps for the collection of evolved <sup>14</sup>CO<sub>2</sub> and tetraglyme/ethylene glycol traps for <sup>14</sup>C volatile capture.

## MATERIALS AND METHODS

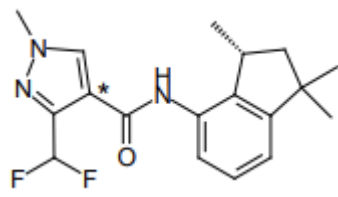
### I. Test items

The test item inpyrfluxam was used in two  $^{14}\text{C}$ -labelled forms, [phenyl- $^{14}\text{C}$ ] inpyrfluxam (PH) and [pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam (PY). Test substances were stored in a freezer (ca.  $-5\text{ }^{\circ}\text{C}$ ) when not in use. HSE notes that the radiolabels are placed in the most stable parts of the respective labelled molecules as per OECD 308 guidelines, indicated by the asterisks in the diagrams below.

**Table B.8.2.2.3.1-01  $^{14}\text{C}$ -labelled test materials**

<b>1. Test Material</b>	[phenyl- $^{14}\text{C}$ ] inpyrfluxam (PH-label)
<b>Lot/Batch:</b>	CFQ41803
<b>Specific activity:</b>	4.51 GBq/mmol
<b>Purity:</b>	Radiochemical purity 98.17% prior to dosing. The chirality was determined to be 100 % <i>R</i> -isomer.
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	stable
<b>Structure:</b>	 <p>*Denotes radiolabelling position</p>
<b>2. Test Material</b>	[pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam (PY-label)
<b>Lot/Batch:</b>	CFQ41802
<b>Specific activity:</b>	2.11 GBq/mmol
<b>Purity:</b>	Radiochemical purity 95.90% prior to dosing. The chirality was determined to be 100 % <i>R</i> -isomer.
<b>CAS#:</b>	Not assigned



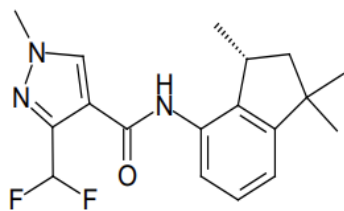
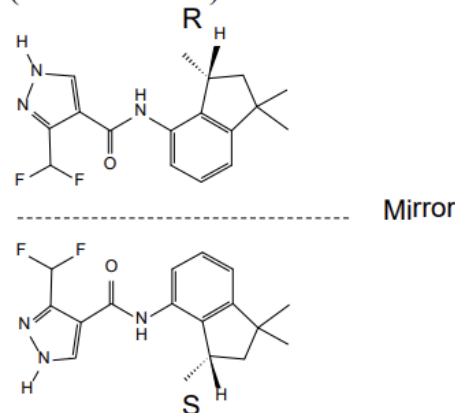
<b>Stability of compound:</b>	stable
<b>Structure:</b>	 <p>*Denotes radiolabelling position</p>

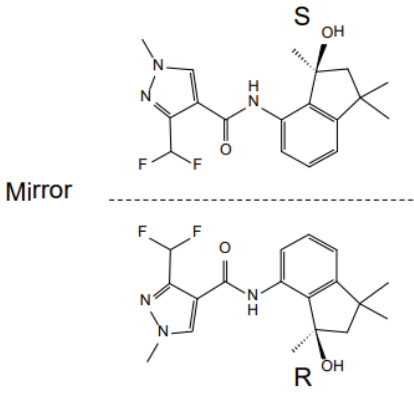
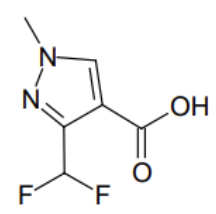
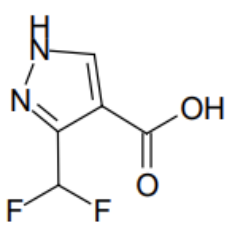
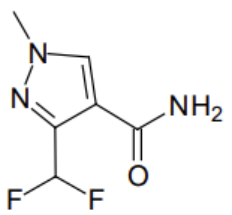
## II. Reference materials

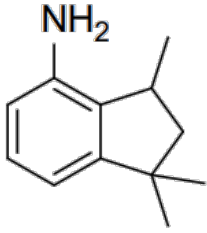
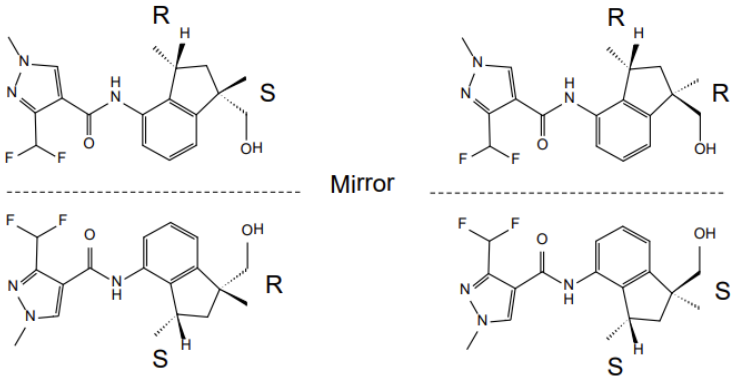
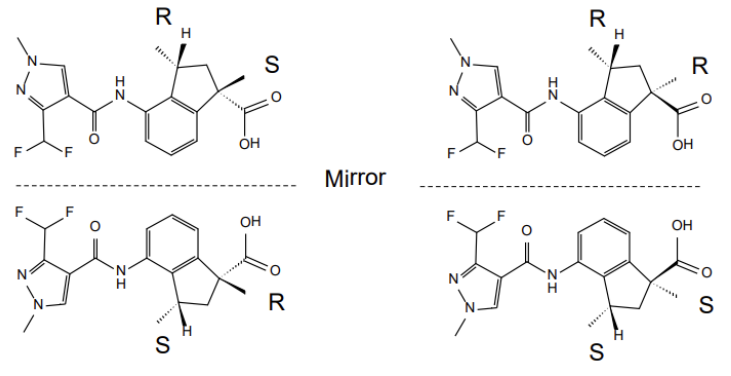
Several reference standards were analysed in this study, and all had a purity or radiochemical purity > 95 %. Standards were prepared in acetonitrile or acetonitrile/water solutions for use in HPLC and/or TLC analysis.

HSE considers the range of reference compounds used to be acceptable for identifying the rate and route of degradation of inpyrfluxam in water-sediment systems.

**Table B.8.2.3.1-02 Unlabelled metabolite reference item details for characterisation of any degradation products**

1	<b>Code Name</b>	inpyrfluxam
	<b>Chemical Name</b>	3-(difluoromethyl)-1-methyl-N-[(3'R)-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	
2	<b>Code Name</b>	N-des-Me-S-2840
	<b>Chemical Name</b>	3-(difluoromethyl)-N-[1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	<p>(2 Enantiomers)</p>  <p>Mirror</p>
3	<b>Code Name</b>	3'-OH-S-2840
	<b>Chemical Name</b>	3-(difluoromethyl)-N-[3'-hydroxy-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1-methyl-1H-pyrazole-4-carboxamide

	<b>Chemical Structure</b>	 <p>Mirror</p>
4	<b>Code Name</b>	DFPA
	<b>Chemical Name</b>	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
	<b>Chemical Structure</b>	
5	<b>Code Name</b>	N-des-Me-DFPA
	<b>Chemical Name</b>	3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid
	<b>Chemical Structure</b>	
6	<b>Code Name</b>	DFPA-CONH <sub>2</sub>
	<b>Chemical Name</b>	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	

7	<b>Code Name</b>	ATMI
	<b>Chemical Name</b>	(3'RS)-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-amine
	<b>Chemical Structure</b>	
8	<b>Code Name</b>	1'-CH <sub>2</sub> OH-S-2840
	<b>Chemical Name</b>	-
	<b>Chemical Structure</b>	
9	<b>Code Name</b>	1'-COOH-S-2840
	<b>Chemical Name</b>	-
	<b>Chemical Structure</b>	

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### III. Sediment/water system

For B.8.2.2.3.1, two test systems (sediment and water) were collected from the top 0-5 cm layer (posthole digger, Golden Lake) and 0-2.5 cm layer (collection tool not specified, Taunton River). The sediment was thoroughly mixed and passed through a 2-mm mesh sieve with a minimum of air-drying. The sediment and water were stored in the dark before being used. Characteristics of the test systems used are summarised in Table B.8.2.2.3.1-03. HSE notes that by OECD 308 guidelines, all information relating to the collection of water-sediment systems should be provided. Therefore no specification of the Taunton river collection tool is viewed as a deviation from OECD 308 guidelines by HSE. HSE considers this as a minor deviation, and not to impact the study validity as the choice of collection tool is not expected to impact system properties.

HSE notes that the Taunton River sediment was sampled at a maximum depth of 2.5 cm, below the minimum recommended depth of 5 cm. However, on this occasion, this is deemed a minor deviation from the guidance as bacterial activity and biomass are generally highest near the sediment surface and decrease with depth, and biomass measurements for Taunton River taken at the beginning and end of the study show that the system is viable throughout. Therefore, HSE considers this deviation does not impact the overall study outcomes.

HSE notes that the oxygen saturation, pH and temperature of the water were not measured directly at the collection sites in accordance with OECD 308. HSE considers that this requirement may be required to check parameters have not been affected during shipping and transport (prior to sending the sample for analysis); identifying environmental factors that could potentially affect the rate or extent of biodegradation of the test substance, and in the case of pH, ensure that two distinct test systems are used. Nevertheless, the dates chosen are unlikely to invalidate the study. The two sediments were characterised with respect to textural class, pH, organic carbon (OC), cation exchange capacity and mineral composition in a separate GLP study (Agvise Laboratories, USA) shortly after collection and are suitably different. Furthermore, the microbial biomass of the sediments were measured at the start of the experiment and confirmed that they were biologically active post collection and handling. Therefore, HSE considers this to be a minor deviation that will not have an impact on the overall study outcomes.

The sediments characteristics are summarised in Table B.8.2.2.3.1-03.

**Table B.8.2.2.3.1-03 Chemical and physical characteristics of test sediments**

<b>Sediment characteristic</b>	<b>Golden Lake</b>	<b>Taunton River</b>
Sampling location	Steele Co., ND; GPS [REDACTED] [REDACTED] [REDACTED]	Taunton River, MA; GPS [REDACTED] [REDACTED] [REDACTED]
<b>USDA Particle size distribution</b>		
% sand (50 µm - 2 mm)	80	58
% silt (2 µm - 50 µm)	15	36
% clay <2 µm	5	6
pH (H <sub>2</sub> O)	7.8	5.9
% Moisture 1/3 bar	20.4	39.4
Cation exchange capacity (meq/100g)	13	6.5
% Organic carbon (Walkley Black)	1.6	3.7
% Organic Matter	2.8	6.3
USDA Textural class	Loamy sand	Sandy loam
Bulk density (disturbed) (g/cm <sup>3</sup> )	1.01	0.81
<b>Microbial Biomass Carbon (µg/g dry weight)</b>		
0 DAT	389	401
112 DAT; untreated control	444	503
112 DAT; solvent control	460	478
112 DAT; inpyrfluxam control	456	480

HSE notes that the exact conditions of storage, such as containers used, are not reported. However, the applicant has reported that, prior to dosing, sediments were stored in the dark and in accordance with 10381-6 (1993), which indicates transport in similar conditions (kept in the dark with free access of air). Furthermore, biomass measurements demonstrate that the soil is biologically active at the start and end of the study.

The total timeframe between sample collection and use (start of acclimation) was 34 days for Golden Lake and 15 days for Taunton River. The applicant stated that the sediment and water were stored in the dark at 4°C except during the aerobic aquatic test system preparation where it was stored at ambient temperatures. Test system preparation was 2 days (20/08/2014 – 21/08/2014) for Golden Lake and 2 days (08/09/2014 and 09/9/14) for Taunton River. HSE notes that the storage period of the Golden Lake system exceeded the maximum recommended period of 4 weeks by 6 days. The OECD 308 states that use of freshly sampled sediment and water is strongly recommended, as recent studies have shown that storage at 4°C can lead to a decrease of the organic carbon content of the sediment which may possibly result in a decrease of microbial activity.

Nevertheless, the microbial biomass at 0 DAT was 389.0 µg microbial carbon/g dry sediment for Golden Lake. This corresponds to biomass of 2.43% as total organic carbon, demonstrating that the sediments are biologically active. Therefore, HSE deems this a minor deviation that is not expected to have any impact on the study outcome.

Furthermore, both sediments qualify as coarse sediments, and neither qualify as fine. By OECD 308 guidelines, at least one coarse and one fine sediment should be used. HSE view this is a significant deviation, however, the applicant has provided a second aerobic water-sediment study (B.8.2.2.3/02) . Considering the studies in tandem, the applicant has met the requirements for a minimum of one fine sediment and one coarse sediment. Therefore HSE does not consider this deviation to void the validity of the study.

HSE notes that the applicant has not provided pesticide histories for the sampling sites. While this is not a requirement by OECD 308 guidelines, it is desired. The applicant has stated that pesticide application would not normally be expected at the sampling sites. HSE therefore does not consider the omission of pesticide histories as a deviation.

## **STUDY DESIGN AND METHODS**

### **I. Experimental Conditions**

A beaker (cylinder internal diameter of 5.2 cm) was charged with 50 g (dry weight) of sediment and 165 mL of water, giving a sediment layer of 2.3 cm and 3.7 cm for the Golden Lake and Taunton River systems respectively and a water surface area of

about 21.6 cm<sup>2</sup> (water column depth of about 6.3 cm (Golden Lake) and 5.1 cm (Taunton River). Both systems had water : sediment (w : w) ratios of 3.3 : 1. HSE notes that the applicant has not provided volume : volume ratios, as is required by OECD 308 and EPA 835.4300 . HSE has calculated water : sediment (v : v) ratios by assuming water densities for both systems as 1.0 g/cm<sup>3</sup> , and taking the sediment bulk disturbed densities. This gives water : sediment (v : v) ratios of 3.3 : 1 for Golden Lake, and 2.7 : 1 for Taunton River. HSE notes that the ratio for Taunton River therefore falls below the OECD 308 and EPA 835.4300 minimum ratio of 3 : 1.

The aerobic aquatic test systems were dosed with a final water concentration of 0.018 µg/mL for both radiolabels in the Golden Lake systems, and a final water concentration of 0.015 µg/mL and 0.014 µg/mL for the PH-label and PY-label in the Taunton River systems, respectively. This gave an aqueous concentration equivalent to an accidental overspray of the maximum label rate applied to a 100 cm deep pond. HSE accepts that concentrations used were appropriate for evaluating the degradation of inpyrfluxam in the two water sediment systems.

The test systems were incubated at 20 ± 2°C in the dark with a constant humidified air flow to ensure aerobic conditions, for a maximum of 112 days and were periodically collected and extracted. The test systems were equipped with 1 M NaOH traps for the collection of evolved CO<sub>2</sub> and tetraglyme/ethylene glycol traps for <sup>14</sup>C volatile capture. Following set up of the test system an incubation period of 26-27 days was performed for the Golden Lake system and 7-8 days for the Taunton River system before dosing. HSE notes that for Taunton river, this is a contradiction of the value of 7-8 days provided in the KCA table, section 3.1. Nevertheless, this is considered to be a minor deviation as it is within the 1-4 week acclimation period stated recommended in OECD 308.

It is also noted that the pH, oxygen concentration in water, redox potential of the sediment and water, and total organic carbon were not reported during the acclimation phase as required by OECD 308 guidelines. HSE considered this to be a major deviation from guidelines, as these measurements are important in ensuring that the system has reached or approached equilibrium with respect to oxygen concentration and redox potential before addition of the test substance. Additionally, while measurements on the oxygen concentration in water as well as the measurement of redox potential in both water and sediment are little relevant to predict microbial viability of the test system, these parameters are relevant to check the prerequisites of the test, namely an aerobic layer of water overlying a sediment layer with a gradient in oxygen status. A request for additional information was sent to the applicant, and these are addressed in the 'Results – physical characteristics of the test system' section below.



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## II. Sampling

Duplicate soil samples were taken at 0, 3, 7, 14, 30, 63 and 112 days after treatment (DAT). The trap solutions were analysed at the same points immediately after removal.

The applicant indicates that, where the samples were not actively worked up, they were kept frozen. This contradicts other information in the report and the MCA stating that samples were analysed immediately after removal from the incubator and were not stored prior to any of the physical-chemical property or chemical analyses. Furthermore, the specific storage conditions of the sample extracts were not reported, such as the temperature and duration of freezing, and it is unclear how the applicant determined the stability of the frozen samples after thawing.

Clarification on this point was requested in an RAI and now addressed by the applicant, who stated that ‘Samples were kept frozen when not actively being worked up’ should read “Sample extracts were kept frozen when not actively being worked up”. Samples were not taken and frozen, only extracts.

Additionally, a storage stability test (KCA 7.1.2.2.1\_10) demonstrates that the compounds are stable over the study duration. Therefore, HSE accepts that this contradiction has been resolved.

## III. Description of analytical procedures

The physical parameters of the aerobic systems (oxygen concentration, redox and pH) were measured and the water decanted into a pre-weighed 250 mL bottle (pore water stays with the sediment), and the weights were recorded. Water and sediment phases were analysed separately.

The water phase and all extracts were analysed by liquid scintillation counting (LSC). The water phase was subjected to Solid Phase Extraction (SPE) and the organosoluble fraction was analysed by HPLC after concentration. Confirmation was performed by 2D-TLC.

The sediment samples were extracted with acetone, twice with acetone:water (3:2, v/v) and with acetone:water:HCl (c) (60:40:1, v/v/v). Activity in the neutral extracts were analysed by HPLC and confirmed by 2D-TLC after concentration. The acidic extracts contained < 4% AR and therefore no further analysis was performed.

Representative Post-extracted solids (PES) at 112 DAT were subjected to further additional sequential solvent extractions with ethyl acetate, dioxane and hexane. Total radioactivity in PES was determined by combustion. The  $^{14}\text{CO}_2$  collected in the NaOH trapping solution and organic volatiles collected in tetraglyme/ethylene glycol traps were quantified by LSC.

The potential isomerisation from [ $^{14}\text{C}$ ] inpyrfluxam was evaluated using chiral HPLC analysis on the extracts obtained from the 112 DAT samples.

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#### **IV. Limits of detection and quantification**

The limit of detection (LOD) for LSC was determined to be 11 dpm (detections per minute), and the limit of quantification (LOQ) was determined to be 43 dpm. Radioactivity dosing to the samples was a minimum of 1,091,200 dpm for the pyrazolyl label, and 2,426,400 dpm for the phenyl label. This gives LOD and LOQ values below 0.01 % AR, well below the OECD 308 guideline of 1 % AR. However, HSE notes that the applicant has given a higher LOD, of ~1.9 % AR, which they attribute to matrix effects. This is larger than the OECD 308 guideline value. HSE deem that this does not affect the data handling procedures for inpyrfluxam as all values are in excess of the ~1.9 % AR LOD. Therefore HSE considers this as a minor deviation. HSE further notes however, that the applicant has not provided a corresponding LOQ value in line with the higher LOD value. HSE regards this as a major deviation from OECD 308 guidelines, and will require an LOQ value in % AR that accounts for the aforementioned matrix effects in order to evaluate the degradation of inpyrfluxam in water-sediment systems. The applicant has later confirmed in an RAI that the maximum LOQ for LSC is 1.88 %.

HSE notes that a LOD and LOQ value for HPLC had not been provided by the applicant. These have since been provided in an RAI giving an LOD << 1 % AR, and a maximum LOQ of 1.01 %.

### **RESULTS AND DISCUSSION**

#### **I. Physical characteristics of the test system**

Surface water in the Golden Lake system was maintained under aerobic conditions throughout the duration of the experimental phase with average oxygen levels at 7 mg/L and positive redox potentials for both labels.

Surface water and sediment pH values were generally consistent for the study duration.

The sediments were less aerobic, with average redox potential measurements of -14 to -241 mV from 0 to 63 DAT in Golden Lake PH sediment and -175 to -276 mV from 0 to 33 DAT in the Golden Lake PY sediment. However, the final timepoints (112 DAT for Golden Lake PH sediment, and 63 and 112 DAT for Golden Lake PY sediment) were inconsistent to previous redox potentials and appeared to increase in aerobicity.

Whilst at several timepoints the value of redox potential can still be classed as anaerobic, HSE could not rule out the effect that the instability of the measured sediment redox potentials might have on the degradation of the test substance, particularly as the concentrations of inpyrfluxam in the whole system increased at the last time points (see tables B.8.2.2.3.1-09 to B.8.2.2.3.1-12).

Furthermore, the applicant did not provide redox potentials at the start of acclimation (prior to 0 DAT), therefore HSE was unable to assess whether the systems

equilibrated sufficiently before the start of the experiments. HSE therefore requested that the applicant submit values for parameters measured during the acclimation phase in a request for additional information (RAI).

The applicant has since provided dissolved oxygen, redox potentials, and pH measurements for the Golden Lake and Taunton River systems during the acclimation periods on 15/09/2014. This corresponds to 3 weeks into the acclimation of the Golden Lake system, and 1 week into the acclimation of the Taunton River system. These are included below in Table B.8.2.2.3.1-04. HSE accepts that the negative redox potentials suitably demonstrate anaerobicity has been achieved. Total organic carbon (TOC) data was not provided, and the applicant states this is applicable only to open ecosystems, while this is a closed ecosystem with no carbon renewal and sequestration. HSE therefore accepts the omission of TOC measurements.

The applicant also confirmed in a response to an RAI that in both systems, “characteristics were monitored at each sample time following application. Although some variation occurred, this was relatively minor and the patterns of the changes in oxygen content and redox potentials in the water and sediment phases suggested that changes were as a result of application and subsequent metabolism of the test item followed by some recovery to the original acclimated levels”. HSE therefore accepts that the systems have been sufficiently acclimated in line it OECD 308 guidelines before the start of the experiments.

**Table B.8.2.2.3.1-04 Oxygen, Redox, and pH values during the acclimation phase (provided in by applicant following RAI)**

Sample	Dissolved oxygen water (ppm)	Golden Lake			
		Redox Pot. (mV)		pH	
		Water	Sediment	Water	Sediment
A-104	8.72	108	-200	7.85	7.14
A-105	8.78	179	-214	7.95	7.17
A-110	8.70	179	-180	7.95	7.15
		Taunton River			
A-96	8.51	150	-18	7.09	6.69
A-99	8.62	50	-132	6.82	6.65
A-100	8.68	108	-156	6.62	6.64

HSE has also asked the applicant to explain the increase in aerobicity in the Golden Lake sediment layer at 63 and 112 DAT. The applicant confirmed that the sediment surface appears to have become less reduced, but did not identify why this may have occurred. Consequently, the impact on degradation remains unknown.

The increase in parent mass at the final time point increases the uncertainty of the  $DT_{90}$  and the kinetic fits around the final time point, particularly for SFO, which was ultimately selected by the applicant. However, it is clear from the applicant fitting that the persistence criteria were going to be met regardless. Therefore, this investigation has not been pursued by HSE.

It is also noted that the test systems were shaken during the incubations to keep the amount of dissolved oxygen in the water as high as possible. This is a deviation to OECD 308, which states that 'disturbance of the sediment is undesirable and should be avoided as far as possible'. However, the applicant stated that the shaking was not so robust that the sediment was stirred up. Furthermore, the disruption to the redox potentials was observed abruptly in later timepoints, which is not consistent with a gradual increase in disruption that may be observed from gentle agitation from the start of incubation. Therefore, HSE considers this to be a minor deviation which does not impact the study.

## **II. Data**

No significant change in the microbial biomass carbon was recognized between the initiation and termination of the incubation (Table B.8.2.2.3.1-03). Thus, HSE is satisfied that microbial viability was proved to be satisfactorily maintained during the incubation period.

It was confirmed that no isomerisation of [ $^{14}C$ ] inpyrfluxam occurred during incubation period based on chiral HPLC analysis.

The distribution and mass balance of applied radioactivity of [ $^{14}C$ ] inpyrfluxam in water phase, extractable, sediment-bound and volatile fractions are summarised in Tables.B.8.2.2.3.1-05 to B.8.2.2.3.1-08. The quantification of inpyrfluxam and the metabolites in the whole system is summarised in table B.8.2.2.3.1-09 to table B.8.2.2.3.1-12

**Table 8.2.2.3.1-05 Summary of the mass balance data for the Golden Lake PH-label as percent of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	89.3	86.8	88.0	51.5	49.8	50.6	37.3	39.1	38.2	33.7	34.9	34.3
Neutral Extract	12.3	13.1	12.7	43.9	46.5	45.2	58.4	57.5	57.9	60.0	60.2	60.1
Acidic Extract	0.0	0.0	0.0	0.6	0.7	0.7	1.0	1.0	1.0	1.5	1.4	1.4
Total Ext.	12.3	13.2	12.7	44.6	47.2	45.9	59.3	58.5	58.9	61.5	61.6	61.6
Sediment-bound	0.0	0.0	0.0	0.6	0.7	0.6	1.3	1.2	1.3	2.0	2.1	2.1
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
Total balance	101.6	100	100.8	96.7	97.7	97.2	98.0	98.9	98.5	97.4	98.7	98.0
Fraction	Days After Treatment (DAT)											
	30			63			112					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Water Phase	30.5	30.7	30.6	24.3	23.6	24	21.5	22.8	22.2			
Neutral Extract	61.5	63.7	62.6	66.1	66.6	66.4	68.6	67.7	68.2			
Acidic Extract	2.2	2.3	2.2	2.9	3.1	3.0	3.8	3.1	3.4			
Total Ext.	63.6	66	64.8	69	69.7	69.3	72.4	70.8	71.6			
Sediment-bound	2.8	2.9	2.9	4.3	4.0	4.2	5.0	5.2	5.1			
Volatiles ( $^{14}\text{CO}_2$ )	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4			
Total balance	97.2	99.9	98.6	98	97.5	97.7	99.3	99.2	99.2			

NA: not analysed

**Table B.8.2.2.3.1-06 Summary of the mass balance data for the Golden Lake PY-label as percent of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	81.9	79.1	80.5	52.5	51.2	51.8	35.6	40.7	38.1	33.4	33.3	33.4
Neutral Extract	18.4	19.8	19.1	46.6	46.5	46.6	61.7	56.9	59.3	63.5	62.1	62.8
Acidic Extract	0.0	0.0	0.0	0.8	0.7	0.7	1.0	1.0	1.0	1.7	1.6	1.6
Total Ext.	18.4	19.8	19.1	47.4	47.2	47.3	62.7	57.9	60.3	65.2	63.6	64.4
Sediment-bound	0.0	0.0	0.0	0.5	0.4	0.4	1.0	1.0	1.0	2.2	2.0	2.1
Volatiles ( <sup>14</sup> CO <sub>2</sub> )	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
Total balance	100.4	98.9	99.6	100.3	98.8	99.6	99.4	99.7	99.5	100.8	99.0	99.9
Fraction	Days After Treatment (DAT)											
	30			63			112					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Water Phase	30.8	33.6	32.2	22.5	23.1	22.8	22.5	21.3	21.9			
Neutral Extract	64.0	59.8	61.9	69.6	67.8	68.7	66.7	66.1	66.4			
Acidic Extract	2.1	1.9	2.0	3.5	3.1	3.3	3.4	3.7	3.5			
Total Ext.	66.1	61.7	63.9	73.1	70.9	72.0	70.1	69.9	70			
Sediment-bound	2.7	2.8	2.8	3.6	3.6	3.6	5.9	5.6	5.7			
Volatiles ( <sup>14</sup> CO <sub>2</sub> )	0.1	0.1	0.1	0.2	0.2	0.2	0.4	0.4	0.4			
Total balance	99.8	98.3	99.0	99.4	97.7	98.6	98.9	97.2	98.0			

NA: not analysed

**Table B.8.2.2.3.1-07 Summary of the mass balance data for the Taunton River PH-label as percent of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	67.7	63.3	65.5	40.5	36.3	38.4	31.9	31.2	31.6	24.0	21.9	22.9
Neutral Extract	30.8	33.3	32.0	55.8	60.9	58.3	65.3	68.1	66.7	73.7	77.4	75.5
Acidic Extract	0.1	0.1	0.1	0.6	0.7	0.7	0.6	0.6	0.6	0.8	1.2	1.0
Total Ext.	30.9	33.3	32.1	56.4	61.6	59.0	65.9	68.7	67.3	74.5	78.5	76.5
Sediment-bound	0.1	0.1	0.1	0.3	0.3	0.3	0.5	0.4	0.5	0.6	0.9	0.8
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
Total balance	98.7	96.7	97.7	97.2	98.2	97.7	98.3	100.5	99.4	99.2	101.4	100.3
Fraction	Days After Treatment (DAT)											
	30			63			112					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Water Phase	17.1	15.8	16.5	8.0	10.0	9.0	6.1	6.3	6.2			
Neutral Extract	78.9	81.2	80.1	85.4	83.2	84.3	86.6	85.9	86.2			
Acidic Extract	1.2	1.2	1.2	2.7	2.5	2.6	4.1	3.6	3.9			
Total Ext.	80.1	82.4	81.2	88.1	85.7	86.9	90.6	89.5	90.1			
Sediment-bound	1.1	1.0	1.0	2.1	2.5	2.3	2.8	2.5	2.6			
Volatiles ( $^{14}\text{CO}_2$ )	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2			
Total balance	98.4	99.4	98.9	98.4	98.4	98.4	99.5	98.3	98.9			

NA: not analysed

**Table B.8.2.2.3.1-08 Summary of the mass balance data for the Taunton River PY-label as percent of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	65.3	74.3	69.8	36.1	36.5	36.3	32.3	35.2	33.8	28.1	26.6	27.4
Neutral Extract	34.1	22.8	28.4	61.2	59.3	60.3	68.4	63.4	65.9	70.8	73.1	72.0
Acidic Extract	0.0	0.0	0.0	0.5	0.4	0.4	0.4	0.4	0.4	0.7	0.6	0.7
Total Ext.	34.1	22.8	28.4	61.7	59.7	60.7	68.8	63.8	66.3	71.5	73.8	72.6
Sediment-bound	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.2	0.4	0.4	0.4
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total balance	99.3	97.1	98.2	97.9	96.3	97.1	101.4	99.2	100.3	100.0	100.8	100.4
Fraction	Days After Treatment (DAT)											
	30			63			112					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Water Phase	21.5	20.3	20.9	11.7	11.2	11.5	6.8	6.3	6.5			
Neutral Extract	75.8	76.1	76.0	84.7	84.2	84.4	89.9	86.0	88.0			
Acidic Extract	1.0	1.1	1.0	2.1	2.0	2.0	4.3	4.1	4.2			
Total Ext.	76.8	77.2	77.0	86.7	86.1	86.4	94.2	90.1	92.1			
Sediment-bound	0.7	0.8	0.7	1.5	1.4	1.4	2.8	2.5	2.7			
Volatiles ( $^{14}\text{CO}_2$ )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Total balance	99.1	98.3	98.7	100.0	98.7	99.4	103.8	98.9	101.4			

NA: not analysed



**Table B.8.2.2.3.1-09 Radioactivity distribution from the water and sediment of Golden Lake PH-label total activity as percent of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	86.3	85.5	85.9	48.6	47.0	47.8	34.2	36.2	35.2	28.9	31.4	30.2
Inpyrfluxam (sediment)	12.3	13.1	12.7	43.2	45.9	44.5	57.3	56.5	56.9	60.0	60.2	60.1
Inpyrfluxam (total)	98.6	98.6	98.6	91.8	92.9	92.3	91.5	92.7	92.1	88.9	91.6	90.3
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.8	1.6	1.2	2.8	2.1	2.5
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	2.2	1.3	1.7	1.0	0.9	1.0	0.7	0.9	0.8	0.8	0.7	0.7
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	0.8	0.6	0.7	1.1	1.0	1.0	0.0	0.0	0.0
N-des-Me-S-2840 (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others (Total)*	0.8	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total*	101.6	99.9	100.7	93.5	94.5	93.5	94.1	96.2	95.1	92.5	94.4	93.4

Table B.8.2.2.3.1-09 continued									
Fraction	Days After Treatment (DAT)								
	30			63			112		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	25.1	27.4	26.2	17.4	15.9	16.7	12.3	12.5	12.4
Inpyrfluxam (sediment)	58.7	60.9	59.8	57.7	44.6	51.2	63.7	60.3	62.0
Inpyrfluxam (total)	83.8	88.3	86	75.1	60.5	67.9	76	72.8	74.4
1'-COOH-S-2840 total** (water)	3.0	1.5	2.2	6.4	5.3	5.9	7.9	9.6	8.8
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	2.1	7.5	4.8	2.6	3.9	3.2
3'-OH-S-2840 (water)	0.9	0.6	0.7	0.6	0.5	0.6	0.6	0.5	0.5
3'-OH-S-2840 (sediment)	2.8	2.8	2.8	3.7	6.3	5.0	2.4	2.8	2.6
N-des-Me-S-2840 (sediment)	0.0	0.0	0.0	0.0	4.1	2.1	0.0	0.7	0.3
Others (Total) *	0.0	0.0	0.0	2.5	4.0	3.3	0.0	0.0	0.0

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

\* Includes 1'-CH<sub>2</sub>OH-S-2840 and unknowns, none of which individually exceeded 4% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

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HSE notes that the Golden Lake PH-label total AR recovery falls below the OECD 308 guideline of 90 – 110 % for 63 days and 112 days. HSE does not consider this to void the validity of the study, as the values are marginally below the 90 % guideline, at 89.5 % (63 DAT) and 89.9 % (112 DAT).

**Table B.8.2.2.3.1-10 Radioactivity distribution from the water and sediment of Golden Lake PY-label total activity as percent of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	79.0	76.0	77.5	39.7	52.8	46.3	33.1	35.9	34.5	29.4	26.3	27.9
Inpyrfluxam (sediment)	18.4	19.8	19.1	45.3	45.2	45.2	58.8	55.1	56.9	60.1	62.1	61.1
Inpyrfluxam (total)	97.4	95.8	96.6	85	98	91.5	91.9	91	91.4	89.5	88.4	89
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.9	1.1	1.0	1.1	1.6	1.3	1.2	4.3	2.7
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	2.9	1.4	2.2	1.5	2.0	1.8	1.6	1.8	1.7	1.4	1.6	1.5
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	1.3	1.3	1.3	2.9	1.9	2.4	3.3	0.0	1.7
N-des-Me-S-2840 (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others (Total) *	0.0	1.7	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total*	100.4	98.8	99.6	88.7	102.5	95.6	97.5	96.2	96.8	95.4	94.3	94.9

Table B.8.2.2.3.1-10 continued

Fraction	Days After Treatment (DAT)								
	30			63			112		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	20.9	26.6	23.7	14.5	15.3	14.9	11.4	10.5	10.9
Inpyrfluxam (sediment)	57.0	53.0	55.0	57.0	52.8	54.9	58.5	59.2	58.9
Inpyrfluxam (total)	77.9	79.6	78.7	71.5	68.1	69.8	69.9	69.7	69.8
1'-COOH-S-2840 total** (water)	4.4	4.0	4.2	5.5	5.0	5.3	10.3	9.6	10.0
1'-COOH-S-2840 total** (sediment)	2.8	2.6	2.7	2.5	3.5	3.0	3.3	2.7	3.1
3'-OH-S-2840 (water)	0.7	1.2	1.0	0.8	0.6	0.7	0.5	0.6	0.6
3'-OH-S-2840 (sediment)	4.1	4.2	4.2	5.1	4.8	4.9	3.6	3.6	3.6
N-des-Me-S-2840 (sediment)	0.0	0.0	0.0	0.0	4.6	2.3	1.2	0.6	0.9
Others (Total) *	0.0	0.0	0.0	6.5	3.2	4.8	0.0	0.4	0.2
Total*	90.0	91.5	90.8	92.0	89.7	90.8	88.8	87.3	88.0

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

\* Includes 1'-CH<sub>2</sub>OH-S-2840 and unknowns, none of which individually exceeded 2.6% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.2.3.1-11 Radioactivity distribution from the water and sediment of Taunton River PH-label total activity as percent of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	66.1	61.6	63.9	39.4	36.1	37.8	28.9	28.6	28.8	22.2	18.6	20.4
Inpyrfluxam (sediment)	30.8	33.3	32.0	55.8	60.0	57.9	64.3	67.0	65.7	73.7	75.5	74.6
Inpyrfluxam (total)	96.9	94.9	95.9	95.2	96.1	95.7	93.2	95.6	94.5	95.9	94.1	95
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.9	1.6
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	1.5	1.7	1.6	1.0	0.9	1.0	1.2	1.1	1.1	0.5	0.5	0.5
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	0.0	0.9	0.4	1.0	1.1	1.0	0.0	1.9	1.0
N-des-Me-S-2840 (Total)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others (Total) *	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total*	98.5	96.6	97.5	96.2	97.9	97.0	95.4	97.8	96.6	97.7	98.4	98.0

Table B.8.2.2.3.1-11 continued									
Fraction	Days After Treatment (DAT)								
	30			63			112		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	15.9	14.0	14.9	7.3	7.7	7.5	5.1	5.7	5.4
Inpyrfluxam (sediment)	69.1	77.3	73.2	72.6	72.0	72.3	81.6	82.8	82.2
Inpyrfluxam (total)	85	91.3	88.1	79.9	79.7	79.8	86.7	88.5	87.6
1'-COOH-S-2840 total** (water)	1.2	0.9	1.0	0.8	2.3	1.6	0.6	0.6	0.7
1'-COOH-S-2840 total** (sediment)	3.3	0.0	1.6	3.8	4.1	4.0	2.2	1.0	1.6
3'-OH-S-2840 (water)	0.0	0.6	0.3	0.0	0.1	0.1	0.4	0.2	0.3
3'-OH-S-2840 (sediment)	4.5	3.9	4.2	6.0	4.8	5.4	2.7	2.1	2.4
N-des-Me-S-2840 (Total)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others (Total) *	2.1	0.0	1.0	3.0	2.3	2.6	0.0	0.0	0.0
Total*	95.9	96.7	96.3	93.5	93.3	93.4	92.7	92.3	92.5

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

\* Includes ATMI, which never exceeded 3.0% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.2.2.3.1-12 Radioactivity distribution from the water and sediment of Taunton River PY-label total activity as percent of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			3			7			14		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	62.7	71.2	66.9	32.9	33.1	33.0	23.9	34.3	29.1	23.3	23.1	23.2
Inpyrfluxam (sediment)	34.1	22.8	28.4	59.7	57.7	58.7	65.5	61.3	63.4	67.2	71.8	69.5
Inpyrfluxam (total)	96.8	94	95.3	92.6	90.8	91.7	89.4	95.6	92.5	90.5	94.9	92.7
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	2.6	3.2	2.9	1.8	1.7	1.8	0.7	1.0	0.8	1.9	1.6	1.8
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	1.5	1.6	1.6	2.9	2.0	2.5	3.6	1.3	2.4
N-des-Me-S-2840 (Total)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others (Total) *	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.8	0.6	2.1	1.0	1.5
Total*	99.3	97.1	98.2	96.0	94.2	95.1	93.5	99.4	96.5	98.1	98.8	98.5



Table B.8.2.2.3.1-12 continued									
Fraction	Days After Treatment (DAT)								
	30			63			112		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	19.1	13.6	16.4	10.7	9.1	9.9	5.7	5.5	5.6
Inpyrfluxam (sediment)	67.7	65.2	66.5	76.6	78.0	77.3	86.3	82.8	84.6
Inpyrfluxam (total)	86.8	78.8	82.9	87.3	87.1	87.2	92	88.3	90.2
1'-COOH-S-2840 total** (water)	0.3	1.6	0.9	0.0	0.6	0.3	0.0	0.0	0.0
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.6	0.9
3'-OH-S-2840 (water)	0.8	0.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (sediment)	6.0	6.0	6.0	7.0	4.3	5.7	2.4	2.6	2.5
N-des-Me-S-2840 (Total)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others (Total) *	3.0	6.3	4.7	2.0	2.8	2.3	0.9	0.9	0.9
Total*	96.9	93.6	95.2	96.3	94.8	95.5	96.5	92.4	94.5

Chromatographically, 1'-CH<sub>2</sub>OH-S-2840A, 1'-COOH-S-2840A, 1'-CH<sub>2</sub>OH-S-2840B and 1'-COOH-S-2840B elute close together between ~32-34 minutes by HPLC and tentative assignments have been made. A definitive assignment was made by 2D-TLC analysis.

\* Includes DFPA-CONH<sub>2</sub>, DFPA and total unknowns which never individually exceeded 3.8% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.2.3.1-13 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Golden Lake, phenyl label)**

DAT	0			3			7		
Sample No.	1	2	Avg.	9	11	Avg.	7	15	Avg.
pH (water phase)	7.74	7.82	7.78	7.97	8.15	8.06	8.16	8.25	8.21
pH (sediment phase)	7.35	7.38	7.37	7.55	7.68	7.62	7.48	7.65	7.57
Dissolved Oxygen (ppm)	8.33	8.28	8.31	7.65	7.9	7.78	7.46	7.63	7.55
Redox potential (mV water phase)	185	175	180	10	20	15	110	27	69
Redox potential (mV sediment phase)	-210	-235	-223	-176	-196	-186	-193	-203	-198
DAT	14			30					
Sample No.	10	12	Avg.	5	14	Avg.			
pH (water phase)	8.3	8.23	8.27	8.01	8.15	8.08			
pH (sediment phase)	7.69	7.57	7.63	7.45	7.49	7.47			
Dissolved Oxygen (ppm)	7.83	8	7.92	8.4	8.53	8.47			
Redox potential (mV water phase)	76	29	53	183	96	140			
Redox potential (mV sediment phase)	-264	-218	-241	-251	-173	-212			
DAT	63			112					
Sample No.	8	18	Avg.	17	20	Avg.			
pH (water phase)	7.13	7.47	7.30	8.11	8.1	8.11			
pH (sediment phase)	7.34	7.33	7.34	7.68	7.76	7.72			
Dissolved Oxygen (ppm)	8.88	8.98	8.93	8.98	8.87	8.93			
Redox potential (mV water phase)	155	257	206	163	244	204			
Redox potential (mV sediment phase)	-5	-23	-14	109	158	134			

**Table B.8.2.3.1-14 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Golden Lake, pyrazolyl label)**

DAT	0			3			7		
Sample No.	24	25	Avg.	39	43	Avg.	30	38	Avg.
pH (water phase)	7.71	7.64	7.68	8.2	8.21	8.21	8.2	8.17	8.17
pH (sediment phase)	7.37	7.39	7.38	7.67	7.54	7.61	7.76	7.84	7.80
Dissolved Oxygen (ppm)	8.23	8.36	8.30	7.16	7.52	7.34	7.42	7.03	7.23
Redox potential (mV water phase)	124	190	157	29	40	35	29	33	31
Redox potential (mV sediment phase)	-200	-150	-175	-246	-225	-236	-241	-222	-232
DAT	14			30					
Sample No.	29	35	Avg.	32	41	Avg.			
pH (water phase)	8.12	8.24	8.18	7.99	8.27	8.13			
pH (sediment phase)	7.81	7.85	7.83	7.59	7.7	7.65			
Dissolved Oxygen (ppm)	7.89	5.23	6.56	7.3	8.65	7.98			
Redox potential (mV water phase)	47	7	27	130	95	113			
Redox potential (mV sediment phase)	-219	-235	-227	-268	-284	-276			
DAT	63			112					
Sample No.	31	34	Avg.	27	33	Avg.			
pH (water phase)	7.31	7.23	7.27	8.27	8.14	8.21			
pH (sediment phase)	7.34	7.26	7.30	7.64	7.65	7.65			
Dissolved Oxygen (ppm)	8.84	8.89	8.87	9.07	9.03	9.05			
Redox potential (mV water phase)	271	268	270	248	252	250			
Redox potential (mV sediment phase)	73	-8	33	166	179	173			

**Table B.8.2.3.1-15 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Taunton River, phenyl label)**

DAT	0			3			7		
Sample No.	47	48	Avg.	60	65	Avg.	55	63	Avg.
pH (water phase)	7.04	6.70	6.87	7.89	7.94	7.92	8.12	8.08	8.10
pH (sediment phase)	6.74	6.68	6.71	6.78	6.77	6.78	6.5	6.57	6.54
Dissolved Oxygen (ppm)	8.31	8.25	8.28	3.75	2.38	3.07	4.33	2.81	3.57
Redox potential (mV water phase)	195	161	178	-25	-174	-100	4	35	20
Redox potential (mV sediment phase)	-133	-133	-133	-145	-225	-185	-176	-207	-192
DAT	14			30					
Sample No.	58	64	Avg.	51	57	Avg.			
pH (water phase)	8.4	8.42	8.41	5.94	5.93	5.94			
pH (sediment phase)	6.95	6.93	6.94	7.06	7.04	7.05			
Dissolved Oxygen (ppm)	8.11	8.17	8.14	8.68	8.47	8.58			
Redox potential (mV water phase)	31	25	28	175	206	191			
Redox potential (mV sediment phase)	-168	-171	-170	-172	-133	-153			
DAT	63			112					
Sample No.	56	62	Avg.	53	59	Avg.			
pH (water phase)	4.24	4.36	4.30	3.99	3.81	3.90			
pH (sediment phase)	6.72	7.05	6.89	6.51	6.55	6.53			
Dissolved Oxygen (ppm)	8.77	8.67	8.72	9.15	8.88	9.02			
Redox potential (mV water phase)	388	270	329	483	330	407			
Redox potential (mV sediment phase)	-123	-134	-129	-75	-114	-95			

**Table B.8.2.2.3.1-16 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Taunton River, pyrazolyl label)**

DAT	0			3			7		
Sample No.	70	71	Avg.	84	88	Avg.	85	86	Avg.
pH (water phase)	7.22	7.04	7.13	8.05	7.88	7.97	8.44	8.38	8.41
pH (sediment phase)	6.80	6.79	6.80	6.83	6.76	6.80	6.66	6.55	6.61
Dissolved Oxygen (ppm)	8.24	8.14	8.19	2.9	3.19	3.05	1.32	4.15	2.74
Redox potential (mV water phase)	205	176	191	-189	-199	-194	-182	-66	-124
Redox potential (mV sediment phase)	-144	-302	-223	-187	-197	-192	-195	-203	-199
DAT	14			30					
Sample No.	72	76	Avg.	75	80	Avg.			
pH (water phase)	8.74	8.63	8.69	5.85	5.84	5.85			
pH (sediment phase)	6.97	6.81	6.89	7.5	7.38	7.44			
Dissolved Oxygen (ppm)	7.67	8.24	7.96	8.05	7.93	7.99			
Redox potential (mV water phase)	20	29	25	168	154	161			
Redox potential (mV sediment phase)	-207	-192	-200	-188	-143	-166			
DAT	63			112					
Sample No.	73	82	Avg.	77	87	Avg.			
pH (water phase)	4.74	4.63	4.69	3.44	3.63	3.54			
pH (sediment phase)	7.17	7.26	7.22	6.5	6.76	6.63			
Dissolved Oxygen (ppm)	8.72	8.58	8.65	8.72	8.66	8.69			
Redox potential (mV water phase)	259	263	261	280	292	286			
Redox potential (mV sediment phase)	-136	-140	-138	-43	-80	-62			

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### III. Mass balance

The material balance in the test vessels ranged from 97.2% to 101.6% AR (phenyl label, Golden Lake), 96.7% to 101.4% AR (phenyl label, Taunton River), 97.2% to 100.8%, (pyrazolyl label, Golden Lake), 97.1% to 103.8% AR (pyrazolyl label, Taunton River). Average material balance for the study was  $98.6 \pm 1.4\%$  AR (phenyl-label) and  $99.2 \pm 1.0\%$  AR (pyrazolyl-label) for the Golden Lake system, and  $98.8 \pm 1.2\%$  AR (phenyl-label) and  $99.4 \pm 1.9\%$  AR (pyrazolyl-label) for the Taunton River system. Results of the distribution of radioactivity are presented in Table B.8.2.2.3.1-05 to Table B.8.2.2.3.1-08.

### IV. Bound residues

The radioactivity remaining in sediment following the neutral and acidic extractions, post extraction sediment (PES) or sediment-bound radioactivity, considered minor, reaching 6% of the AR (Golden Lake) and 3% of the AR (Taunton River) by the end of the study (112 DAT). Additional extractions of the Golden Lake PES extracted low amounts of activity from the sediment (~2% AR). No further analysis was performed on the PES.

### V. Volatilisation

The cumulative production of  $^{14}\text{CO}_2$  was insignificant reaching a maximum of 0.4% AR at 112 DAT in the Golden Lake system.

### VI. Metabolites

Two metabolites were observed above 5% AR: 3'-OH-S-2840 (max. 6.8% AR), and 1'-COOH-S-2840, (max. 13.1% AR). N-demethylation of the pyrazolyl ring to produce N-des-Me-S-2840 was minor as well as hydrolysis of the amide bond to produce the pyrazolyl derivatives DFPA and DFPA-CONH<sub>2</sub>.

Generally, all metabolites were observed to be declining in both sediments by the end of the study (112 DAT) except 1'-COOH-S-2840 in the Golden Lake sediment.

The aerobic aquatic metabolism degradation pathway of inpyrfluxam is summarized in Figure B.8.2.2.3.1-01.

#### *Enantiomeric ratio changes - 1'-COOH-S-2840A and B*

The amounts of isomers A and B were recorded at each timepoint in all 3 water-sediment systems. Changes in enantiomeric excess of 1'-COOH-S-2840 in the total systems were considered by HSE in accordance with the principles outlined in the EFSA Guidance document, 'Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers' (2019) and the 'GB Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers'.

While the applicant stated that the 1'methyl group was oxidized preferentially to the 1'-COOH-S-2840B isomer versus the 1'-COOH-S-2840A isomer, the ratio between the isomers remained approximately racemic throughout the study and any shifts observed were less than the 10 % change specified in the EFSA guidance document. See Table B.8.2.2.3.1-17 for details.

**Table B.8.2.2.3.1-17 Changes in enantiomeric excess of 1'-COOH-S-2840A and B**

	<b>1'-COOH-S-2840A (% AR)</b>	<b>1'-COOH-S-2840B (% AR)</b>	<b>Enantiomeric excess</b>	<b>Change in enantiomeric excess</b>
<b>Golden Lake PH (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	-
<b>3</b>	0	0	0	0
<b>7</b>	0.4	0.8	-0.4	-0.4
<b>14</b>	1.2	1.3	-0.1	0.3
<b>30</b>	1	1.2	-0.2	-0.1
<b>62</b>	4.2	6.5	-2.3	-2.1
<b>112</b>	3.7	8.3	-4.6	-2.3
<b>Golden Lake PY (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	-
<b>3</b>	0.6	0.4	0.2	0.2
<b>7</b>	0.5	0.8	-0.3	-0.5
<b>14</b>	1.1	1.6	-0.5	-0.2
<b>30</b>	2.9	4	-1.1	-0.6
<b>62</b>	2.8	5.5	-2.7	-1.6
<b>112</b>	4.3	8.6	-4.3	-1.6
<b>Taunton River PH (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	NA
<b>3</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>14</b>	0.6	1	-0.4	-0.4
<b>30</b>	0.7	1.9	-1.2	-0.8
<b>62</b>	1.6	3.9	-2.3	-1.1
<b>112</b>	0.6	1.6	-1	1.3
<b>Taunton River PY (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	NA
<b>3</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>14</b>	0	0	0	0
<b>30</b>	0	0.9	-0.9	-0.9
<b>62</b>	0	0.3	-0.3	0.6
<b>112</b>	0.3	0.6	-0.3	0

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## KINETIC ASSESSMENT

The applicant stated that the kinetic evaluation conducted within this study did not meet the current guidance and as such, a kinetic re-evaluation of the data was conducted.

The data from this study was used to determine the degradation/ dissipation half-lives of inpyrfluxam in water/sediment. The data was analysed by the applicant using the CAKE v3.7 (2023) software package according to guidance provided by FOCUS (2014) based on level P-1 kinetics (single compartment kinetics). As part of the independent validation, HSE has repeated the applicant's modelling using KinGUI v2.1 (2014) software package.

HSE notes that the applicant did not add the percentage of active substance found in the sediment at 0 days back to the water phase day 0 value, as recommended by FOCUS guidance. This led to differences between the water phase dissipation ( $\text{DissT}_{50}$  and  $\text{DissT}_{90}$ ) values calculated by HSE and the applicant, albeit the applicant's kinetic fittings led to a more conservative estimate of  $\text{DissT}_{50}$  and  $\text{DissT}_{90}$ . Nevertheless, as a result of these differences in data handling, the kinetic fittings for the water compartments come from the independent HSE assessment.

### Materials and methods

The degradation of inpyrfluxam in water/ sediment systems was performed at  $20 \pm 2$  °C, thus normalisation was not required. The data from these studies were sufficient to allow for the calculation of kinetic endpoints for inpyrfluxam, in combination with data from a separate water-sediment study in B.8.2.2.2.3.2 (KCA 7.2.2.3/02).

The results obtained with the two differently labelled test items were pooled and regarded as true replicates for kinetic evaluation in each test system. Total system  $\text{DT}_{50}$  and  $\text{DT}_{90}$  values of inpyrfluxam were calculated for comparison with relevant study triggers and persistence criteria, and water phase  $\text{DissT}_{50}$  and  $\text{DissT}_{90}$  values calculated for use as modelling endpoints. There was no significant decline of detected metabolites 1'-COOH-S-2840, 3'-OH-S-2840, DFPA and DFPA-CONH<sub>2</sub> observed within the study, so a kinetic analysis of these metabolites was not performed.

At Level P-I, persistence endpoints were derived from the kinetic models that provided the best fit to the measured total system data. The goodness-of-fit was evaluated by visual assessment,  $\chi^2$  minimum error, and type-I-error rate (t-test). Modelling endpoints were derived preferably from the SFO model fitted to water data only.

In the first instance, the data were directly fitted, un-weighted, with the complete usable data set and unconstrained initial concentration ( $M_0$ ). The acceptability of



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kinetic fits was judged both visually and according to the  $\chi^2$  error and the t-test functions as recommended by FOCUS (2014).

As no clear decline phase is observed in the sediment for inpyrfluxam, a kinetic analysis of this compartment has not been conducted by the applicant. HSE accepts this decision, and proposes the use of 1000 day default for the sediment DissT<sub>50</sub> modelling endpoints. This will be used to account for potential accumulation in sediment in a simple and conservative way as part of the exposure assessment.

The applicant did not provide a kinetic assessment for the metabolites, and HSE considers that such assessments are not typically required for UK first-tier surface water assessments. In these assessments, maximum initial PEC<sub>sw</sub> or a total dose approach (for GAPs with multiple applications) are usually applied. The main rationale for performing a kinetic assessment of metabolites would be to rule out the potential for accumulation in the sediment. However in this case, there was no significant decline in the major metabolites 1'-COOH-S-2840 (A and B isomers combined) and 3'-OH-S-2840 in the sediment. Specifically, 1'-COOH-S-2840 (A and B) showed evidence of persistence in both water and sediment, with concentrations either increasing or failing to show a significant decline across all five water-sediment systems. In the water phase, 1'-COOH-S-2840 peaked at 10.0% at study end (Golden Lake, PY label, 112 DAT), while in the sediment phase it peaked at 4.8% at 63 DAT then declined to 3.2% at study end (112 DAT), though this decline only occurred in 1 out of the 5 sediment systems. It showed a lack of decline in the other 4 sediments.

Similarly, for 3'-OH-S-2840, while it showed a slow decline in the water phase (albeit <5%), it exhibited a lack of significant decline in the sediment. In the water compartment, it peaked at 2.9% at 0 DAT (Taunton River PY label) and declined slowly to 0.3% at study end (112 DAT). In the sediment, it peaked or did not show a decline in 3 out of 5 systems, with the maximum occurrence at 6% (Taunton River PY label, 30 DAT). For the remaining 2 systems, the decline phase was only observed at 2 time points, which was insufficient to derive reliable degradation kinetics.

Given the limited degradation observed in both water and sediment phases and the lack of a decline for these metabolites, HSE agrees with the applicant that no reliable DT<sub>50</sub> and DT<sub>90</sub> values can be derived from kinetic modelling, and that use of a conservative default DT<sub>50</sub> of 1000 days for both water and sediment is appropriate for the exposure assessment.

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## Results and discussion

### Modelling endpoints

To derive modelling endpoints, the acceptability of the SFO model fit to the water phase only data is considered in the first instance. For the water systems however, due the high  $X^2$  value and poor visual fit, the SFO model was considered unacceptable. The FOMC, DFOP and HS models were ran and compared against each other, and DFOP provided the best visual and statistical fits for both test systems (indicated by the low  $\chi^2$  value). The DFOP model also provides the greatest potential for incorporation into SFO based exposure models, such as the GB spray drift calculators. Therefore, this model was used to derive the modelling endpoints.

The modelling  $DT_{50}$  can be back calculated from the  $DT_{90}$  by dividing the  $DT_{90}$  by 3.32 for the purposes of deriving conservative modelling endpoints; therefore, the appropriate modelling  $DissT_{50}$  is 34.28 days for Golden Lake and 17.38 for Taunton River. Alternatively, where refinement of surface water exposure values is required, full implementation of DFOP kinetics can be applied. All endpoints are included in the tables below.

**Table B.8.2.2.3.1-18 Results of the kinetic analyses to derive parent-only water modelling endpoints for inpyrfluxam in the Golden Lake system (HSE fitting; final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DissT <sub>50</sub> (d)	DissT <sub>90</sub> (d)
Golden Lake Water	SFO	Poor	29.93	M <sub>0</sub> : 88.51 k: 0.11045	- k: <0.05	6.28	20.85
	FOMC	Good	3.567	M <sub>0</sub> : 100.16 $\alpha$ : 0.35346 $\beta$ : 0.40546	N/A	2.48**	273.2
	<b>DFOP</b>	<b>Good</b>	<b>2.433</b>	<b>M<sub>0</sub>: 100.1</b> <b>k<sub>1</sub>: 0.5141</b> <b>k<sub>2</sub>: 0.01084</b> <b>g: 0.6564</b>	- <b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b> -	<b>2.67</b> <b>34.28*</b>	<b>113.8</b>
	HS	Good	3.196	M <sub>0</sub> : 100.2 k <sub>1</sub> : 0.2521	- k <sub>1</sub> : <0.05	2.75 33.13*	110

\*DT<sub>90</sub>/3.32

\*\* Back-calculation could not be undertaken due to the uncertainty of the DT<sub>90</sub> value

SFO: visual fit is poor; the initial measured amount is not well described (>10% residual values). Data points from 14 days after treatment (14 DAT) onwards are underestimated and residuals show clear systematic deviations with 4 consecutive positive residuals. The DissT<sub>50</sub> is overestimated and the DissT<sub>90</sub> is significantly underestimated. Although the t-test is passed, the  $\chi^2$  error is > 15% and supports the overall conclusion on the poor SFO fit.

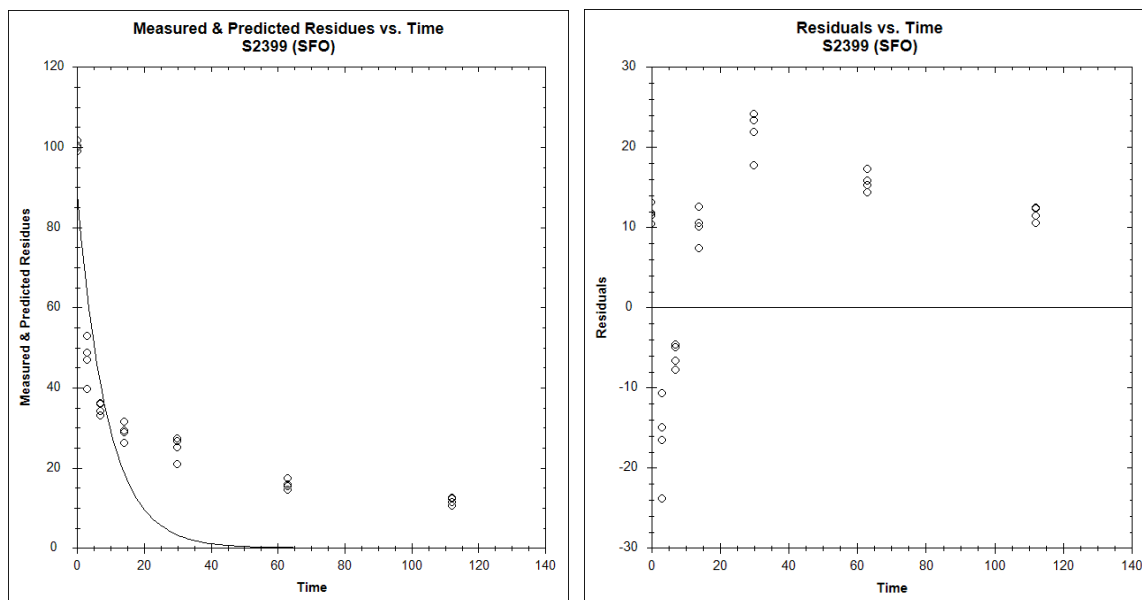
FOMC: Good visual fit & good description of the initial mass.  $\chi^2$  error acceptable. However, dissipation at final time point (112 DAT) has been underestimated.

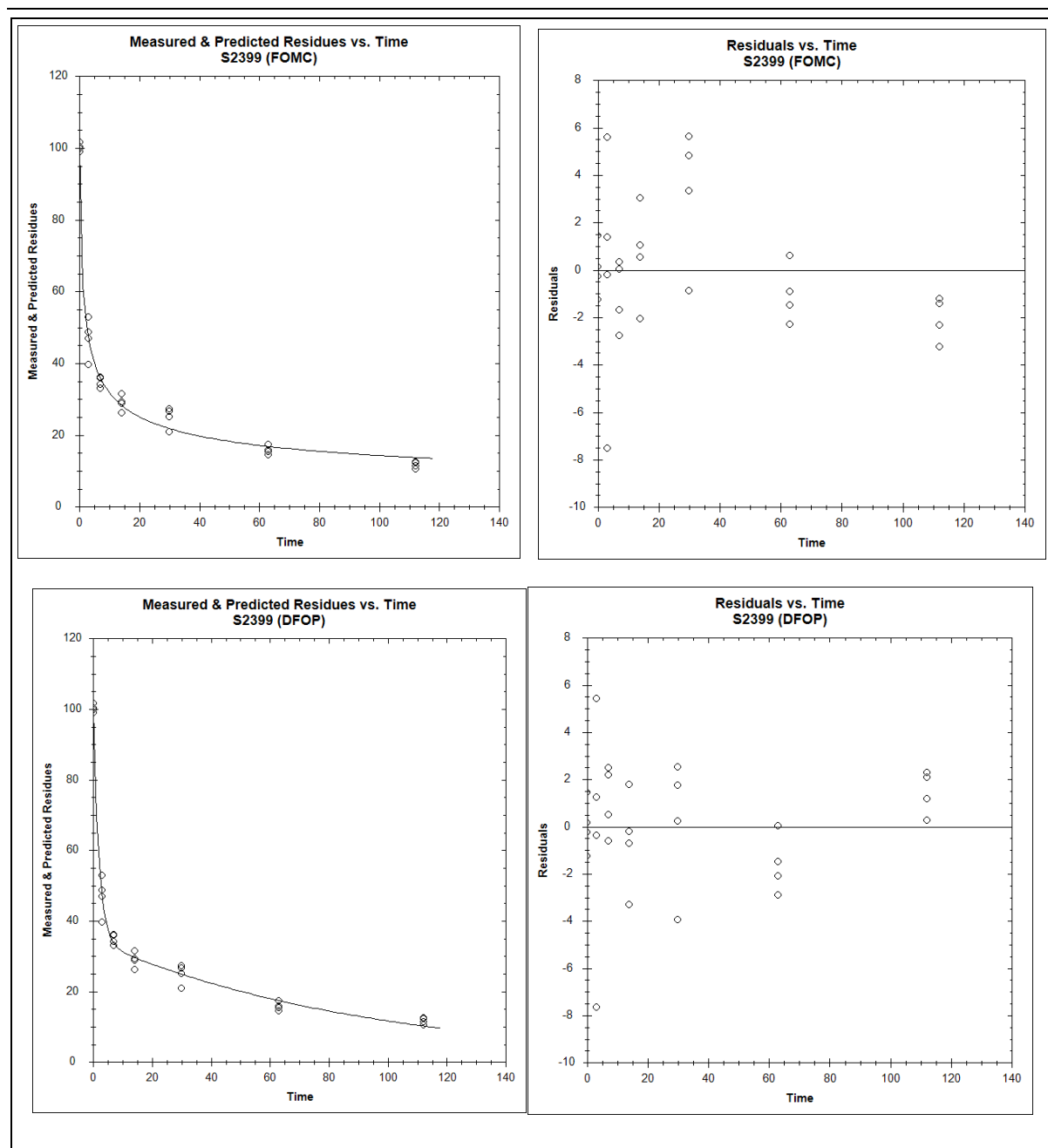
DFOP: Good visual fit, similar to FOMC. The timepoints are well described with an acceptable description of the initial mass, DissT<sub>50</sub> and DissT<sub>90</sub>. No systematic deviation of the residuals.  $\chi^2$  error good and lowest out of all models. t-test passed for k<sub>1</sub> and k<sub>2</sub>.

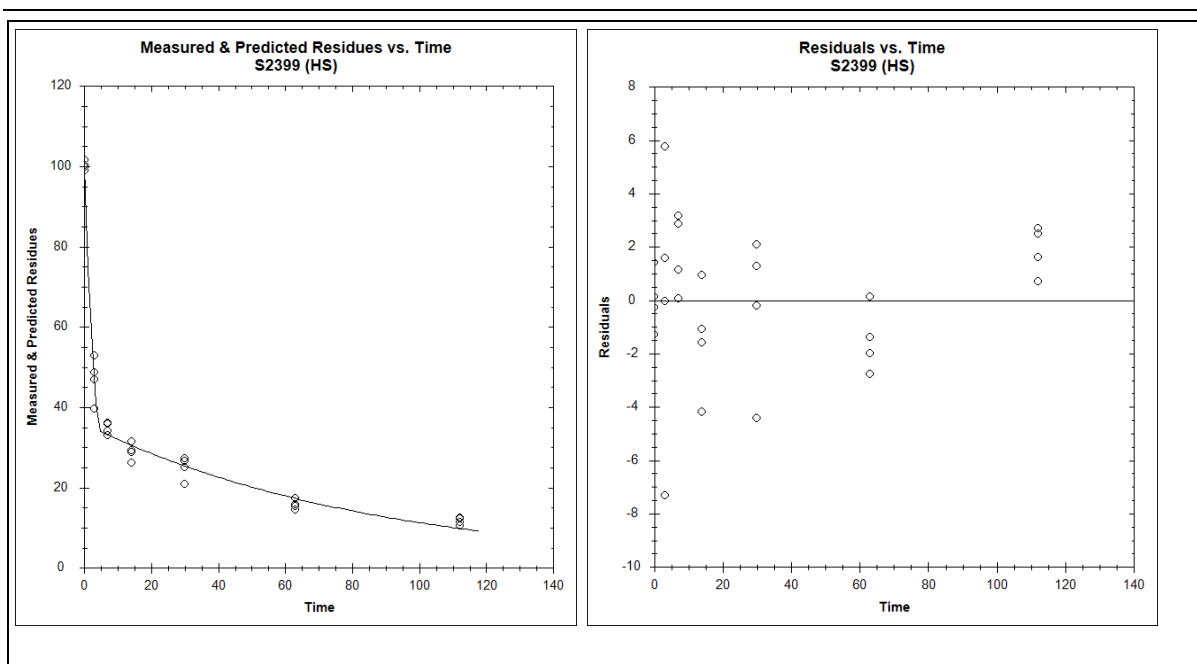
HS: Good visual fit, similar to FOMC and DFOP. The timepoints are well described, acceptable description of the initial mass, DissT<sub>50</sub> and DissT<sub>90</sub>. No systematic deviation of the residuals.  $\chi^2$  error good, t-test passed. However, The HS model does not further improve the visual and statistical fit.

Conclusion: DFOP kinetics selected for modelling endpoints. DissT<sub>50</sub> = 2.67 (Pseudo DissT<sub>50</sub> = 34.28 days), DissT<sub>90</sub> = 113.8.

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**







**Table 8.2.2.3.1-19 Results of the kinetic analyses to derive parent-only water modelling endpoints for inpyrfluxam in the Taunton River system (HSE fitting; final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DissT <sub>50</sub> (d)	DissT <sub>90</sub> (d)
Taunton River Water	SFO	Poor	29.66	M <sub>0</sub> : 91.95 k: 0.1976	- k: <0.05	3.51	11.65
	FOMC	Good	5.275	M <sub>0</sub> : 97.93 $\alpha$ : 0.43541 $\beta$ : 0.37197	N/A	1.46 22.07*	73.28
	DFOP	Good	4.076	<b>M<sub>0</sub>: 97.96</b> <b>k<sub>1</sub>: 0.7645</b> <b>k<sub>2</sub>: 0.0197</b> <b>g: 0.6882</b>	- <b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b> -	<b>1.63</b> <b>17.38*</b>	<b>57.71</b>
	HS	Good	4.26	M <sub>0</sub> : 97.98 k <sub>1</sub> : 0.3396 k <sub>2</sub> : 0.0202 t <sub>b</sub> : 3.6	- k <sub>1</sub> : <0.05 k <sub>2</sub> : <0.05 -	2.04	57.11

**\*DissT<sub>90</sub>/3.32**

SFO: visual fit is poor; the initial measured amount is not well described (>10% residual values). The data points from 14 DAT onwards are underestimated, with residuals showing clear systematic deviations with 5 consecutive positive residuals. The DissT<sub>50</sub> is overestimated and the DissT<sub>90</sub> is significantly underestimated. Although the t-test is passed, the  $\chi^2$  error is > 15% and supports the overall conclusion on the poor SFO fit.

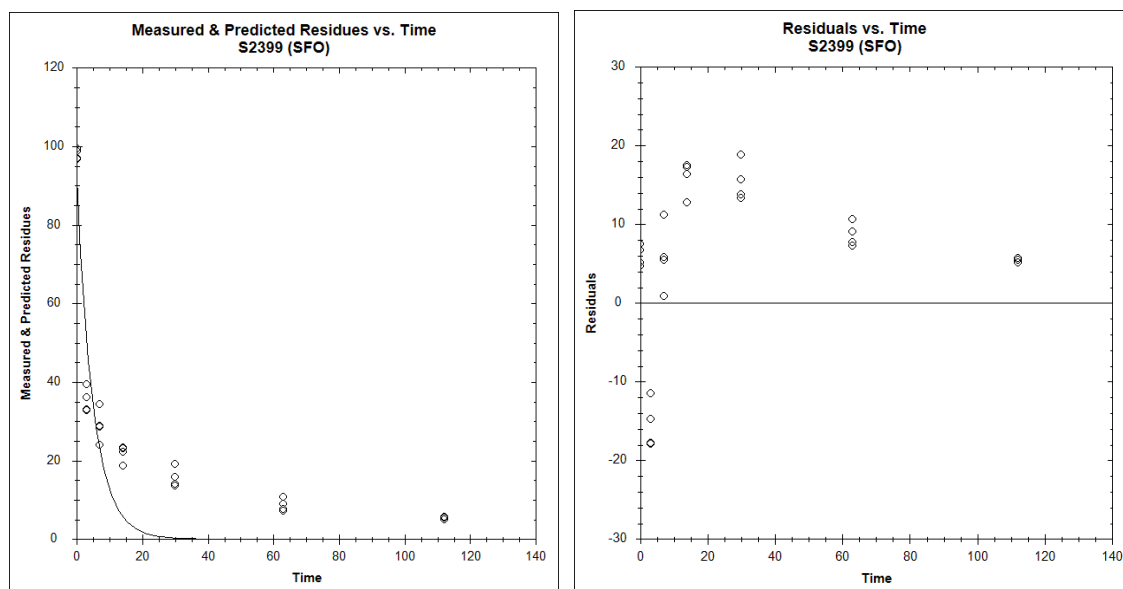
FOMC: Good visual fit & good description of the initial mass.  $\chi^2$  error acceptable. However, dissipation at final time point (112 DAT) has been underestimated.

DFOP: Good visual fit, similar to FOMC. The timepoints are well described with an acceptable description of the initial mass, DissT<sub>50</sub> and DissT<sub>90</sub>. No systematic deviation of the residuals.  $\chi^2$  error lowest out of all models and t-test passed for  $k_1$  and  $k_2$ .

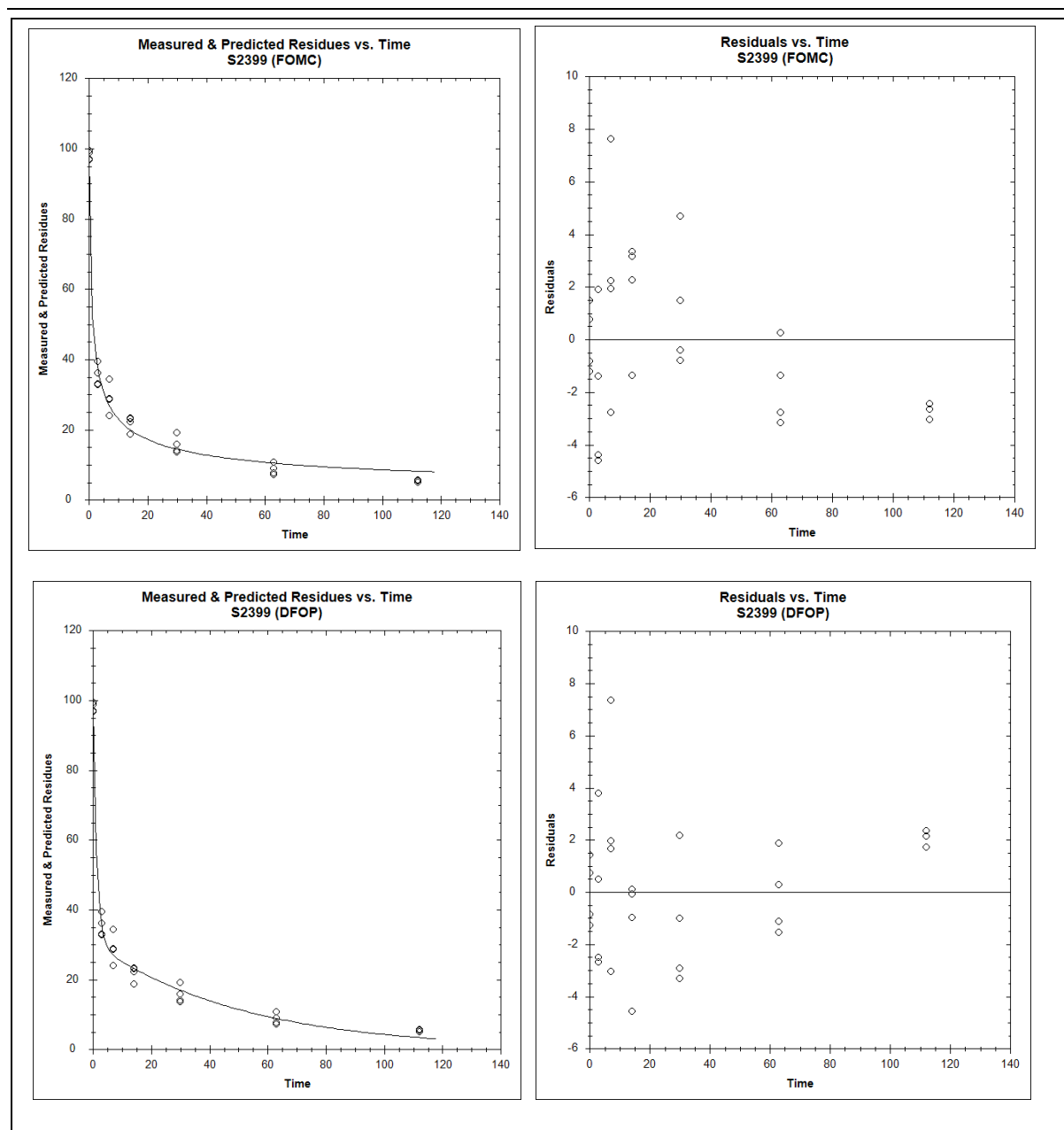
HS: Good visual fit, similar to FOMC and DFOP. The timepoints are well described, with acceptable description of the initial mass, DissT<sub>50</sub> and DissT<sub>90</sub>. No systematic deviation of the residuals.  $\chi^2$  error good, t-test passed. The HS model does not further improve the visual and statistical fit.

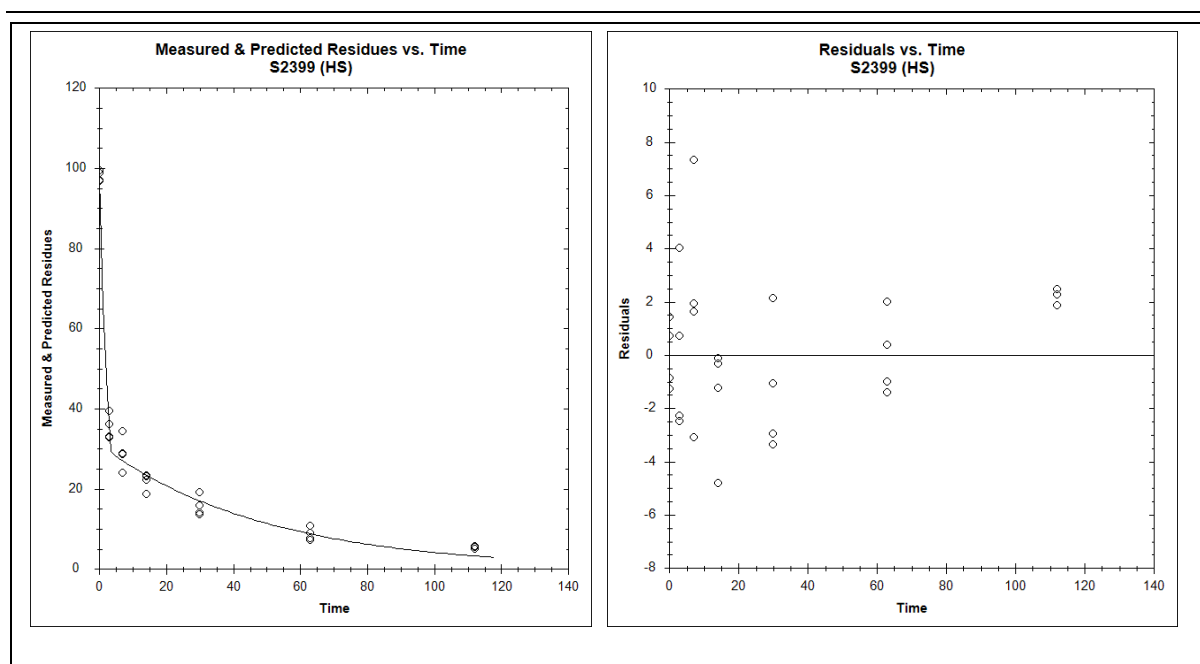
Conclusion: DFOP kinetics selected for modelling endpoints. DissT<sub>50</sub> = 1.63, (Pseudo DissT<sub>50</sub> = 17.38 days), DissT<sub>90</sub> = 57.71.

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**









### Triggering/ persistence endpoints

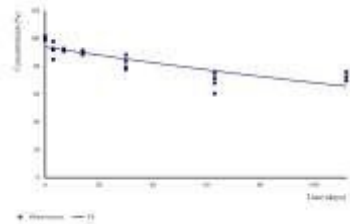
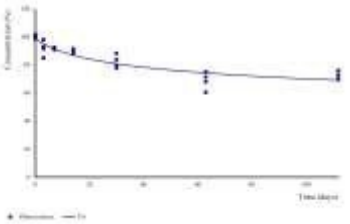
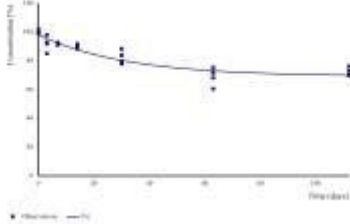
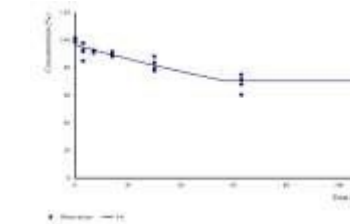

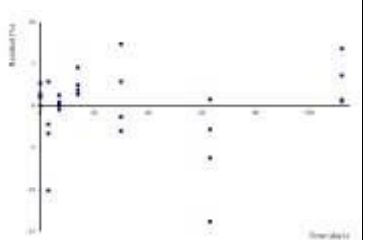
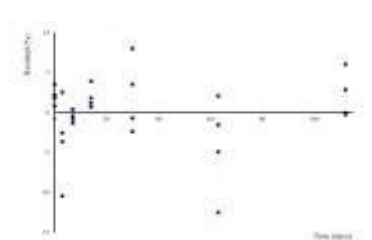
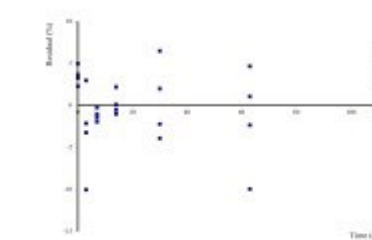
The inpyrfluxam persistence endpoints for evaluation of degradation in the whole system are shown in Tables B.8.2.2.3.1-20 (Golden Lake) to B.8.2.2.3.1-21 (Taunton River).

For the Golden Lake total system, the applicant states that the SFO model provided the best visual and statistical fit for triggering, and they have therefore selected this model to provide the persistence endpoints. The applicant's results are presented in table 8.2.2.3/1-23.

Whilst the  $\chi^2$  error is acceptable (<15%) and the t-test for the k-value is passed, HSE notes that minimal degradation occurred across the total test system, the visual fit is relatively poor, with a poor description of the initial time point ( $M_0$ ). The model also fails to intercept at 62 and 112 DAT, with an under and over-estimation of degradation at these time points respectively. As neither the  $DT_{50}$  nor  $DT_{90}$  are reached within the study period, greater weight may be placed between the differences in measured and modelled data, particularly at the end of the study, suggesting that degradation would be significantly overestimated if the model were extrapolated over a long period. HSE therefore considers that the DFOP model should be used to derive triggering/persistence endpoints for the Golden lake system. While k values fail the t-test, this is expected due to the minimal degradation during the study period.

All three biphasic models tested by the applicant resulted in  $DT_{50}$  values greater than 1000 d and  $DT_{90}$  values > 10,000 d. It is clear that the substance is very persistent in this test system, and no further kinetic fitting was undertaken by HSE.

**Table B.8.2.2.3.1-20 Results of the kinetic analyses to derive parent-only, total-system persistence endpoints for inpyrfluxam in the Golden Lake system as presented by the applicant.**

<b>Kinetic Model:</b>	Parent: SFO	Parent: FOMC	Parent: DFOP	Parent: HS
<b>Visual Fit:</b>	Good	Good	Good	Good
<b>M<sub>0</sub>:</b>	94	98.9	98.1	96.6
<b>Chi-sq error:</b>	4.38	2.95	2.9	2.28
<b>Rate Parameters: value / probability (trigger: 0.05)</b>	k: 0.00319 p < 0.01	α: 0.139 95th %ile CI does not contain 0	k <sub>1</sub> : 0.0318 p = 0.132	k <sub>1</sub> : 0.00555 p < 0.01
		β: 9.27 90th %ile CI contains 0	k <sub>2</sub> : 2.02E-14 p = 0.5	k <sub>2</sub> : 6.56E-12 p = 0.5
<b>g/t<sub>b</sub>:</b>	N/A	N/A	g: 0.295	t <sub>b</sub> : 57
<b>DT<sub>50</sub> (days):</b>	217	1345	>10,000	>10,000
<b>DT<sub>90</sub> (days):</b>	721	>10,000	>10,000	>10,000
<b>Representative DT<sub>50</sub> (days):</b>	217	>10,000	>10,000	>10,000
<b>Fitted vs Observed:</b>				
<b>Residuals:</b>				

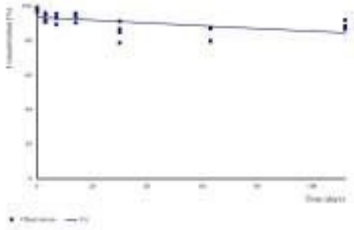
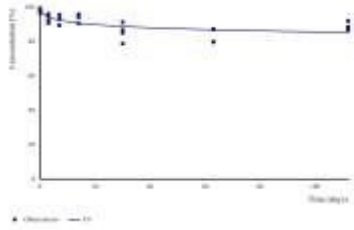
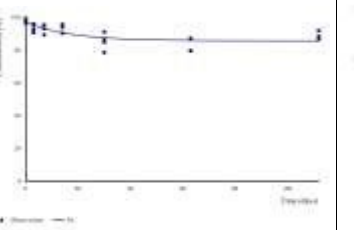
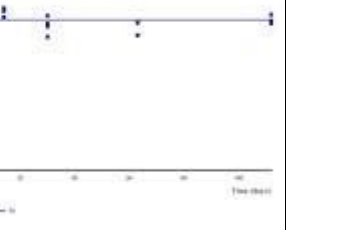
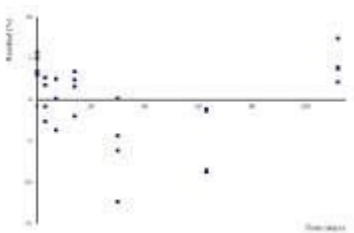

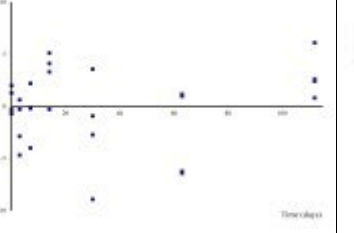
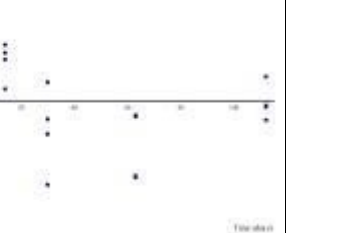
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Similarly to the Golden Lake system, the applicant states that the SFO model provided the best visual and statistical fit for triggering and persistence values from the Taunton River Total system data, therefore this model was selected to provide the persistence endpoints. The applicant's results are presented in table B.8.2.2.3.1-21.

Again however, HSE notes that degradation is limited, the SFO visual fit is relatively poor, with a poor description of the initial time point ( $M_0$ ) and an under and over-estimation of degradation at 62 and 112 DAT respectively.

Biphasic models were considered, with a smaller  $\chi^2$  error moving from SFO to FOMC to DFOP and HS. However, all three biphasic models tested by the applicant resulted in  $DT_{50}$  and  $DT_{90}$  values  $> 10,000$  d. It is clear that the substance is also very persistent in this test system and no further kinetic fitting was undertaken by HSE.

**Table B.8.2.2.3.1-21 Results of the kinetic analyses to derive parent-only, total-system persistence endpoints for inpyrfluxam in the Taunton River system as presented by the applicant.**

<b>Kinetic Model:</b>	Parent: SFO	Parent: FOMC	Parent: DFOP	Parent: HS
<b>Visual Fit:</b>	Acceptable	Good	Good	Acceptable
<b>M<sub>0</sub>:</b>	93.7	98	97.4	98
<b>Chi-sq error:</b>	3.23	2.41	2.29	3.65
<b>Rate Parameters: value / probability (trigger: 0.05)</b>	k: 0.000914 p < 0.01	$\alpha$ : 0.0306 95th %ile CI does not contain 0	k <sub>1</sub> : 0.061 p = 0.126	k <sub>1</sub> : 0.015 p = 0.11
		$\beta$ : 1.27 90th %ile CI contains 0	k <sub>2</sub> : 1.45E-15 p = 0.5	k <sub>2</sub> : 4.6E-56 p = 0.5
<b>g/t<sub>b</sub>:</b>	N/A	N/A	g: 0.118	t <sub>b</sub> : 6.4
<b>DT<sub>50</sub> (days):</b>	758	>10,000	>10,000	>10,000
<b>DT<sub>90</sub> (days):</b>	2518	>10,000	>10,000	>10,000
<b>Representative DT<sub>50</sub> (days):</b>	758	>10,000	>10,000	>10,000
<b>Fitted vs Observed:</b>				
<b>Residuals:</b>				

### Kinetic assessment conclusions

The modelling endpoints from this study are summarised in table B.8.2.2.3.1-22. For complete endpoints across both water-sediment studies, see table B.8.2.2.3.2-25 to B.8.2.2.3.2-28.

**Table B.8.2.2.3.1-22 Summary of modelling endpoints for inpyrfluxam from study 1**

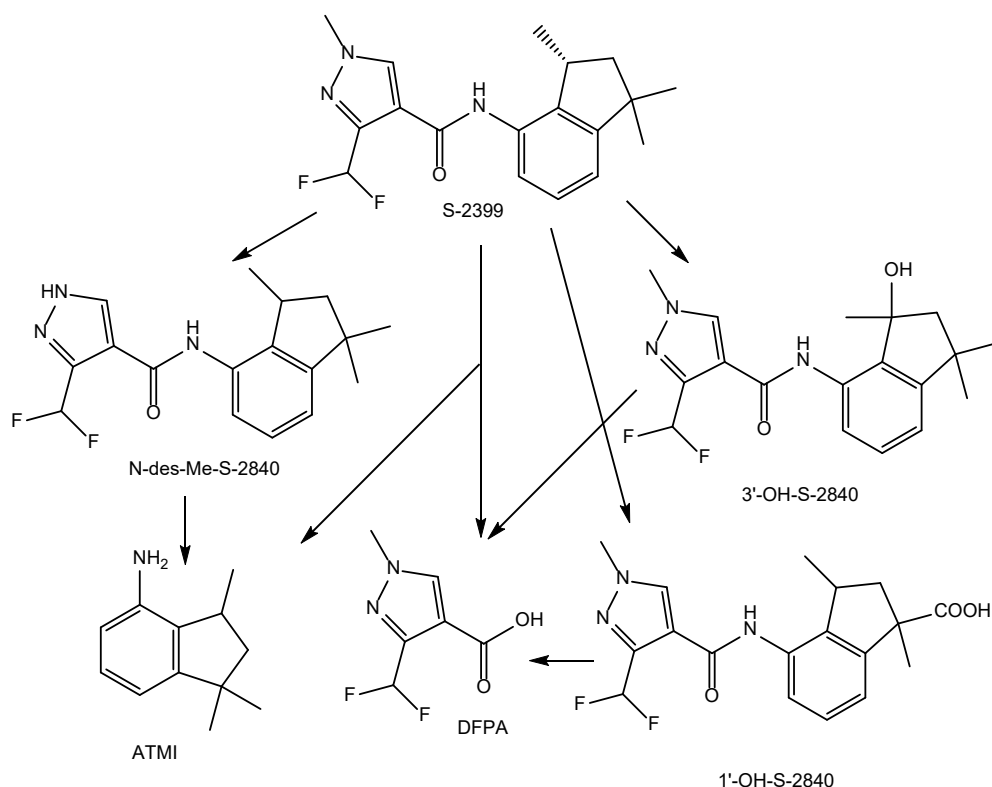
System	Phase	pH	Temp (°C)	<i>Diss</i> <i>T</i> <sub>50</sub> (d)	<i>DissT</i> <sub>90</sub> (d)	St. (χ <sup>2</sup> )	Parameters	Model
Golden Lake	Water	7.8 8.0 (mean)	20 ± 2	2.67 34.28 <sub>a</sub>	113.8	2.43 3	M <sub>0</sub> : 100.1 k <sub>1</sub> : 0.5141 k <sub>2</sub> : 0.01084 g: 0.6564	DFOP
	Sediment	N/A 7.8		1000 <sup>b</sup>	3320	N/A	N/A	Default
Taunton River	Water	5.9 6.6 (mean)		1.63 17.38 <sub>a</sub>	57.71	4.07 6	M <sub>0</sub> : 97.96 k <sub>1</sub> : 0.7645 k <sub>2</sub> : 0.0197 g: 0.6882	DFOP
	Sediment	N/A 5.9		1000 <sup>b</sup>	3320	N/A	N/A	Default
Maximum	Water			34.28	113.8			
	Sediment			1000 <sup>c</sup>	3320			

<sup>a</sup>Diss  $T_{90}$ /3.32

<sup>b</sup>No significant decline was noted in the Golden Lake and Taunton River systems. Use of 1000 day default for sediment Diss  $T_{50}$  is suggested.

Inpyrfluxam was observed to partition significantly from the water phase into the sediment phase in the water sediment laboratory study (B.8.2.2.3.1, KCA 7.2.2.3/01). As such, the sediment compartment is considered to be the relevant compartment against which to assess inpyrfluxam persistence criteria.

The very persistent trigger in freshwater sediment has been determined as a  $DT_{50}$  value > 180 days, according to the criteria in Regulation (EC) No 1107/2009. Following determination of the total system trigger/persistence endpoints, where the  $DT_{50}$  was in excess of 1000 d and  $DT_{90}$  in excess of 10,000 d based on applicant fitting, inpyrfluxam can be observed to exceed this trigger value in both the Golden Lake and Taunton River systems.



**Figure B.8.2.3.1-01 Applicant's proposed aerobic aquatic degradation pathways of inpyrfluxam**

HSE accepts the applicants proposed degradation pathway. HSE notes that the applicant has named 1'-COOH-S-2840 as 1'-OH-S-2840 on the diagram provided (above). This is considered to be a misprint

## CONCLUSIONS

Inpyrfluxam degraded slowly in two sediment/water systems (Golden Lake and Taunton River) under aerobic aquatic conditions. The majority of the dose remained unchanged after 112 days of aerobic aquatic exposure and ultimate mineralization to bound residues and CO<sub>2</sub> was minor. Whole system aerobic aquatic half-lives were estimated at >10,000 d (DFOP) and 758 d (SFO) for Golden Lake and Taunton River, respectively.  $\chi^2$  error values were 2.90 and 3.23, respectively. Metabolite formation was primarily to 1'-COOH-S-2840 and 3'-OH-S-2840, formed at average maximums of 13.1 % and 6.8% AR, respectively. No significant degradation of the metabolites was observed within the duration of the study

**B.8.2.2.3.2. Water / sediment – aerobic study 2**

<b>Data Point:</b>	KCA 7.2.2.3/02 and KCA 7.2.2.3/04
<b>Report Author:</b>	██████ & ██████
<b>Report Year:</b>	2017a
<b>Report Title:</b>	S-2399: Degradation under Aerobic Aquatic Conditions – Rate Study; Amended report #1  Kinetic analysis: Recalculation of the degradation rate of S-2399 (inpyrfluxam) in aquatic systems according to FOCUS Kinetics Guidance
<b>Study Number:</b>	VP-39270
<b>Test Centre:</b>	Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568 U.S.A.
<b>Guideline(s) followed in study:</b>	OECD 308, Environmental Fate Data Requirement, 40 CFR 158.290, OCSP 835.4300 Aerobic Aquatic Metabolism  Kinetic analysis: FOCUS kinetic guidance
<b>Previous evaluation:</b>	New data, submitted for purpose of review
<b>GLP:</b>	Underlying study: Yes  Kinetic analysis: N/A

<b>Deviation</b>	<b>HSE assessment of deviation</b>
Sampling site pesticide history not provided.	While desired, this is not a requirement by OECD 308. The applicant has later provided a pesticide history for the Sharkey system, confirming that no inpyrfluxam has been applied in the catchment area prior to sampling. Pesticide histories were not available for the Goose River of Wewaeantic systems.
Conditions of water-sediment storage during transport, such as containers used, are not reported.	Minor omission. Sediments were stored in the dark and in accordance with 10381-6 (1993).
The difference in pH between the Sharkey sediment and Wewaeantic River sediment is <2.	Minor deviation. Sediments were suitably different to each other in respect of other characteristics. Not expected to significantly affect study outcomes.



Water-sediment ratio is below OECD 308 3:1 minimum guideline ratio.	Minor deviation. Not expected to influence the outcome of the test.
Redox, pH, and O <sub>2</sub> concentration not provided for the acclimation period	Major deviation. These values are required to determine that the systems have approached equilibrium prior to test substance dosing. These have been requested in an RAI and subsequently provided by the applicant.
Discrepancy in stated period of acclimation for the Weweantic system, which was either 11 days or 4 days. The latter is well under the acclimation period recommended in OECD 308 guidelines of 1-2 weeks.	Major deviation, as an actual acclimation duration could not be determined. This is required in order to evaluate the duration against OECD 308 guidelines. This has been requested from the applicant subsequently provided.
The study was extended beyond the recommended 100 days to 111 days.	Minor deviation. Period of extension was minimal.
LOQ value for HPLC analysis not provided	Major deviation from OECD 308 guidelines. This is required to evaluate degradation rates of inpyrfluxam in water-sediment systems. This has been requested from the applicant subsequently provided.
<p style="text-align: center;"><b>HSE conclusion on deviations</b></p> <p>LOQ value for HPLC analysis is now provided. Redox, pH, and O<sub>2</sub> concentration for the acclimation period was only provided for 1 day vs total period, but justification from applicant is accepted. Clarification on the acclimation duration for the Weweantic River system now provided and acclimation period is acceptable. Other deviations are not considered by HSE to void the validity of the study, and therefore further clarification is not required.</p>	

## INTRODUCTION

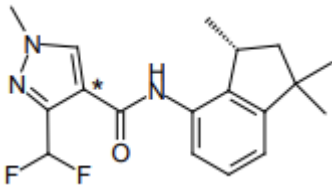
The biotransformation of inpyrfluxam in three water/sediment systems (Goose River, Sharkey and Weweantic River) was investigated under aerobic aquatic condition using [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PY-label). The aerobic aquatic test systems (50 g of dry weight sediment with approximately 115-149 mL of water) were dosed at dry sediment concentrations at ca. 0.05 mg/kg (Goose River), 0.06 mg/kg (Sharkey) and 0.06 mg/kg (Weweantic River). The test systems were incubated at 20±2°C in the

dark for a maximum of 111 days and were periodically collected and extracted. The test systems were equipped with NaOH traps for the collection of evolved  $^{14}\text{CO}_2$  and tetraglyme/ethylene glycol traps for  $^{14}\text{C}$  volatile capture.

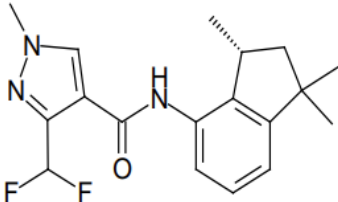
## MATERIALS AND METHODS

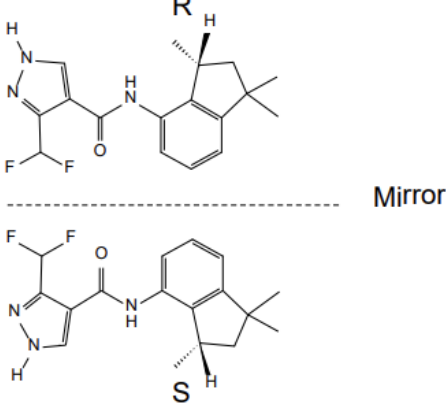
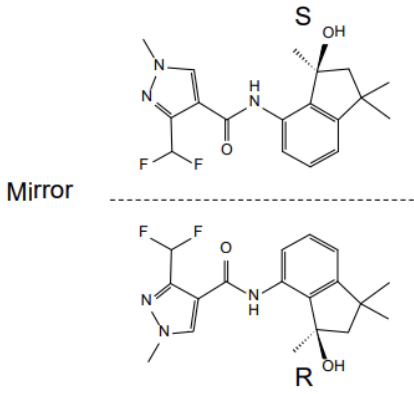
### I. Materials

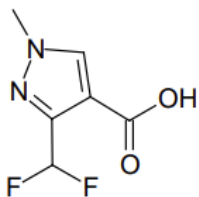
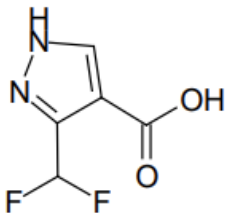
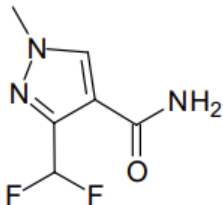
**Table B.8.2.2.3.2-01  $^{14}\text{C}$ -labelled test materials**

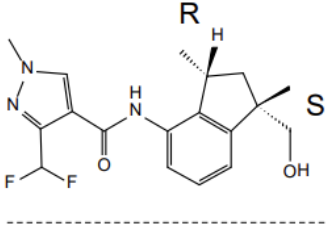
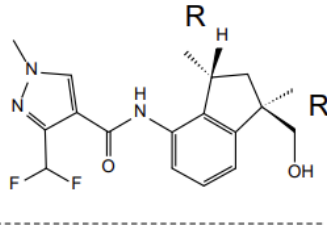
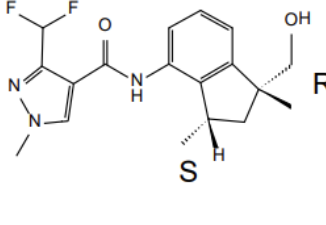
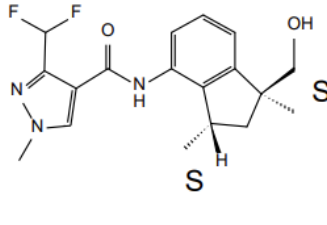
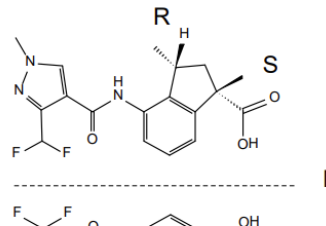
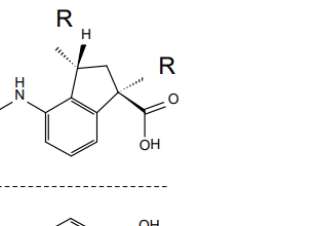
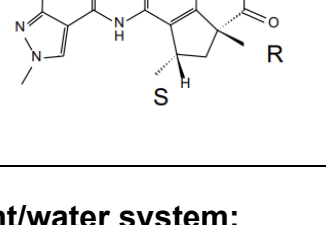
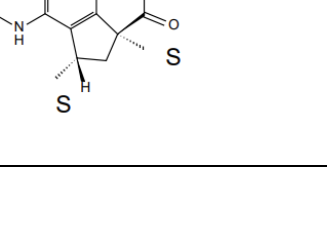
<b>Test Material</b>	[pyrazolyl-4- $^{14}\text{C}$ ] inpyrfluxam (PY-label)
<b>Lot/Batch:</b>	CFQ41802
<b>Specific activity:</b>	2.11 GBq/mmol
<b>Purity:</b>	Radiochemical purity 98.9% at dosing. The chirality was determined to be 100 % <i>R</i> -isomer.
<b>CAS#:</b>	Not assigned
<b>Stability of compound:</b>	stable
<b>Structure:</b>	 <ul style="list-style-type: none"> <li>• Denotes radiolabeling position</li> </ul>

**Table B.8.2.2.3.2-02 Unlabelled metabolite reference item details for characterisation of any degradation products**

<b>1</b>	<b>Code Name</b>	Inpyrfluxam
	<b>Chemical Name</b>	3-(difluoromethyl)-1-methyl-N-[(3'R)-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	

2	<b>Code Name</b>	N-des-Me-S-2840
	<b>Chemical Name</b>	3-(difluoromethyl)-N-[1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	<p>(2 Enantiomers)</p> 
3	<b>Code Name</b>	3'-OH-S-2840
	<b>Chemical Name</b>	3-(difluoromethyl)-N-[3'-hydroxy-1',1',3'-trimethyl-2',3'-dihydro-1'H-inden-4'-yl]-1-methyl-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	
4	<b>Code Name</b>	DFPA
	<b>Chemical Name</b>	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid

	<b>Chemical Structure</b>	
5	<b>Code Name</b>	N-des-Me-DFPA
	<b>Chemical Name</b>	3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid
	<b>Chemical Structure</b>	
6	<b>Code Name</b>	DFPA-CONH <sub>2</sub>
	<b>Chemical Name</b>	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
	<b>Chemical Structure</b>	
7	<b>Code Name</b>	1'-CH <sub>2</sub> OH-S-2840
	<b>Chemical Name</b>	-

	<b>Chemical Structure</b>	  <div style="text-align: center;">Mirror</div>  
8	<b>Code Name</b>	1'-COOH-S-2840
	<b>Chemical Name</b>	-
	<b>Chemical Structure</b>	  <div style="text-align: center;">Mirror</div>  

### I. Sediment/water system:

Three test systems (sediment and water) were collected. The water samples were collected with a bucket. The sediment was collected from the top 0-5 cm layer (posthole digger, Goose River), 0-7.6 cm layer (shovel, Sharkey) and 0-7.6 cm layer (Weweantic River), up to 1 inch of leaf litter was removed from the surface of the Weweantic River sediment before sampling. The sampling collection methods are suitable by OECD 308 guidelines, with the applicant having collected sediment from the upper layer to a depth of 5 – 10 cm.

Each sediment was thoroughly mixed and passed through a 2-mm mesh sieve with a minimum of air-drying. HSE notes that the exact conditions of storage during transport, such as containers used, have not reported. However, the applicant has reported that, prior to dosing, sediments were stored in the dark and in accordance with 10381-6 (1993), which indicates transport in similar conditions (kept in the dark

with free access to air). Therefore, this is deemed to be a minor deviation which does not affect study outcomes. The sediments' characteristics are summarised in Table B.8.2.2.3.2-03.

The OECD 308 guidance states that one sediment should have a high organic carbon content and fine texture, the other a low organic carbon content and coarse texture. The Goose River sediment has a fine texture (clay and silt 75%) and a high organic carbon content (OC) (3.5 %). The Sharkey sediment has a fine texture (clay and silt 79%) and a moderately high OC content (2.4%). The Weweantic River sediment has a coarse texture (clay and silt 3%) and a low OC content (0.9%). From this, HSE calculated that the difference in organic carbon between Goose River and Weweantic River as 2.6% (above the guideline value of 2 %), and the difference between Sharkey and Weweantic River as 1.5%. Furthermore, the difference in texture between Goose River and Weweantic River is 72%, and the difference between Sharkey and Weweantic River is 76%, which exceeds the guideline 20%.

It is noted that the difference in pH between the Sharkey sediment and Weweantic River sediment is <2%. However, given the marked difference in texture and sediment pH of the test systems, HSE is of the opinion that the test systems were suitably different from each other and this slight deviation from the guidelines is not expected to significantly affect the outcomes of the study. Furthermore, the Goose River characteristics varies sufficiently from that of the Weweantic River system, and a further two systems are presented in section B.8.2.2.3.1 . HSE therefore confirms that the selection and range of the water-sediment systems are appropriate.

**Table B.8.2.2.3.2-03 Chemical and Physical characteristics of test sediments**

<b>Sediment characteristic</b>	<b>Goose River</b>	<b>Sharkey</b>	<b>Weweantic River</b>
Sampling location	Grand Forks, ND, N 47°43.779' W 97°37.312'	Deer Creek, MS; GPS N 33° 26.743 min, W 90° 58.471 min	Wareham, MA; GPS N 41° 45.527 min, W 70° 44.550 min
<b>USDA Particle size distribution</b>			
% sand (50 µm - 2 mm)	25	21	97
% silt (2 µm - 50 µm)	42	21	3
% clay <2 µm	33	58	0
pH (H <sub>2</sub> O)	7.9	6.5	5.7
% Moisture 1/3 bar	49.9	52.5	10.6

Cation exchange capacity (meq/100g)	22.7	30.9	3.8
% Organic carbon	3.5	2.4	0.9
% Organic Matter	6.0	4.2	1.6
USDA Textural class	Clay loam	Clay	Sand
<b>Microbial Biomass Carbon (µg/g dry weight)</b>			
0 DAT	72.9	67.9	10.4
111 DAT; untreated control	73.6	60.1	< 0.1
111 DAT; solvent control	31.4	48.9	< 0.1
111 DAT; inpyrfluxam control	45.3	69.8	3.5

HSE notes that pesticide histories of the sites were not provided. HSE considers this as a minor omission, as while desired by OECD 308, it is not required. Furthermore, while pesticide contamination is possible, no direct application would be expected for these sampling sites. The applicant has later clarified that a pesticide history is available for the Sharkey system, and that there was no exposure to inpyrfluxam previous to the sampling.

## STUDY DESIGN

### I. Experimental Conditions

The aerobic aquatic test systems consisted of an incubation apparatus, with an airtight chamber and air inlet and outlet valves. Each contained 50 g of dry weight sediment, and water collected from the same location in a sediment:water ratios of 0.30 (w:w) (Goose River), 0.29 (w:w) Sharkey and 0.33 (w:w) (Weweantic River). The average sediment:water volume ratios were 1:1.93 in the Goose River systems, 1:2.46 in the Sharkey systems, and 1:3.14 in the Weweantic River systems. HSE notes that the Goose River and Sharkey systems fall below the 1:3 (v : v) OECD 308 and EPA 835.4300 minimum guideline thresholds. HSE does not believe that this will impact the determined endpoints. Chamber air was continuously evacuated through the chamber air outlet, which was connected to a vacuum source, at a steady rate of approximately 10 mL per minute. The chamber inlet air was bubbled through a water trap to humidify the air before entering the chamber. The continuous airflow was used to maintain aerobic conditions as well as to evacuate any radiolabelled volatiles into the outlet traps. The evacuated air passed through a volatiles trap (Tetraglyme or ethylene glycol) and two consecutive NaOH traps to capture any <sup>14</sup>CO<sub>2</sub> present in the

chamber's air. Before opening the chamber for each sampling, airflow was increased for a length of time sufficient to evacuate any lingering volatile degradation products through the traps. The incubation apparatus was incubated in the dark at  $20 \pm 2$  °C on a platform that was continuously shaken.

The total timeframe between sample collection and use (start of acclimation) was 14 days for Goose River, 14 days for Sharkey and 7 days for the Weweantic system. The applicant stated that the sediment and water were stored in the dark at 4°C except during the aerobic aquatic test system preparation where it was stored at ambient temperatures. Test system preparation was 4 days for Goose River (11/08/2016 – 15/08/2016), 2 days for Sharkey (08/08/2016 – 10/08/2016) and 1 day (12/08/2016) for Taunton River. Test substances were dosed on 23/08/2016 (0 DAT).

The applicant stated a pre-incubation period of 8-12 days was performed for the Goose River system, 14 days for the Sharkey sediment system and 4 days for the Weweantic River system before dosing. However, HSE notes that there is a discrepancy in the stated period of acclimation for the Weweantic system from the dates provided above, which suggests an acclimation period of 11 days. Given that the stated 4 day acclimation period is well under that recommended in the OECD 308 guidelines of 1-2 weeks, HSE requested clarification from the applicant on the correct period for the Weweantic River system. The correct acclimation period of 11 days has been confirmed by the applicant in their response to the RAI.

HSE also requested measurements of pH, O<sub>2</sub> concentration, total organic carbon of the water and sediment, oxygen concentration and redox readings during acclimation so that the suitability of the incubation period could be evaluated. In their response, the applicant stated that measurements taken during the acclimation phase only occurred on one day, on 18/08/2016. However, given that acclimation periods of 1-2 weeks were used and is within the usual period of the guidelines. As such, they reason that the incubation period can be considered acceptable regardless of whether acclimation phase system measurements are reported or not, and that the deviation could be considered minor. HSE accepts the applicant's reasoning and agrees that the lack of measurements taken during acclimation can be considered a minor deviation which does not affect study outcomes.

In terms of the measurements taken, the applicant provided the data in table B.8.2.2.3.2-04 below and state that the water phase was aerobic and oxygenated and the sediment phase showed an acceptable level of anaerobicity. They also assert that the TOC is useful for understanding the health of open ecosystems, but as these systems were closed ecosystems with no carbon renewal and sequestration done by NaOH, TOC was not evaluated.



**Table B.8.2.2.3.2-04 Water and sediment parameters taken on 1 day of acclimation phase (18/08/2016)**

		<b>Goose River</b>			
<b>Sample</b>	<b>Dissolved oxygen water (ppm)</b>	<b>Redox Pot. (mV)</b>		<b>pH</b>	
		<b>Water</b>	<b>Sediment</b>	<b>Water</b>	<b>Sediment</b>
<b>Acc-100</b>	8.74	169	8	8.57	7.28
<b>Acc-102</b>	8.67	98	-74	8.56	7.25
<b>Acc-106</b>	8.59	92	-109	8.45	7.37
		<b>Sharkey</b>			
<b>Acc-79</b>	8.73	167	110	8.01	6.81
<b>Acc-80</b>	8.77	129	102	7.37	6.32
<b>Acc-81</b>	8.65	208	128	6.94	6.16
		<b>Weweantic</b>			
<b>Acc-129</b>	8.68	112	-90	7.11	6.76
<b>Acc-130</b>	8.49	115	-61	6.73	6.63
<b>Acc-131</b>	8.38	89	-63	6.40	6.61

HSE accepts that the water phase was suitably aerobic and the sediment suitably anaerobic based on Table B.8.2.2.3.2-04, with the exception of the Sharkey system sediment which had positive redox values of 102 to 128 mV. Nevertheless, the redox potentials of the sediment taken from 0 DAT to 111 DAT were all negative and generally consistently anaerobic, ranging from -63 to -215 mV. Therefore, HSE agrees that reasonable stability had been reached in all three systems in accordance with the test guidelines.

## **II. Test substance application rate**

The systems were dosed with [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam at dry sediment concentrations at approximately 0.05 mg/kg, 978,222 dpm, (Goose River), 0.06 mg/kg, 1,118,684 dpm (Sharkey) and 0.06 mg/kg, 1,153,800 dpm (Weweantic River). This corresponds to an accidental overspray of the maximum label rate applied to a 100 cm deep pond, as required in the EPA 835.4300 guideline. This application rate is higher than the dosing based on predicted environmental emissions required by the OECD 308 guideline.

## **III. Sampling**

The test systems were incubated at 20 ± 2°C in the dark for a maximum of 111 days and were periodically collected and extracted. HSE notes that the study was extended beyond the recommended 100 days in the OECD 308 guidelines. However,

as the period of extension was minimal, HSE considers this to be a minor deviation that will not have an impact on the overall study outcomes.

Duplicate samples were removed for analysis at: 0, 1, 3, 7, 15, 30, 45, 62, 76, 91 and 111 days after treatment (DAT). The test systems were equipped with 1 M NaOH traps for the collection of evolved  $^{14}\text{CO}_2$  and tetraglyme/ethylene glycol traps for  $^{14}\text{C}$  volatile capture. Untreated control soils, organic solvent controls and non-radiolabelled inpyrfluxam samples were used to measure the effect on the microbial biomass at the end of sampling.

The applicant indicates that, where the samples were not actively worked up, they were kept frozen. This contradicts other information in the report and the MCA stating that samples were analysed immediately after removal from the incubator and were not stored prior to any of the physical-chemical property or chemical analyses. Furthermore, the specific storage conditions of the sample extracts were not reported, such as the temperature and duration of freezing, and it is unclear how the applicant determined the stability of the frozen samples after thawing.

Clarification on this point was requested in an RAI and now addressed by the applicant, who stated that ‘Samples were kept frozen when not actively being worked up’ should read ‘Sample extracts were kept frozen when not actively being worked up’. Samples were not taken and frozen, only extracts.

Additionally, a storage stability test (KCA 7.1.2.2.1\_10) demonstrates that the compounds are stable over the study duration. Therefore, HSE accepts that this contradiction has been resolved.

#### **IV. Description of analytical procedures**

The physical parameters of the aerobic systems (oxygen concentration, redox and pH) were measured and the water separated from the sediment by decanting the water into a separate bottle.

The water phase and all eluents were analysed by liquid scintillation counting (LSC). The water phase was centrifuged and decanted through a glass funnel packed loosely with glass wool, adjusted to pH 5 and subjected to solid phase extraction (SPE) with water and acetonitrile (average recovery 97.2%). Representative samples were analysed by LSC, thin layer chromatography-autoradiography (TLC) and HPLC-RAM.

The sediment was initially extracted with neutral organic solvents to yield a neutral extract and then, if needed, and acidic solvent mix was used. For the neutral extract, the sediment remaining in the test vessel after the water was decanted off was extracted with acetone, twice with acetone:water (3:2, v/v) and with

acetone:water:HCl (c) (60:40:1, v/v/v) and the extracts were pooled and analysed by LSC, TLC-autoradiography and HPLC-RAM .

The sediment for the 62 and 111 DAT samples were also extracted by acidic solvent 60:40:1 acetone/water/concentrated HCl (v/v/v). The acidic extract was rotary-evaporated to remove the acetone, adjusted to pH 5 and subjected to SPE with water and acetonitrile. The activity of the extracts was determined by LSC. The extracts were analysed by HPLC-RAM and representative samples by TLC. Selected 62 and 111 DAT samples from the acidic extraction were analysed by HPLC.

Representative post-extracted solids (PES) at 111 DAT were subjected to further additional sequential solvent extractions with ethyl acetate, dioxane and hexane, and with a dismembrator (5:1 acetone:0.5 M HCl) for comparison, this was done for a single replicate for each water-sediment system.

The radioactivity was determined by LSC and the extract was analysed by HPLC-RAM. Total radioactivity in PES was determined by combustion. The Goose River PES was finally subjected to a humin, humic acid and fulvic acid fractionation. The PES was shaken with dilute NaOH for ca. 16 hours on a reciprocating shaker. The sediment debris pellet and aqueous supernatant were separated by centrifugation. The sediment debris pellet was rinsed with water and centrifuged. The water added to the NaOH solution. The NaOH solution contains the humic acids and fulvic acids associated radioactivity and the sediment debris represent the humin fraction. The humin portion was weighed and sub-samples were analysed by combustion/LSC. The radioactivity present in the NaOH extract was further fractionated into fulvic acid and humic acid associated radioactivity by acidification to pH ~1 using hydrochloric acid to precipitate the humic acids.

Following centrifugation, the soluble fulvic materials and the precipitated humic substances were separated. The supernatant, which contains the fulvic acids associated radioactivity, was measured and its content of radioactivity determined by LSC. The precipitated fraction (humic acids) was weighed and sub-samples were analysed by combustion/LSC.

The  $^{14}\text{CO}_2$  collected in the NaOH trapping solution and organic volatiles collected in tetraglyme/ethylene glycol traps were quantified by LSC.

The potential isomerisation from [ $^{14}\text{C}$ ] inpyrfluxam was evaluated using chiral HPLC analysis on the extracts obtained from the 111 DAT samples.

## **V. Limits of detection and quantification**

The limit of detection (LOD) for LSC was determined to be 11 dpm (detections per minute), and the limit of quantification (LOQ) was determined to be 43 dpm. Radioactivity dosing to the samples was 978,222 dpm, 1,118,684 dpm, and

1,153,800 dpm respectively, for the Goose River, Sharkey, and Weweantic River test systems. This gives LOD and LOQ values below 0.01 % AR, well below the OECD 308 guideline of 1 % AR. HSE notes that these measurements should be provided by the applicant in % AR.

The LOD was evaluated for every HPLC analysis as a percent of the AR. The average LOD as a percent of the AR for the study was 0.89% of the AR  $\pm$  0.16%. The minimum and maximum values were 0.48 to 1.45%, respectively. Practically, this means no area of radioactivity > 1.45% of the AR was uncharacterized.

The applicant did not provide a corresponding LOQ value in line with the HPLC LOD value. This was regarded by HSE as a major deviation from OECD 308 guidelines, and requested the LOQ value for HPLC in an RAI. This has now been addressed and the applicant confirmed that the LOQ was 0.22 % AR for acidic extracts, 0.82 – 1.80 % AR for neutral extracts and 0.56 – 1.85 % AR for water extracts (pyrazolyl label). They state that although the LOQ is > 1% AR for some samples/intervals, the integrity of the study is not affected. HSE agrees with this assessment. Furthermore, they confirmed that the LOD varied according to matrix and analyst judgement, but that detectable radioactivity was generally clearly distinguishable and LOD << LOQ.

The applicant also provided exact LOD and LOQ values for LSC, in addition to the LOQ requested for HPLC. These are reported as < 1 % AR for the LOD, and for LOQ 0.31 – 0.38 % AR for acidic extracts, 0.92 – 1.08 % AR for neutral extracts and 0.27 – 0.31 % AR for water extracts (pyrazolyl label).

## RESULTS AND DISCUSSION

### I. Physical characteristics of the test system

#### Goose River parameters

Measurements of pH values in the water were consistent, ranging from 7.67 to 8.81 between 0 to 111 DAT. Sediment pH values were also fairly consistent, ranging from 6.70 to 7.47.

Concerning dissolved oxygen in the water, with the exception of 7 DAT, all other samples ranged from just slightly under 7 ppm to 9.48 ppm from 0 to 111 DAT, in line with recommendations in the guidelines of 7-10 mg/L O<sub>2</sub> content. It is noted that the O<sub>2</sub> content at 7 DAT ranges from 3.70 to 5.81 ppm, however HSE does not consider this dip to significantly impact study outcomes.

Redox potentials in the water were more aerobic than the sediment with positive values, with the exception of 0, 1 and 3 DAT where redox potentials had negative values ranging from -3 to -34 mV. The applicant clarified that the accuracy of the redox values for 1 and 3 DAT could not be verified due to improper calibration of the

electrode. Nevertheless, HSE accepts the applicants' conclusion that the water phase was generally aerobic.

Redox potentials in the sediment were all negative and generally consistently anaerobic, with redox potentials ranging from -81 to -266 mV.

### **Sharkey parameters**

Measurements of pH values in the water were fairly consistent, ranging from 7.16 to 8.78 between 0 to 111 DAT. Sediment pH values were also consistent, ranging from 6.37 to 7.69.

For dissolved oxygen, all samples ranged from just slightly under 7 ppm (lowest reading 6.27 ppm) to 9.48 ppm from 0 to 111 DAT, in line with recommendations in the guidelines of 7-10 mg/L O<sub>2</sub>.

Redox potentials in the water were more aerobic than the sediment with positive values, with the exception of 3 DAT where one replicate had a negative redox potential of -25 mV. Nevertheless, HSE accepts the applicants' conclusion that the water phase was generally consistently aerobic.

Redox potentials in the sediment were all negative and generally consistently anaerobic, with redox potentials ranging from -63 to -215 mV.

### **Weweantic River parameters**

There was a greater variation of pH values in the water compared to the other systems, ranging from 6.16 to 8.85 between 0 to 111 DAT. Sediment pH values were also variable, ranging from 6.45 to 8.58. However, looking at the system properties, the Weweantic River sediment has a very low clay content (0%) as well as a low organic carbon content (0.9%), meaning it has a low capacity for adsorption and may be poor at buffering changes in pH. Therefore, HSE finds the variation in pH values acceptable.

For dissolved oxygen, with the exception of 7 DAT, all samples ranged from 7.62 ppm to 9.61 ppm from 0 to 111 DAT, in line with recommendations in the guidelines of 7-10 mg/L O<sub>2</sub>. It is noted that the O<sub>2</sub> content at 7 DAT ranges from 0.3 to 1.82 ppm, however HSE does not consider this dip to significantly impact study outcomes.

Redox potentials in the water were more aerobic than the sediment with positive values, with the exception of 1 and 7 DAT which each had one negative replicate values of -42 and -97 mV respectively. Nevertheless, the other replicates had positive redox values, and HSE accepts the applicants' conclusion that the water phase was generally aerobic.

Redox potentials in the sediment were all negative and generally consistently anaerobic, with redox potentials ranging from -25 to -294 mV.

## **II. Biological Viability of the Test System**

The applicant has stated that no significant change in the microbial biomass carbon was recognized between the initiation and termination of the incubation (Table B.8.2.2.3.2-03). Thus, microbial viability was proved to be satisfactorily maintained during the incubation period. HSE disagrees with this assessment, and notes that for the Goose River sample, a significant decrease is observed for the inpyrfluxam control and the solvent control, relative to the untreated control. This possibly indicates that the solvent has an adverse affect on biomass, however this affect is not as marked in the Sharkey sample. HSE notes that a decrease in biomass is likely to increase the length of the determined endpoints, leading to a more conservative assessment for the parent compound. As such, the reduced biomass does not compromise the validity of the experiments. Furthermore, five water-sediment systems were tested across the two studies, which reducing the likelihood that relevant metabolites were not detected due to the variability across the systems.

The applicant has stated that the values for the Weweantic River are considered not atypical for the type of sediment, having consulted with Smithers Viscient who routinely uses the Weweantic River sediment. Viscient confirmed that as a low organic carbon sandy sediment, “the microbial biomass value of ~0.1% of its OC is not atypical of this sediment”.

HSE notes that there is no advice in the test guidelines regarding how the biomass results during the study should be used to interpret the results, nor is there criteria regarding a threshold microbial biomass measurement as a percentage of organic carbon like the OECD 307 guidelines. The applicant also has not provided a sterile control to determine extent that biodegradation has on overall degradation, or account for any abiotic processes.

Nevertheless, HSE does not consider this to significantly impact the outcomes of the study, as any lack of degradation of the parent compound in the sediment on account of the low microbial biomass can represent a more conservative estimate of degradation.

## **III. Isomerisation**

It was confirmed that no isomerisation of [<sup>14</sup>C] inpyrfluxam occurred during incubation period based on chiral HPLC analysis.

## **IV. Distribution of radioactivity**

The distribution and mass balance of applied radioactivity of [<sup>14</sup>C] inpyrfluxam in water phase, extractable, sediment-bound and volatile fractions are summarised in

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Tables B.8.2.2.3.2-05 to B.8.2.2.3.2-07. The quantification of inpyrfluxam and the metabolites in the neutral extracts and water phase is summarised in Tables B.8.2.2.3.2-08 to B.8.2.2.3.2-10. The acidic sediment extracts at 62 and 111 DAT showed only the presence of inpyrfluxam, and 3'-OH-S-2840 and other unknowns in only very low percentage of applied radioactivity (Table B.8.2.2.3.2-11).

**Table B.8.2.2.3.2-05 Summary of the mass balance data for the Goose River system as percentage of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			1			3			7		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	91.4	90.8	91.1	57.9	64.5	61.2	42.7	45.1	43.9	30.7	33.4	32.0
Neutral Extract	6.6	7.2	6.9	37.7	30.1	33.9	53.3	48.8	51.1	61.0	59.1	60.0
Acidic Extract	0.1	0.1	0.1	0.8	0.7	0.8	2.1	2.1	2.1	4.0	3.4	3.7
Total Ext.	6.6	7.3	7.0	38.4	30.8	34.6	55.4	50.9	53.2	65.0	62.5	63.8
Sediment-bound	0.1	0.1	0.1	0.8	0.8	0.8	2.6	0.2	1.4	5.5	4.6	5.0
Volatiles ( <sup>14</sup> CO <sub>2</sub> )	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total balance	98.2	98.2	98.2	97.1	96.2	96.6	100.6	96.3	98.5	101.2	100.4	100.8
Fraction	Days After Treatment (DAT)											
	15			30			45			62		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	19.7	18.4	19.1	15.0	10.3	12.6	7.9	8.6	8.3	7.4	7.8	7.6
Neutral Extract	68.6	70.7	69.7	75.7	77.8	76.7	78.0	78.7	78.3	73.9	75.7	74.8
Acidic Extract	5.9	5.5	5.7	5.1	5.0	5.0	5.1	5.2	5.1	6.6	8.0	7.3
Total Ext.	74.5	76.2	75.3	80.8	82.8	81.8	83.0	83.8	83.4	80.6	83.7	82.1
Sediment-bound	6.8	6.1	6.4	6.1	6.4	6.3	7.7	6.7	7.2	7.9	7.7	7.8



Volatiles ( $^{14}\text{CO}_2$ )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2
Total balance	101.1	100.7	100.9	101.9	99.6	100.7	98.6	99.2	98.9	96.1	99.4	97.7
Fraction	Days After Treatment (DAT)											
	76			91			111					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Water Phase	8.1	5.7	6.9	6.3	6.8	6.5	6.9	6.6	6.8			
Neutral Extract	75.8	78.0	76.9	75.0	73.9	74.5	71.6	69.0	70.3			
Acidic Extract	6.8	7.5	7.2	10.3	9.8	10.0	9.9	9.6	9.8			
Total Ext.	82.6	85.5	84.1	85.3	83.7	84.5	81.5	78.6	80.0			
Sediment-bound	9.7	9.3	9.5	9.4	10.5	10.0	12.5	12.7	12.6			
Volatiles ( $^{14}\text{CO}_2$ )	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.5	0.5			
Total balance	100.7	100.8	100.7	101.4	101.4	101.4	101.4	98.4	99.9			

NA: not analysed

**Table B.8.2.2.3.2-06 Summary of the mass balance data for the Sharkey system as percentage of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			1			3			7		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	89.6	92.8	91.2	64.4	66.3	65.3	46.7	53.7	50.2	43.5	46.1	44.8
Neutral Extract	8.1	7.6	7.9	31.9	29.9	30.9	41.4	44.1	42.7	52.8	48.8	50.8
Acidic Extract	0.0	0.0	0.0	0.3	0.3	0.3	1.1	1.0	1.0	3.1	3.3	3.2
Total Ext.	8.1	7.6	7.9	32.3	30.2	31.2	42.4	45.1	43.8	55.9	52.1	54.0
Sediment-bound	0.0	0.0	0.0	0.4	0.2	0.3	0.6	0.5	0.6	1.6	1.6	1.6
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total balance	97.7	100.5	99.1	97.0	96.7	96.8	89.8	99.3	94.5	100.9	99.8	100.4
Fraction	Days After Treatment (DAT)											
	15			30			45			62		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	37.7	39.2	38.5	28.6	28.9	28.7	15.9	15.3	15.6	13.8	10.0	11.9
Neutral Extract	56.8	55.4	56.1	62.4	71.6	67.0	78.3	78.2	78.3	76.8	80.9	78.9
Acidic Extract	3.4	3.8	3.6	4.0	3.3	3.7	3.5	3.0	3.3	5.4	4.4	4.9
Total Ext.	60.3	59.2	59.7	66.4	74.9	70.6	81.8	81.2	81.5	82.2	85.4	83.8
Sediment-bound	1.9	2.2	2.0	2.4	3.4	2.9	3.1	3.5	3.3	3.2	4.1	3.7
Volatiles ( $^{14}\text{CO}_2$ )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Total balance	99.9	100.6	100.2	97.4	107.2	102.3	100.9	100.1	100.5	99.3	99.6	99.4
Fraction	Days After Treatment (DAT)											
	76			91			111					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Water Phase	12.0	10.6	11.3	10.5	9.0	9.8	10.1	10.5	10.3			
Neutral Extract	80.1	81.7	80.9	81.3	81.6	81.4	79.2	79.7	79.5			
Acidic Extract	5.2	5.3	5.3	6.3	6.0	6.2	5.9	5.1	5.5			
Total Ext.	85.3	87.1	86.2	87.6	87.6	87.6	85.1	84.8	85.0			
Sediment-bound	3.6	3.5	3.5	4.0	4.5	4.2	4.2	5.3	4.8			
Volatiles ( $^{14}\text{CO}_2$ )	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1			
Total balance	101.0	101.2	101.1	102.2	101.1	101.6	99.6	100.8	100.2			

NA: not analysed

**Table B.8.2.2.3.2-07 Summary of the mass balance data for the Weweantic River system as percentage of Applied Radioactivity**

Fraction	Days After Treatment (DAT)											
	0			1			3			7		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	79.7	76.9	78.3	65.4	63.9	64.7	54.4	56.5	55.5	41.7	44.4	43.1
Neutral Extract	18.7	21.2	20.0	31.4	34.3	32.9	43.7	43.2	43.4	56.1	54.4	55.2
Acidic Extract	0.0	0.0	0.0	0.3	0.2	0.3	0.5	0.5	0.5	1.1	1.1	1.1
Total Ext.	18.7	21.2	20.0	31.7	34.5	33.1	44.1	43.7	43.9	57.3	55.5	56.4
Sediment-bound	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.2	0.4	0.3	0.4
Volatiles ( $^{14}\text{CO}_2$ )	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total balance	98.5	98.2	98.3	97.1	98.5	97.8	98.6	100.6	99.6	99.4	100.2	99.8
Fraction	Days After Treatment (DAT)											
	15			30			45			62		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Water Phase	37.9	41.0	39.5	34.1	33.5	33.8	24.6	23.6	24.1	23.5	22.3	22.9
Neutral Extract	60.6	57.7	59.2	64.3	61.8	63.0	69.9	71.3	70.6	68.9	71.2	70.1
Acidic Extract	1.4	1.3	1.3	2.0	1.9	2.0	2.5	2.2	2.4	4.2	3.7	4.0
Total Ext.	62.0	59.0	60.5	66.3	63.7	65.0	72.4	73.5	73.0	73.1	75.0	74.0
Sediment-bound	0.6	0.4	0.5	0.8	0.7	0.8	1.1	1.0	1.1	1.3	1.3	1.3

Volatiles ( $^{14}\text{CO}_2$ )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total balance	100.5	100.4	100.4	101.2	97.9	99.5	98.1	98.1	98.1	98.0	98.5	98.2
<b>Fraction</b>	<b>Days After Treatment (DAT)</b>											
	<b>76</b>			<b>91</b>			<b>111</b>					
<b>Sample</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>			
Water Phase	19.4	19.7	19.6	16.6	19.5	18.1	15.6	15.1	15.4			
Neutral Extract	72.8	73.9	73.4	75.5	71.1	73.3	73.3	73.5	73.4			
Acidic Extract	5.1	4.8	4.9	6.3	7.1	6.7	7.4	6.7	7.0			
Total Ext.	77.9	78.7	78.3	81.8	78.2	80.0	80.6	80.2	80.4			
Sediment-bound	2.0	1.9	1.9	2.1	2.4	2.3	3.1	3.3	3.2			
Volatiles ( $^{14}\text{CO}_2$ )	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
Total balance	99.3	100.3	99.8	100.6	100.1	100.4	99.4	98.7	99.0			

NA: not analysed

HSE notes that total mass balance AR recoveries are suitably within the 90- 110 % OECD 308 guideline

**Table B.8.2.3.2-08 Radioactivity distribution from the water and sediment of Goose River system (excluding acidic sediment extract) as percentage of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			1			3			7		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	92.3	93.5	92.9	56.0	63.2	59.6	43.4	43.8	43.6	29.8	32.9	31.4
Inpyrfluxam (sediment)	6.6	7.2	6.9	37.7	30.1	33.9	52.8	48.2	50.5	60.3	58.4	59.3
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	0.0	0.0	0.0	0.6	0.5	0.6	0.4	0.3	0.3	0.0	0.4	0.2
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.6	0.5	0.7	0.7	0.7
Others (Total) *	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total*	98.8	100.7	99.8	94.3	93.8	94.0	97.1	92.9	95.0	90.8	92.4	91.6
Fraction	Days After Treatment (DAT)											
	15			30			45			62		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	18.0	16.0	17.0	11.9	7.0	9.5	5.9	5.7	5.8	4.4	4.4	4.4
Inpyrfluxam (sediment)	67.4	69.6	68.5	74.5	76.1	75.3	74.8	74.2	74.5	69.8	71.9	70.9

1'-COOH-S-2840 total** (water)	1.0	1.5	1.3	1.7	1.8	1.8	2.1	2.5	2.3	2.5	2.9	2.7
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	1.3	2.4	1.9	2.0	3.1	2.5
3'-OH-S-2840 (water)	0.6	0.3	0.4	0.3	0.2	0.2	0.0	0.0	0.0	0.3	0.0	0.1
3'-OH-S-2840 (sediment)	1.3	1.2	1.2	1.2	1.7	1.4	1.8	2.0	1.9	2.2	2.3	2.2
Others (Total) *	0.0	0.0	0.0	0.2	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Total*	88.2	88.4	88.3	89.8	87.2	88.5	86.0	86.8	86.4	81.1	84.6	82.8
<b>Fraction</b>	<b>Days After Treatment (DAT)</b>											
	<b>76</b>			<b>91</b>			<b>111</b>					
<b>Sample</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>			
Inpyrfluxam (water)	4.3	2.8	3.6	2.1	2.3	2.2	2.2	1.7	1.9			
Inpyrfluxam (sediment)	71.0	73.7	72.3	68.9	68.0	68.5	64.3	62.0	63.2			
1'-COOH-S-2840 total** (water)	3.5	2.4	3.0	3.2	3.9	3.5	3.8	3.3	3.6			
1'-COOH-S-2840 total** (sediment)	2.3	2.2	2.3	3.2	3.1	3.1	4.3	4.2	4.2			
3'-OH-S-2840 (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
3'-OH-S-2840 (sediment)	2.1	2.2	2.1	2.8	2.9	2.9	2.9	2.8	2.9			

Others (Total) *	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Total*	83.1	83.3	83.2	80.3	80.1	80.2	77.7	74.0	75.8	

\* Includes DFPA and total other unknowns which never individually exceed 0.2% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.2.3.2-09 Radioactivity distribution in the water and sediment of Sharkey system (excluding acidic sediment) as percentage of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			1			3			7		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	86.0	92.0	89.0	63.7	64.5	64.1	44.0	53.9	49.0	42.1	44.0	43.1
Inpyrfluxam (sediment)	8.1	7.6	7.9	31.9	29.5	30.7	40.9	43.5	42.2	52.1	48.0	50.0
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	1.0	1.1	1.0	0.5	1.1	0.8	0.4	0.8	0.6	0.7	0.6	0.6
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	0.0	0.4	0.2	0.5	0.6	0.5	0.7	0.8	0.7
Others (Total) *	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total*	95.2	100.7	97.9	96.2	95.5	95.8	85.8	98.8	92.3	95.7	93.4	94.5



Fraction	Days After Treatment (DAT)											
	15			30			45			62		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	35.7	37.8	36.8	25.0	24.7	24.8	11.5	10.4	11.0	8.9	5.1	7.0
Inpyrfluxam (sediment)	55.9	54.2	55.0	61.1	70.2	65.6	74.9	75.4	75.1	72.0	77.4	74.7
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	2.6	2.4	2.6	3.3	2.1	2.7	2.6	3.6	3.1
1'-COOH-S-2840 total** (sediment)	0.3	0.0	0.2	0.0	0.0	0.0	1.6	1.2	1.4	3.0	1.7	2.4
3'-OH-S-2840 (water)	0.9	0.6	0.7	0.4	0.4	0.4	0.0	0.0	0.0	0.2	0.0	0.1
3'-OH-S-2840 (sediment)	0.6	0.8	0.7	1.3	1.4	1.3	1.9	1.6	1.7	1.4	1.8	1.6
Others (Total)*	0.0	0.3	0.2	0.9	0.6	0.7	0.5	0.8	0.7	1.3	0.7	1.0
Total*	93.4	93.8	93.6	91.3	99.8	95.5	93.8	91.6	92.7	89.4	90.3	89.9
Fraction	Days After Treatment (DAT)											
	76			91			111					
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg			
Inpyrfluxam (water)	6.6	5.4	6.0	5.1	4.0	4.6	4.2	4.1	4.1			
Inpyrfluxam (sediment)	74.8	77.9	76.4	74.7	76.3	75.5	73.5	74.4	74.0			
1'-COOH-S-2840 total** (water)	3.9	3.5	3.7	3.2	4.0	3.7	4.3	4.1	4.2			

1'-COOH-S-2840 total** (sediment)	2.7	1.4	2.0	2.7	2.0	2.3	3.0	1.7	2.4
3'-OH-S-2840 (water)	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (sediment)	1.8	1.8	1.8	2.4	2.8	2.6	2.7	2.6	2.7
Others (Total)*	1.9	1.9	1.8	3.1	1.4	2.2	1.1	2.5	1.8
Total*	91.7	91.9	91.8	91.2	90.6	90.9	88.7	89.5	89.1

\* Includes DFPA and total other unknowns which never individually exceed 2.4% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.2.3.2-10 Radioactivity distribution in water and sediment from Weweantic River system (excluding acidic sediment extract) as percentage of applied radioactivity**

Fraction	Days After Treatment (DAT)											
	0			1			3			7		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	79.3	76.0	77.6	64.8	63.2	64.0	55.3	55.7	55.5	39.6	40.6	40.1
Inpyrfluxam (sediment)	18.7	21.2	20.0	31.4	34.3	32.9	43.1	42.7	42.9	55.4	54.2	54.8
1'-COOH-S-2840 total** (water)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3'-OH-S-2840 (water)	1.7	1.7	1.7	0.8	1.6	1.2	0.7	0.5	0.6	0.6	0.6	0.6
3'-OH-S-2840 (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.5	0.5	0.7	0.2	0.5
Others (Total)*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.5	0.3
Total*	99.7	98.9	99.3	97.0	99.0	98.0	99.7	99.6	99.6	96.3	96.1	96.2
Fraction	Days After Treatment (DAT)											
	15			30			45			62		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
Inpyrfluxam (water)	35.0	36.9	36.0	29.4	27.9	28.6	22.7	19.9	21.3	16.9	18.0	17.5
Inpyrfluxam (sediment)	59.4	57.1	58.2	63.0	60.8	61.9	68.9	69.8	69.4	66.4	67.7	67.0

1'-COOH-S-2840 total** (water)	0.9	0.9	0.9	2.4	2.7	2.6	1.9	1.5	1.7	4.6	2.2	3.4
1'-COOH-S-2840 total** (sediment)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.8	0.7
3'-OH-S-2840 (water)	0.7	0.8	0.8	0.8	0.6	0.7	0.4	0.4	0.4	0.5	0.7	0.6
3'-OH-S-2840 (sediment)	1.3	0.6	0.9	1.3	1.0	1.1	0.9	1.6	1.2	1.1	1.5	1.3
Others (Total)*	1.9	0.7	1.3	0.9	1.7	1.3	0.7	0.9	0.8	1.3	1.8	1.6
Total*	99.1	97.0	98.1	97.8	94.6	96.2	95.5	94.0	94.8	91.5	92.7	92.1
<b>Fraction</b>	<b>Days After Treatment (DAT)</b>											
	<b>76</b>			<b>91</b>			<b>111</b>					
<b>Sample</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Avg</b>			
Inpyrfluxam (water)	15.2	12.0	13.6	12.0	11.4	11.7	10.6	11.6	11.1			
Inpyrfluxam (sediment)	70.6	72.7	71.7	73.7	69.1	71.4	70.0	70.2	70.1			
1'-COOH-S-2840 total** (water)	3.2	3.3	3.2	1.7	3.5	2.6	2.4	1.7	2.0			
1'-COOH-S-2840 total** (sediment)	0.4	0.0	0.2	0.0	0.4	0.2	1.2	0.5	0.9			
3'-OH-S-2840 (water)	0.3	0.3	0.3	0.4	0.2	0.3	0.0	0.0	0.0			
3'-OH-S-2840 (sediment)	0.9	0.8	0.9	1.5	1.6	1.6	1.2	2.0	1.6			

Others (Total)*	1.6	2.3	2.1	1.6	1.7	1.7	2.3	1.5	1.9
Total*	92.3	91.5	91.9	91.0	87.9	89.4	87.7	87.5	87.6

\* Includes DFPA, DFPA-CONH<sub>2</sub>, N-des-Me-S-2840 and total other unknowns which never individually exceed 1.3% in the whole system

\*\* As the sum of both isomers = 1'-COOH-S-2840 A + 1'-COOH-S-2840 B

**Table B.8.2.2.3.2-11 Radioactivity distribution in acidic sediment extracts as percent of applied radioactivity**

System	Goose River						Sharkey					
Fraction	Days After Treatment (DAT)											
	62			111			62			111		
Sample	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg	Rep 1	Rep 2	Avg
3'-OH-S-2840	0.0	0.0	0.0	0.0	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0
inpyrfluxam	6.6	6.9	6.8	9.9	9.1	9.5	5.4	4.2	4.8	5.9	4.2	5.0
Total other unknowns	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.9	0.4
Total	6.6	6.9	6.8	9.9	9.6	9.8	5.4	4.4	4.9	5.9	5.1	5.5
System	Weweantic River											
Fraction	Days After Treatment (DAT)											
	62						111					
Sample	Rep 1		Rep 2		Avg		Rep 1		Rep 2		Avg	
3'-OH-S-2840	0.0		0.0		0.0		0.0		0.0		0.0	
inpyrfluxam	4.2		3.1		3.7		7.2		6.7		6.9	
Total other unknowns	0.0		0.6		0.3		0.2		0.0		0.1	
Total	4.2		3.7		4.0		7.4		6.7		7.0	

**Table B.8.2.3.2-12 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Goose River)**

<b>DAT</b>	<b>0</b>			<b>1</b>			<b>3</b>			<b>7</b>		
<b>Sample No.</b>	26	27	Avg.	28	47	Avg.	33	37	Avg.	32	36	Avg.
<b>pH (water phase)</b>	8.68	8.75	8.72	8.6	8.81	8.71	8.52	8.54	8.53	8.59	8.54	8.57
<b>pH (sediment phase)</b>	7.20	7.18	7.19	7.25	7.37	7.31	7.47	7.31	7.39	7.23	7.43	7.33
<b>Dissolved Oxygen (ppm)</b>	8.43	8.41	8.42	8.77	8.83	8.80	6.95	6.97	6.96	3.7	5.81	4.76
<b>Redox potential (mV water phase)<sup>1</sup></b>	6	-48	-21	-3	-28	-16	-29	-34	-32	90	117	104
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-215	-217	-216	-169	-266	-218	-258	-266	-262	-81	-83	-82
<b>DAT</b>	<b>15</b>			<b>30</b>			<b>45</b>			<b>62</b>		
<b>Sample No.</b>	30	40	Avg.	34	43	Avg.	35	39	Avg.	29	44	Avg.
<b>pH (water phase)</b>	8.81	8.76	8.79	7.79	7.74	7.77	7.75	7.81	7.78	7.67	8.14	7.91
<b>pH (sediment phase)</b>	6.99	6.85	6.92	7.15	6.77	6.96	7.14	6.7	6.92	6.76	6.92	6.84
<b>Dissolved Oxygen (ppm)</b>	8.73	8.61	8.67	8.84	8.94	8.89	9.35	9.17	9.26	9.19	9.11	9.15
<b>Redox potential (mV water phase)<sup>1</sup></b>	62	25	44	89	70	80	92	110	101	173	155	164
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-154	-140	-147	-153	-138	-146	-133	-130	-132	-149	-124	-137
<b>DAT</b>	<b>76</b>			<b>91</b>			<b>111</b>					
<b>Sample No.</b>	42	50	Avg.	31	45	Avg.	38	48	Avg.			
<b>pH (water phase)</b>	8.13	7.83	7.98	8.32	8.39	8.36	7.51	8.12	7.82			

<b>pH (sediment phase)</b>	6.95	7.05	7.00	7.39	7.26	7.33	7.16	7.26	7.21
<b>Dissolved Oxygen (ppm)</b>	9.04	8.65	8.85	9.23	9.38	9.31	9.32	9.48	9.40
<b>Redox potential (mV water phase)<sup>1</sup></b>	165	170	168	187	155	171	106	127	117
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-143	-140	-142	-144	-163	-154	-146	-153	-150

<sup>1</sup> The accuracy of the redox values for the 1 DAT and 3 DAT samples cannot be verified because the electrode did not calibrate properly.

A new electrode was purchased and used for all samples on and after 15 DAT.

Conclusion: Water phase was more aerobic (had high oxygen concentrations and more positive redox potentials) than the sediment phase which showed more anaerobic character (more negative redox potentials)

**Table B.8.2.2.3.2-13 Summary of dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Goose River)**

<b>Summary</b>			
	<b>Max</b>	<b>Min</b>	<b>Avg.</b>
<b>pH (water)</b>	8.81	7.51	8.26
<b>pH (sediment)</b>	7.47	6.70	7.13
<b>Dissolved Oxygen (ppm)</b>	9.48	3.70	8.41
<b>Redox potential (water phase)</b>	187	-48	80
<b>Redox potential (sediment phase)</b>	-81	-266	-162



**Table B.8.2.2.3.2-14 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Sharkey)**

<b>DAT</b>	<b>0</b>			<b>1</b>			<b>3</b>			<b>7</b>		
<b>Sample No.</b>	1	2	Avg.	11	24	Avg.	4	18	Avg.	5	17	Avg.
<b>pH (water phase)</b>	8.33	7.66	8.00	8.03	6.89	7.46	8.33	8.23	8.28	8.78	8.69	8.74
<b>pH (sediment phase)</b>	6.42	6.37	6.40	6.54	6.4	6.47	6.48	6.62	6.55	6.93	6.85	6.89
<b>Dissolved Oxygen (ppm)</b>	8.70	8.71	8.71	8.45	8.73	8.59	6.27	7.28	6.78	7.5	7.39	7.45
<b>Redox potential (mV water phase)<sup>1</sup></b>	12	8	10	74	64	69	9	-25	-8	160	125	143
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-63	-63	-63	-115	-96	-106	-146	-178	-162	-95	-100	-98
<b>DAT</b>	<b>15</b>			<b>30</b>			<b>45</b>			<b>62</b>		
<b>Sample No.</b>	3	19	Avg.	10	16	Avg.	7	21	Avg.	9	20	Avg.
<b>pH (water phase)</b>	8.63	8.61	8.62	8.38	8.65	8.52	7.21	7.45	7.33	7.31	7.16	7.24
<b>pH (sediment phase)</b>	7.08	7.01	7.05	7.23	7.15	7.19	7.69	7.28	7.49	7.19	7.19	7.19
<b>Dissolved Oxygen (ppm)</b>	8.75	8.79	8.77	8.56	8.95	8.76	8.80	8.94	8.87	8.86	8.96	8.91
<b>Redox potential (mV water phase)<sup>1</sup></b>	176	77	127	159	14	87	21	95	58	208	188	198
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-147	-176	-162	-215	-200	-208	-176	-158	-167	-197	-134	-166
<b>DAT</b>	<b>76</b>			<b>91</b>			<b>111</b>					
<b>Sample No.</b>	8	25	Avg.	6	15	Avg.	12	14	Avg.			
<b>pH (water phase)</b>	7.54	7.23	7.39	7.46	7.41	7.44	7.47	7.52	7.5			
<b>pH (sediment phase)</b>	7.27	7.21	7.24	7.27	7.28	7.28	7.37	7.35	7.36			

<b>Dissolved Oxygen (ppm)</b>	8.85	8.9	8.88	9.3	9.18	9.24	9.35	9.16	9.26
<b>Redox potential (mV water phase)<sup>1</sup></b>	232	189	211	235	215	225	179	163	171
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-163	-129	-146	-183	-186	-185	-160	-188	-174

<sup>1</sup> The accuracy of the redox values for the 1 DAT and 3 DAT samples cannot be verified because the electrode did not calibrate properly. A new electrode was purchased and used for all samples on and after 15 DAT.

Conclusion: Water phase was more aerobic (had high oxygen concentrations and more positive redox potentials) than the sediment phase which showed more anaerobic character (more negative redox potentials)

**Table B.8.2.2.3.2-15 Summary of dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Sharkey)**

<b>Summary</b>			
	Max	Min	Avg.
<b>pH (water)</b>	8.78	6.89	7.86
<b>pH (sediment)</b>	7.69	6.37	7.01
<b>Dissolved Oxygen (ppm)</b>	9.35	6.27	8.56
<b>Redox potential (water phase)</b>	235	-25	117
<b>Redox potential (sediment phase)</b>	-63	-215	-149

**Table B.8.2.3.2-16 Dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Weweantic River)**

<b>DAT</b>	<b>0</b>			<b>1</b>			<b>3</b>			<b>7</b>		
<b>Sample No.</b>	53	56	Avg.	57	64	Avg.	62	71	Avg.	55	65	Avg.
<b>pH (water phase)</b>	6.72	6.67	6.70	7.41	7.24	7.33	7.81	7.76	7.79	8.09	8.18	8.14
<b>pH (sediment phase)</b>	6.45	6.66	6.56	7.22	7.03	7.13	7.15	7.16	7.16	7.37	7.42	7.40
<b>Dissolved Oxygen (ppm)</b>	8.80	8.82	8.81	8.47	8.8	8.64	7.62	7.64	7.63	0.3	1.82	1.06
<b>Redox potential (mV water phase)<sup>1</sup></b>	52	19	36	-42	35	-4	3	8	6	28	-97	-35
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-165	-25	-95	-149	-220	-185	-267	-257	-262	-151	-171	-161
<b>DAT</b>	<b>15</b>			<b>30</b>			<b>45</b>			<b>62</b>		
<b>Sample No.</b>	54	69	Avg.	60	66	Avg.	61	68	Avg.	58	70	Avg.
<b>pH (water phase)</b>	8.69	8.74	8.72	8.74	8.85	8.80	6.16	6.48	6.32	6.71	6.58	6.65
<b>pH (sediment phase)</b>	7.83	8.09	7.96	8.55	8.58	8.57	7.82	7.68	7.75	7.80	8.05	7.93
<b>Dissolved Oxygen (ppm)</b>	8.83	8.75	8.79	9.05	9.09	9.07	9.51	9.35	9.43	8.83	8.91	8.87
<b>Redox potential (mV water phase)<sup>1</sup></b>	25	33	29	17	29	23	215	195	205	178	185	182
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-252	-266	-259	-256	-294	-275	-155	-248	-202	-140	-160	-150
<b>DAT</b>	<b>76</b>			<b>91</b>			<b>100</b>					
<b>Sample No.</b>	67	74	Avg.	72	75	Avg.	59	63	Avg.			
<b>pH (water phase)</b>	6.36	6.44	6.40	6.56	5.53	6.05	4.11	4.26	4.2			

<b>pH (sediment phase)</b>	8.02	7.52	7.77	7.83	7.78	7.81	6.95	6.92	6.94
<b>Dissolved Oxygen (ppm)</b>	8.92	9.04	8.98	9.61	9.29	9.45	8.75	9.06	8.91
<b>Redox potential (mV water phase)<sup>1</sup></b>	207	203	205	202	251	227	325	355	340
<b>Redox potential (mV sediment phase)<sup>1</sup></b>	-158	-135	-147	-48	-118	-83	-73	-62	-68

<sup>1</sup> The accuracy of the redox values for the 1 DAT and 3 DAT samples cannot be verified because the electrode did not calibrate properly. A new electrode was purchased and used for all samples on and after 15 DAT.

Conclusion: Water phase was more aerobic (had high oxygen concentrations and more positive redox potentials) than the sediment phase which showed more anaerobic character (more negative redox potentials)

**Table B.8.2.3.2-17 Summary of dissolved oxygen, redox potential, and pH measurements for the aerobic aquatic study (Weweantic River)**

<b>Summary</b>			
	<b>Max</b>	<b>Min</b>	<b>Avg.</b>
<b>pH (water)</b>	8.85	4.11	7.00
<b>pH (sediment)</b>	8.58	6.45	7.54
<b>Dissolved Oxygen (ppm)</b>	9.61	0.30	8.15
<b>Redox potential (water phase)</b>	355	-97	110
<b>Redox potential (sediment phase)</b>	-25	-294	-171

## V. Mass Balance

All three systems had acceptable mass balances. The average mass balance was  $99.5 \pm 1.9\%$  AR for the Goose River system,  $99.6 \pm 3.1\%$  for the Sharkey system and  $99.2 \pm 1.1\%$  for the Weweantic River system. These values are within the OECD 308 Guidelines recommended range of 90 – 110 % and are therefore accepted by HSE.

## VI. Bound Residues

The radioactivity remaining in sediment following the neutral and acidic extractions was 13% of the AR (Goose River), 5 % of the AR (Sharkey) and 3% of the AR (Weweantic River) by the end of the study (111 DAT). Additional extractions (ethyl acetate extract, 4.5% of the AR; dioxane and hexane extract, <1% of the AR; dismembrator extract, 6.4% of the AR) of the Goose River and fractioning into humin (5.5% of AR), humic acid (0.8% of AR) and fulvic acid (1.0% of AR) fractions was performed.

## VII. Volatilisation

The total amount of carbon dioxide generated during the study phase was negligible, reaching 0.5% of the AR in the Goose River system, 0.1% in the Sharkey system and 0.0% in the Weweantic River system.

## VIII. Metabolites

Two metabolites were observed above 2.5% AR: 3'-OH-S-2840, and 1'-COOH-S-2840. The maximum observed 3'-OH-S-2840 and 1'-COOH-S-2840 (as 1'-COOH-S-2840 A + 1'-COOH-S-2840 B) were 2.9 % AR and 8.1 % AR respectively at 111 DAT in the Goose River system and 2.8 % AR at 91 DAT and 7.3 % AR at 111 DAT respectively in the Sharkey system. In the Weweantic River system, the maximum observed 3'-OH-S-2840 and 1'-COOH-S-2840 were 2.2 % AR and 5.2 % AR at 62 DAT.

### *Enantiomeric ratio changes - 1'-COOH-S-2840A and B*

The amounts of isomers A and B were recorded at each timepoint in all 3 water-sediment systems. Changes in enantiomeric excess of 1'-COOH-S-2840 in the total systems were considered by HSE in accordance with the principles outlined in the EFSA Guidance document, 'Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers' (2019) and the 'GB Guidance on the environmental fate and behaviour of plant protection products that include stereoisomers'.

While the applicant stated that the 1'methyl group was oxidized preferentially to the 1'-COOH-S-2840B isomer versus the 1'-COOH-S-2840A isomer, the ratio between

the isomers remained approximately racemic throughout the study and any shifts observed were less than the 10 % change specified in the EFSA guidance document. See Table B.8.2.2.3.2-18 for details.

**Table B.8.2.2.3.2-18 Changes in enantiomeric excess of 1'-COOH-S-2840A and B**

	1'-COOH-S-2840A (% AR)	1'-COOH-S-2840B (% AR)	Enantiomeric excess	Change in enantiomeric excess
<b>Goose River (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	NA
<b>1</b>	0	0	0	0
<b>3</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>15</b>	0.4	0.9	-0.5	-0.5
<b>30</b>	0.8	1.0	-0.2	0.3
<b>45</b>	1.9	2.3	-0.4	-0.2
<b>62</b>	2.2	3.0	-0.8	-0.4
<b>76</b>	2.5	2.8	-0.3	0.5
<b>91</b>	3.4	3.3	0.1	0.4
<b>111</b>	3.3	4.5	-1.2	-1.3
<b>Sharkey (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	NA
<b>1</b>	0	0	0	0
<b>3</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>15</b>	0	0.2	-0.2	-0.2
<b>30</b>	1.2	1.4	-0.2	0
<b>45</b>	1.5	2.5	-1.0	-0.8
<b>62</b>	3.0	2.5	0.5	1.5
<b>76</b>	2.8	2.9	-0.1	-0.6
<b>91</b>	2.9	3.1	-0.2	-0.1
<b>111</b>	2.4	4.1	-1.7	-1.5
<b>Weweantic River (total system – combined neutral and water extracts)</b>				
<b>0</b>	0	0	0	NA
<b>1</b>	0	0	0	0
<b>3</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>15</b>	0	0.9	-0.9	-0.9
<b>30</b>	1.2	1.4	-0.2	0.7
<b>45</b>	1.1	0.6	0.5	0.7
<b>62</b>	1.9	2.2	-0.3	-0.8
<b>76</b>	1.9	1.5	0.4	0.7
<b>91</b>	1.6	1.2	0.4	0

111	2.1	0.8	1.3	0.9
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## KINETIC ANALYSIS

The applicant initially submitted aquatic degradation kinetics using PestDF, a method aligned with NAFTA guidance and not in accordance with FOCUS kinetics. Following a request at sift, the applicant resubmitted the kinetic evaluation of B.8.2.2.3.1 and B.8.2.2.3.2 under Report Number: 20063620.UK0 – 5338 ‘Recalculation of the degradation rate of inpyrfluxam (S-2399) in aquatic systems according to FOCUS Kinetics Guidance’. The data from this study was used to derive appropriate degradation/ dissipation half-lives of inpyrfluxam in water/sediment.

The data was analysed by the applicant using the CAKE v3.7 (2023) software package according to guidance provided by FOCUS (2014) based on level P-1 kinetics (single compartment kinetics). As part of the independent validation, HSE has repeated the applicant’s modelling using KinGUII v2.1 (2014) software package.

HSE notes that no sediment dissipation modelling was performed for inpyrfluxam. As no decline phase was observed in the sediment phase, this is correct by FOCUS guidance.

The whole system data were considered appropriate for calculation of persistence endpoints ( $DT_{50}$  and  $DT_{90}$ ) for comparison with relevant study triggers and persistence criteria, and water phase  $DissT_{50}$  and  $DissT_{90}$  values calculated for use as modelling endpoints. As no clear decline phase is observed in the sediment, a kinetic analysis of this compartment has not been conducted by the applicant. HSE accepts this decision, and proposes the use of 1000 day default for the sediment  $DissT_{50}$  modelling endpoints. This will be used to account for potential accumulation in sediment in a simple and conservative way as part of the exposure assessment.

HSE notes that the applicant did not add the percentage of active substance found in the sediment at 0 days back to the water phase day 0 value, as recommended by FOCUS guidance. This led to differences between the dissipation ( $DissT_{50}$  and  $DissT_{90}$ ) values calculated by HSE and the applicant, albeit noted that the applicant’s kinetic fittings led to a more conservative estimate of  $DissT_{50}$  and  $DissT_{90}$ . As a result of these differences in data handling, the kinetic fittings for the water compartments come from the independent HSE assessment.

## Materials and methods

Similar methods to the water-sediment study in kinetic evaluation in B.8.2.2.3.01 were used when deriving modelling and persistence endpoints for this study. See B.8.2.2.3.1 ‘KINETIC ANALYSIS - Materials and Methods’ section for details.

## Results and discussion

### Modelling endpoints

To derive modelling endpoints, the acceptability of the SFO model fit to the water phase only data is considered in the first instance. For the water systems however, due the high  $\chi^2$  value and poor visual fit, the SFO model was considered unacceptable. Because >90% dissipation occurred in the water phase in the study period, FOMC, DFOP and HS models were run and compared against each other. The DFOP model provided the best visual and statistical fits for both test systems (indicated by the low  $\chi^2$  value). Therefore, this model was used to derive the modelling endpoints.

The modelling  $DT_{50}$  can be back calculated from the  $DT_{90}$  by dividing the  $DT_{90}$  by 3.32 for the purposes of deriving conservative modelling endpoints; therefore, the appropriate modelling  $DissT_{50}$  is 8.58 days for Goose River, 29.59 for Weweantic River, and 17.44 for the Sharkey water system. Alternatively, where refinement of surface water exposure values is required, full implementation of DFOP kinetics can be applied. All endpoints are included in the tables below.

**Table B.8.2.2.3.2-19 Results of the kinetic analyses to derive parent-only water modelling endpoints for inpyrfluxam in the Goose River system (HSE fitting; final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	$DissT_{50}$ (d)	$DissT_{90}$ (d)
Goose River Water	SFO	Poor	24.9	$M_0$ : 85.22 $k$ : 0.1676	- $k$ : <0.05	4.14	13.74
	FOMC	Acceptable	9.13	$M_0$ : 96.75 $\alpha$ : 0.71709 $\beta$ : 1.32514	N/A	2.16	31.54
	DFOP	Acceptable	7.14	<b><math>M_0</math>: 98.0</b> <b><math>k_1</math>: 1.1610</b> <b><math>k_2</math>: 0.0548</b> <b><math>g</math>: 0.5232</b>	- <b><math>k_1</math>: &lt;0.05</b> <b><math>k_2</math>: &lt;0.05</b> -	<b>1.77</b> <b>8.58*</b>	<b>28.49</b>
	HS	Acceptable	14.09	$M_0$ : 93.46 $k_1$ : 0.3017 $k_2$ : 0.0456 $t_b$ : 0.52	- $k_1$ : <0.05 $k_2$ : <0.05 -	2.30 9.63*	31.98



\*DissT<sub>90</sub>/3.32

SFO: Poor visual fit. Data points from 14 days after treatment (DAT) onwards are underestimated and residuals show clear systematic deviations with 7 consecutive positive residuals. The initial measured amount is not well described (with some slightly under 10% and some over 10% residual value). The DissT<sub>50</sub> is slightly overestimated and DissT<sub>90</sub> is underestimated.  $\chi^2$  error is >15% and supports the overall conclusion on the poor SFO fit.

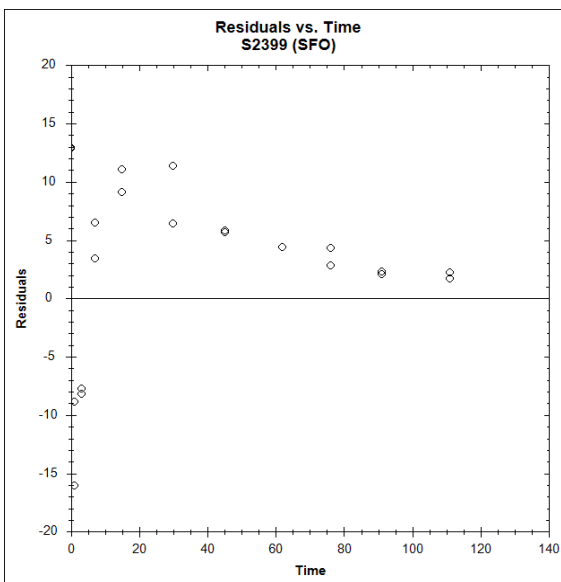
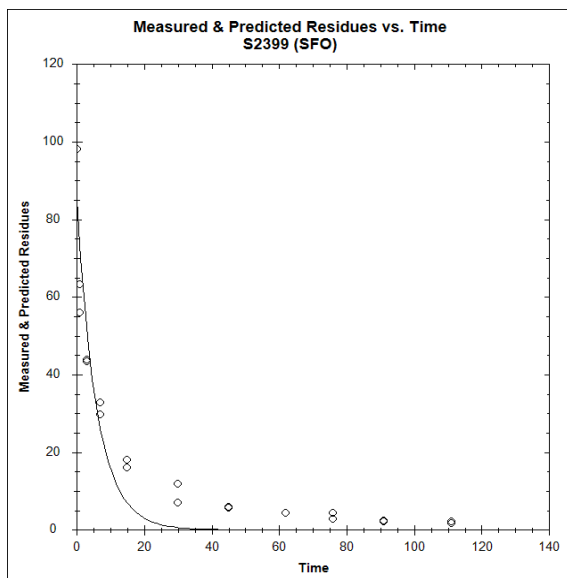
FOMC: provides an acceptable and improved visual fit from SFO, residuals are generally small but are not randomly distributed as data points from 30 DAT onward are overestimated. The measured initial amount and the expected DT<sub>50</sub> are well met.  $\chi^2$  error acceptable.

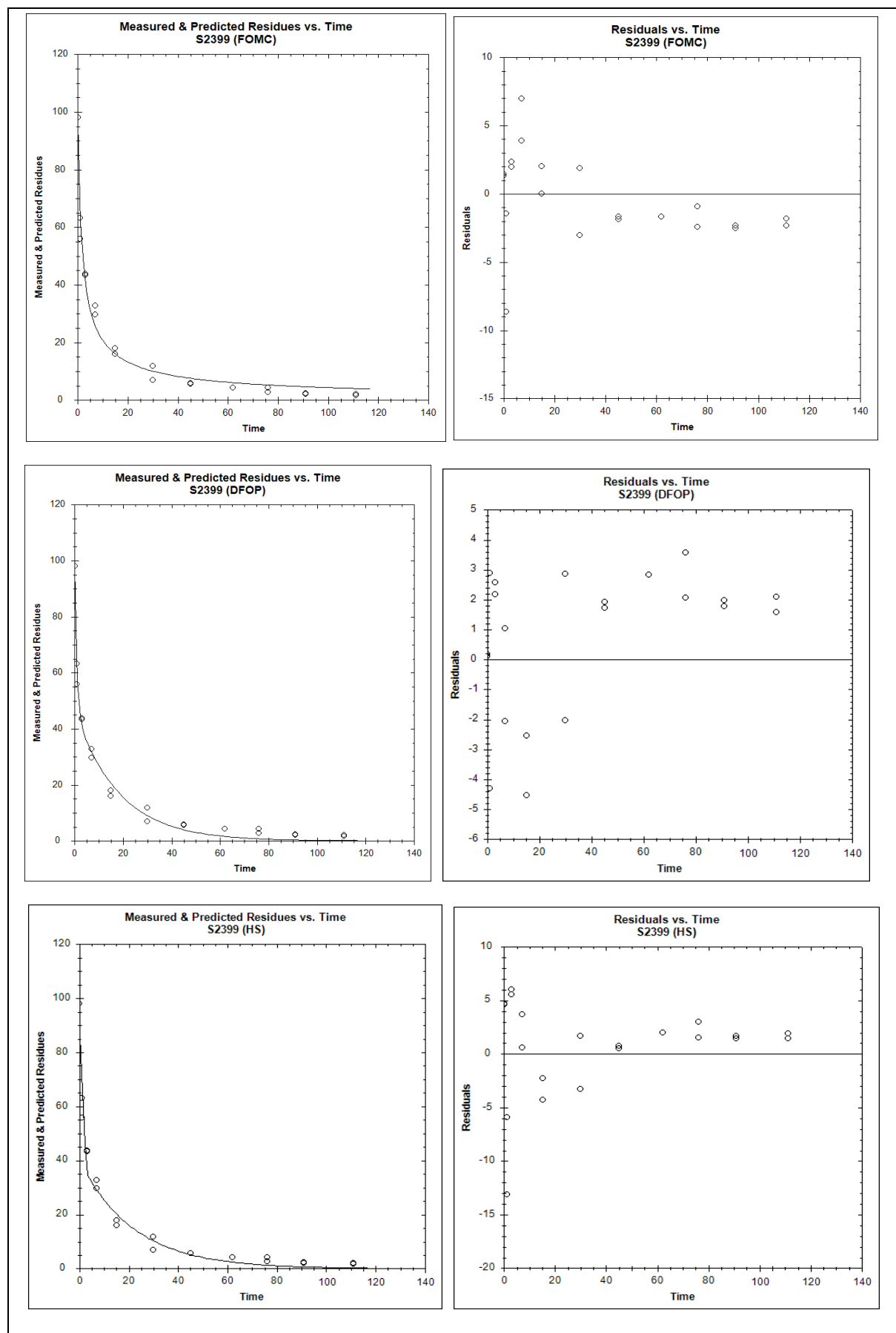
DFOP: Acceptable visual fit, good description of the initial mass & mass around the DissT<sub>50</sub>. Residuals are generally small but are not randomly distributed from 45 DAT onward as data points are overestimated (noting this only affects datapoints beyond the DT<sub>90</sub>).  $\chi^2$  error acceptable, t-test passed.

HS: Acceptable visual fit, but no improvement from DFOP. Good description of the initial mass & mass around the DissT<sub>50</sub>. Residuals are generally small but are not randomly distributed from 62 DAT onward as data points are overestimated from this point (noting this only affects datapoints beyond the DT<sub>90</sub>).

Conclusion: DFOP kinetics selected for modelling endpoints (DissT<sub>50</sub> = 1.77 days (Pseudo DissT<sub>50</sub> = 8.58 days), DissT<sub>90</sub> = 28.49 days).

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**





**Table B.8.2.3.2-20 Results of the kinetic analyses to derive parent-only water modelling endpoints for inpyrfluxam in the Weweantic River system (HSE fitting; final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DissT <sub>50</sub> (d)	DissT <sub>90</sub> (d)
Weweantic River Water	SFO	Poor	25.66	M <sub>0</sub> : 69.85 k: 0.0291	- K: <0.05	23.84	79.21
	FOMC	Acceptable	8.34	M <sub>0</sub> : 97.01 $\alpha$ : 0.36939 $\beta$ : 0.69046	N/A	3.82	351.1
	DFOP	Good	6.55	<b>M<sub>0</sub>: 97.35</b> <b>k<sub>1</sub>: 0.8887</b> <b>k<sub>2</sub>: 0.0162</b> <b>g: 0.5109</b>	- <b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b> -	<b>3.04</b> <b>29.59*</b>	<b>98.23</b>
	HS	Good	10.47	M <sub>0</sub> : 92.20 k <sub>1</sub> : 0.2046 k <sub>2</sub> : 0.0147 t <sub>b</sub> : 3.87	- k <sub>1</sub> : <0.05 k <sub>2</sub> : <0.05 -	3.39 32.17*	106.8

\*DissT<sub>90</sub>/3.32

SFO: Poor visual fit. The initial mass is overestimated and data points from 1 to 30 DAT are overestimated, while 45 to 111 DAT are underestimated. DissT<sub>50</sub> and DissT<sub>90</sub> are also over and underestimated, respectively.  $\chi^2$  error is >15% and supports the overall conclusion on the poor SFO fit.

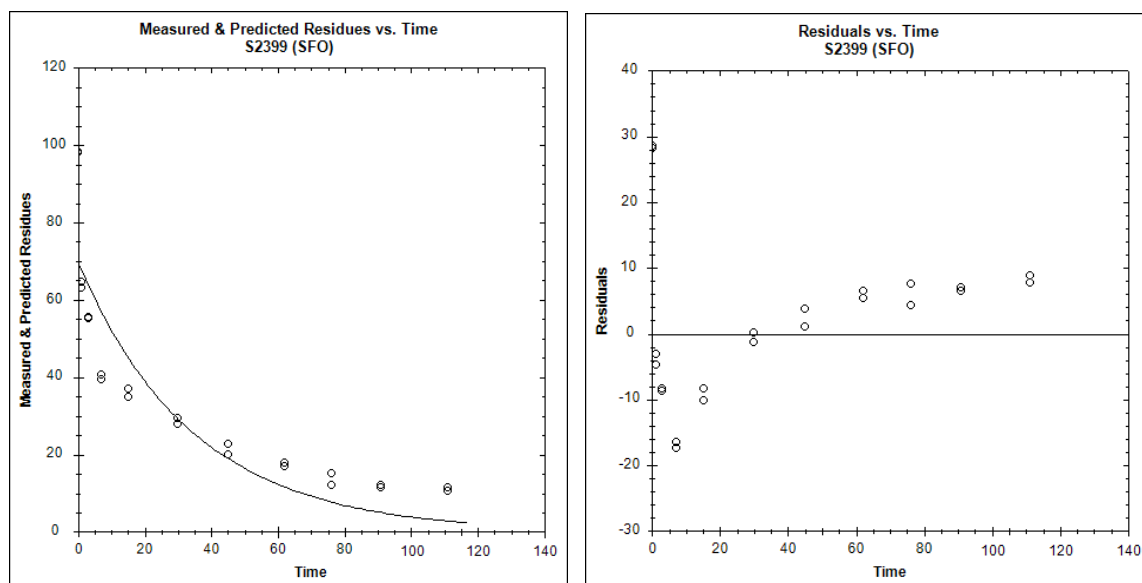
FOMC: Acceptable and improved visual fit from SFO. Residuals are generally small but not randomly distributed, as

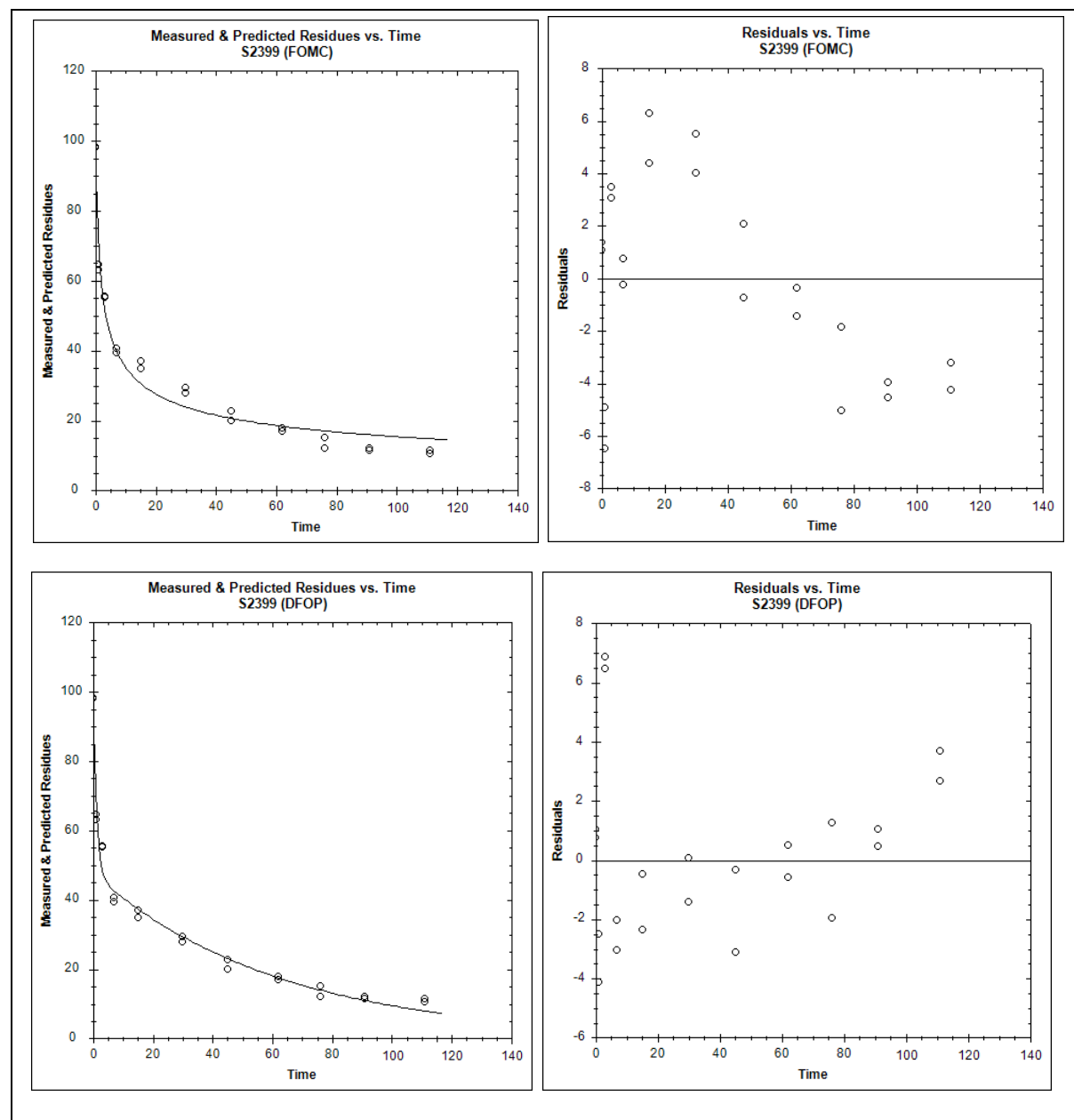
DFOP: Good and improved visual fit from FOMC. The data points are well described with an acceptable description of the initial mass & DissT<sub>50</sub>. DissT<sub>90</sub> is slightly underestimated.  $\chi^2$  error is low, t-test has passed.

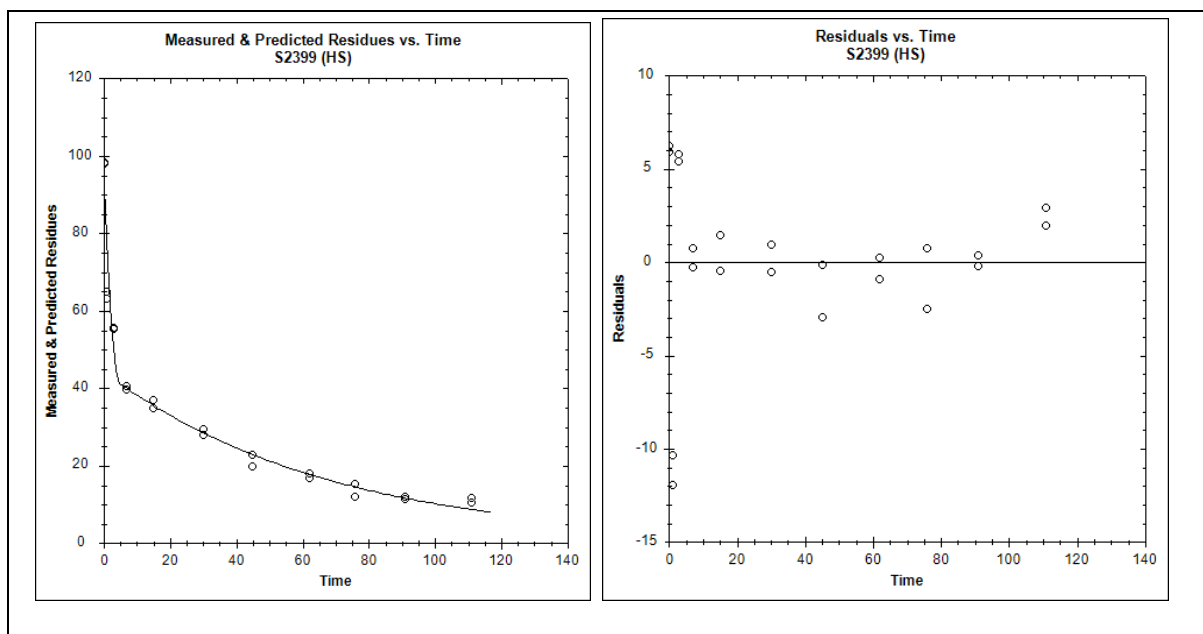
HS: Good visual fit but no improvement to DFOP. The data points are well described with an acceptable description of the initial mass & DissT<sub>50</sub>. DissT<sub>90</sub> is slightly underestimated.  $\chi^2$  error is acceptable, t-test has passed.

Conclusion: DFOP kinetics selected for modelling endpoints (DissT<sub>50</sub> = 3.04 days (Pseudo DissT<sub>50</sub> = 29.59 days), DissT<sub>90</sub> = 98.23 days).

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**







**Table B.8.2.3.2-21 Results of the kinetic analyses to derive parent-only water modelling endpoints for inpyrfluxam in the Sharkey system (HSE fitting; final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DissT <sub>50</sub> (d)	DissT <sub>90</sub> (d)
Sharkey Water	SFO	Poor	25.47	M <sub>0</sub> : 74.69 k: 0.04896	- k: <0.05	14.16	47.03
	FOMC	Poor	16.26	M <sub>0</sub> : 96.12 $\alpha$ : 0.53337 $\beta$ : 1.34974	N/A	3.6	99.84
	DFOP	Good	4.78	<b>M<sub>0</sub>: 99.09</b> <b>k<sub>1</sub>: 1.3497</b> <b>k<sub>2</sub>: 0.0292</b> <b>g: 0.4585</b>	- <b>k<sub>1</sub>: &lt;0.05</b> <b>k<sub>2</sub>: &lt;0.05</b> -	<b>3.17</b> <b>17.44*</b>	<b>57.89</b>
	HS	Good	4.69	M <sub>0</sub> : 99.05 k <sub>1</sub> : 0.4352 k <sub>2</sub> : 0.0294 t <sub>b</sub> : 1.493	- k <sub>1</sub> : <0.05 k <sub>2</sub> : <0.05 -	2.97 17.39*	57.72

\*DissT<sub>90</sub>/3.32

SFO: Visual fit is poor; the initial measured amount is not well described (>10% residual values). The data points from 15 DAT onwards are underestimated, with residuals showing clear systematic deviations with 7 consecutive positive residuals. The DissT<sub>50</sub> is overestimated and the DissT<sub>90</sub> is underestimated.  $\chi^2$  error is >15% and supports the overall conclusion on the poor SFO fit.

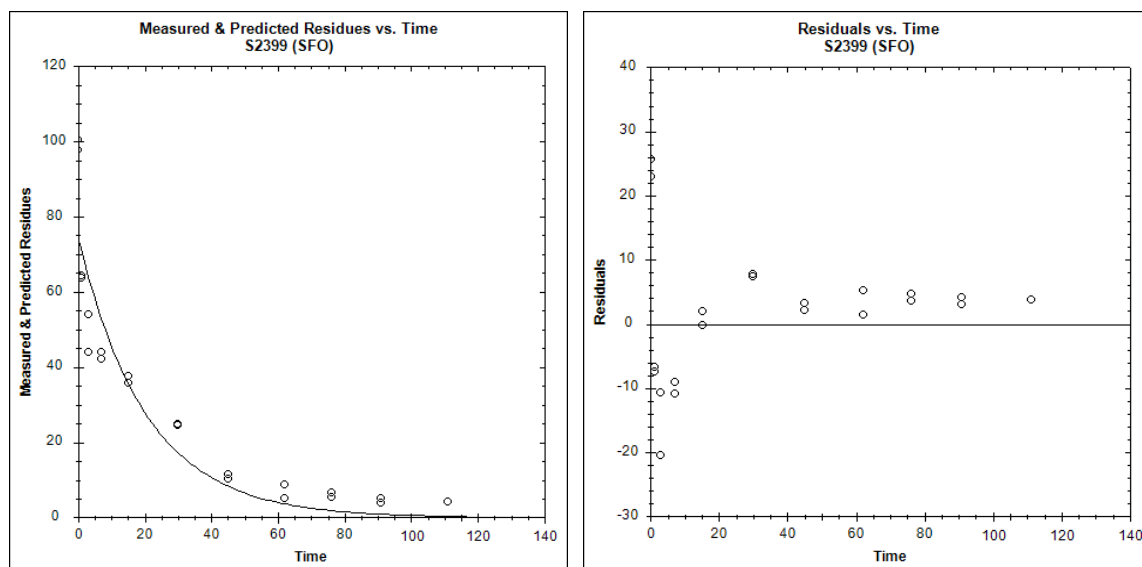
FOMC: Visual fit is poor; no improvement from SFO. Data points at 7 – 30 DAT are underestimated, and 45 – 111 DAT are overestimated, meaning DissT<sub>50</sub> and DissT<sub>90</sub> are under and overestimated, respectively.  $\chi^2$  error level is not acceptable (>15%).

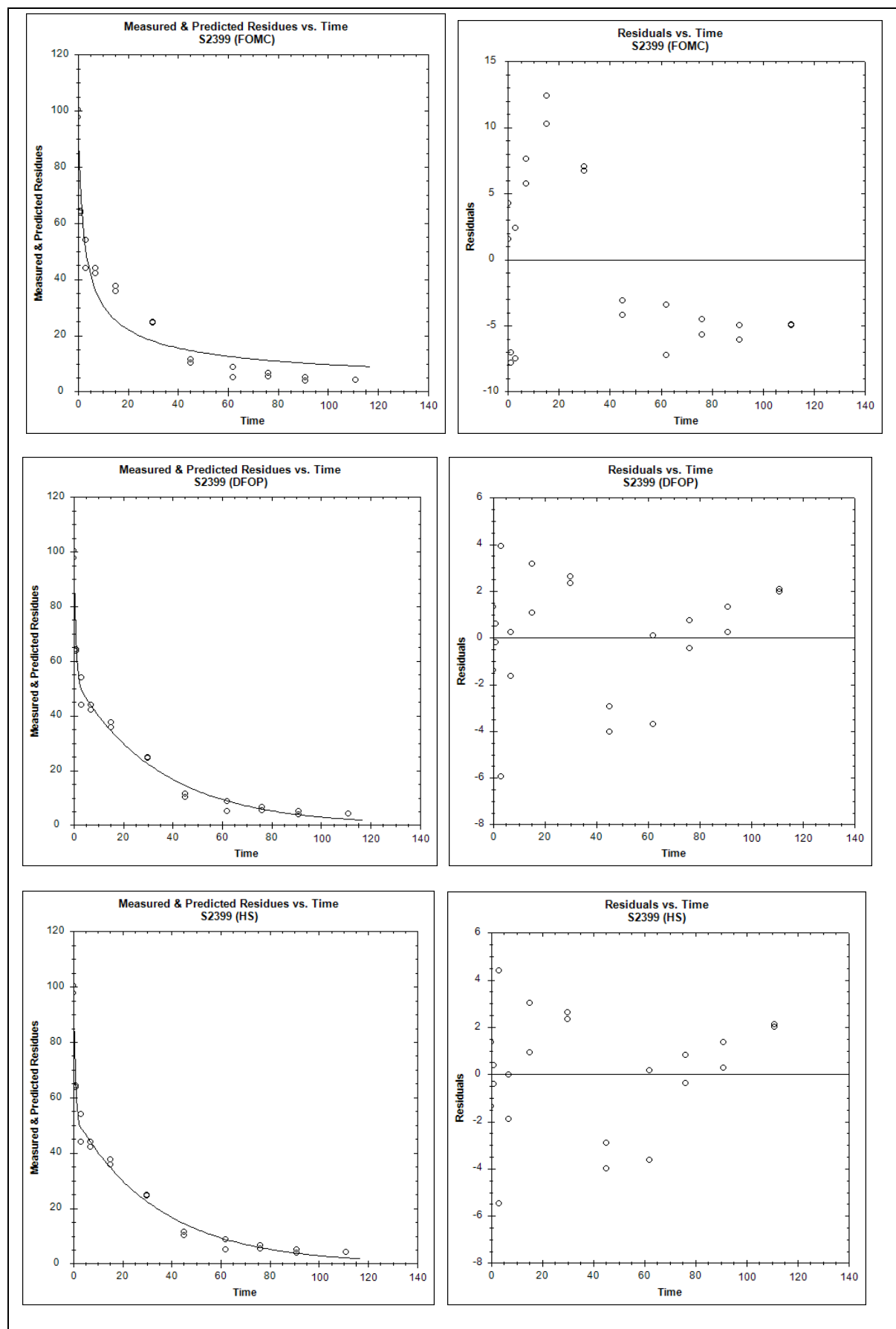
DFOP: Good and improved visual fit from FOMC. Residuals are generally small and randomly distributed. The data points are well described with an acceptable description of the initial mass & DissT<sub>50</sub>.  $\chi^2$  error is very low, t-test has passed.

HS: Good visual fit but insignificant improvement to DFOP visually and statistically. The data points are well described with an acceptable description of the initial mass & DissT<sub>50</sub>.  $\chi^2$  error is very low, t-test has passed.

Conclusion: DFOP kinetics selected for modelling endpoints (DissT<sub>50</sub> = 13.17 days (Pseudo DissT<sub>50</sub> = 17.44 days), DissT<sub>90</sub> = 57.89 days).

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**







The inpyrfluxam persistence endpoints for evaluation of degradation in the whole system are shown in Tables B.8.2.2.3.2-22 to B.8.2.2.3.2-24. Where HSE agrees with the applicant's approach and validated their results, the applicant's results are presented.

In all systems, neither the DT<sub>50</sub> nor DT<sub>90</sub> is reached in the experimental period. As such, greater weight may be placed on the differences between the measured and modelled data, particularly towards the end of the study. Although the DT<sub>50</sub> and DT<sub>90</sub> are extrapolated well beyond the study duration, it does not appear that there is systematic over or underestimation of degradation at all time points for the chosen models, and residuals are <10%. Therefore, HSE agrees with the applicant's selection of the SFO in all cases. In addition it is noted that irrespective of the kinetic model chosen, the persistence criteria would be exceeded in all test systems.

**Table B.8.2.2.3.2-22 Applicant supplied results of the kinetic analyses to derive parent-only, total-system persistence endpoints for inpyrfluxam in the Goose River system (final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
Goose River total system	<b>SFO</b>	<b>Very good**</b>	<b>1.99</b>	<b>M<sub>0</sub>: 94.0</b> <b>k: 0.00327</b>	- k: p<0.01	<b>212</b>	<b>705</b>
	FOMC	Very good**	1.90	M <sub>0</sub> : 95.1 $\alpha$ : 0.4 $\beta$ : 80.9	N/A	377	>10,000
	DFOP	Very good**	1.45	M <sub>0</sub> : 97.2 k1: 0.269 k2: 0.00282 g: 0.0631	- k1: 0.0763 k2: p<0.01 -	222	792
	HS	Very good**	1.47	M <sub>0</sub> : 96.5 k1: 0.00918 k2: 0.00277 t <sub>b</sub> : 9.69	- k1: p<0.01 k2: p<0.01 -	228	809

\*\*HSE disagrees with applicant's assessment. Detailed below where relevant.

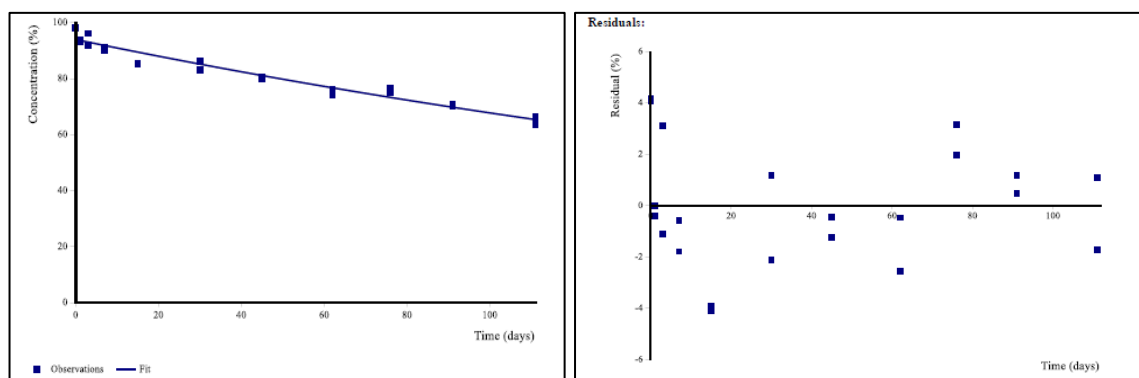
SFO: Applicant states very good visual fit, however HSE describes it as 'good' as whilst the data points are generally well described, there is a slight underestimation of the initial mass. Residuals are generally small and randomly distributed. DT<sub>50</sub> and DT<sub>90</sub> are outside the scope of this study.  $\chi^2$  error is very low, t-test has passed.

FOMC: Applicant states that visual fit is very good, however HSE disagrees and describes it as 'good' as whilst the data points are generally well described, there is a slight overestimation of the final data point at 111 DAT. Residuals are generally small and randomly distributed. DT<sub>50</sub> and DT<sub>90</sub> are outside the scope of this study.  $\chi^2$  error is very low. Overall, FOMC does not provide a better visual fit and only a slightly improved statistical fit to SFO.

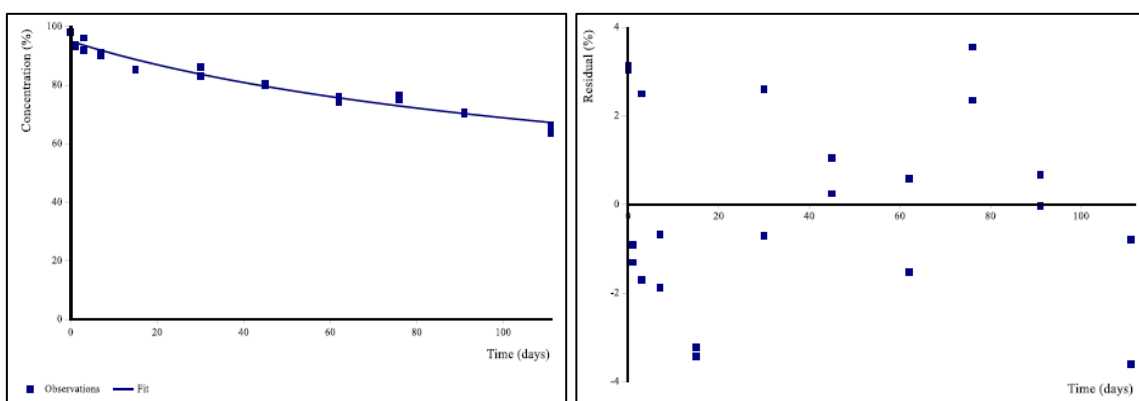
Conclusion: Accept applicants' SFO kinetics for persistence endpoints (DT<sub>50</sub> = 212 days, DT<sub>90</sub> = 705 days).

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**

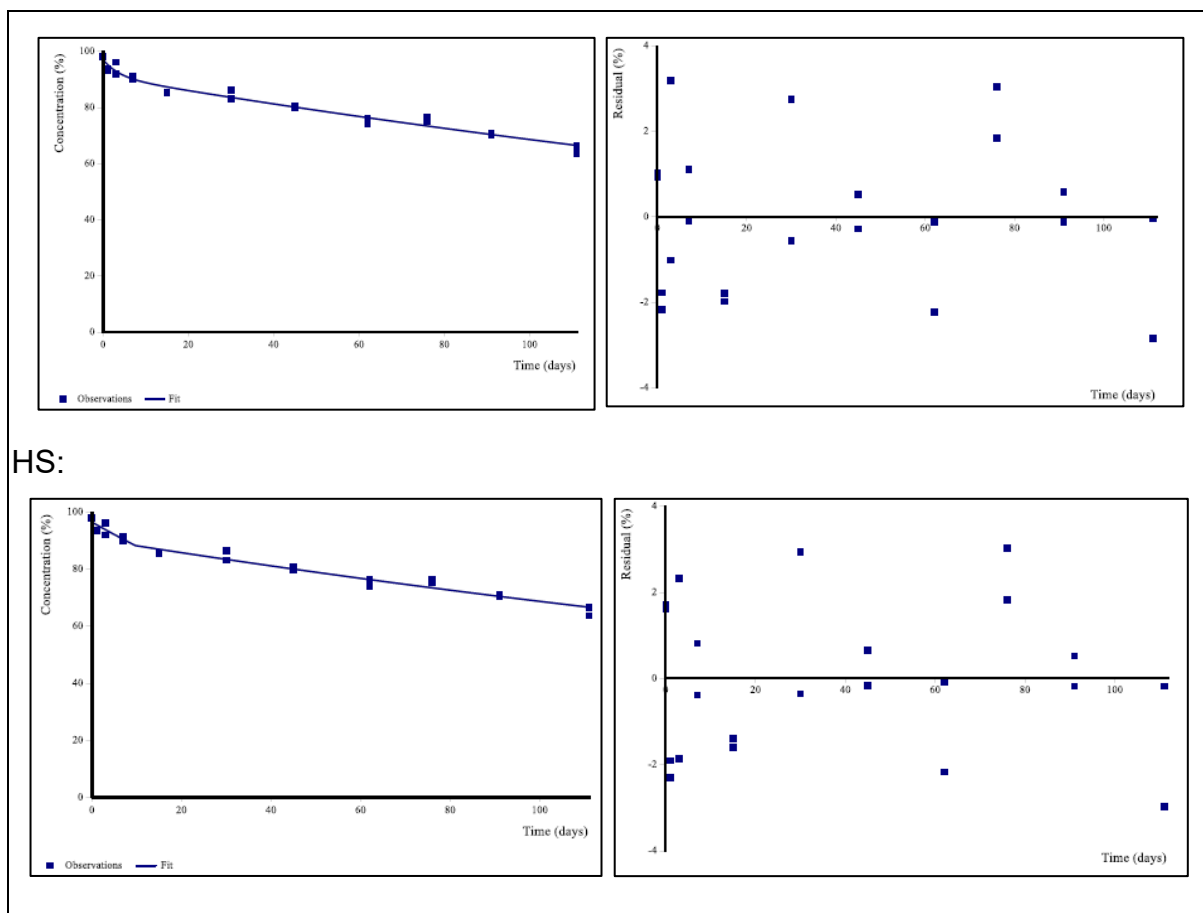
SFO:



FOMC:



DFOP:

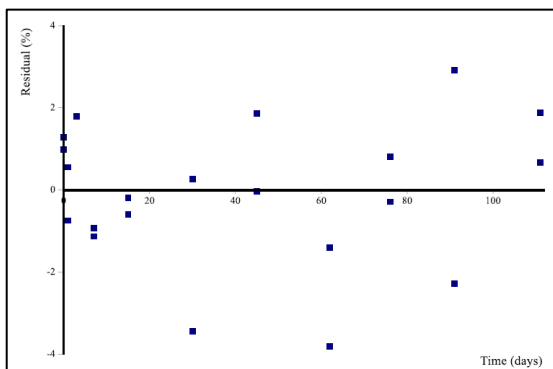
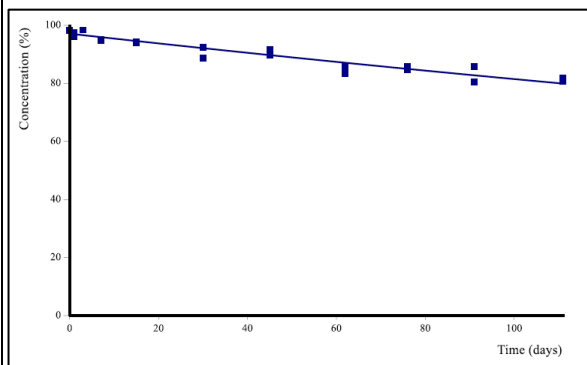


**Table B.8.2.2.3.2-23 Applicant supplied results of the kinetic analyses to derive parent-only, total-system persistence endpoints for inpyrfluxam in the Wewaeantic River system (final selected fit shown in bold).**

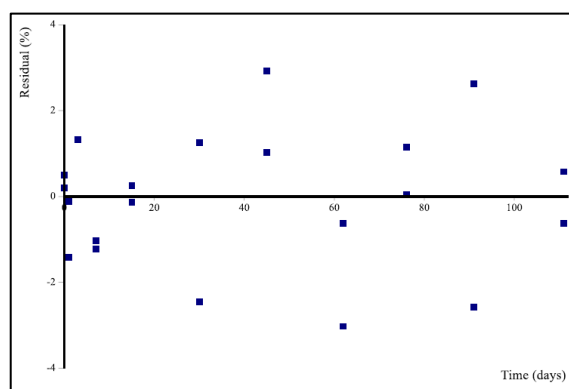
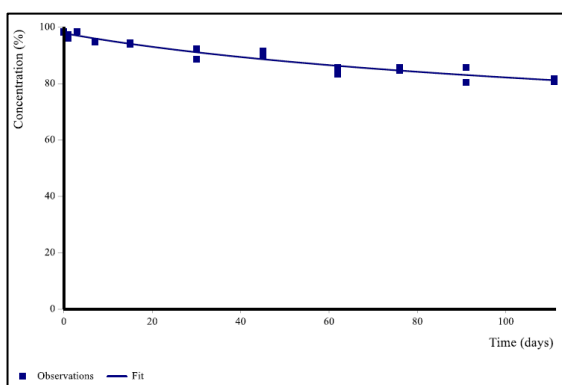
System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
Wewaeantic River total system	<b>SFO</b>	<b>Very good</b>	<b>1.13</b>	<b>M<sub>0</sub>: 97.1</b> <b>k: 0.00176</b>	- k: p<0.01	<b>395</b>	<b>1312</b>
	FOMC	Very good	0.957	M <sub>0</sub> : 97.9 $\alpha$ : 0.185 $\beta$ : 63.6	N/A	2636	>10,000
	DFOP	Very good	1.01	M <sub>0</sub> : 97.9 k <sub>1</sub> : 0.0265 k <sub>2</sub> : 0.00103 g: 0.075	- k <sub>1</sub> : 0.325 k <sub>2</sub> : 0.239 -	595	2153
				M <sub>0</sub> : 97.9	-		

	HS	Very good	1.03	k <sub>1</sub> : 0.00282 k <sub>2</sub> : 0.00145 t <sub>b</sub> : 23.4	k <sub>1</sub> : p<0.01 k <sub>2</sub> : p<0.01 -	457	1570
<p>SFO: Good visual fit visually and statistically. Residuals are generally small and randomly distributed. DT<sub>50</sub> and DT<sub>90</sub> are extrapolated beyond the duration of this study. <math>\chi^2</math> error is very low, t-test has passed.</p> <p>FOMC: Residuals are generally small and randomly distributed. DT<sub>50</sub> and DT<sub>90</sub> are extrapolated beyond the duration of this study. <math>\chi^2</math> error is very low. Overall, FOMC does not provide a better visual fit and only a slightly improved statistical fit to SFO.</p> <p>Conclusion: Accept applicants' SFO kinetics for persistence endpoints (DT<sub>50</sub> = 395 days, DT<sub>90</sub> = 1312 days).</p> <p><b>Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS</b></p>							

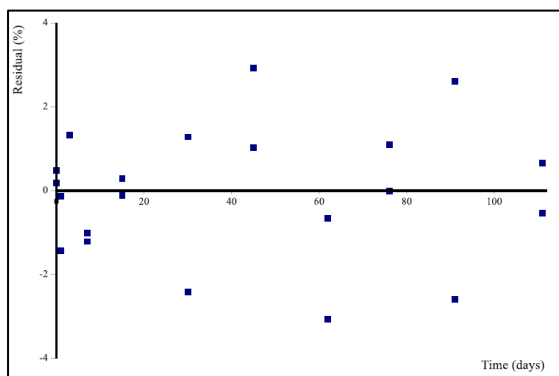
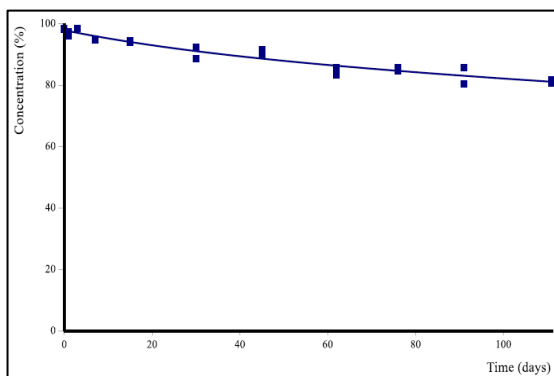
SFO:



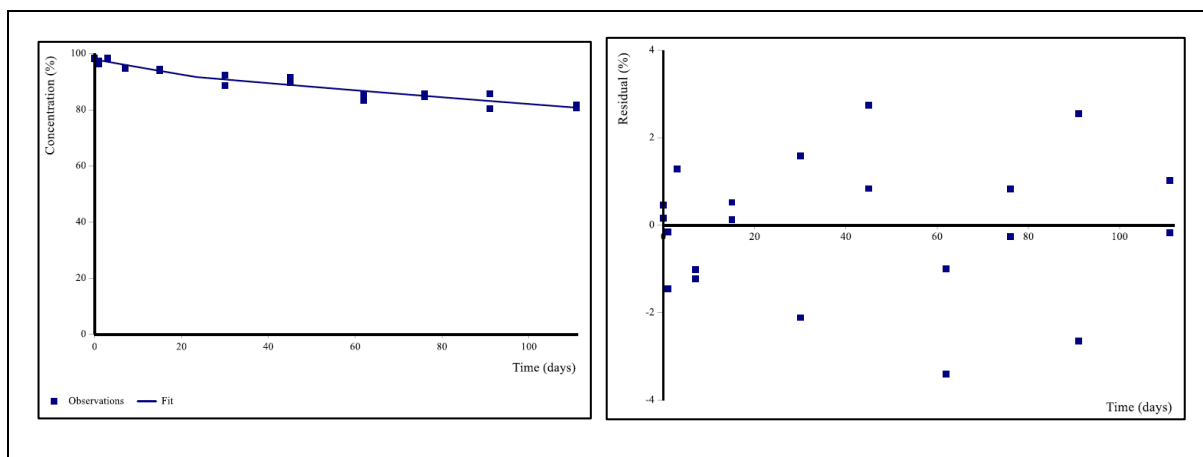
FOMC:



DFOP:



HS:



**Table B.8.2.2.3.2-24 Applicant supplied results of the kinetic analyses to derive parent-only, total-system persistence endpoints for inpyrfluxam in the Sharkey system (final selected fit shown in bold).**

System	Model	Visual Assessment	$\chi^2$ error (%)	Estimated Parameters	Prob>t	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)
Sharkey total system	<b>SFO</b>	<b>Very good</b>	<b>1.71</b>	<b>M<sub>0</sub>: 94.8</b> <b>k: 0.0019</b>	- k: p<0.01	<b>364</b>	<b>1209</b>
	FOMC	Very good	1.65	M <sub>0</sub> : 95.6 $\alpha$ : 0.218 $\beta$ : 72.1	N/A	>10,000	>10,000
	DFOP	Very good	1.14	M <sub>0</sub> : 99.1 k <sub>1</sub> : 1.48 k <sub>2</sub> : 0.0017 g: 0.0577	- k <sub>1</sub> : 0.216 k <sub>2</sub> : p<0.01 -	374	1323
	HS	Very good	1.12	M <sub>0</sub> : 99.1 k <sub>1</sub> : 0.0439 k <sub>2</sub> : 0.0169	- k <sub>1</sub> : 0.0828 k <sub>2</sub> : p<0.01	376	1329
				t <sub>b</sub> : 1.41	-		

\*ln(2)/k2

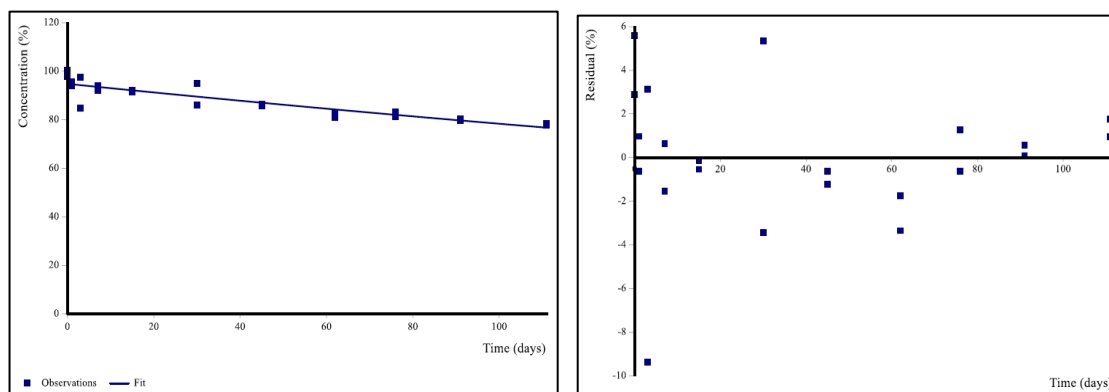
SFO: Good visual fit visually and statistically. Residuals are generally small and randomly distributed. DT<sub>50</sub> and DT<sub>90</sub> are extrapolated beyond the duration of this study.  $\chi^2$  error is very low, t-test has passed.

FOMC: Residuals are generally small and randomly distributed. DT<sub>50</sub> and DT<sub>90</sub> are extrapolated beyond the duration of this study.  $\chi^2$  error is very low. Overall, FOMC does not provide a better visual fit and only a slightly improved statistical fit to SFO.

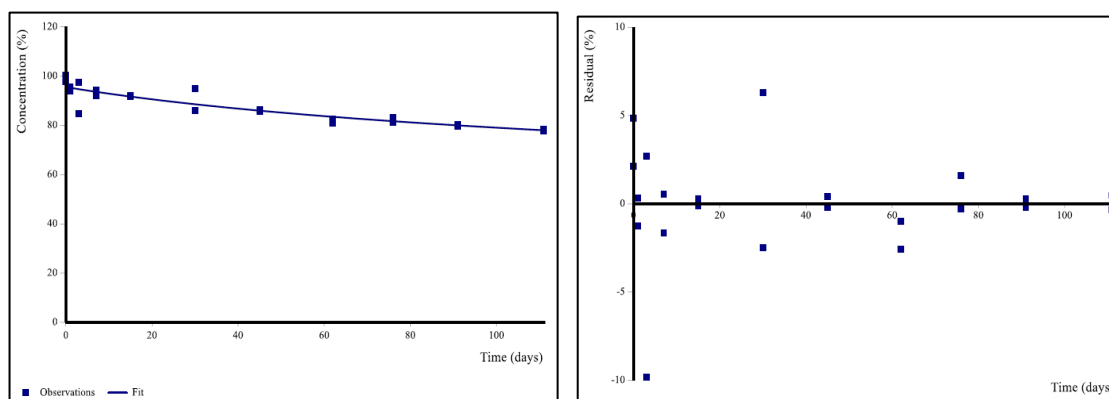
Conclusion: Accept applicants' SFO kinetics for persistence endpoints (DT<sub>50</sub> = 364 days, DT<sub>90</sub> = 1209 days).

**Kinetic fits for: Row 1; SFO: Row 2; FOMC: Row 3; DFOP: Row 4; HS**

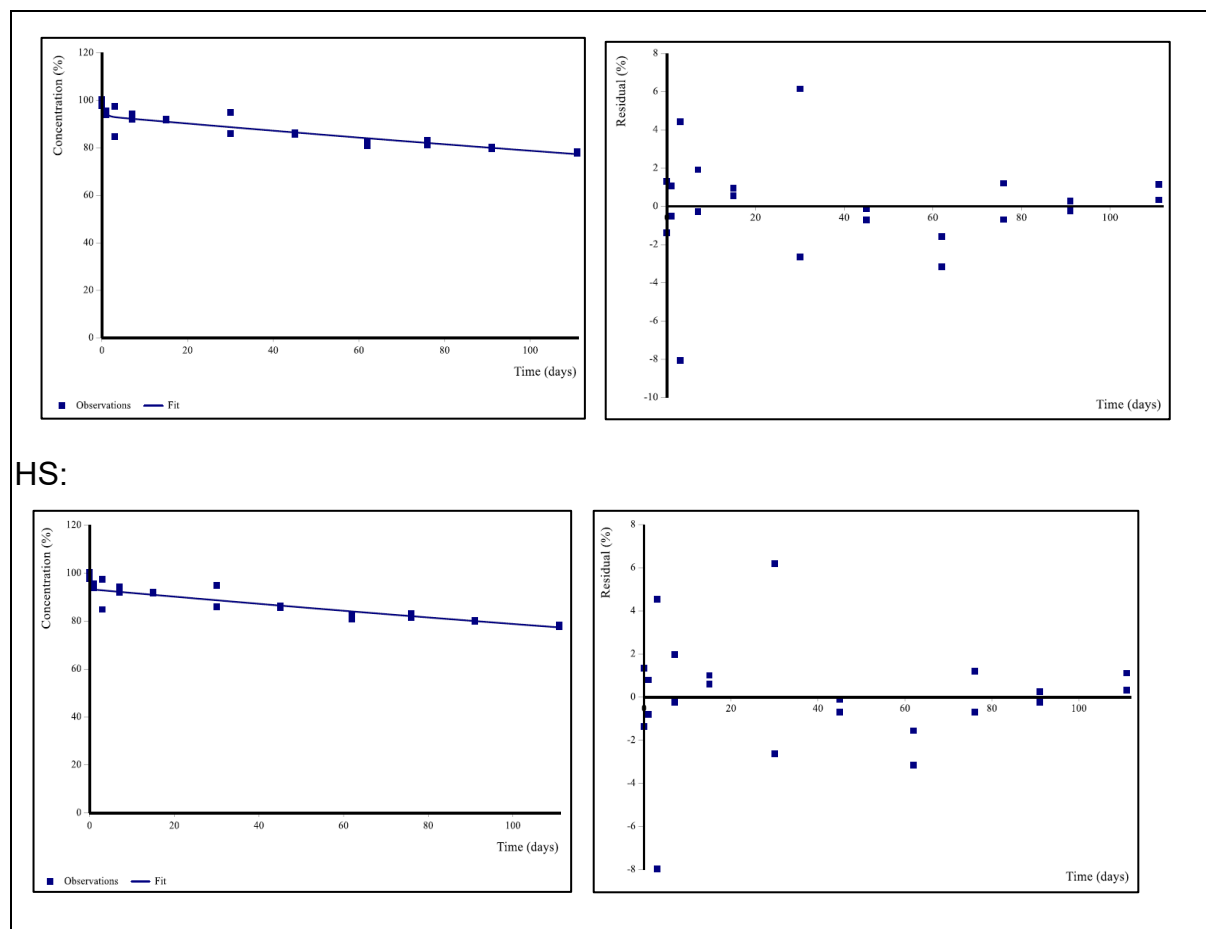
SFO:



FOMC:



DFOP:



## CONCLUSION

The degradation pattern of inpyrfluxam was similar in all three sediment systems. Inpyrfluxam declined to 80.0, 85.0, and 80.4 % of the AR (total inpyrfluxam in total extract) by the end of the 111 DAT study in the Goose River, Sharkey and Weweantic River sediment systems, respectively. Aerobic total-system half-lives were estimated at 212, 364 and 395 days (SFO fit, CAKE 3.7, verified with KinGUI). Metabolite formation was primarily to 1'-COOH-S-2840 and 3'-OH-S-2840, formed at maximum levels of 8.1% AR and 2.9 % AR respectively for the Goose River system, 7.3% AR and 2.8 % AR respectively for the Sharkey system and 5.2 % AR and 2.2 % AR respectively for the Weweantic River system during the study.

HSE deems this water-sediment study acceptable in accordance with the OECD 308 Guidelines.



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### Conclusions of kinetic analysis from both water sediment studies

The modelling and triggering/persistence endpoints across both water sediment studies are summarised in Tables B.8.2.2.3.2-25 and B.8.2.2.3.2-26 respectively.

Inpyrfluxam was observed to partition significantly from the water phase into the sediment phase in the water sediment laboratory study. As such, the sediment compartment is considered to be the relevant compartment against which to assess inpyrfluxam persistence criteria.

The very persistent trigger in freshwater sediment has been determined as a  $DT_{50}$  value > 180 days, according to the criteria in Regulation (EC) No 1107/2009. Following determination of the total system trigger/persistence endpoints in Table B.8.2.2.3.2-26, inpyrfluxam can be observed to exceed this trigger value in all five systems.

HSE notes that the applicant did not update their exposure assessment in the CP to reflect their modelling endpoints from their revised kinetics report. Furthermore, for the  $DissT_{50}$  of inpyrfluxam, the applicant incorrectly used a geomean of the total system  $DT_{50}$  rather than the longest non-normalised  $DissT_{50}$  from the water phase to calculate  $PEC_{sw}$  values. They also used the maximum % of metabolites 1'-COOH-S-2840 and 3'-OH-S-2840 in the total system instead of the % observed in individual water or sediment compartments. As a result, the endpoints selected by HSE for the exposure assessment differ from those of the applicant.

However, since there were already discrepancies between the dissipation values calculated by HSE and those in the applicant's revised report, it was considered unnecessary to request a repeat of the exposure assessment.

**Table B.8.2.2.3.2-25 Summary of modelling endpoints for inpyrfluxam across both water-sediment studies**

System	Phase	pH	Temp p (°C)	DT <sub>50</sub> /DissT <sub>50</sub> (d)	DT <sub>90</sub> /DissT <sub>90</sub> (d)	St. (X <sup>2</sup> )	Parameters	Model
Golden Lake	Water	8.0 (mean)	20 ± 2	2.67 34.28 <sup>(a)</sup>	113.8	2.43 3	M <sub>0</sub> : 100.1 k <sub>1</sub> : 0.5141 k <sub>2</sub> : 0.01084 g: 0.6564	DFOP
	Sediment	N/A 7.8		1000 <sup>(b)</sup>	3320	N/A	N/A	Default
Taunton River	Water	6.6 (mean)	20 ± 2	1.63 17.38 <sup>(a)</sup>	57.71	4.07 6	M <sub>0</sub> : 97.96 k <sub>1</sub> : 0.7645 k <sub>2</sub> : 0.0197 g: 0.6882	DFOP
	Sediment	N/A 5.9		1000 <sup>(b)</sup>	3320	N/A	N/A	Default
Goose River	Water	8.3 (mean)	20 ± 2	1.77 8.58 <sup>(a)</sup>	28.49	7.14	M <sub>0</sub> : 98.0 k <sub>1</sub> : 1.1610 k <sub>2</sub> : 0.0548 g: 0.5232	DFOP
	Sediment	N/A 7.9		1000 <sup>(b)</sup>	3320	N/A	N/A	Default
Weweantic River	Water	7.0 (mean)	20 ± 2	3.04 29.59 <sup>(a)</sup>	98.23	6.55	M <sub>0</sub> : 97.35 k <sub>1</sub> : 0.8887 k <sub>2</sub> : 0.0162 g: 0.5109	DFOP
	Sediment	N/A 5.7		1000 <sup>(b)</sup>	3320	N/A	N/A	Default
Sharkey	Water	7.9 (mean)	20 ± 2	3.17 17.44 <sup>(a)</sup>	57.89	4.78	M <sub>0</sub> : 99.09 k <sub>1</sub> : 1.3497 k <sub>2</sub> : 0.0292 g: 0.4585	DFOP
	Sediment	N/A 6.5		1000 <sup>(b)</sup>	3320	N/A	N/A	Default
Maximum	Water			34.28	113.8			
	Sediment			1000 <sup>(b)</sup>	3320			

a) DissT<sub>90</sub>/3.32

b) No significant decline was noted in the sediment compartments. Use of 1000 day default for sediment DissT<sub>50</sub> is suggested.

**Table B.8.2.2.3.2-26 Summary of modelling endpoints for 1'-COOH-S-2840 across both water-sediment studies**

<b>Metabolite 1'-COOH-S-2840 (A + B combined) modelling endpoints <sup>(a)</sup></b>				Distribution: max in water 10 % after 112 days; Golden Lake PY label <sup>(b)</sup>  Max. sed 4.8 % after 63 days, Golden Lake PH label <sup>(b)</sup>  Max in total system 13.1 % after 112 days; Golden Lake PY label <sup>(b)</sup>					
				<b>Water</b>			<b>Sediment</b>		
<b>Water / sediment system</b>	<b>pH water phase (mean)</b>	<b>pH sed</b>	<b>t. °C <sup>(c)</sup></b>	<b>DisT<sub>50</sub> / DisT<sub>90</sub></b>	<b>St. (X<sup>2</sup>)</b>	<b>Kinetic model</b>	<b>DisT<sub>50</sub> / DisT<sub>90</sub></b>	<b>St. (X<sup>2</sup>)</b>	<b>Kinetic model</b>
<b>Golden Lake</b>	7.8 8.0	N/A 7.8	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Taunton River</b>	5.9 6.6	N/A 5.9	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Goose River</b>	7.9 8.3	N/A 7.9	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Weweantic River</b>	5.7 7.0	N/A 5.7	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Sharkey</b>	6.5 7.9	N/A 6.5	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Maximum (non-normalised) <sup>(c)</sup></b>				1000 / 3320			1000 / 3320		

a) DT<sub>50</sub> and DT<sub>90</sub> values could not be reliably determined from kinetic modelling. A conservative default of 1000 days is recommended for use in the exposure assessment.

b) Mean of two replicates. Value for isomers are combined (1'-COOH-S-2840 A + B).

c) Normalisation of temperature not required as systems were incubated at a constant temperature of 20 ±2 °C.

**Table B.8.2.2.3.2-27 Summary of modelling endpoints for 3'-OH-S-2840 across both water-sediment studies**

<b>Metabolite 3'-OH-S-2840 (A + B combined) modelling endpoints <sup>(a)</sup></b>				Distribution: max in water 2.9 % after 0 days; Taunton River PY label <sup>(b)</sup>  Max. sed 6.0 % after 30 days, Taunton River PY label <sup>(b)</sup>  Max in total system 6.8 % after 30 days; Taunton River PY label <sup>(b)</sup>					
				<b>Water</b>			<b>Sediment</b>		
<b>Water / sediment system</b>	<b>pH water phase (mean)</b>	<b>pH sed</b>	<b>t. °C <sup>(c)</sup></b>	<b>DisT<sub>50</sub> / DisT<sub>90</sub></b>	<b>St. (X<sup>2</sup>)</b>	<b>Kinetic model</b>	<b>DisT<sub>50</sub> / DisT<sub>90</sub></b>	<b>St. (X<sup>2</sup>)</b>	<b>Kinetic model</b>
<b>Golden Lake</b>	7.8 8.0	N/A 7.8	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Taunton River</b>	5.9 6.6	N/A 5.9	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Goose River</b>	7.9 8.3	N/A 7.9	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Weweantic River</b>	5.7 7.0	N/A 5.7	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Sharkey</b>	6.5 7.9	N/A 6.5	20 ±2	1000 / 3320	N/A	N/A	1000 / 3320	N/A	N/A
<b>Maximum (non-normalised) <sup>(c)</sup></b>				1000 / 3320			1000 / 3320		

a) DT<sub>50</sub> and DT<sub>90</sub> values could not be reliably determined from kinetic modelling. A conservative default of 1000 days is recommended for use in the exposure assessment.

b) Mean of two duplicate samples analysed at the time point.

c) Normalisation of temperature not required as systems were incubated at a constant temperature of 20 ±2 °C

**Table B.8.2.2.3.2-28 Summary of trigger/persistence endpoints for inpyrfluxam across both water-sediment studies**

System	Phase	pH water (mean)	pH sed	Temp (°C)	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	St. (X <sup>2</sup> )	Parameters	Model
Golden Lake	Total	8.0	7.8	20 ± 2	>10,000	>10,000	2.90	M <sub>0</sub> : 98.1 k <sub>1</sub> : 0.0318 k <sub>2</sub> : 2.02E-014 g: 0.295	DFOP
Taunton River	Total	6.6	5.9		758	2518	3.23	M <sub>0</sub> : 93.7 k: 0.000914	SFO
Goose River	Total	8.3	7.9		212	705	1.99	M <sub>0</sub> : 94.0 k: 0.00327	SFO
Wewantic River	Total	7.0	5.7		395	1312	1.13	M <sub>0</sub> : 97.1 k: 1.13	SFO
Sharkey	Total	7.9	6.5		364	1209	1.17	M <sub>0</sub> : 94.8 k: 0.0019	SFO
Maximum	Total				10,000	10,000			

**DEGRADATION PATHWAY**

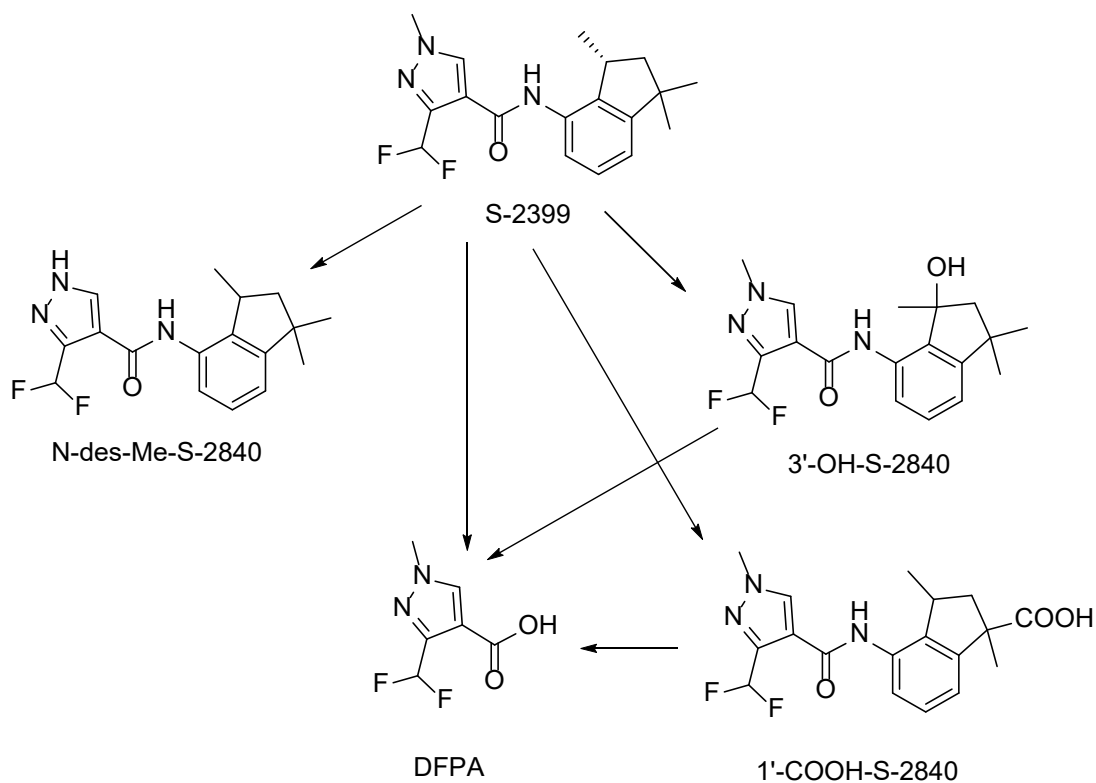
The following pathway of degradation has been proposed by the applicant:

- Inpyrfluxam was degraded by multiple pathways. Two major metabolites were observed: 3'-OH-S-2840, representing oxidation of the 3-carbon of the indene ring and 1'-COOH-S-2840A&B, representing oxidation of the 1'methyl group of the indene ring.
- Overall, the 1'methyl group was oxidized preferentially to the 1'-COOH-S-2840B isomer versus the 1'-COOH-S-2840A isomer.
- N-demethylation of the pyrazolyl ring to produce N-des-Me-S-2840 was minor as well as hydrolysis of the amide bond to produce the pyrazolyl derivatives, DFPA-CONH<sub>2</sub> and DFPA.
- Mineralization (degradation to bound residues unrelated to parent and <sup>14</sup>CO<sub>2</sub> formation) was insignificant.

- No metabolite modelling has been provided by the applicant. Therefore a default DT<sub>50</sub> value of 1000 d will be used for all metabolites breaching the trigger for inclusion in the exposure assessments.

HSE accepts the applicants proposed degradation pathway.

The aerobic aquatic metabolism degradation pathway of inpyrfluxam is summarised in figure B.8.2.2.3.2-01 below.



**Figure B.8.2.2.3.2-01 Proposed aerobic aquatic degradation pathways of inpyrfluxam.**

**B.8.2.2.3.3. Water / sediment – anaerobic study**

<b>Data Point:</b>	KCA 7.2.2.3/03
<b>Report Author:</b>	
<b>Report Year:</b>	2017b
<b>Report Title:</b>	14C]S-2399: Degradation under Anaerobic Aquatic Conditions; Amended Report 1
<b>Study number</b>	Study No: VP-38548 Report No: TPM-0036
<b>Guideline(s) followed in study:</b>	OECD 308 Aerobic and Anaerobic Transformation in Aquatic Sediment Systems.  OCSP 835.4400 Anaerobic Aquatic Metabolism (US EPA)
<b>GLP?</b>	Yes

**THIS HAS NOT BEEN CONSIDERED AS THE ENDPOINTS FROM THIS STUDY ARE NOT USED IN THE EXPOSURE ASSESSMENT. ADDITIONALLY, THE STUDY DID NOT INDICATE THE FORMATION OF ANY NOVEL MAJOR METABOLITES. THE STUDY SUMMARY IS PRESENTED FOR INFORMATION PURPOSES ONLY.**

The transformation of inpyrfluxam in two water/sediment systems (Sharkey and Golden Lake) was investigated under anaerobic aquatic conditions using [phenyl-<sup>14</sup>C] inpyrfluxam (PH-label) and [pyrazolyl-4-<sup>14</sup>C] inpyrfluxam (PY-label). The anaerobic aquatic test systems (50 g of dry weight sediment with 181 mL water for Sharkey and 175 mL water for Golden Lake) were dosed after the anaerobic conditions were established. The concentrations applied to the Sharkey phenyl and pyrazolyl systems were 0.019 and 0.020 µg/mL, respectively, and 0.014 µg/mL for both radiolabels in the Golden Lake systems. The test systems were incubated at 20 ± 2°C in the dark for a maximum of 180 days in the Sharkey system and 112 days in the Golden Lake system and were periodically collected and extracted. Physical parameters of the anaerobic system (O<sub>2</sub> concentration, redox and pH) were measured at each sampling point. Evolved <sup>14</sup>CO<sub>2</sub> and <sup>14</sup>CH<sub>4</sub> was collected using NaOH traps and analysed by oxidation of a sample of the biometer flask headspace gas.

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Activity in the water phase and all extracts was counted by LSC. Water and sediment extracts were analysed by HPLC and 2D-TLC to identify and quantify [ $^{14}\text{C}$ ] inpyrfluxam and [ $^{14}\text{C}$ ] labelled metabolites.

Post-extracted sediments (PES) at 180 DAT (Sharkey system) was subjected to additional solvent extractions, then fractionated into humin, humic acid and fulvic acid. Total radioactivity in PES was determined by combustion.

Inpyrfluxam degraded slowly in both sediment/water systems (Sharkey and Golden Lake) under anaerobic aquatic conditions. The majority of the dose remained unchanged after 180 days (Sharkey) or 112 days (Golden Lake) of anaerobic aquatic exposure (87 – 91% AR). 3'-OH-S-2840 formed at  $\geq 5\%$  at three consecutive time points (14 to 63 DAT, Golden Lake PY-label), but decreased to  $< 5\%$  at study end. No other significant metabolites were formed, and ultimate mineralization to bound residues and  $\text{CO}_2$  was minor. 3'-OH-S-2840 was the only metabolite found consistently in both sediments and both labels (max 5.3%). A variety of other transitory metabolites were seen (ATMI, DFPA-CONH<sub>2</sub>, DFPA, N-des-Me-S-2840 and 1'-COOH-S-2840) in very low amounts  $< 5\%$ . Kinetic analysis was not validated or conducted by HSE, as the study was not considered relevant for the exposure assessment.



### **B.8.2.3. Degradation in the saturated zone**

No data provided, and none required with regards to the saturated zone.

#### **B.8.2.3.1. Water treatment procedures**

Article 4 Section 3 (b) refers to the impact of water treatment processes on water-borne residues of active substances and metabolites, i.e. the capability of water-treatment processes to form potentially harmful substances when degrading the water-borne residue. At present there is no definitive approach to consider the above whilst HSE considers the relevance of the ECHA/EFSA guidance for use under the GB regulatory scheme. Whilst GB is no longer part of the EU, a historical perspective of a.s. evaluations made before and after EU Exit indicates it is common for applicants to address this Article of Regulation 1107/2009 by consideration of the following:

- An initial screening step based on examination of substance structure for potential formation of harmful degradates, metabolites and residues;
- If these harmful components are predicted, then risk management based approaches should be invoked.
- If risk management leads to severe restrictions, then applicants should consider the generation of degradation data to disprove the prediction.
- If the prediction is confirmed, modelling or monitoring data showing levels of these harmful degradates are below 0.1 µg/L will have to be generated by the applicant for the restrictions to be lifted.

The applicant submitted the following consideration of water treatment processes in Section CP 9.4:

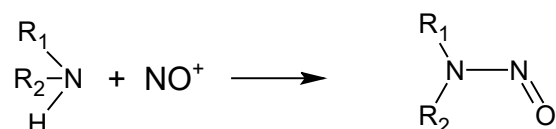
#### ***Possible formation of nitroamines and specific harmful substances during water treatment***

*N-nitrosamines are formed in the reaction of an electrophilic substitution of organic nitrogen with a nitrosating compound (NOx) and mainly from the secondary amines<sup>1</sup>.*

#### ***General formation reaction of nitrosamines***

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<sup>1</sup> - [http://ec.europa.eu/health/scientific\\_committees/consumer\\_safety/docs/sccs\\_o\\_090.pdf](http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_090.pdf)

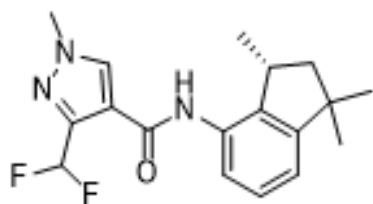


*Inpyrfluxam possesses three nitrogen atoms, but two of these are part of the pyrazole ring and will not partake in secondary amine reactions. The third nitrogen atom is an amide function and hence also not a secondary amine. In addition, the major metabolites 1'-COOH-S-2840 and 3'-OH-S-2840, contain the same structure in respect of the nitrogen atoms. Hence neither the parent or the exposed metabolites will act as secondary amines.*

*Furthermore, the yield of formation of nitroamines is very low and will not exceed 2% even by direct exposition to nitrosating compounds such as by chloramination. Bond et al <sup>2</sup> showed in experimental work that for water treatment chlorination, ozonation and UV irradiation do practically not form any significant nitroamines, even from the secondary amines. Bond et al also showed that chlorination before chloramine (nitrosating agent) addition was an effective way of limiting nitrosamine formation for most of the secondary amines.*

*Water treatment processes in general are not known to result in formation of other specific harmful substances. Hence, no nitroamines or other specific harmful substances are to be expected to be formed from inpyrfluxam or its metabolites during drinking water treatment processes.*

The points discussed by the applicant have been evaluated below. The structure of inpyrfluxam has been considered.



**Figure B.8.2.3.1-01 Structure of inpyrfluxam.**

The applicant's consideration addresses the initial screening step above by considering the potential for inpyrfluxam to form nitrosamines and concludes that the structure of inpyrfluxam makes this unlikely. HSE Chemistry colleagues have agreed with the applicant that the two rings in the pyrazole ring are stable and therefore would be unlikely to form nitrosamines under normal conditions, while the amide

<sup>2</sup> -

[https://spiral.imperial.ac.uk/bitstream/10044/1/14083/2/Water%20Science%20and%20Technology%20Water%20Supply\\_11\\_3\\_2011.pdf](https://spiral.imperial.ac.uk/bitstream/10044/1/14083/2/Water%20Science%20and%20Technology%20Water%20Supply_11_3_2011.pdf)

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group is even more stable. It is not considered likely therefore that inpyrfluxam or its major metabolites 1'-COOH-S-2840 and 3'-OH-S-2840 would form nitrosamines during water treatment.

It is noted that while ozonation had been addressed, chlorination and other potential processes have not.

Due to the lack of agreed guidance in this area, this has been concluded to be an open point. Confirmatory data will be required to address this when the relevant GB guidance is noted.

Following the ECP meeting, a concern was identified that that inpyrfluxam is hydrophilic, exhibits limited hydrolysis and photolysis, and is highly persistent. In combination, these properties may present challenges for water treatment systems. This includes potentially limited removal by activated carbon filtration, with potential implications for drinking water quality.

#### **B.8.2.4. Persistence in water**

The applicant considered whether inpyrfluxam fulfils the persistence or very persistent criteria within the PBT and vPvB assessments, which are defined according to Section 3.7.2.1. and 3.7.3.1, respectively, of Annex II of Regulation 1107/2009 as follows:

*An active substance, safener or synergist fulfils the persistence criterion where:*

- *The half-life in marine water is higher than 60 days,*
- *The half-life in fresh or estuarine water is higher than 40 days,*
- *The half-life in marine sediment is higher than 180 days,*
- *The half-life in fresh or estuarine water sediment is higher than 120 days*

*An active substance, safener or synergist fulfils the 'very persistent' criterion where:*

- *the half-life in marine, fresh- or estuarine water is higher than 60 days,*
- *the half-life in marine, fresh- or estuarine water sediment is higher than 180 days*

The relevant endpoints for the persistence assessment were identified based on the DG SANCO working document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides" [SANCO 2012. DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides". Brussels: European Commission Health and Consumers Directorate-General. Report 25.09.2012 - rev. 3.]. According to this document, degradation half-lives in the whole system of aerobic aquatic studies shall be compared with trigger values relevant for P and vP assessment for the degrading compartment. Additionally, data on aquatic photolysis should also be considered when relevant.

Inpyrfluxam was found to be hydrolytically stable at all pH values tested (pH 4-9) at 50°C [see B.8.2.1.1].

A direct photolysis study was conducted in sterilized pH 7 buffer [see B.8.2.1.2], and an indirect study in sterilized natural water [see B.8.2.1.3]. Degradation of inpyrfluxam in the irradiated buffer was negligible. Degradation of inpyrfluxam in irradiated sterilized natural water gave a DT<sub>50</sub> of 74.4 days for the pyrazolyl label, and 37.6 days for the phenyl label.

In an aerobic water/sediment study under dark conditions, inpyrfluxam was observed to partition from the water phase to the sediment phase [see KCA 7.2.2.3/01 & 02]. The maximum dissipation half-life of inpyrfluxam from the water phase is 34.3 days, and the sediment phase is therefore assumed to be the relevant compartment for consideration against the persistence triggers. No degradation was observed in the sediment compartment and a default DT<sub>50</sub> of 1000d can be assumed.

The degradation half-life of inpyrfluxam in the whole system was found to be >10,000

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days (maximum dissipation half-life, Golden lake system).

According to the DG SANCO working document, degradation half-lives in the whole system should be compared with trigger values relevant for P and vP assessment for the degrading compartment. Considering the maximum DegT<sub>50</sub> of >10,000 days and the trigger values of 120 and 180 days for the sediment compartment, inpyrfluxam fulfils the criterion for both P and vP.

The aqueous photolysis studies demonstrated that direct photolysis in the water phase is not a major dissipation pathway for inpyrfluxam from the aquatic environment. Indirect photolysis is a more important contributor to dissipation. As these laboratory studies are not representative of environmental conditions, they have not been taken into account in the persistence assessment. While field studies are more representative of environmental conditions, these have not been provided. Therefore direct and indirect photolysis do not change the conclusion of inpyrfluxam as both P and vP.

### B.8.3. Route and rate of degradation in air (Data Requirement 7.3.1)

Based on the low vapour pressure of inpyrfluxam at  $3.8 \times 10^{-8}$  Pa at 20°C and short DT<sub>50</sub> in air (0.233 days, see section B.8.3.1), HSE agrees with the applicant's conclusion that short-range transport via air is not expected to be significant, and any amount that may enter the air will likely degrade rapidly, minimising the potential exposure or drift within the immediate application area. Long-range transport is addressed in section B.8.3.1.

#### B.8.3.1 Photochemical oxidative degradation

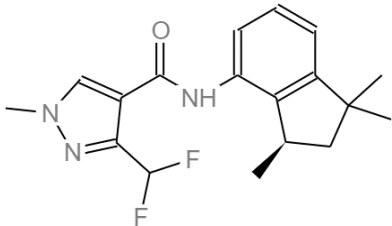
<b>Report:</b>	KCA 7.3.1/1
<b>Title</b>	Photochemical oxidative degradation of S-2399 (QSAR estimates)
<b>Author</b>	[REDACTED] (2017)
<b>Document No.:</b>	TPP-0025
<b>Guidelines</b>	None
<b>GLP?</b>	No – this is a QSAR modelling estimation of parameters therefore GLP is not required
<b>Acceptability?</b>	Yes
<b>Study relied upon?</b>	Yes
<b>Previous evaluations</b>	None – report submitted as part of a new active substance registration.

#### INTRODUCTION

The applicant estimated the degradation rates for reactions of inpyrfluxam with hydroxyl radicals in the atmosphere. These were calculated using the AOPWIN tool based on Atkinson's increment method. Table B.8.3.1/1-01 summarises details related to the active substance.

**Table B.8.3.1-01: Summary of the active substance**

<b>Common name</b>	Inpyrfluxam
<b>Internal code</b>	inpyrfluxam
<b>SMILE notation</b>	<chem>O=C(c1cn(C)nc1C(F)F)Nc2cccc3c2C(C)CC3(C)C</chem>

<b>Molar mass</b>	333.38 g/mol
<b>Empirical formula</b>	C <sub>18</sub> H <sub>21</sub> F <sub>2</sub> N <sub>3</sub> O <sub>1</sub>
<b>Structure</b>	

## METHODS

The applicant used AOPWIN v.1.92 within the EPISuite tool to estimate the degradation rate for reactions of inpyrfluxam with hydroxyl radicals based on the structural formula. Assuming a pseudo-first order reaction, the degradation half-life via this reaction route is calculated by accounting for the diurnally and seasonally averaged concentration of hydroxyl radicals in the troposphere.

HSE assessed the applicant's QSAR estimation by also using AOPWIN v 1.92 to estimate values. HSE notes that the SMILE notation used by the applicant does not specify that inpyrfluxam is only the R-isomer. HSE does not consider this as a deviation, as chirality does not affect the structure-activity relationship described by the Atkinson method. HSE agreed with the applicant's processes and input values; as such, the obtained values presented in the following sections are those provided by the applicant.

## RESULTS

The overall hydroxyl rate constant was determined to be  $45.8565 \times 10^{-12}$  cm<sup>3</sup>/molecule/s. The weighted global average tropospheric hydroxyl radical concentration is  $1.5 \times 10^6$  mol/cm<sup>3</sup> for a 12-hour period. This gives a half-life of 0.233 days (12 hour day). For ozone attack degradation of inpyrfluxam, AOPWIN could not approximate a degradation rate as it is neither an olefin or an acetylene.

## CONCLUSION

The half-life for inpyrfluxam degradation by hydroxyl radicals is 0.233 days (12 hour day). The HSE evaluator agrees and accepts the applicant's calculated half-life. It can be concluded that inpyrfluxam will be degraded by photochemical processes in the troposphere. Due to this degradation in air, it can be concluded that there is a low risk of long-range transport of inpyrfluxam.

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#### **B.8.4. Monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products**

As a new active substance, there are no monitoring data available for inpyrfluxam.



## B.8.5. References Relied On

### B.8.5.1. Literature search

A literature review has been carried out for the active substance inpyrfluxam (S-2399). One literature search was submitted to address all areas of the RAR. HSE has assessed the suitability of the mechanics of the literature search in line with EFSA guidance on conducting literature searches (EFSA Journal 2011). The literature review was conducted in accordance with Article 8(5) of Regulation No. 1107/2009 at the time of completion, and was conducted to comply with the EFSA guidance document as published in EFSA Journal 2011; 9(2):2092.

The key objective of the submitted literature review was to identify scientific peer reviewed open literature on the active substance inpyrfluxam (S-2399) and relevant metabolites, which may affect the assessment of human health, animal health and/or the environment.

In this section the conduct of the literature search methods in relation to fate and behaviour studies has been evaluated; the conclusions of which are presented here. Key information from this report has been summarised below.

<b>Report:</b>	<b>KCA 9.1 TPT-0133</b>
<b>Title</b>	• Literature Review Report
<b>Guidelines:</b>	• Submission of scientific peer-reviewed open literature under Regulation (EC) No 1107/2009
<b>GLP:</b>	• No

#### Deviations

No deviations identified by HSE.

#### HSE assessment of deviations

HSE considers that the literature search performed is acceptable by Article 8(5) of Regulation No. 1107/2009

One literature search was submitted to address all areas of the RAR. HSE has assessed the suitability of the mechanics of the literature search in line with EFSA guidance on conducting literature searches (EFSA, 2011).

HSE finds that the process used was acceptable (discussed in further detail below). One relevant literature article was identified by the applicant with regards to all specialist areas. This article was classed as relevant to environmental fate and behaviour.

The process of selection of relevant scientific peer-reviewed open literature was based on a single-concept search in the CAS and Dialog platform databases. The time period was limited to studies published July 2013 up to July 2023.

The literature search was performed to cover the 10 years prior to the submission of the new active substance dossier for Inpyrfluxam (S-2399) which was submitted for review in July, 2023. Patents and conference papers were excluded as these were not expected to contain information that was both relevant and reliable. HSE considers this time period and breadth of search as acceptable.

This report summarises the search for published information on inpyrfluxam (S-2399) and the metabolites given in Table B.8.5.1-1 below.

The search terms used to identify the active substance and metabolites, and databases used during the literature search are given in Table B.8.5.1-1. Parameters of the search are given in Table B.8.5.1-2. Databases contained within the CAS and Dialog platforms are given in Table B.8.5.1-3.

**Table B.8.5.1-01 Search terms and databases used for literature search**

Common name	Synonyms	Search engine	Fields searched
Inpyrfluxam	( <i>R</i> )-3-(difluoromethyl)-1-methyl- <i>N</i> -(1,1,3-trimethyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-1 <i>H</i> -pyrazole-4-carboxamide  1352994-67-2  inpyrfluxam	CAS (Sci-finder n)  Proquest Dialogue	Full Text
3'-OH-S-2840	3-(difluoromethyl)- <i>N</i> -(3-hydroxy-1,1,3-trimethyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci-finder n)  Proquest Dialogue	Full Text
1'-CH <sub>2</sub> OH-S-2840A	3-(difluoromethyl)- <i>N</i> -((1 <i>R</i> ,3 <i>S</i> )-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide  3-(difluoromethyl)- <i>N</i> -((1 <i>S</i> ,3 <i>R</i> )-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci-finder n)  Proquest Dialogue	Full Text
1'-CH <sub>2</sub> OH-S-2840B	3-(difluoromethyl)- <i>N</i> -((1 <i>R</i> ,3 <i>R</i> )-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci-finder n)  Proquest Dialogue	Full Text

	3-(difluoromethyl)-N-((1S,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide		
N-des-Me-S-2840	3-(difluoromethyl)-N-(1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-1'-CH <sub>2</sub> OH-S-2840	3-(difluoromethyl)-N-((1R,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide  3-(difluoromethyl)-N-((1S,3R)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-1'-CH <sub>2</sub> OH-S-2840	3-(difluoromethyl)-N-((1R,3R)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide  3-(difluoromethyl)-N-((1S,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
7'-OH-inpyrfluxam	(R)-3-(difluoromethyl)-N-(7-hydroxy-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-CH <sub>2</sub> OH-3'-OH-S-2840	N-[(1RS,3RS;1RS,3SR)-2,3-dihydro-1,3-dimethyl-3-hydroxy-1-(hydroxymethyl)-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
glucuronide of 1'-CH <sub>2</sub> OH-3'-OH-S-2840	Glucuronide of N-[(1RS,3RS;1RS,3SR)-2,3-dihydro-1,3-dimethyl-3-hydroxy-1-(hydroxymethyl)-1H-inden-4-yl]-1-methyl-3-(difluoromethyl)-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
ATMI	(R)-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-amine  125349-37-3	CAS (Sci-finder n)	Full Text

		Proquest Dialogue	
1'-COOH-S-2840A	(1S,3R)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid  (1R,3S)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid	CAS (Sci-finder n)  Proquest Dialogue	Full Text
1'-COOH-S-2840B	(1R,3R)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid  (1S,3S)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid	CAS (Sci-finder n)  Proquest Dialogue	Full Text
1',1'-bis(CH <sub>2</sub> OH)-S-2840	<i>N</i> -(1,1-bis(hydroxymethyl)-3-methyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-3-(difluoromethyl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci-finder n)  Proquest Dialogue	Full Text
N-des-Me-1',1'-bis(CH <sub>2</sub> OH)-S-2840	<i>N</i> -(1,1-bis(hydroxymethyl)-3-methyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-3-(difluoromethyl)-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci-finder n)  Proquest Dialogue	Full Text
N-des-Me-1'-CH <sub>2</sub> OH-3'-OH-S-2840	3-(difluoromethyl)- <i>N</i> -(3-hydroxy-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -inden-4-yl)-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci-finder n)  Proquest Dialogue	Full Text
N-des-Me-1'-COOH-S-2840  NDM-1'-COOH-S-2840	4-(3-(difluoromethyl)-1 <i>H</i> -pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -indene-1-carboxylic acid	CAS (Sci-finder n)  Proquest Dialogue	Full Text
DFPA	3-(difluoromethyl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxylic acid  176969-34-9	CAS (Sci-finder n)	Full Text

		Proquest Dialogue	
N-des-Me-DFPA	3-(difluoromethyl)-1 <i>H</i> -pyrazole-4-carboxylic acid  151734-02-0	CAS (Sci- finder n)  Proquest Dialogue	Full Text
DFPA-CONH <sub>2</sub>	3-(difluoromethyl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamide  925689-10-7	CAS (Sci- finder n)  Proquest Dialogue	Full Text
1'-CH <sub>2</sub> OH-S-2840-sulfate	(4-(3-(difluoromethyl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -inden-1-yl)methyl hydrogen sulfate	CAS (Sci- finder n)  Proquest Dialogue	Full Text
Glu-1'-CH <sub>2</sub> OH-S-2840	6-((4-(3-(difluoromethyl)-1-methyl-1 <i>H</i> -pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1 <i>H</i> -inden-1-yl)methoxy)-3,4,5-trihydroxytetrahydro-2 <i>H</i> -pyran-2-carboxylic acid	CAS (Sci- finder n)  Proquest Dialogue	Full Text
3'-OH-S-2840-dehydrate	3-(difluoromethyl)-1-methyl- <i>N</i> -(1,1,3-trimethyl-1 <i>H</i> -inden-4-yl)-1 <i>H</i> -pyrazole-4-carboxamide	CAS (Sci- finder n)  Proquest Dialogue	Full Text

**Table B.8.5.1-02 Reporting/Overview of the search process for scientific peer-reviewed open literature in bibliographic databases**

	<b>CAS Databases</b>	<b>Dialog Databases</b>
<b>Justification for choice of the database:</b>	See Appendix 2	See Appendix 2
<b>Date of the search:</b>	14 January 2025	14 January 2025
<b>Date span of the search:</b>	July 2013-July 2023	July 2013-July 2023
<b>Date of the latest database update included in the search:</b>	See Table 3	See Table 3
<b>Fields searched</b>	Full text	Full text
<b>Number of summary records retrieved after removing duplicates</b>	160	192
<b>Total number of summary records retrieved after removing duplicates</b>	352	

**Table B.8.5.1-03 Databases searched within CAS and Dialog platforms**

<b>CAS DATABASES:</b>	<b>FREQUENCY OF UPDATES</b>
SciFinder-n	Updated daily
<b>DIALOG DATABASES:</b>	<b>FREQUENCY OF UPDATES</b>
AGRICOLA	All PROQUEST databases are current and updated regularly, except as noted in Appendix 2.
AGRIS	
Analytical Abstracts	
Aqualine	
Aquatic Science & Fisheries Abstracts (ASFA)	
BIOSIS Toxicology	
CAB ABSTRACTS	
Ecology Abstracts	
Embase	
Endocrinology Abstracts	
Environment Abstracts	
FSTA	
GEOBASE	
GeoRef	
MEDLINE	
Meteorological & Geostrophysical Abstracts	
Oceanic Abstracts	
Pollution Abstracts	
ToxFile	
Toxicology Abstracts	
TOXLINE	
Water Resources Abstracts	

HSE considers that the use of the CAS and Dialog platforms covers an acceptably wide range of search engines across all disciplines. Findings of the literature review, are given in Table B.8.5.1-04.

**Table B.8.5.1-04 Findings of the literature review**

<b>Summary of the review</b>	<b>n</b>	<b>Justification</b>
Total number of summary records retrieved from search	352	Appendix 1
Number of summary records excluded after rapid assessment for relevance (by title/abstract)	349	Appendix 5
Number of summary records of potential/unclear relevance assessed in further detail (by abstract/full-text)	3	Appendices 3 and 4
Number of studies excluded from the risk assessment after detailed assessment of full-text documents (i.e. not relevant)	2	
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1	
Number of studies included in the dossier as supporting information (reliability criteria 1-2)	1	

352 articles of potential relevance to the regulatory data package for the active substance were identified and investigated first by rapid assessment. The review process identified three records of potential relevance. Two of these were excluded after a detailed assessment, leaving one study included in the dossier as supporting information (See Appendix 5, B.8.5.2).

The applicant states that a reliability assessment for relevant studies was carried out according to Klimisch et al. (1997)<sup>3</sup>

HSE notes that at the date that this literature review was completed, the Klimisch criteria was not considered acceptable by EFSA. Later, in response to an EFSA Q&A, EFSA deemed that the Klimisch approach could be considered. HSE therefore reviews study reliability based on the following criteria:

- Is the study free from bias?
- Do the findings reflect facts?
- Have the studies been conducted to suitable guidelines?
- Have adequate controls been included?
- Is the study fit for purpose?
- statistical power
- verification of measurement methods and data
- control of experimental variables that could affect measurements
- uniformity among substances with similar attributes and effects

<sup>3</sup>Klimisch, H-J., Andreae, M. & Tillmann, U. (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology 25 pp 1-5



**Conclusion**

The applicant states that a review of the published literature for inpyrfluxam (S-2399) identified one article that is both relevant and reliable with respect to the regulatory data package (See Appendix 5, B.8.5.2).

HSE agrees with the applicant, and considers this literature review to be acceptable by Article 8(5) of Regulation No. 1107/2009.

### Appendix 1: Reporting/Overview of the search process for scientific peer-reviewed open literature in bibliographic databases

	<b>CAS Databases</b>	<b>Dialog Databases</b>
<b>Justification for choice of the database:</b>	Appendix 2	Appendix 2
<b>Date of the search:</b>	14 January 2025	14 January 2025
<b>Date span of the search:</b>	July 2013-July 2023	July 2013-July 2023
<b>Date of the latest database update included in the search:</b>	Table 3	Table 3
<b>Fields searched</b>	Full text	Full text
<b>Number of summary records retrieved after removing duplicates</b>	160	192
<b>Total number of summary records retrieved after removing duplicates</b>	352	

### Appendix 2: Justification for choice of databases used

#### CAS DATABASES

<b>Provider</b>	<b>Database</b>	<b>Justification</b>
CAS	SciFinder-n	SciFinder-n, a resource from the Chemical Abstracts Service (CAS), a division of the American Chemical Society (ACS), is a curated database of chemical and bibliographic information. It is a core research tool for chemistry, biochemistry, chemical engineering, materials science, nanotechnology, physics, environmental science and other science and engineering disciplines.

## DIALOG DATABASES

The applicant has stated that Dialog is the premier online retrieval service with the most comprehensive content collection and most powerful search language available. Dialog is the worldwide leader in providing online-based information in science. The database holds data from more than 800 million unique records of key information, accessible via the Internet. Content areas include, but are not limited to, biomedical research, biotechnology, chemicals, environment, food and agriculture, medicine and science and technology. HSE considers that Dialog provides a comprehensive search tool for identifying relevant literature.

Provider	Database	Justification
Dialog	AGRICOLA (AGRICultural OnLine Access)	AGRICOLA (AGRICultural OnLine Access) is an extensive international bibliographic database consisting of records for literature citations of journal articles, monographs, theses, patents, translations, microforms, audiovisuals, software and technical reports. Available since 1970, AGRICOLA serves as a document locator and bibliographic access and control system for the U.S. National Agricultural Library (NAL) collection, but since 1984 the database has also included some records produced by cooperating institutions for documents not held by NAL.
Dialog	AGRIS International	AGRIS International is the international information system for agricultural sciences and technology. The AGRIS International database has served since 1974 as a comprehensive inventory of worldwide agricultural literature which reflects research results, food production, and rural development to help users identify problems involved in all aspects of world food supply. Emphasis in AGRIS International is non-U.S. This file corresponds in part to the printed publication, Agrindex, published monthly by the Food and Agriculture Organization (FAO) of the United Nations. AGRIS is a cooperative, decentralised system in which over 100 national and multinational centers take part. It collects and makes available current information on the agricultural literature of the world appearing in journals, books, reports, and conference papers. Each country which participates in AGRIS does so by submitting information about documents published within its own territories. All contributing sources are of non-U.S. origin. FAO acts as a coordinating agency within this global information system, facilitating the exchange of agricultural information to its member countries.

<b>Provider</b>	<b>Database</b>	<b>Justification</b>
Dialog	Analytical Abstracts	Analytical Abstracts covers all aspects of analytical chemistry in a wide variety of areas including general applications, biochemistry and clinical chemistry, industrial and applied science, environmental science, agriculture and food, pharmaceuticals and instrumentation.
Dialog	Aqualine	Aqualine contains abstracts and bibliographic citations from approximately 300 journals as well as from conference proceedings, scientific reports, books and theses. Major subjects of coverage include water resources and supplies management, water legislation, water quality, potable water distribution, wastewater collection, water treatment technologies, wastewater and sewage treatment, and ecological and environmental effects of water pollution. Previously published by the well-known and respected WRc in England, Aqualine is now produced in joint cooperation with WRc and CSA.
Dialog	ASFA (Aquatic Sciences and Fisheries Abstracts)	ASFA (Aquatic Sciences and Fisheries Abstracts) series is the premier international reference in the field of aquatic resources. Since 1966 input to ASFA has been provided by a growing international network of information centres monitoring more than 5,000 serial publications, books, reports, conference proceedings, translations and limited distribution literature. ASFA is a component of the Aquatic Sciences and Fisheries Information System (ASFIS), formed by four United Nations agency sponsors of ASFA and a network of international and national partners.
Dialog	BIOSIS® Toxicology	BIOSIS® Toxicology is a subset of BIOSIS® Previews, with a focus on toxicology and related topics. Records are drawn from journal articles, conference papers, monographs and book chapters, notes, letters, and reports, as well as original research. U.S. patent records are also included. Abstracts are available for records beginning in 1976.
Dialog	CAB Abstracts	The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including biotechnology, forestry, and veterinary medicine. Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents. Bibliographic information, indexing terms, abstracts and CAS Registry Numbers are searchable.

<b>Provider</b>	<b>Database</b>	<b>Justification</b>
Dialog	Ecology Abstracts	Ecologists will find in this journal the essence of current ecology research across a wide range of disciplines, reflecting recent advances in light of growing evidence regarding global environmental change and destruction. Ecology Abstracts focuses on how organisms of all kinds - microbes, plants, and animals - interact with their environments and with other organisms. Included are relevant papers on evolutionary biology, economics, and systems analysis as they relate to ecosystems or the environment. With coverage ranging from habitats to food chains, from erosion to land reclamation, the journal provides an important cross-section of current findings in target research areas. Detailed information on resource and ecosystems management and modelling contributes to the journal's practical value, as does material on the impact of climate, water resources, soil, and man or growing environmental problems such as depletion, erosion, and pollution all topics which are covered in depth. Comprehensive, yet carefully focused coverage makes this an essential resource for scientists concerned with preserving the environment.
Dialog	Embase	The Excerpta Medica database covers worldwide literature in the biomedical and pharmaceutical fields, including biological science, biochemistry, human medicine, forensic science, paediatrics, pharmacy, pharmacology and drug therapy, pharmacoeconomics, psychiatry, public health, biomedical engineering and instrumentation and environmental science. Sources for EMBASE include more than 4,000 journals from approximately 70 countries, monographs, conference proceedings, dissertations and reports.
Dialog	Endocrinology Abstracts	Part of the ProQuest Biological & Health Database Cluster covering human, animal, and plant science, with specific information on AIDS and HIV, algology, animal behaviour, aquatic and marine science, bacteriology, biotechnology, genetics, immunology, neurosciences, toxicology, virology and zoology.

<b>Provider</b>	<b>Database</b>	<b>Justification</b>
Dialog	Environment Abstracts	Environment Abstracts (formerly Environment Abstracts published by LexisNexis) encompasses all aspects of the impact of people and technology on the environment and the effectiveness of remedial policies and technologies. As of 1994, the database also provides expanded coverage of energy-related issues. Environment Abstracts provides access to more than 950 journals published in the U.S. and abroad. The database also covers conference papers and proceedings, special reports from international agencies, non-governmental organizations, universities, associations and private corporations. Other materials selectively indexed include significant monographs, government studies and newsletters. Environment Abstracts customers will also receive access to Sustainability Science Abstracts and EIS: Digests of Environmental Impact Statements. Environment Abstracts also includes a special collection of over 4,000 full text government reports.
Dialog	FSTA®	FSTA® is produced by IFIS (UK) - core food information, an independent, not-for-profit organisation whose primary objective is to provide quality information products and services designed to meet the needs of all those working in the food sector. FSTA® is the largest and most respected collection of food science, food technology and food related human nutrition abstracts, providing content since 1969. It is compiled by a team of specialist scientists dedicated to producing a database of consistent high quality and timeliness. Continual development of coverage allows FSTA® to maintain its position as the market-leading food science database. There are more than 109,000 patent records including more than 11,000 Japanese patents. FSTA® covers journal articles (approximately 80%), patents, theses, standards, legislation, books, reviews and conference proceedings.
Dialog	GEOBASE	GEOBASE is a unique bibliographic database covering worldwide research literature since 1980 in physical and human geography, earth and environmental sciences, ecology, and related disciplines. In addition to providing comprehensive coverage of the core scientific and technical periodicals, Geobase has a unique coverage of non-English language and less readily available publications. Over 2,000 journals are fully covered with an additional 3,000 having partial coverage. Over 2,000 books, monographs, conference proceedings, and reports are also included.

<b>Provider</b>	<b>Database</b>	<b>Justification</b>
Dialog	GeoRef	Established by the American Geosciences Institute (AGI) in 1966, GeoRef is AGI's most comprehensive geosciences database with worldwide coverage growing by more than 100,000 references a year. The database contains more than 4 million references to geoscience journal articles, books, maps, conference papers, reports and theses.
Dialog	MEDLINE (Medical Literature, Analysis, and Retrieval System Online)	MEDLINE is produced by the U.S. National Library of Medicine (NLM) and is the U.S. National Library of Medicine's premier bibliographic database that contains more than 15 million references to journal articles in life sciences with a concentration on biomedicine. The broad coverage of the database includes basic biomedical research and the clinical sciences since 1950 including nursing, dentistry, veterinary medicine, pharmacy, allied health and pre-clinical sciences. MEDLINE also covers life sciences that are vital to biomedical practitioners, researchers and educators, including some aspects of biology, environmental science, marine biology, plant and animal science as well as biophysics and chemistry. Increased coverage of life sciences began in 2000. MEDLINE is indexed using NLM's controlled vocabulary, MeSH® (Medical Subject Headings). Approximately 400,000 records are added per year, of which more than 76% are in English.
Dialog	Meteorological and Geoastrophysical Abstracts	Meteorological and Geoastrophysical Abstracts provides current citations in English for the most important meteorological and geoastrophysical research published in worldwide literature sources since 1966 to the present. Over 200 sources, including technical journals, monographs, proceedings, reviews and annual publications are scanned for relevant literature. Subject coverage includes meteorology (weather and climate), astrophysics, physical oceanography, hydrosphere and hydrology, environmental sciences, and glaciology. Content from American Meteorological Society, published by CSA.
Dialog	Oceanic Abstracts	This database is a comprehensive, leading source of information on the living and non-living resources of oceans. The database provides editorially curated A&I resources pertaining to the marine and brackish water environment, covering topics such as marine biology and physical oceanography, fisheries, aquaculture, meteorology and geology, and environmental, technological, and legislative issues and policy.

Provider	Database	Justification
Dialog	Pollution Abstracts	Pollution Abstracts provides fast access to the environmental information necessary to ensure ongoing compliance and handle emergency situations more effectively. Pollution Abstracts combines information on scientific research and government policies in a single resource. Topics of growing concern are extensively covered from the standpoints of atmosphere, emissions, mathematical models, effects on people and animals, toxicology and health and environmental action in response to global pollution issues. To ensure comprehensive coverage, material from conference proceedings and hard-to-find documents has been summarised along with information from primary journals in the field. Published since 1966 by CSA (Cambridge Scientific Abstracts).
Dialog	SciSearch	Science Citation Index, one of the largest multidisciplinary scientific databases, is an international index to the literature covering virtually every subject area within the broad fields of science, technology and biomedicine. Records include references from over 5,600 scientific, technical and medical journals are contained in the database.



Provider	Database	Justification
Dialog	ToxFile	ToxFile covers 1965 to the present of the toxicological, pharmacological, biochemical and physiological effects of drugs and other chemicals: adverse drug reactions, chemically induced diseases, carcinogenesis, mutagenesis, teratogenesis, environmental pollution, pesticides, waste disposal, radiation, and food contamination. ToxFile includes toxicology records derived from MEDLINE and also includes citations referred to as TOXNET records from the following organizations and data repositories: Aneuploidy File (ANEUPL), International Labor Office (CIS), Toxicology Research Projects (CRISP), Developmental and Reproductive Toxicology (DART), Environmental Mutagen Information Center File (EMIC), Epidemiology Information System (EPIDEM), Environmental Teratology Information Center File (ETICBACK), Federal Research in Progress (FEDRIP), Health Aspects of Pesticides Abstract Bulletin (HAPAB), Toxicological Aspects of Environmental Health (HEEP), Hazardous Materials Technical Center File (HMTc), National Institute for Occupational Safety and Health (NIOSH), Toxicology Document and Data Repository (NTIS), Pesticides Abstracts (PESTAB), Poisonous Plants Bibliography (PPBIB), Swedish National Chemicals Inspectorate (RISKLINE), and Toxic Substances Control Act Test Submissions (TSCATS).
Dialog	Toxicology Abstracts	Toxicology Abstracts is the only comprehensive print resource for professionals in this field who must be aware of every new finding. Specifically focused to meet the needs of toxicologists, Toxicology Abstracts covers issues from social poisons and substance abuse to natural toxins, from legislation and recommended standards to environmental issues. Surveying the literature for toxicology studies of industrial and agricultural chemicals, household products, pharmaceuticals, and myriad other substances, each issue publishes information concerning the in vivo effects of toxic substances. Topics of current concern such as the effects of alcohol and smoking, drug abuse, hydrocarbon studies, nitrosamines, radiation and radioactive materials, and much more are extensively examined. Toxicity testing methodology and analytical procedures for toxic substances are also covered. Through many years of delivering crucial information on the tough, far-reaching issues of toxicology, Toxicology Abstracts has become the single most widely used journal in this field.

Provider	Database	Justification
Dialog	TOXLINE	Bibliographic citations to toxicological, pharmacological, biochemical and physiological effects of drugs and other chemicals. Coverage is international but contains primarily English language items; Updates are monthly, with about 9,300 new citations added each month; the file contains over 2.4 million records. The records are derived from about 16 secondary sources.
Dialog	Water Resources Abstracts	Water Resources Abstracts offers a comprehensive range of water-related topics summarising the world's technical and scientific literature on water-related topics covering the characteristics, conservation, control, pollution, treatment, use and management of water resources in the life and physical sciences, as well as the engineering and legal aspects of the conservation, control, use, and management of water. The database was originally produced by the U.S. Geological Survey starting in 1968 when it was generally known as Selected Water Resources Abstracts. Since 1994, Water Resources Abstracts has been produced by CSA (Cambridge Scientific Abstracts), which broadened the scope by including more material published outside the U.S.A. This database, which concentrates on water supply and water treatment, complements the Aquatic Sciences & Fisheries Abstracts database, ASFA, where there is greater coverage of the marine environment and biological material.

**Appendix 3: Publications excluded from the risk assessment after detailed assessment of documents**

Number	Author	Year	Title	Reference	Meet relevance criteria	Meet Reliability Criteria	Basis for relevance/reliability decision (title, abstract or full article)	Comments
					Y or N	Score		
1.	Irie, Y., & Nakae, H.	2023	Warning about use of pesticides containing inpyrfluxam for suicide	Acute Medicine and Surgery, 10(1), e834	N	N/A	Full text	Describes a case report of poisoning following ingestion of 125 ml Kename (37% inpyrfluxam flowable formulation, 67% water and surfactants), where the patient died. However, it does not describe any inpyrfluxam specific clinical methods to treat poisoning symptoms other than the usual supportive methods such as gastric lavage and continuous hemodialysis.

2.	Machino, S., Yokoyama, Y., Egawa, T., Satoh, H., Miyajima, K., Yoshida, M., Asano, S., & Ozawa, S.	2021	Case analysis of kinetics investigations in toxicity studies of pesticides to identify the nonlinearity of internal exposure and the influences of nonlinearity on the toxicological interpretation of pesticide residue	Regulatory Toxicology and Pharmacology, 124, 104958	N	N/A	Full text	Presents case analysis of kinetics to identify nonlinearity of internal exposure and its influence on the toxicological interpretation of pesticide residue. As the data were obtained from risk assessment reports published by the Food Safety Commission it implies that regulatory studies for inpyrfluxam described in dossiers were used for the analysis, i.e. no new data.
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**Appendix 4: Relevant studies after detailed assessment of full-text documents for relevance:**

<b>Number</b>	<b>Author</b>	<b>Year</b>	<b>Title</b>	<b>Reference</b>
1.	Adachi, T., Suzuki, Y., & Fujisawa, T.	2021	Photodegradation of an anilide fungicide inpyrfluxam in water and nitrate aqueous solution	Journal of Agricultural and Food Chemistry, 69(44), 12966-12973

**Relevant studies after detailed assessment of full-text documents for relevance (cont.):**

<b>Meet relevance criteria</b>	<b>Meet Reliability Criteria</b>	<b>Basis for relevance/ reliability decision (title, abstract or full article)</b>	<b>Comments</b>	<b>EU data point</b>
<b>Y or N</b>	<b>Score</b>			
Y	2	Full article	The study was not conducted under GLP, however, the authors follow the OECD 316 guideline for direct photolysis of chemicals in water. The authors also investigate the effects of indirect photolysis (for which there is no official OECD guideline method) through the formation of hydroxyl radicals in the presence of nitrate ions. All studies were conducted using radiolabelled test items and full mass balance tables are reported. Reaction kinetics were calculated according to FOCUS (2014).	7.2.1.2 & 7.2.1.3

**Appendix 5: All other studies considered to be non-relevant after rapid assessment; (ordered by title)**

<b>Number</b>	<b>Title</b>
1.	1,2-Disubstituted bicyclo[2.1.1]hexanes as saturated bioisosteres of ortho-substituted benzene
2.	19-(Benzyloxy)-19-oxojolkinolide B (19-BJB), an ent-abietane diterpene diepoxide, inhibits the growth of bladder cancer T24 cells through DNA damage
3.	2016 Central Italy Earthquakes: comparison between GPS signals and low-cost distributed MEMS arrays
4.	2-Oxabicyclo[2.1.1]hexanes as saturated bioisosteres of the ortho-substituted phenyl ring
5.	3,3-Difluoroallyl ammonium salts: highly versatile, stable and selective gem-difluoroallylation reagents
6.	53BP1 regulates heterochromatin through liquid phase separation
7.	A complex of BRCA2 and PP2A-B56 is required for DNA repair by homologous recombination
8.	A damaged genome's transcriptional landscape through multilayered expression profiling around in situ-mapped DNA double-strand breaks
9.	A Dynamic Fusion Pathfinding Algorithm Using Delaunay Triangulation and Improved A-Star for Mobile Robots
10.	A flower pollination algorithm for flexible job shop scheduling with fuzzy processing time
11.	A genome-wide screening uncovers the role of CCAR2 as an antagonist of DNA end resection
12.	A Green Route for the Synthesis of 3-(Difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic Acid
13.	A kinome-targeted RNAi-based screen links FGF signaling to H2AX phosphorylation in response to radiation
14.	A long noncoding RNA sensitizes genotoxic treatment by attenuating ATM activation and homologous recombination repair in cancers
15.	A method for the examination of sdhi fungicide resistance mechanisms in phytopathogenic fungi using a heterologous expression system in Sclerotinia sclerotiorum
16.	A new activated carbon for CO <sub>2</sub> capture from coal-fired boiler flue gas
17.	A non-canonical, interferon-independent signaling activity of cGAMP triggers DNA damage response signaling
18.	A One-Pot Photochemical Method for the Generation of Functionalized Aminocyclopentanes
19.	A partially pixel-parallel DROIC for MWIR imagers with columnwise residue quantization
20.	A Portable Gait Asymmetry Rehabilitation System for Individuals with Stroke Using a Vibrotactile Feedback
21.	A Portable Platform for Evaluation of Visual Performance in Glaucoma Patients
22.	A potential new role of ATM inhibitor in radiotherapy: suppressing ionizing Radiation-Activated EGFR

Number	Title
23.	A room-oriented artificial bee colony algorithm for optimizing the patient admission scheduling problem
24.	A scalable CRISPR/Cas9-based fluorescent reporter assay to study DNA double-strand break repair choice
25.	A short G1 phase imposes constitutive replication stress and fork remodelling in mouse embryonic stem cells
26.	A statistical approach to determine fluxapyroxad and its three metabolites in soils, sediment and sludge based on a combination of chemometric tools and a modified quick, easy, cheap, effective, rugged and safe method
27.	A unique tolerizing dendritic cell phenotype induced by the synthetic triterpenoid CDDO-DFPA (RTA-408) is protective against EAE
28.	Aberrant topoisomerase-1 DNA lesions are pathogenic in neurodegenerative genome instability syndromes
29.	ABL1 kinase as a tumor suppressor in AML1-ETO and NUP98-PMX1 leukemias
30.	Abrogation of Cellular Senescence Induced by Temozolomide in Glioblastoma Cells: Search for Senolytics
31.	Acquired Bartter-like Syndrome Presenting with Polyuria and Reversible Hypokalemia Associated with Colistin Use in a Critically Ill Pediatric Patient
32.	Active hyperspectral imaging using a quantum cascade laser (QCL) array and digital-pixel focal plane array (DFPA) camera
33.	ADAR-mediated RNA editing of DNA:RNA hybrids is required for DNA double strand break repair
34.	Agricultural transformation and market innovation: Theory, concepts, and definitions
35.	Altered gene expression by sedaxane increases PSII efficiency, photosynthesis and growth and improves tolerance to drought in wheat seedlings
36.	Amino acid substitutions responsible for different Qol and SDHI sensitivity patterns in Puccinia horiana, the causal agent of chrysanthemum white rust
37.	Amorphous Gadolinium Aluminate as a Dielectric and Sulfur for Indium Phosphide Passivation
38.	An Algorithm Developed for Smallsats Accurately Retrieves Landsat Surface Reflectance Using Scene Statistics
39.	An Optimal Scheme for UWSAN of Hotspots Issue Based on Energy-Efficient Novel Watchman Nodes
40.	Anodal Transcranial Direct Current Stimulation Shows Minimal, Measure-Specific Effects on Dynamic Postural Control in Young and Older Adults: A Double Blind, Sham-Controlled Study
41.	Application of Severe Accident Analysis Codes for Plant Calculations
42.	Applications for CO <sub>2</sub> -Activated Carbon Monoliths: I. Gas Storage
43.	Applications for CO <sub>2</sub> -Activated Carbon Monoliths: II. EDLC Electrodes
44.	Applying Reconstructed Daily Water Storage and Modified Wetness Index to Flood Monitoring: A Case Study in the Yangtze River Basin

Number	Title
45.	ARF triggers senescence in Brca2-deficient cells by altering the spectrum of p53 transcriptional targets
46.	Assessing the Impact of Family Planning Advice on Unmet Need and Contraceptive Use among Currently Married Women in Uttar Pradesh, India
47.	Assessment of packed bed bioreactor systems in the production of viral vaccines
48.	Associate Editor's Commentary: Measuring Responsibility
49.	Association of parent's body height towards adolescents body height
50.	Ataxia telangiectasia mutated inhibitor-loaded copper sulfide nanoparticles for low-temperature photothermal therapy of hepatocellular carcinoma
51.	ATM Alters the Otherwise Robust Chromatin Mobility at Sites of DNA Double-Strand Breaks (DSBs) in Human Cells
52.	ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks
53.	ATM phosphorylates PP2A subunit A resulting in nuclear export and spatiotemporal regulation of the DNA damage response
54.	ATM specifically mediates repair of double-strand breaks with blocked DNA ends
55.	Atomic-scale investigations on the wet etching kinetics of Ge versus SiGe in acidic H <sub>2</sub> O <sub>2</sub> solutions: a post operando synchrotron XPS analysis
56.	ATR kinase regulates its attenuation via PPM1D phosphatase recruitment to chromatin during recovery from DNA replication stress signalling
57.	Azo-incorporating Increases Inhibitory Activity of Succinate Dehydrogenase
58.	Bacterial Genotoxins Promote Inside-Out Integrin $\beta$ 1 Activation, Formation of Focal Adhesion Complexes and Cell Spreading
59.	Bioinspired Synthesis of a Sedaxane Metabolite Using Catalytic Vanadyl Acetylacetonate and Molecular Oxygen
60.	Boronic ester functionalised 1,8-diboryl-naphthalene scaffolds: fluoride versus oxide chelation
61.	BRCT-domain protein BRIT1 influences class switch recombination
62.	C16orf72/HAPSTR1/TAPR1 functions with BRCA1/Senataxin to modulate replication-associated R-loops and confer resistance to PARP disruption
63.	Castration-resistant prostate cancer: Androgen receptor inactivation induces telomere DNA damage, and damage response inhibition leads to cell death
64.	Cell death during crisis is mediated by mitotic telomere deprotection
65.	Characterization of the VisdhC and VisdhD genes in <i>Venturia inaequalis</i> , and sensitivity to fluxapyroxad, pydiflumetofen, inpyrfluxam, and benzovindiflupyr



Number	Title
66.	CHD7 and 53BP1 regulate distinct pathways for the re-ligation of DNA double-strand breaks
67.	Chronic exposure to Cytolethal Distending Toxin (CDT) promotes a cGAS-dependent type I interferon response
68.	Cip29 is phosphorylated following activation of the DNA damage response in <i>Xenopus</i> egg extracts
69.	Comparative analysis of forestry policy and implementation during the AK Party period in Turkey
70.	Comparison of current peanut fungicides against <i>Athelia rolfsii</i> through a laboratory bioassay of detached plant tissues
71.	Comparison of morphological and functional outcomes of mouse sciatic nerve repair with three biodegradable polymer conduits containing poly(lactic acid)
72.	Computation-Directed Molecular Design, Synthesis, and Fungicidal Activity of Succinate Dehydrogenase Inhibitors
73.	Concentration and turnover of dissolved free polyamines on the South Coast of Lake Erie
74.	Condenser-side integration of a simple solar-type waste heat recovery device in a thermal plant
75.	Contracting CAG/CTG repeats using the CRISPR-Cas9 nickase
76.	Coordinated nuclease activities counteract Ku at single-ended DNA double-strand breaks
77.	Correlation between molar activity, injection mass and uptake of the PARP targeting radiotracer (18F)olaparib in mouse models of glioma
78.	Crosstalk between SUMOylation and ubiquitylation controls DNA end resection by maintaining MRE11 homeostasis on chromatin
79.	Crystal structure and molecular docking studies of new pyrazole-4-carboxamides
80.	Curative effects of fungicides against <i>Venturia inaequalis</i> causing apple scab
81.	CX-5461 activates the DNA damage response and demonstrates therapeutic efficacy in high-grade serous ovarian cancer
82.	Dam Safety Evaluation Based on Multiple Linear Regression and Numerical Simulation
83.	Dataset on the relationship between students' attitude towards, and performance in mathematics word problems, mediated by active learning heuristic problem-solving approach
84.	Dense carbon monoliths for supercapacitors with outstanding volumetric capacitances
85.	Design, synthesis and antifungal activity of novel pyrazole amides derivatives
86.	Design, synthesis and antifungal evaluation of novel pyrazole carboxamides with diarylamines scaffold as potent succinate dehydrogenase inhibitors
87.	Design, synthesis and antifungal/anti-oomycete activity of pyrazolyl oxime ethers as novel potential succinate dehydrogenase inhibitors

Number	Title
88.	Design, synthesis and bioactivity of novel fluoropyrazole hydrazides
89.	Design, synthesis and biological activity of N-sulfonyl aromatic amide derivatives
90.	Design, synthesis and biological evaluation of pyrazole-aromatic containing carboxamides as potent SDH inhibitors
91.	Design, synthesis and fungicidal activity of pyrazole-thiazole carboxamide derivatives
92.	Design, synthesis, and antifungal activities of novel aromatic carboxamides containing a diphenylamine scaffold
93.	Design, Synthesis, and Antifungal Activities of Novel Carboxamides Derivatives Bearing a Chalcone Scaffold as Potential SDHIs
94.	Design, Synthesis, and Antifungal Activities of Novel Pyrazole-4-Carboxamide Derivatives Containing Ether Group as Potential Succinate Dehydrogenase Inhibitors
95.	Design, synthesis, and antifungal activity of carboxamide derivatives possessing 1,2,3-triazole as potential succinate dehydrogenase inhibitors
96.	Design, Synthesis, and Bioactivity of Novel Quinazolinone Scaffolds Containing Pyrazole Carbamide Derivatives as Antifungal Agents
97.	Design, synthesis, and biological activity of novel aromatic amide derivatives containing sulfide and sulfone substructures
98.	Design, Synthesis, and Evaluation of Antifungal Bioactivity of Novel Pyrazole Carboxamide Thiazole Derivatives as SDH Inhibitors
99.	Design, Synthesis, and Evaluation of the Antifungal Activity of Novel Pyrazole-Thiazole Carboxamides as Succinate Dehydrogenase Inhibitors
100.	Design, Synthesis, and Herbicidal Activity of Naphthalimide-Aroyl Hybrids as Potent Transketolase Inhibitors
101.	Design, synthesis, and insecticidal activity of a novel series of flupyrimin analogs bearing 1-aryl-1H-pyrazol-4-yl subunits
102.	Design, Synthesis, Fungicidal and Insecticidal Activities of Novel Diamide Compounds Combining Pyrazolyl and Polyfluoro-Substituted Phenyl into Alanine or 2-Aminobutyric Acid Skeletons
103.	Design, synthesis, insecticidal activities, and molecular docking of novel pyridylpyrazolo carboxylate derivatives
104.	Detection of pesticide residues in imported agricultural products in Tokyo and an alteration to testing portions for international harmonization
105.	Determination of 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid and its impurities in pesticide intermediate by reversed phase high performance liquid chromatography
106.	Development and functional characterization of murine tolerogenic dendritic cells
107.	Development of a 3D Hybrid Finite-Discrete Element Simulator Based on GPGPU-Parallelized Computation for Modelling Rock Fracturing Under Quasi-Static and Dynamic Loading Conditions

Number	Title
108.	Development of a Practical Process for the Large-Scale Preparation of the Chiral Pyridyl-Backbone for the Crabtree/Pfaltz-Type Iridium Complex Used in the Industrial Production of the Novel Fungicide Inpyrfluxam
109.	Development of Cyanopyrazoles as Building Blocks to Fungicide Fluxapyroxad and Analogues
110.	Direct formation of amide/peptide bonds from carboxylic acids: no traditional coupling reagents, 1-pot, and green
111.	Discovery of a Fungicide Candidate Targeting Succinate Dehydrogenase via Computational Substitution Optimization
112.	Discovery of a novel agricultural fungicide, inpyrfluxam
113.	Discovery of Highly Efficient Novel Antifungal Lead Compounds Targeting Succinate Dehydrogenase: Pyrazole-4-carboxamide Derivatives with an N-Phenyl Substituted Amide Fragment
114.	Discovery of N-(4-fluoro-2-(phenylamino)phenyl)-pyrazole-4-carboxamides as potential succinate dehydrogenase inhibitors
115.	Discovery of N-benzoxazol-5-yl-pyrazole-4-carboxamides as nanomolar SQR inhibitors
116.	Discovery of Novel Aminocyclobutanecarboxylic Acid Derivatives as Succinate Dehydrogenase Inhibitors
117.	Discovery of novel iminosydnone compounds with insecticidal activities based on the binding mode of triflumezopyrim
118.	Discovery of Novel Piperidinylthiazole Derivatives As Broad-Spectrum Fungicidal Candidates
119.	Discovery of Novel Pyrazole Carboxylate Derivatives Containing Thiazole as Potential Fungicides
120.	Discovery of Potent Succinate-Ubiquinone Oxidoreductase Inhibitors via Pharmacophore-linked Fragment Virtual Screening Approach
121.	Discrete flower pollination algorithm for patient admission scheduling problem
122.	Dissecting the early steps of MLL induced leukaemogenic transformation using a mouse model of AML
123.	Dissipation, metabolism, accumulation, processing and risk assessment of fluxapyroxad in cucumber and cowpea vegetables from field to table
124.	Dissolved free amino acids and polyamines are two major dissolved organic nitrogen sources for marine bacterioplankton in the northern slope of the South China Sea
125.	DNA damage-encouraged Mn-As-based nanoreactors reshape intratumoral cell phenotypes to recover immune surveillance and potentiate anti-tumor immunity
126.	DNA damage-induced metaphase I arrest is mediated by the spindle assembly checkpoint and maternal age
127.	DNA damage-induced transcription stress triggers the genome-wide degradation of promoter-bound Pol II
128.	DNA double-strand break repair pathway regulates PD-L1 expression in cancer cells
129.	DNA repair inhibitors sensitize cells differently to high and low LET radiation

Number	Title
130.	DNA-damage-induced differentiation of leukaemic cells as an anti-cancer barrier
131.	Dual-wavelength dual-cavity spectrometer for NO <sub>2</sub> detection in the presence of aerosol interference
132.	Effect of ATM and HDAC inhibition on etoposide-induced DNA damage in porcine early preimplantation embryos
133.	Effective Monitoring of Fluxapyroxad and Its Three Biologically Active Metabolites in Vegetables, Fruits, and Cereals by Optimized QuEChERS Treatment Based on UPLC-MS/MS
134.	Effects of confining pressure on the permeability of three rock types under compression
135.	Effects of fungicides on four native generalist phytoseiid species (Acari: Phytoseiidae)
136.	Effects of Fungicides on Four Native Generalist Phytoseiid Species (Acari: Phytoseiidae)
137.	Eksplorasi Tumbuhan Paku (Pteridophyta) di STL Ulu Terawas, Musi Rawas, Sumatera Selatan
138.	Electrocardiographic diagnosis of atrial infarction in patients with acute inferior ST-segment elevation myocardial infarction
139.	Electrochemically-initiated polymerization of reactive monomers via 4-fluorobenzenediazonium salts
140.	Estimating the Hurst parameter from short term volatility swaps: a Malliavin calculus approach
141.	Evaluation of several fungicides on leaf blight and rust disease of Welsh onion for simultaneous control
142.	Evaporation study for real soils based on HYPROP hydraulic functions and micro-CT-measured pore-size distribution
143.	Expanding the Chemical Space of Succinate Dehydrogenase Inhibitors via the Carbon-Silicon Switch Strategy
144.	Expansion of gastric intestinal metaplasia with copy number aberrations contributes to field cancerization
145.	Expedient Discovery for Novel Antifungal Leads Targeting Succinate Dehydrogenase: Pyrazole-4-formylhydrazide Derivatives Bearing a Diphenyl Ether Fragment
146.	Exploration on Damage Mechanism and Equivalent Damage Model of High Arch Dams under Earthquakes
147.	Extensive abdominal sarcoidosis without pulmonary manifestation
148.	Fabrication of superhydrophobic fabric coating using microphase-separated dodecafluoroheptyl-containing polyacrylate and nanosilica
149.	Fabrication of Versatile Pyrazole Hydrazide Derivatives Bearing a 1,3,4-Oxadiazole Core as Multipurpose Agricultural Chemicals against Plant Fungal, Oomycete and Bacterial Diseases
150.	Failure and overall stability analysis on high arch dam based on DFPA code
151.	Fast Semantic Segmentation of Remote Sensing Images Using a Network That Integrates Global and Local Information
152.	Fermentative Biohydrogen Modelling and Optimization Research in Light of Miniaturized Parallel Bioreactors

Number	Title
153.	Field-Effect Transistors Built from All Two-Dimensional Material Components
154.	Finding the Missing Property Concepts in Pesticide-Likeness
155.	Flood risk assessment using deep learning integrated with multi-criteria decision analysis
156.	Fractured Rock Permeability as a Function of Temperature and Confining Pressure
157.	Fungicide isopyrazam degradative response toward extrinsically added fungal and bacterial strains
158.	Fungicide sensitivity of Colletotrichum species causing bitter rot of apple in the Mid-Atlantic U.S.A
159.	Future direction of agrochemical development for plant disease in China
160.	Gait Kinematic and Kinetic Characteristics of Older Adults With Mild Cognitive Impairment and Subjective Cognitive Decline: A Cross-Sectional Study
161.	Gas storage scale-up at room temperature on high density carbon materials
162.	Gene expression profiling of brains from bovine spongiform encephalopathy (BSE)-infected cynomolgus macaques
163.	Genomic Regions Associated with the Position and Number of Hair Whorls in Horses
164.	Grafts of human adipose-derived stem cells into a biodegradable poly (acid lactic) conduit enhances sciatic nerve regeneration
165.	Groundwater potential mapping in the Central Highlands of Vietnam using spatially explicit machine learning
166.	Growing hydrophobicity on a smooth copper oxide thin film at room temperature and reversible wettability transition
167.	Helium integrity testing of single-use vessels
168.	Helium integrity testing of single-use vessels: Helium integrity testing can prevent failures in single-use bioprocessing vessels
169.	HEM: An Improved Parametric Link Prediction Algorithm Based on Hybrid Network Evolution Mechanism
170.	High-Gain Inverters Based on WSe <sub>2</sub> Complementary Field-Effect Transistors
171.	HOMO-LUMO, NBO, NLO, MEP analysis and molecular docking using DFT calculations in DFPA molecule
172.	How can the floor area types of a university campus mitigate the increase of urban air temperature?
173.	How Mental Illness is Perceived by Iranian Medical Students: A Preliminary Study
174.	Human ALS/FTD brain organoid slice cultures display distinct early astrocyte and targetable neuronal pathology
175.	Hydrodynamics in a disposable rectangular parallelepiped stirred bioreactor with elliptic pendulum motion paddle
176.	Identification and characterization of fungicide resistance in botrytis populations from small fruit fields in the Mid-Atlantic United States

Number	Title
177.	Imaging PARP with (18F)rucaparib in pancreatic cancer models
178.	Impact of Q-Time on the Passivation of Al <sub>2</sub> O <sub>3</sub> /p-In <sub>0.53</sub> Ga <sub>0.47</sub> As Interfaces Using Various Surface Treatments
179.	iMUT-seq: high-resolution DSB-induced mutation profiling reveals prevalent homologous-recombination dependent mutagenesis
180.	Increased sensitivity to ionizing radiation by targeting the homologous recombination pathway in glioma initiating cells
181.	Industrial-scale enantioselective hydrogenation using Pfaltz-type catalysts: Chemical development and academic collaborations
182.	Inhibition of histone acetyltransferase function radiosensitizes CREBBP/EP300 mutants via repression of homologous recombination, potentially targeting a gain of function
183.	Insect cell entrapment, growth and recovering using a single-use fixed-bed bioreactor. Scaling up and recombinant protein production
184.	Integration of electrocoagulation, adsorption and wetland technology for jewelry industry wastewater treatment
185.	Ionizing radiation response of primary normal human lens epithelial cells
186.	Isolation and characterization of cellulose nanofibers from Agave gigantea by chemical-mechanical treatment
187.	Kinase inhibitors of DNA-PK, ATM and ATR in combination with ionizing radiation can increase tumor cell death in HNSCC cells while sparing normal tissue cells
188.	Kinase-dead ATR differs from ATR loss by limiting the dynamic exchange of ATR and RPA
189.	Know thy tumour: Biomarkers to improve treatment of molecular radionuclide therapy
190.	Least Square Support Tensor Regression Machine Based on Submatrix of the Tensor
191.	Loop extrusion as a mechanism for formation of DNA damage repair foci
192.	Loss of the abasic site sensor HMCES is synthetic lethal with the activity of the APOBEC3A cytosine deaminase in cancer cells
193.	Low power reconfigurable FP-FFT core with an array of folded DA butterflies
194.	Low-dose tributyltin triggers human chondrocyte senescence and mouse articular cartilage aging
195.	MAD2L2 dimerization and TRIP13 control shieldin activity in DNA repair
196.	Mammalian polymerase [theta] promotes alternative NHEJ and suppresses recombination
197.	Mapping combinatorial drug effects to DNA damage response kinase inhibitors
198.	Measurement of Gender Differences of Gastrocnemius Muscle and Tendon Using Sonomyography during Calf Raises: A Pilot Study

Number	Title
199.	Mechanism of Action of Novel Pyrazole Carboxamide Containing a Diarylamine Scaffold against <i>Rhizoctonia solani</i>
200.	Mechanochemical synthesis of an organometallic compound: a high volume manufacturing method
201.	Medicine manipulation: An alternative to mitigate therapeutic gaps in the Brazilian Unified Health System?
202.	Method for the preparation of 3-fluoroalkyl-1-methylpyrazol-4-carboxylic acid
203.	Mild replication stress causes premature centriole disengagement via a sub-critical Plk1 activity under the control of ATR-Chk1
204.	Mitotic progression following DNA damage enables pattern recognition within micronuclei
205.	Modelling of the temperature distribution of resistance-spot-welded dissimilar metals using the finite element method
206.	Monitoring the extreme flood events in the Yangtze River basin based on GRACE and GRACE-FO satellite data
207.	Monolayer Kagome metals AV3Sb5
208.	Mott insulators with boundary zeros
209.	MRN-dependent and independent pathways for recruitment of TOPBP1 to DNA double-strand breaks
210.	Multilayer ReS2 lateral p-n homojunction for photoemission and photodetection
211.	Multi-omics analysis reveals neoantigen-independent immune cell infiltration in copy-number driven cancers
212.	N-[2-[(3-Chlorophenyl)amino]-phenyl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide: Synthesis, Crystal Structure, Molecular Docking and Biological Activities
213.	N-Ammonium Ylide Mediators for Electrochemical C-H Oxidation
214.	New cytochrome b haplotypes, harboring L299F or N256S + L299F substitutions, were found in azoxystrobin-resistant <i>Puccinia horiana</i> , the causal agent of chrysanthemum white rust
215.	Nitrogen Deposition on Danish Nature
216.	Non-canonical function of DGCR8 in DNA double-strand break repair signaling and tumor radioresistance
217.	Non-Communicable Disease Risk Factors among Employees and Their Families of a Saudi University: An Epidemiological Study
218.	Non-isothermal compositional liquid gas Darcy flow: formulation, soil-atmosphere boundary condition and application to high-energy geothermal simulations
219.	Novel 4-pyrazole carboxamide derivatives containing flexible chain motif: design, synthesis and antifungal activity
220.	Novel aromatic carboxamides from dehydroabietylamine as potential fungicides: Design, synthesis and antifungal evaluation
221.	Nrf2-mediated metabolic reprogramming of tolerogenic dendritic cells is protective against aplastic anemia



Number	Title
222.	Nucleolar release of rDNA repeats for repair involves SUMO-mediated untethering by the Cdc48/p97 segregase
223.	On Smile Properties of Volatility Derivatives: Understanding the VIX Skew
224.	On the Importance of Collaboration in the Development of Sustainable Catalytic Processes: The Case of Inpyrfluxam
225.	One hundred fold increase in current carrying capacity in a carbon nanotube-copper composite
226.	One pot and room temperature photochemical synthesis of high quantum yield NIR emissive Ag <sub>2</sub> S@Ag(In, Zn)S <sub>2</sub> core-shells at the presence of air in water
227.	One-Pot, Three-Step Synthesis of Cyclopropylboronic Acid Pinacol Esters from Synthetically Tractable Propargylic Silyl Ethers
228.	On-farm research identifies options for managing fungicide-resistant aerial blight of soybean
229.	Optimization of HVAC system energy consumption in a building using artificial neural network and multi-objective genetic algorithm
230.	Orally Bioavailable and Blood-Brain Barrier-Penetrating ATM Inhibitor (AZ32) Radiosensitizes Intracranial Gliomas in Mice
231.	Overcoming hypoxia-induced tumor radioresistance in non-small cell lung cancer by targeting DNA-dependent protein kinase in combination with carbon ion irradiation
232.	PAXX promotes KU accumulation at DNA breaks and is essential for end-joining in XLF-deficient mice
233.	Pellino1 regulates reversible ATM activation via NBS1 ubiquitination at DNA double-strand breaks
234.	Persistent DNA damage triggers activation of the integrated stress response to promote cell survival under nutrient restriction
235.	Persistent repair intermediates induce senescence
236.	Pharmaceutical effluent: a critical link in the interconnected ecosystem promoting antimicrobial resistance
237.	Photoanodic pyramid texturization of n-Ge(100) in HCl solution: unexpected anisotropy in the surface chemistry of etching
238.	Photochemical Formal (4 + 2)-Cycloaddition of Imine-Substituted Bicyclo[1.1.1]pentanes and Alkenes
239.	PI3K $\delta$ activates E2F1 synthesis in response to mRNA translation stress
240.	Pilot Randomized Clinical Trial of a Text Messaging-Based Intervention for Smoking Cessation Among Young People Experiencing Homelessness
241.	Positioning of nucleosomes containing $\gamma$ -H2AX precedes active DNA demethylation and transcription initiation
242.	Pre-Exposure to Ionizing Radiation Stimulates DNA Double Strand Break End Resection, Promoting the Use of Homologous Recombination Repair



Number	Title
243.	Preparation method of 3-Difluoromethyl-1-Methyl-1H-Py-4-carboxylic acid
244.	Preprocessing the Nintendo Wii Board Signal to Derive More Accurate Descriptors of Statokinesigrams
245.	Production of recombinant rabies virus glycoprotein by insect cells in a single-use fixed-bed bioreactor
246.	Profiling forest fires along the urban gradient: a Mediterranean case study
247.	Profiling ubiquitin signalling with UBIMAX reveals DNA damage- and SCF $\beta$ -Trcp1-dependent ubiquitylation of the actin-organizing protein Dbn1
248.	Protection of nascent DNA at stalled replication forks is mediated by phosphorylation of RIF1 intrinsically disordered region
249.	Quantitative phosphoproteomics to unravel the cellular response to chemical stressors with different modes of action
250.	Quantum Well InAs/AlSb/GaSb Vertical Tunnel FET With HSQ Mechanical Support
251.	Radiation-induced DNA damage and repair effects on 3D genome organization
252.	Radiofluorination of a highly potent ATM inhibitor as a potential PET imaging agent
253.	Rapid Synthesis of 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives and their Characterization, anticancer and antifungal activities
254.	Regio-controllable [2+2] benzannulation with two adjacent C(sp <sup>3</sup> )-H bonds
255.	Regulation of DNA repair pathway choice in S and G2 phases by the NHEJ inhibitor CYREN
256.	Regulation of Rad52-dependent replication fork recovery through serine ADP-ribosylation of PolD3
257.	Reinforcement Analysis of Toe Blocks and Anchor Cables at the Xiluodu Super-High Arch Dam
258.	Removal of bromide from raw water in drinking industry by electrochemical method with horizontal rotating anode reactor
259.	Replication-associated formation and repair of human topoisomerase III $\alpha$ cleavage complexes
260.	Research and development of a novel fungicide, inpyrfluxam
261.	Research on ground reaction forces and utilized coefficient of friction of turning gait
262.	Research on multiple effects of fixed-asset investment on energy consumption——by three strata of industry in China
263.	Research on the Stability Mechanism of the Surrounding Rock of Gob-Side Entry Retaining by Roof Cutting in Dianping Coal Mine
264.	Rh(III)-Catalyzed C-H Amidation of Arenes with N-Methoxyamide as an Amidating Reagent
265.	RNAPII-dependent ATM signaling at collisions with replication forks
266.	ROBUST TRANSITIVITY OF MAPS OF THE REAL LINE

Number	Title
267.	RPA shields inherited DNA lesions for post-mitotic DNA synthesis
268.	Rsearch on ground reaction forces and utilized coefficient of friction of turning gait
269.	Salivary gland tumors, clinico-epidemiological study and radioanatomy correlation: retrospective study of 148 cases
270.	SDHI Fungicide Toxicity and Associated Adverse Outcome Pathways: What Can Zebrafish Tell Us?
271.	Sedaxane, Isopyrazam and Solatenol™: Novel Broad-spectrum Fungicides Inhibiting Succinate Dehydrogenase (SDH) - Synthesis Challenges and Biological Aspects
272.	Sedaxane-Use of Nuclear Receptor Transactivation Assays, Toxicogenomics, and Toxicokinetics as Part of a Mode of Action Framework for Rodent Liver Tumors
273.	Selective human inhibitors of ATR and ATM render Leishmania major promastigotes sensitive to oxidative damage
274.	Self-Decoupled Dual-Frequency Patch Antennas via Hybrid Coupling Interface Technique
275.	Shallow unloading deformation analysis on Baihetan super-high arch dam foundation
276.	Solving the generalized cubic cell formation problem using discrete flower pollination algorithm
277.	Spatiotemporal distribution and microbial assimilation of polyamines in a mesotrophic lake
278.	Spline interpolation for modelling of accumulated effects of ozone
279.	STAG2 Regulates Homologous Recombination Repair and Sensitivity to ATM Inhibition
280.	Standoff Infrared Measurements of Chemical Plume Dynamics in Complex Terrain Using a Combination of Active Swept-ECQCL Laser Spectroscopy with Passive Hyperspectral Imaging
281.	Stereoselective bioactivity, toxicity and degradation of novel fungicide sedaxane with four enantiomers under rice-wheat rotation mode
282.	Structural investigation and molecular modeling studies of strobilurin-based fungicides active against the rice blast pathogen <i>Pyricularia oryzae</i>
283.	Structure-Based Discovery of Potential Fungicides as Succinate Ubiquinone Oxidoreductase Inhibitors
284.	Studies on the novel pyridine sulfide containing SDH based heterocyclic amide fungicide
285.	Study on Deformation and Control Measures of Columnar Jointed Basalt for Baihetan Super-High Arch Dam Foundation
286.	Study on Optimal Grouting Timing for Controlling Uplift Deformation of a Super High Arch Dam
287.	Study on process control of cracking process in DFPA production line based on DCS
288.	Subatmospheric gas storage and delivery: Past, present and future
289.	Suppressing Non-Uniform Tunneling in InAs/GaSb TFET With Dual-Metal Gate

Number	Title
290.	Surface Modification and Assembly of Transparent Indium Tin Oxide Nanocrystals for Enhanced Conductivity
291.	Synergism between ATM and PARP1 inhibition involves DNA damage and abrogating the G2 DNA damage checkpoint
292.	Synthesis and anti-leukemia activity of phorbol 13,20-diesters and phorbol 12,13,20-triesters
293.	Synthesis and bioactivity of novel sulfonate scaffold-containing pyrazolecarbamide derivatives as antifungal and antiviral agents
294.	Synthesis and biological activity of acyl thiourea containing difluoromethyl pyrazole motif
295.	Synthesis and Biological Activity of Novel Antifungal Leads: 3,5-Dichlorobenzyl Ester Derivatives
296.	Synthesis and Biological Activity of Novel Succinate Dehydrogenase Inhibitor Derivatives as Potent Fungicide Candidates
297.	Synthesis and biological activity of waltherione F-derived diamide derivatives containing 4-quinolone group
298.	Synthesis and biological evaluation of novel benodanil-heterocyclic carboxamide hybrids as a potential succinate dehydrogenase inhibitors
299.	Synthesis and biological evaluation of novel pyrazole carboxamide with diarylamine-modified scaffold as potent antifungal agents
300.	Synthesis and fungicidal activity of difluoromethyl substituted carboxamide derivatives
301.	Synthesis and herbicidal activity evaluation of novel diacylhydrazine derivatives containing pyrazolyl moiety
302.	Synthesis and SWOT analysis of date palm frond ash–Portland cement composites
303.	Synthesis of 3-difluoromethyl-1-methyl-1H-pyrazol-4-carboxylic acid
304.	SYNTHESIS, ANTIFUNGAL ACTIVITY AND QSAR OF NOVEL PYRAZOLE AMIDES AS SUCCINATE DEHYDROGENASE INHIBITORS
305.	Synthesis, antifungal activity and structure-activity relationships of novel 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid amides
306.	Synthesis, biological activity and toxicity to zebrafish of benzamides substituted with pyrazole-linked 1,2,4-oxadiazole
307.	Synthesis, crystal structure and fungicidal activity of 3-(difluoromethyl)-1-methyl-N-((2-(trifluoromethyl)phenyl) carbamoyl)-1H-pyrazole-4-carboxamide
308.	Synthesis, Crystal Structure, Antifungal Activity and Docking Study of Difluoromethyl Pyrazole Derivatives
309.	Synthesis, fungicidal activity and SAR of 2-thiazolamide/pyrazolamide-cyclohexylsulfonamides against Botrytis cinerea
310.	Synthesis, Fungicidal Activity, and Mechanism of Action of Pyrazole Amide and Ester Derivatives Based on Natural Products L-Serine and Waltherione Alkaloids

Number	Title
311.	Synthesis, nematocidal activity and SAR study of novel difluoromethylpyrazole carboxamide derivatives containing flexible alkyl chain moieties
312.	Synthesis, structure, and antifungal activities of 3-(difluoromethyl)-pyrazole-4-carboxylic oxime ester derivatives
313.	Synthetic approaches to the 2015-2018 new agrochemicals
314.	Targeting PFKFB3 radiosensitizes cancer cells and suppresses homologous recombination
315.	Targeting the DNA Damage Response Machinery for Lung Cancer Treatment
316.	TDP1-independent pathways in the process and repair of TOP1-induced DNA damage
317.	Terrestrial ecosystems in a changing environment: a dominant role for water
318.	The agricultural transformation and market integration in ASEAN region responding to food security and inclusiveness concerns: Summary report of a regional policy forum, Phnom Penh, Cambodia, December 13-14, 2018
319.	The association between air pollution and cancers: controversial evidence of a systematic review
320.	The ATR Inhibitor VE-821 Enhances the Radiosensitivity and Suppresses DNA Repair Mechanisms of Human Chondrosarcoma Cells
321.	The CHD6 chromatin remodeler is an oxidative DNA damage response factor
322.	The chemotherapeutic CX-5461 primarily targets TOP2B and exhibits selective activity in high-risk neuroblastoma
323.	The Combination of ATM and Chk1 Inhibitors Induces Synthetic Lethality in Colorectal Cancer Cells
324.	The embryonic developmental effect of sedaxane on zebrafish ( <i>Danio rerio</i> )
325.	The evaluation of the urban parks in Konya province in terms of quality, sufficiency, maintenance, and growth rate
326.	The Fresnel-Fizeau effect and the atmospheric time delay in geodetic VLBI
327.	The function of ER-phagy receptors is regulated through phosphorylation-dependent ubiquitination pathways
328.	The relationship between terrain and rural migration (1965-2013) on the north of Turkey (the case of Kastamonu)
329.	The RSF1 Histone-Remodelling Factor Facilitates DNA Double-Strand Break Repair by Recruiting Centromeric and Fanconi Anaemia Proteins
330.	The shieldin complex mediates 53BP1-dependent DNA repair
331.	The splicing factor XAB2 interacts with ERCC1-XPF and XPG for R-loop processing
332.	Therapeutic targeting of ATR in alveolar rhabdomyosarcoma
333.	Thiamethoxam Toxicity and Effects on Consumption Behavior in <i>Orius insidiosus</i> (Hemiptera: Anthocoridae) on Soybean
334.	Time-varying wetting behavior on copper wafer treated by wet-etching

Number	Title
335.	Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage
336.	Transition of domestic market of pesticides-specialized in fungicides (4)
337.	Translating cell-based regenerative medicines from research to successful products: Challenges and solutions
338.	Treacle controls the nucleolar response to rDNA breaks via TOPBP1 recruitment and ATR activation
339.	Treatment of soybean seeds to control pathogenic fungi and maintain physiological quality
340.	Trend in fungicide development
341.	Triflylpyridinium Enables Rapid and Scalable Controlled Reduction of Carboxylic Acids to Aldehydes using Pinacolborane
342.	TRMM MICROWAVE IMAGER (TMI) ALIGNMENT AND ALONG-SCAN BIAS CORRECTIONS
343.	Tumor-specific radiosensitizing effect of the ATM inhibitor AZD0156 in melanoma cells with low toxicity to healthy fibroblasts
344.	Tuning the physical properties of organic sensitizers by replacing triphenylamine with new donors for dye sensitized solar cells - a theoretical approach
345.	Using inpyrfluxam to control peanut ( <i>Arachis hypogaea</i> L.) foliar and soil-borne diseases
346.	Variegated Pedospheric Matrices Based Pyrzaole Fungicide Chemico-physical and Biological Degradation Elucidation
347.	Wet Chemical Processing of Ge in Acidic H <sub>2</sub> O <sub>2</sub> Solution: Nanoscale Etching and Surface Chemistry
348.	Yield Losses and Control by Sedaxane and Fludioxonil of Soilborne Rhizoctonia, Microdochium, and Fusarium Species in Winter Wheat
349.	γH2AX analysis for monitoring cellular response to DNA double-strand breaks in human osteosarcoma cell

One study from the literature review was considered relevant (see Appendix 4 of the literature review). The evaluation of the review is given below.

#### **B.8.5.2 Studies considered relevant to environmental fate and behaviour from the literature review**

<b>Data Point:</b>	KCA 7.2.1.2/03
<b>Report Author:</b>	Adachi. T., Suzuki, Y. and Fujisawa, T.
<b>Report Year:</b>	2021
<b>Report Title:</b>	Photodegradation of and anilide fungicide S-2399 in water and nitrate aqueous solution  Journal of Agricultural Food Chemistry 2021, 69, 12966-12973
<b>Study number</b>	N/A
<b>Guideline(s) followed in study:</b>	OECD Guideline for the Testing of Chemicals: Phototransformation of Chemicals on Soil Surfaces (Draft Document, January 2002)
<b>GLP?</b>	No

<b>Deviations from guideline</b>	<b>HSE assessment of deviations</b>
N/A	
<b>HSE conclusion</b>  The study is acceptable and can be considered supporting information to the regulatory study reported under KCA 7.2.1.2/02.	

## **INTRODUCTION**

This study was identified by the applicant literature search as being relevant to the environmental fate and behaviour assessment of inpyrfluxam.

The photolytic degradation of inpyrfluxam was studied in phosphate buffer (pH 7). Agricultural fertilisers under the irradiation of sunlight in agricultural fields can be a potential source of hydroxyl radicals, which play an important role in advanced oxidation processes. Different concentrations of aqueous nitrate solution were therefore also included in the study, to estimate the effect of hydroxyl radicals derived from nitrate ions on the rate of degradation.

## STUDY DESIGN

Inpyrfluxam was radiolabelled in both the indane [PH-<sup>14</sup>C] and pyrazole [PY-<sup>14</sup>C] rings. The specific activities of [PY-<sup>14</sup>C] and [PH-<sup>14</sup>C] were 6.33 and 13.5 MBq/mg respectively and radiochemical purities were >97 %.

Phosphate buffer (50 mM, pH 7) was prepared with pure water, alongside three concentrations of nitrate aqueous solution (1, 5 and 50 ppm; pH 7). Solutions were sterilised by autoclaving (1.5 kg/cm<sup>3</sup>; 121 °C; 15 min) at a nominal concentration of 1.0 mg/L. It is not stated that sterility was confirmed.

Cylindrical reaction vessels (100 mL) covered with a quartz glass plate were continuously irradiated with an Atlas Suntest XLS+ 2 kW xenon arc lamp filtered to remove UV light below 290 nm and simulating natural sunlight as described in OECD 316. Light intensity was 42-53 W/m<sup>2</sup> at 300-400 nm. Temperature of solutions was maintained at 25 ± 2 °C. Moistened and CO<sub>2</sub>-free air was gently passed through the headspace of each test solution and entered into a series of traps including those containing ethylene glycol and NaOH (0.5 M) for trapping organic volatiles and <sup>14</sup>CO<sub>2</sub>. A dark control without traps for organic volatiles and CO<sub>2</sub> was also included.

Samples were taken at 0, 1, 2, 3, 7 and 10 days [PY-label] and at 1, 3 and 10 days [PH-label] after application of the test item. It is not stated whether samples were taken in duplicate, but only single measurements are reported.

Samples were analysed by HPLC-UV. Peaks were identified by comparing HPLC and TLC retention times with non-radiolabelled reference standards of inpyrfluxam and 4 metabolites (3'-OH-S-2840, DFPA-CONH<sub>2</sub>, DFPA and ATMI (1,1,3-trimethyl-2,3-dihydro-1H-inden-4-amine)). Amounts of radioactivity in the volatile traps were analysed by LSC. The structures of 3'-OH-S-2840 and DFPA-CONH<sub>2</sub> were confirmed using LC-MS and NMR.

Degradation rates were derived in the paper using CAKE 3.1 and SFO kinetics. Kinetic fits were not presented in the paper but have been derived by HSE below. The reaction rate of photolysis in buffer solution (direct photolysis rate) was subtracted from the total photolysis rate in nitrate solution to give the indirect photolysis rate.

## RESULTS

The UV-vis adsorption spectra of inpyrfluxam measured in pure water and 50 ppm nitrate solution showed adsorption at 250 nm. Limited adsorption occurs at wavelengths >290 nm, with no adsorption above c. 315 nm.

Recovery of both radiolabels ranged between 94.1 and 103.5 % AR. Radioactivity adsorbed to test vessels was <0.3 % AR.

It was reported that in buffered aqueous solution, direct photolysis of inpyrfluxam proceeded mainly by cleavage of the amide linkage and the N-phenyl ring bond to form metabolites DFPA-CONH<sub>2</sub> and DFPA. An alternative route was via oxidation of the 3'-position of the Indane ring to form 3'-OH-S-2840. PY-labelled inpyrfluxam degraded to 74.4 % AR with degradation products 3'-OH-S-2840, DFPA-CONH<sub>2</sub> and DFPA formed at up to 5.6, 5.4 and 6.7 % AR respectively. Radioactivity in the volatile traps was ≤1.6 % AR, most of which was <sup>14</sup>CO<sub>2</sub>. PH-labelled inpyrfluxam degraded to 80.3 % AR after 10 days with formation of 3'-OH-S-2840 at up to 5.6 % AR. Volatiles including CO<sub>2</sub> reached a maximum of 5.0 % AR at study end and thus were higher than for the PY-label. Other unidentified degradates were observed in both radiolabels, but these were <3.8 % AR in the absence of nitrate.

**Table B.8.3.2-1 Distribution of [PY – <sup>14</sup>C] in aqueous buffer**

	% AR						
	Irradiated						Dark
Sampling days	0	1	2	3	7	10	10
Volatile <sup>14</sup> C	n.a.	0.2	0.4	0.6	1.2	1.6	n.a.
NaOH	n.a.	0.2	0.4	0.6	1.2	1.5	n.a.
<sup>14</sup> CO <sub>2</sub>	n.a.	0.2	0.4	0.6	1.2	1.5	n.a.
Others	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
Ethylene glycol	n.a.	n.d.	n.d.	n.d.	n.d.	0.1	n.a.
Aqueous <sup>14</sup> C	99.0	96.5	97.6	98.9	97.7	98.1	99.1
Inpyrfluxam	97.1	91.3	87.5	86.5	77.4	73.4	96.2
3'-OH-S-2840	1.9	2.8	3.5	3.8	5.1	5.6	2.9



DFPA-CONH <sub>2</sub>	n.d.	n.d.	1.4	1.8	5.4	4.7	n.d.
DFPA	n.d.	n.d.	1.8	2.9	5.4	6.7	n.d.
Polars (< 5 min)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others <sup>b</sup>	n.d.	2.4	3.4	3.9	4.4	7.7	n.d.
Adsorbed <sup>14</sup> C	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.	0.3
Total <sup>14</sup> C	99.0	96.7	98.0	99.5	98.9	99.7	99.4

n.a.: not analysed; n.d.: not detected; <sup>b</sup>Consists of multiple components, none of which exceeded 3.8 % AR

**Table B.8.5.2-2: Distribution of [PH – <sup>14</sup>C] in aqueous buffer**

	% AR		
	Irradiated		
Sampling days	0	3	10
Volatile <sup>14</sup> C	n.a.	1.1	5.0
NaOH	n.a.	1.1	4.8
<sup>14</sup> CO <sub>2</sub>	n.a.	1.1	4.8
Others <sup>b</sup>	n.a.	n.d.	n.d.
Ethylene glycol	n.a.	n.d.	0.2
Aqueous <sup>14</sup> C	99.8	100.1	96.1
Inpyrfluxam	97.9	92.5	80.3
3'-OH-S-2840	n.d.	3.1	5.6
ATMI	n.d.	n.d.	n.d.

Polars (< 5 min)	n.d.	n.d.	2.2
Others <sup>b</sup>	1.9	4.5	8.0
Adsorbed <sup>14</sup> C	n.a.	n.a.	<0.1
Total <sup>14</sup> C	99.8	101.2	101.2

n.a.: not analysed; n.d.: not detected; <sup>b</sup>consists of multiple components, none of which exceeded 1.9 % AR

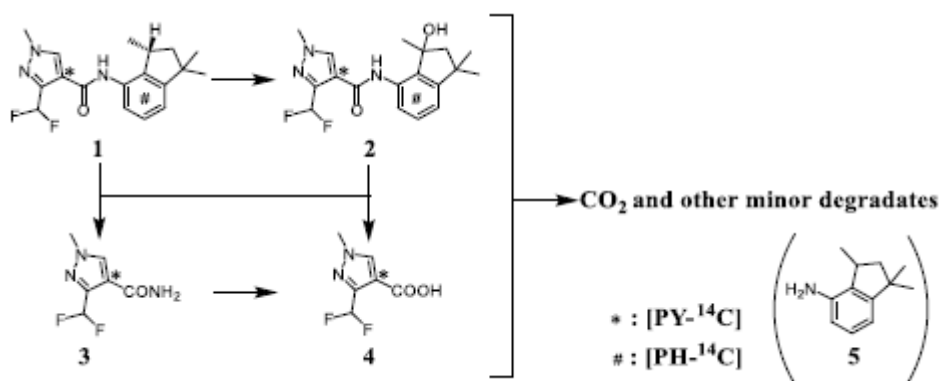
Estimated half-lives for PY-labelled inpyrfluxam were 25.6 days in artificial sunlight and 54.2 days at 30 - 50 °N in summer sunlight. It is not specifically stated that the rate constant for the dark control was subtracted. No DT<sub>50</sub> values were calculated for PH-labelled inpyrfluxam, presumably due to insufficient sampling points.

The article also considered degradation in the presence of nitrate at concentrations of 1, 5 and 10 ppm. Generally inpyrfluxam degradation proceeded more rapidly as the nitrate concentration increased with corresponding increase in metabolite formation; this was postulated to be due to increasing hydroxyl radical concentrations (indirect photolysis). No additional metabolites were identified in the presence of nitrate although metabolites were present at higher levels due to the more rapid degradation. As the data from the experiments including nitrate are not relevant in the regulatory context, these are not reported or considered further.

## CONCLUSION

The photodegradation of inpyrfluxam exposed to artificial sunlight at wavelengths >290 nm was studied over 10 days in buffer solution (pH 7). Information on the methodology used lacks detail, but it is stated that OECD 316 was followed,

In the absence of nitrate ions PY-labelled inpyrfluxam degraded to 74.4 % AR with degradation products 3'-OH-S-2840, DFPA-CONH<sub>2</sub> and DFPA formed at up to 5.6, 5.4 and 6.7 % AR respectively, with formation of <sup>14</sup>CO<sub>2</sub>. PH-labelled inpyrfluxam degraded to 80.3 % AR after 10 days with formation of metabolite 3'-OH-S-2840 at up to 5.6 % AR, while volatiles including CO<sub>2</sub> reached a maximum of 5.0 % AR at study end. The proposed degradation pathway is shown below and is identical to the proposed pathway submitted as part of [REDACTED] (2015), with the exception that ATMI was not considered as part of the regulatory study. ATMI was tested for but not observed in the literature study and it was postulated that this was due to rapid degradation of this metabolite; this is consistent with findings in section B.6 of this dossier, where loss due to rapid degradation was observed in plant metabolism studies.



Key to numbered substances in the proposed degradation scheme	
1	Inpyrfluxam
2	3'-OH-S-2840
3	DFPA-CONH <sub>2</sub>
4	DFPA
5	ATMI (1,1,3-trimethyl-2,3-dihydro-1H-inden-4-amine)*

\*Plant metabolite considered in plant residues studies, B.6 and observed to be rapidly degraded

**Figure B.8.5.2-1: Aqueous photolysis pathway proposed for inpyrfluxam in the literature study (\* [PY-<sup>14</sup>C]; # [PH-<sup>14</sup>C])**

Rate of degradation was calculated as part of the study using CAKE v. 3.1 for PY-radiolabelled inpyrfluxam as 25.6 days in artificial sunlight and 54.2 days at 30 -50 °N in summer sunlight using SFO. The kinetics for artificial light have been briefly checked by HSE using CAKE v. 3.7 to confirm the DT<sub>50</sub>. A slightly shorter DT<sub>50</sub> of 24.5 days was obtained for SFO as the applicant used parent residues at time 0 rather than the mass balance. A good visual and statistical fit was observed ( $\chi^2 = 1.94$  %). The FOMC model was also tested to see if biphasic models resulted in a better fit.

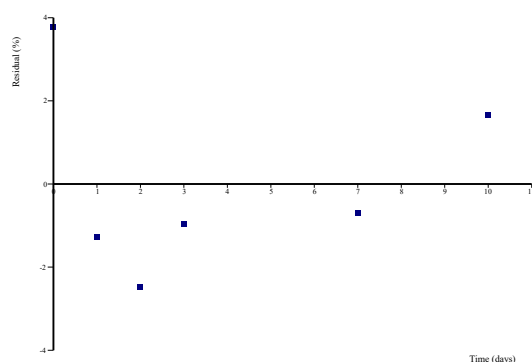
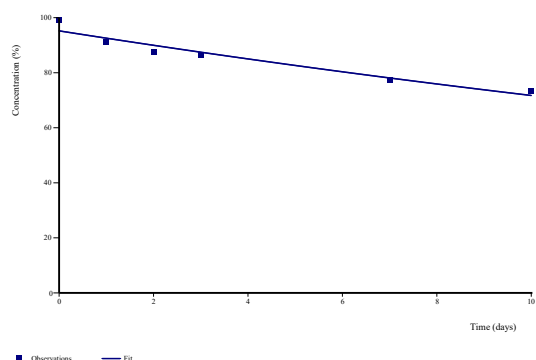
**Table B.8.5.2-3: Rate of degradation for parent-only PYR label in dark control samples - HSE kinetic fitting. Selected best fit in bold.**

<b>PYR label, dark control</b>						
<b>Model</b>	<b>Visual Assessment</b>	<b><math>\chi^2</math> error (%)</b>	<b>Estimated Parameters</b>	<b>Prob&gt;t</b>	<b>DT<sub>50</sub> (d)</b>	<b>DT<sub>90</sub> (d)</b>
SFO	Good	1.94	M <sub>0</sub> : 94.47 k: 0.00271	k: <0.05	24.5	81.3
<b>FOMC</b>	<b>Good</b>	<b>0.835</b>	<b>M<sub>0</sub>: 98.59</b> <b>alpha: 0.1603</b> <b>beta: 1.965</b>	<b>not applicable</b>	<b>147</b>	<b>&gt;10,000</b>
DFOP	Good	0.599	M <sub>0</sub> : 102.6 k <sub>1</sub> : 1.279 x 10 <sup>-1</sup> k <sub>2</sub> : 2.654 x 10 <sup>-3</sup> g: 0.496	k <sub>1</sub> : 0.1229 k <sub>2</sub> : 0.00458 1	26.9	97
<p><b>SFO:</b> The visual fit of the SFO model to the data is good; the decline phase and final time point are well represented, but starting concentrations are under-represented and there are systematic errors. The <math>\chi^2</math> value is low at 1.94 %. The fit is considered to be acceptable. The fit is extrapolated beyond the DT<sub>50</sub> value.</p> <p><b>FOMC:</b> The visual fit of the FOMC model to the data is good and an improvement on SFO; the starting concentrations are better represented with good representation of the decline phase and final time point. The <math>\chi^2</math> value is improved at 0.835 %. The fit is extrapolated beyond the DT<sub>50</sub> value.</p> <p><b>DFOP:</b> The visual fit of the DFOP model to the data is good and an improvement on SFO but very similar to FOMC; the starting concentrations, decline phase and final time point are all well represented. The <math>\chi^2</math> value is improved at 0.599 %. The fit is extrapolated beyond the DT<sub>50</sub> value.</p> <p>The DFOP and FOMC models give a similar visual fit. The DFOP model has the lowest <math>\chi^2</math> value, suggesting that this should be chosen as the best fit model. The DT<sub>50</sub> values are however extrapolated beyond study end, showing that these values are uncertain. The K<sub>1</sub> parameter for the DFOP model fails the t-test, which may suggest that this parameter is not robust when used for long term assessments. The DFOP DT<sub>50</sub> is quite similar to the SFO DT<sub>50</sub> value, however which may provide some reassurance regarding the robustness of the DFOP DT<sub>50</sub>. The FOMC model also provides a good visual and statistical fit but suggests that the DT<sub>50</sub> value may</p>						

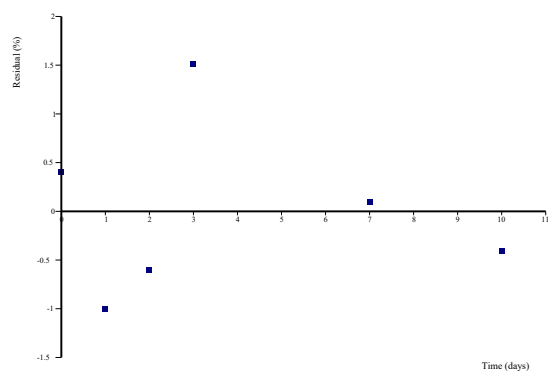
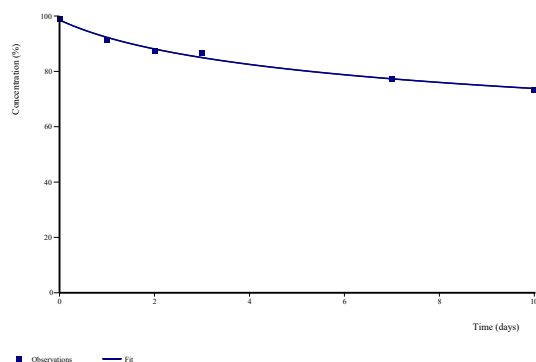
be much longer at 147 d. Given that all 3 models result in a good visual and statistical fit, but result in very different  $DT_{50}$  values, the  $DT_{50}$  value for this study must be considered to be very uncertain. This is probably due to a limited number of data points and extrapolation beyond study end.

**Conclusion: The  $DT_{50}$  value for this study must be considered to be very uncertain. The FOMC model is selected as a conservative choice given this uncertainty ( $DT_{50} = 147$ ,  $DT_{90} = >10,000$ ).**

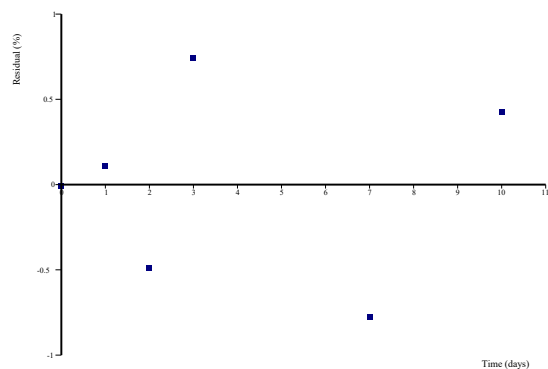
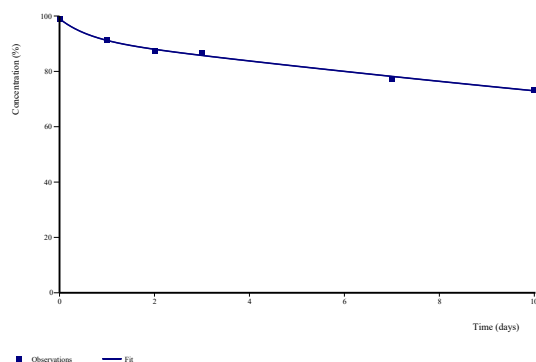
### SFO



### FOMC



### DFOP



### Comparison of regulatory study and literature study

Two regulatory photolysis studies were submitted, a study in sterile aqueous pH 7 buffer (██████ (2015a) and a second study in sterilised natural water (██████ 2015b).

Detail on methodology used in the literature study is limited, but it appears to be approximately in line with OECD 316 Guideline methodology and was conducted in sterile pH 7 buffer. The literature study (10 days) was a slightly shorter duration than the regulatory studies (15 days).

The literature study contrasts with regulatory study in buffer solution, as limited degradation was observed in the regulatory study (inpyrfluxam remaining at study end >99 % AR in the regulatory study compared to 72.4 % remaining in the literature study). No DT<sub>50</sub> value was calculated for the regulatory study in pH 7 buffer due to the insignificant degradation of inpyrfluxam, while in contrast for the literature review study a DT<sub>50</sub> value of 147 days was calculated. The results from the studies are compared below, including the regulatory study in sterilised natural water (74.4 to 37.6 days) for further comparison.

**Table B.8.5.2-4 Summary of trigger endpoints for the indirect photolysis of inpyrfluxam following 16 days of continuous irradiation**

	<b>DT<sub>50</sub> in Suntest (days)<sup>1</sup></b>	<b>OECD (30-50 °N, summer)<sup>2</sup> (days)</b>
Regulatory study in sterilised pH 7 buffer (██████ M., 2015a KCA 7.2.1.2/021		
No degradation observed, concluded that aqueous photolysis in sterile buffer is negligible		
Regulatory study in sterilised natural water (██████ M., 2015b KCA 7.2.1.2/02)		
PYR label	74.4	145
PH label	37.6	73
Literature study in sterilised pH7 buffer (Adachi <i>et al.</i> KCA 7.2.1.2/03)		
PYR label	147	246-310 <sup>3</sup>

PH label	Not calculated
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<sup>1</sup> Continuous Suntest irradiation

<sup>2</sup> Average summer irradiation in the 300-400 nm range at 30-50 °N latitude

<sup>3</sup> Equivalent DT<sub>50</sub> calculated using  $T_s = T_a \times I_a/I_s$  where  $T_a$  is the Suntest DT<sub>50</sub> in hours,  $I_a$  is the average light intensity of 42-53 W/m<sup>2</sup> and  $I_s$  is the OECD day of 603 W\*h/m<sup>2</sup>/day

It is unclear from the information provided why degradation occurred more rapidly in the literature study compared with the regulatory study using sterile buffer. Light intensities in the 290-400 nm range are similar (61.3 W/m<sup>2</sup> for the regulatory study and 42-53 W/m<sup>2</sup> in the literature study). It is noted that sterility was not checked in the literature review study and therefore it is possible that microbial degradation was occurring and contributing to the faster degradation compared to the regulatory study. The absence of replicates also means that errors and inconsistencies in the analytical method which could be responsible for the differences between the two studies might be less apparent. The DT<sub>50</sub> value for the literature study was however considered to be uncertain as it was extrapolated beyond study end. Although other FOCUS kinetic models tested (SFO and DFOP) indicated even faster degradation, this may partially explain the discrepancy.

Negligible degradation was observed in the regulatory study in buffer solution with no metabolites formed. The literature review study and the regulatory study conducted in sterile natural water indicate the same route of degradation, with the main metabolite formed being 3'-OH-S-2840. This metabolite formed at similar levels in both studies (maximum of 6.8-8.6 % AR in the regulatory study, compared to 5.6 % AR for the literature study). Metabolites DFPA-CONH<sub>2</sub> and DFPA were also observed at similar levels (maximums of 3.4 and 4.7 % AR respectively in the regulatory study and 5.4 and 6.7 % AR in the literature study). All metabolites are present at well below the trigger of 10 % AR, but as 73- 80 % AR is still present at study end, there is potential for metabolites to continue to increase if the study were continued. Metabolite DFPA-CONH<sub>2</sub> was decreasing by study end, while the rate of increase of DFPA had slowed by study end and the metabolite was still well below 10 % AR. It is considered unlikely therefore that DFPA-CONH<sub>2</sub> and DFPA would be considered major metabolites had the study been continued. As 3'-OH-S-2840 was close to the regulatory trigger in the natural water study by [REDACTED] its potential to be a major metabolite is already being further considered.

The study with natural water is considered to be more representative of environmental exposure than the studies using buffer solution and so is referred to in preference. The results of this literature study corroborate the degradation profile of the standard OECD 316 studies submitted as part of this dossier and confirm the conclusion that aqueous photolysis may contribute somewhat to inpyrfluxam overall

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degradation in water. As the literature study does not give any new information, the study is included as supporting evidence only.



## 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 7.1.1.1/01		2017	<p>Aerobic Soil Metabolism of [Phenyl-<sup>14</sup>C] S-2399 and [Pyrazolyl-4-<sup>14</sup>C] S-2399 Amended Report</p> <p>VP-38556, Sumitomo Chemical Co. Ltd. Report No: TPM-0023</p> <p>Valent Technical Center (VTC), 6560 Trinity Court, Dublin, CA 94568</p> <p>Yes</p> <p>Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
<b>KCA 7.1.1.1/02</b>	████████	2017a	<p>S-2399: Degradation under Aerobic Conditions in Soil Rate Studies</p> <p>VP-38898, Sumitomo Chemical Co. Ltd. Report No: TPM-0044</p> <p>Valent Technical Center (VTC), 6560 Trinity Court, Dublin, CA 94568</p> <p>Yes</p> <p>Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation
<b>KCA 7.1.2.1.2/01</b>	████████	2017a	<p>[<sup>14</sup>C]3'-OH-S-2840: Aerobic Soil Metabolism Study</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
			3201399, Sumitomo Chemical Co. Ltd. Report No: TPM-0033  Yes  Unpublished					
<b>KCA 7.1.2.1.2/02</b>		2017b	[ <sup>14</sup> C]1'-COOH-S-2840: Aerobic Soil Metabolism Study  3201400, Sumitomo Chemical Co. Ltd. Report No: TPM-0049  Yes  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
<b>KCA 7.1.1.2_01</b>		2017	S-2399: Anaerobic Soil Metabolism  VP-39081, Sumitomo Chemical Co. Ltd. Report No: TPM-0040  Valent Technical Center (VTC), 6560 Trinity Court, Dublin, CA 94568  Yes  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation
<b>KCA 7.1.1.3/01</b>		2014	Photodegradation of [ <sup>14</sup> C] S-2399 in/on Soil by Artificial Sunlight	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
			<p>2454W-1, Sumitomo Chemical Co. Ltd. Report No: TPM-0005</p> <p>PTRL West (a division of EAG, Inc.) 625-B Alfred Nobel Drive Hercules, CA 94547</p> <p>Yes</p> <p>Unpublished</p>					

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 7.1.1.1_01, KCA 7.1.1.1_02	[REDACTED] & [REDACTED]	2023	Recalculation of the laboratory aerobic degradation rate of S-2399 (inpyrfluxam) in soil according to FOCUS Kinetics Guidance  20063620.UK0 – 5243  Exponent International Ltd. The Lenz, Hornbeam Business Park Harrogate, Yorkshire, HG2 8RE, UK  Yes  Unpublished	N	N	Not applicable	Sumitomo chemical	None – data for first authorisation

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 7.1.2.1.1_03	[REDACTED] & [REDACTED]	2023	Recalculation of the laboratory aerobic degradation rate of the metabolites of S-2399 (inpyrfluxam) in soil according to FOCUS Kinetics Guidance  20063620.UK0 – 9637  Exponent International Ltd. The Lenz, Hornbeam Business Park Harrogate, Yorkshire, HG2 8RE, UK  Yes  Unpublished	N	N	Not applicable	Sumitomo chemical	None – data for first authorisation


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 7.1.2.2.1/06	[REDACTED] and [REDACTED]	2018a	Normalisation of S-2399 DT <sub>50</sub> data from terrestrial field dissipation studies in North America  1403863.UK0-2381 Exponent International Ltd. The Lenz, Hornbeam Business Park Harrogate, Yorkshire, HG2 8RE, UK  Yes  Unpublished	N	N	Not applicable	Sumitomo chemical	None – data for first authorisation
KCA 7.2.2.1/11	[REDACTED] and [REDACTED]	2024	The relevance of North American field dissipation studies on	N	N	Not applicable	Sumitomo chemical	None – data for first authorisation




<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
			<p>S-2399 to EU conditions</p> <p>2006362.UK0-9770</p> <p>Exponent International Ltd.</p> <p>The Lenz, Hornbeam Business Park</p> <p>Harrogate, Yorkshire, HG2 8RE, UK</p> <p>Yes</p> <p>Unpublished</p>					
				N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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 7.1.2.2.1/05		2017b	<p>S-2399: Terrestrial Field Soil Dissipation of S-2399 on Bare Ground in Ontario, Canada.</p> <p>V-14-38593, Sumitomo Chemical Co. Ltd. Report No: TPR-0033</p> <p>Valent U.S.A. LLC; Valent Technical Center</p> <p>6560 Trinity Court</p> <p>Dublin, California 94568 U.S.A.</p> <p>Yes</p> <p>Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
<b>KCA 7.1.2.2.1/07</b>	[REDACTED]	2018	Soil dissipation study after application of S-2399 at four different locations in Europe – 2016/2018  267-2016, Sumitomo Chemical Co. Ltd. Report No: TPR-0085  Yes  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation
<b>KCA 7.1.2.2.1/08</b>	[REDACTED] and [REDACTED]	2018	Normalisation of data from four field studies in Europe and determination of normalised field DT <sub>50</sub> values for S-2399 and metabolites	N	N	Not applicable	Sumitomo chemical	None – data for first authorisation

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
			1403863.UK0-7449, Sumitomo Chemical Co. Ltd. Report No: TPR-0079  Yes  Unpublished					
<b>KCA</b> <b>7.1.2.2.1/12</b>	 &	2023	Recalculation of the field dissipation/degradation rate of S-2399 (inpyrfluxam) in soil according to FOCUS Kinetics Guidance.  20063620.UK0 – 8865	N	N	Not applicable	Sumitomo chemical	None – data for first authorisation

<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
			Exponent International Ltd. The Lenz, Hornbeam Business Park Harrogate, Yorkshire, HG2 8RE, UK  Yes  Unpublished					
<b>KCA 7.1.2.2.2/01</b>		2018	Soil accumulation study after application of S-2399 at four different locations in Europe – 2016/2021 (Interim Report)  268-2016, Sumitomo Chemical Co. Ltd. Report No: TPR-0089	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation


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
			TESTAPI, 464 Sarré, 49350 GENNES, France					
<b>KCA 7.1.2.2.2/02</b>		2023	<p>Soil accumulation study after application of S-2399 at four different locations in Europe – 2016/2021 (Final Report)</p> <p>268-2016, Sumitomo Chemical Co. Ltd. Report No: TPR-0090</p> <p>TESTAPI, 464 Sarré, 49350 GENNES, France</p> <p>Yes</p> <p>Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation


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 7.1.2.2.1/10		2018	<p>Storage Stability of S-2399 and its metabolites 3'-OH-S-2840, 1'-COOH-S-2840A and 1'-COOH-S-2840B in Soil under Deep Frozen Conditions</p> <p>Eurofins Agrosience Services EcoChem GmbH, Eutinger Str. 24, D-75223, Niefern-Öschelbronn, Germany</p> <p>S16-04056, Sumitomo Chemical Co. Ltd. Report No: TPR-0088</p> <p>Yes</p> <p>Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation


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 7.1.2.2.1/09	████████	2017	<p>S-2399: Freezer Storage Stability of S-2399, 3'-OH-S-2840, 1'-COOH-S-2840-A, and 1'-COOH-S-2840-B in Soils</p> <p>VP-39033, Sumitomo Chemical Co. Ltd. Report No: TPR-0064</p> <p>Valent U.S.A. LLC, 6560 Trinity Court, Dublin, California 94568</p> <p>Yes</p> <p>Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation



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 7.1.3.1.1/01	[REDACTED] & 2016	2016	[ <sup>14</sup> C] S-2399: Adsorption/Desorption in Soil  3201088, Sumitomo Chemical Co. Ltd. Report No: TPM-0018  Smithers Viscient (ESG) Ltd., 108 Woodfield Drive, Harrogate, North Yorkshire HG1 4LS, UK  Yes  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation
KCA 7.1.3.1.2/02	[REDACTED]	2017	[ <sup>14</sup> C] 3'-OH-S-2840: Adsorption/Desorption in Soil.	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
			3201398, Sumitomo Chemical Co., Ltd. Report TPM-0032  Smithers Viscient (ESG) Ltd., 108 Woodfield Drive, Harrogate, North Yorkshire HG1 4LS  Yes  Unpublished					
<b>KCA</b> <b>7.1.3.1.2/01</b>		2017a	[ <sup>14</sup> C]1'-COOH-S-2840A and B: Adsorption/Desorption in Soil  3201397, Sumitomo Chemical Co., Ltd. Report TPM-0034	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
			Smithers Viscient (ESG) Ltd., 108 Woodfield Drive, Harrogate, North Yorkshire HG1 4LS  Yes  Unpublished					
<b>KCA 7.3.1/01</b>		2017	S-2399: Stability in air  1403863.UK0 – 0889, Sumitomo Chemical Co., Ltd. Report TPP-0025	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
			Exponent International Ltd. The Lenz, Hornbeam Business Park, Harrogate, Yorkshire, HG2 8RE, UK  Yes  Unpublished					
<b>KCA</b> <b>7.2.1.1/01</b>		2016	[ <sup>14</sup> C]S-2399: Hydrolysis at pH 4, 7 and 9  12709.6427, Sumitomo Chemical Co., Ltd. Report TPM-0030  Smithers Viscient, 790 Main Street, Wareham, Massachusetts, USA, Study No.	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation


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
			Yes Unpublished					
KCA 7.2.1.2/01		2015a	Photodegradation of [ <sup>14</sup> C]S-2399 in Sterilized pH 7 Buffer by Artificial Sunlight  2642W. Sumitomo Chemical Co., Ltd. Report: TPM-0008 PTRL West, 625-B Alfred Nobel Drive, Hercules, CA 94547, USA.  Yes Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

<b>Data Point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate Study Y/N</b>	<b>Data Protection Claimed Y/N</b>	<b>Justification if Data Protection is claimed</b>	<b>Owner</b>	<b>Previous evaluation</b>
<b>KCA 7.2.1.2/02</b>		2015b	Photodegradation of [ <sup>14</sup> C]S-2399 in Sterilized Natural Water by Artificial Sunlight  2644W, Sumitomo Chemical Co., Ltd. Report: TPM-0010  PTRL West, 625-B Alfred Nobel Drive, Hercules, CA 94547, USA.  Yes  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation
<b>KCA 7.2.2.1/01</b>		2016	S-2399 TGAI - Determination of the Biodegradability of a Test Substance Based	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
			<p>on OECD Method 301B (CO2 Evolution Test)</p> <p>13048.6940, Sumitomo Chemical Co., Ltd. Report: TPM-0019</p> <p>Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571, USA</p> <p>Yes</p> <p>Unpublished</p>					
<b>KCA</b> <b>7.2.2.2/01</b>			<p>[<sup>14</sup>C]S-2399: Aerobic Mineralisation in Surface Water</p> <p>3201629, Sumitomo Chemical Co., Ltd.</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
			Report: TPM-0048 Smithers Viscient, 108 Woodfield Drive, Harrogate North Yorkshire, HG1 4LS, UK  Yes  Unpublished					
<b>KCA 7.2.2.3/01</b>		2017b	S-2399: Degradation under Aerobic Aquatic Conditions Amended Report 1  VP-38790, Sumitomo Chemical Co., Ltd. Report: TPM-0041  Valent Technical Centre, 6560 Trinity Court,	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation



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
			Dublin, CA 94568, USA  Yes Unpublished					
<b>KCA 7.2.2.3/02</b>		2017a	S-2399: Degradation under Aerobic Aquatic Conditions – Rate Study; Amended Report 1  VP-39270, Sumitomo Chemical Co., Ltd. Report: TPM-0050  Valent Technical Centre, 6560 Trinity Court, Dublin, CA 94568, USA  Yes  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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KCA 7.2.2.3/04	[REDACTED] & [REDACTED]	2023	<p>Recalculation of the degradation rate of S-2399 (Inpyrfluxam) in aquatic systems according to FOCUS Kinetics Guidance</p> <p>20063620.UK0 – 5338, Sumitomo Chemical Co., Ltd.</p> <p>Exponent International Ltd., The Lenz, Hornbeam Business Park, Harrogate, Yorkshire, HG2 8RE, UK</p> <p>Yes (from underlying studies KCA 7.2.2.3/01 and 02) Unpublished</p>	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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
<b>KCA 9.1</b>	Exponent International Ltd.	2018  Updated to 2023	Dossier for the evaluation of the active substance Inpyrfluxam (S-2399) Literature review report (Fulfilling EU data points: KCA Section 9/ KCP Section 11)  1403863.UK0 – 4472 Sumitomo Chemical Co., Ltd. Report No:TPT-0133  Exponent International Ltd. Harrogate, HG2 8RE, UK  Non GLP  Unpublished	N	Y	Data for first authorisation	Sumitomo chemical	None – data for first authorisation

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