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ISOFLUCYPRAM

Volume 3 – B.8 (AS)

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B.8. ENVIRONMENTAL FATE AND BEHAVIOUR

INTRODUCTION

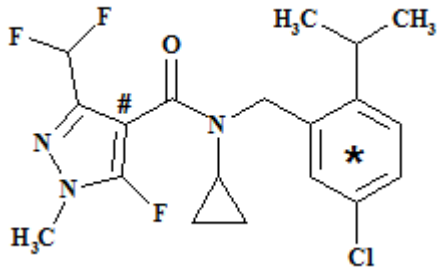
Isoflucypram (CAS-No. 1255734-28-1) is a new fungicidal active substance developed by Bayer.
This draft assessment report (DAR) supports the application for regulatory approval of Isoflucypram in Europe under Regulation (EC) No 1107/2009. Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides with application to cereal crops (wheat, triticale, rye, barley and oats) evaluated as part of this submission. Isoflucypram is an SDH inhibitor fungicide the application scope of isoflucypram-containing products on cereals with only one foliar spray at a maximum of 75 g a.s./ha at BBCH 39-69.

This document MCA Section 8 summarises all data on the fate of isoflucypram in all environmental compartments, assessment relating to the non-approval criteria for the persistence element of POP, PBT or vPvB as well as definitions of residues for risk assessments which are relevant for the approval of Isoflucypram alongside the proposed intended uses, including the representative uses, under Regulation (EC)
No 1107/2009 in accordance with the requirements laid down in the Commission Regulation (EU) No 283/2013. Endpoints for the use in the MCP document are also generated here. The study summaries included by the applicant to address the environmental fate risk assessment have been taken from the final reports submitted to the RMS. The UKRMS has assessed each final report in detail and in its entirety. Where detail is not of sufficient quantity or quality or, for example mean values were presented but duplicate values were available in the report, these have been added by the RMS. All final reports have been conducted to Good Laboratory Practice where applicable and sufficient Quality Assurance has been performed on all final reports. As such the data contained are considered to be reliable by the UKRMS. Kinetic modelling submitted by the applicant has been validated by the UK RMS using a separate model to that used by the applicant.

Throughout the development of isoflucypram the following synonyms may have been used and also referred to in individual study reports: Bayer Code: BCS-CN88460, BCS-CN88460-a.s., ´460 and the Bayer-internal short Code: ISY. All chemical substances described by either of these codes refer to the same chemical name and structural formula. A full list of common names is provided in table B.8.3 below, for the evaluation the Bayer common name Isoflucypram and code BCS-CN88460 are used. For the metabolites codes M12 are used for BCS-CN88460-carboxylic acid, M10 for BCS-CN88460-lactic acid and M11 *for* BCS-CN88460-desmethyl-carboxylic acid are used unless both name and code are presented.

The studies concerning the fate and behaviour of isoflucypram in the environment were conducted using two different radiolabel positions, [chlorophenyl-UL-^{14}C] and [pyrazole-4-^{14}C], as well as unlabelled isoflucypram. These radiolabel positions are sufficient to define the route of degradation of isoflucypram. The structure of isoflucypram and the positions of the different radiolabels are as follows:

Table B.8-1. Isoflucypyram label positions.

Structural formula of isoflucypram: *: ^{14}C-labeling position of the **phenyl-label** (short form used in this summary) = [chlorophenyl-UL-^{14}C]isoflucypram #: ^{14}C-labeling position of the **pyrazole-label** (short form used in this summary) = [pyrazole-4-^{14}C]isoflucypram	

Labelling Strategy:
Isoflucypram contains a phenyl and a pyrazole ring. The studies concerning the fate and behaviour of isoflucypram in the environment were all conducted using the ^{14}C-labeling position of the pyrazole-label ([pyrazole-4-^{14}C]isoflucypram). All studies showed no cleavage of the molecule. *M12)* was found as the only major metabolite. The majority of studies are only conducted with radio-labelling in the pyrazole ring with a single soil degradation study in the phenyl ring to confirm that no novel metabolites to the pyrazole ring are formed. Taking in to account all of the radiolabelled studies presented, the RMS is content with this approach.

Soil:
As mentioned above all soil metabolism studies were performed using the pyrazole-label. 1 study has been performed using the phenyl label to confirm the degradation of the whole molecule and that no cleavage of the molecule occurs.

In soil metabolism studies under aerobic conditions the metabolites were formed possibly via carboxylation of isoflucypram to result in M12 as major metabolite, hydroxylation of M12 to result in M10 and demethylation of M12 to result in M11 (see Figure B.8-1). No cleavage of the molecule was observed.
An addition aerobic soil metabolism study with the phenyl-label was also performed using one soil. In this study M12 was also found as major metabolite. In this study no split of the molecule could be observed.

No metabolite applied soil studies were submitted, but the RMS considers that sufficient information on metabolites can be obtained from the isoflucypram- applied studies.

Under anaerobic soil conditions using the pyrazole-label no degradation products > 5% AR were found.

In the soil pyrazole-label photolysis study no products> 5% AR were found.

Therefore, in soil it is proposed that the degradation pathway and main metabolites are addressed using the mentioned pyrazole-label position.

Water:
All aqueous studies were performed using the pyrazole-label.

Isoflucypram was hydrolytically stable in sterile aqueous buffer solutions at three pH values (pH 4, 7 and 9) in the laboratory in the dark. No degradation products of isoflucypram were observed.

In the aquatic photolysis study also no degradation products of isoflucypram > 10% AR were observed and identified. The total unidentified residues amounted to a maximum of 2.7% AR in irradiated samples.

In the aerobic mineralisation in surface water study under aerobic conditions (pelagic test) isoflucypram was stable in all test systems. No degradation products were formed in any test systems in this study.

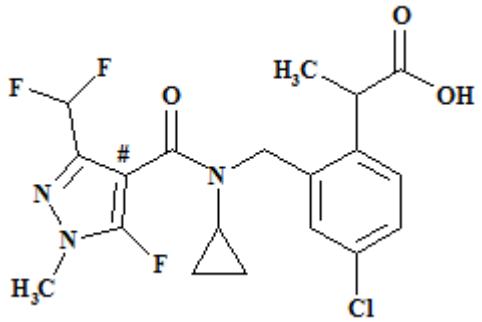
Degradation of isoflucypram in the total system was accompanied by the formation of one degradation product identified as M12 with a maximum occurrence of 6.6% AR. The total unidentified residues amounted to a maximum of 12.4% AR and no single component exceeded 4.6% AR at any sampling interval in both water/sediment systems (see Figure B.8-2).

Therefore the RMS is content that in water and water/sediment the entire pathway and all possible main metabolites are addressed using the mentioned pyrazole-label position only.

The results of the studies are summarised in the following sections B.8.1 to B.8.4. The proposed degradation pathways in soil, water and sediment are given in Figure B.8-1 and Figure B.8-2, respectively.

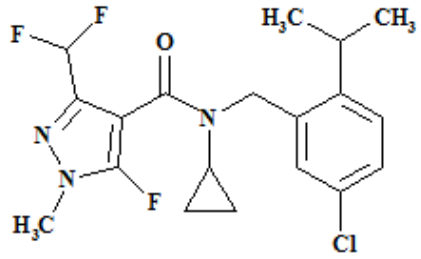
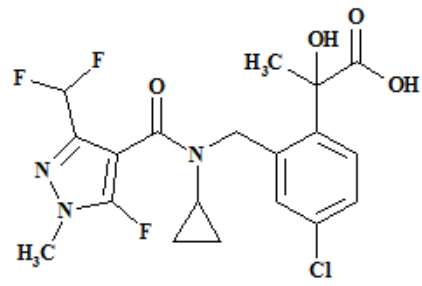
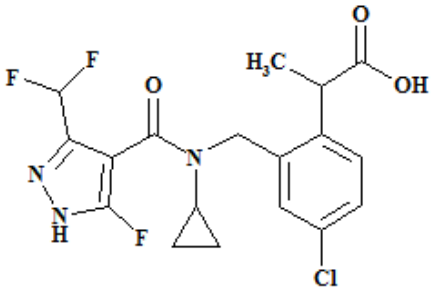
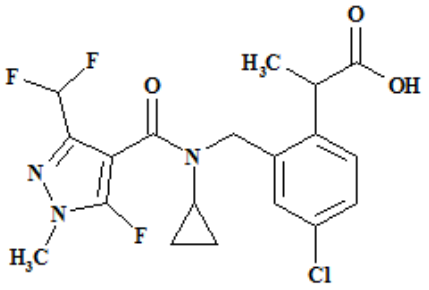
In addition, studies have been performed with the radiolabelled metabolite M12. A structural diagram is provided in table B.8-2.

Table B.8-2: Label position of the major metabolite M12.

Structural formula of M12 *(*BCS-CN88460-carboxylic acid*)*: #: ^{14}C-labeling position of the **pyrazolyl-labelled** BCS-CN88460-carboxylic acid (short form used in this summary) = [pyrazole-4-^{14}C]BCS-CN88460-carboxylic acid	

In original reports study authors may have used different names or codes for degradation products of isoflucypram. In this summary, a single name or a single code is used for each degradation product. In order to present a common system of nomenclature for the evaluation in the dossier a list of the metabolites observed in environmental fate testing is included here (see following table B.8-3).

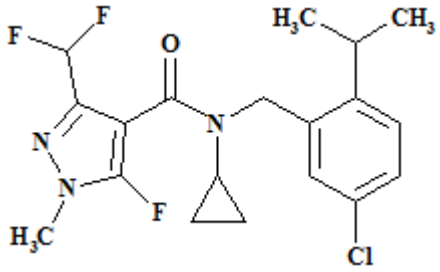
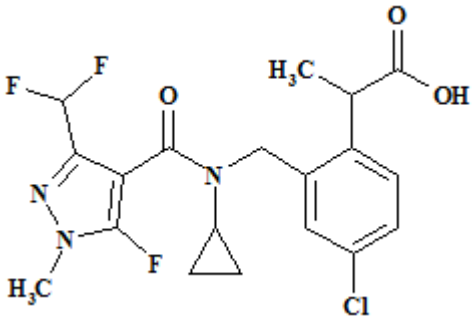
Table B.8-3: Isoflucypram: Substances and environmental fate metabolites; structures, codes, synonyms

No.	Structure Empirical formula / nominal mass	Name / Code no. (synonyms)	Description	Compound found in
a.s.	 *$C_{19}H_{21}ClF_3N_3O$* *[399] nominal mass* *399.84 g/mol (molecular weight)*	**isoflucypram** BCS-CN88460 *CAS: 1255734-28-1* ISY LYAM823-1-2	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide *[IUPAC]* 1H-pyrazole-4-carboxamide, N-[[5-chloro-2-(1-methyl-ethyl)phenyl]methyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl- *[CA]*	soil: aerobic & anaerobic, field dissipation, photolysis water: hydrolysis, photolysis, water-sediment
M10	 *$C_{19}H_{19}ClF_3N_3O_4$* *[445]*	**BCS-CN88460-lactic acid** ROI 3 M10	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}-2-hydroxypropanoic acid *[IUPAC]*	soil: met., aerobic water: -
M11	 *$C_{18}H_{17}ClF_3N_3O_3$* *[415]*	**BCS-CN88460-desmethyl-carboxylic acid** BCS-CX99799 ROI 5 M11	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1H-pyrazol-4-yl]carbonyl}amino)methyl]phenyl}propanoic acid *[IUPAC]*	soil: met., aerobic water: -
M12	 *$C_{19}H_{19}ClF_3N_3O_3$* *[429]*	BCS-CN88460-carboxylic acid BCS-CY26497 MXM 7275-1-5 ROI 1 M12	2-{4-chloro-2-[(cyclopropyl{[3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazol-4-yl]-carbonyl}amino)-methyl]phenyl}-propanoic acid *[IUPAC]*	soil: met., aerobic water: met., aerobic

Compounds addressed in this document with environmental fate studies

In addition to the active substance, environmental fates studies were performed with the following metabolite as it was considered important due to the amounts which were found during the course of environmental fate studies with isoflucypram, details are included in table B.8-4.

Table B.8-4: Active substance and metabolite addressed in this document with environmental fate studies

Compound / Codes	Chemical structure	Explanation for consideration
Isoflucypram, BCS-CN88460		active substance
M12 *(*BCS-CN88460-carboxylic acid*)*		occurrence in - soil (> 5% AR, increasing at study end) - water-sediment (water layer > 5% AR, increasing at study end)

% AR = % of applied radioactivity

Figure B.8-1: Proposed degradation pathway of isoflucypram in soil

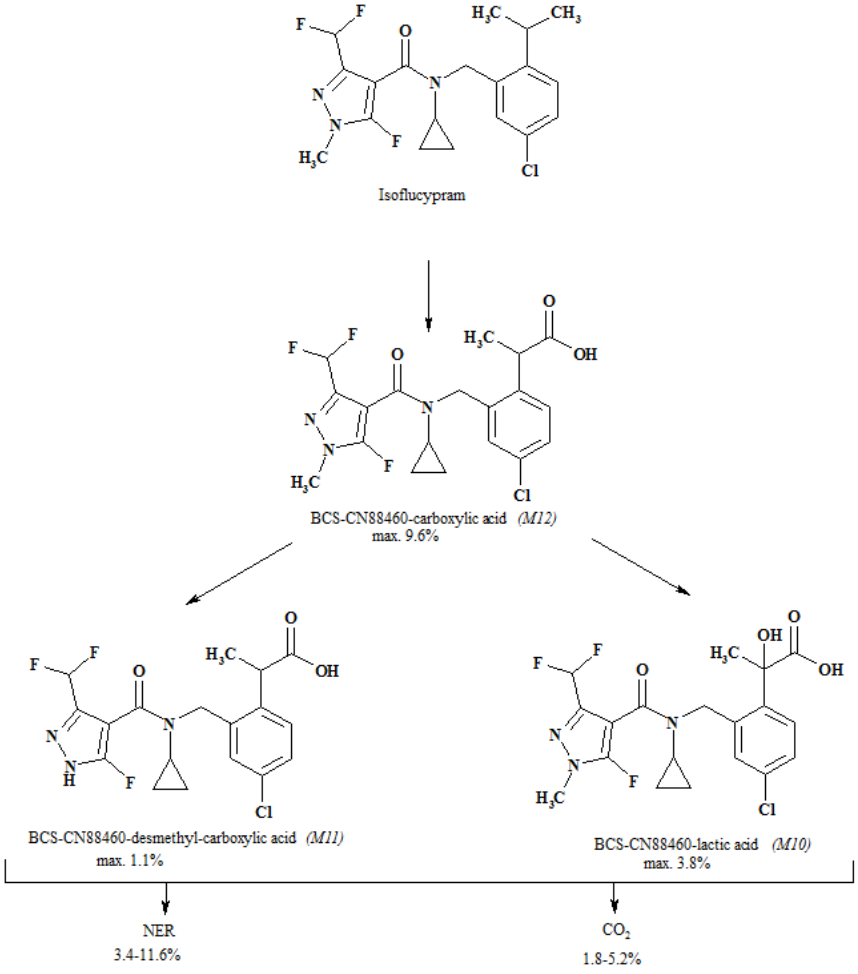
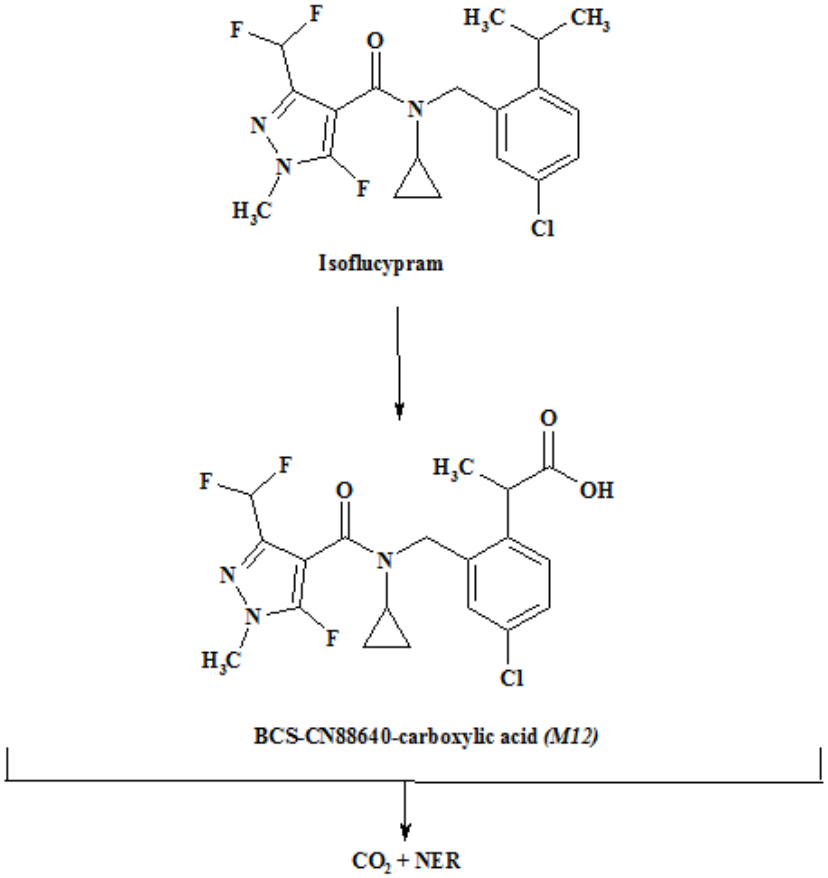


Figure B.8-2: Proposed degradation of isoflucypram in aerobic aquatic water/sediment systems



Details of the literature search undertaken are summarized in MCA Section 10 and summarised in section B.8.5.

B.8.1. FATE AND BEHAVIOUR IN SOIL

B.8.1.1. Route and rate of degradation in soil

Introduction: Route of degradation in soil

The route of degradation of isoflucypram in soil was studied using two different radiolabel positions, phenyl- and pyrazole-label. The studies have been performed in a number of soils in the laboratory at slightly different temperatures and at different soil moistures.

For isoflucypram three laboratory soil degradation studies have been performed Hellpointner, E.; Junge, T.; (2014) see section B.8.1.1.1.1, Gabbert, D.; McConnell, L. L.; Arthur, E. L.; (2017) see section B.8.1.1.1.2 and Heinemann, O.; Kasel, D.; (2017) see section B.8.1.1.1.3. Only 1 major metabolite was produced and sufficient number of data points were available from the parent assessment that no metabolite applied studies were performed.

A single Anaerobic degradation study has been performed Heinemann, O.; Kasel, D.; (2015) see section B.8.1.1.2.1 and a single photolysis is soil study has been assessed Heinemann, O.; (2013) see section B.8.1.1.3.1.

Kinetic assessments of the laboratory data were performed with in each of the studies, but these are presented centrally by the UK RMS in section B.8.1.1.4.1 for ease of reading.

A single terrestrial field dissipation study has been provided by the applicant, Heinemann, O.; Junge, T.; (2017), see section B.8.1.1.5.1. Assessments of non-normalized and normalized kinetics are presented in section B.8.1.1.5.2.

The proposed degradation pathway of isoflucypram in soil is shown in Figure B.8-1 above and a summary of the overall degradation in soil is provided in section B.8.1.1.6 including the endpoints agreed by the UK RMS for modelling and persistence endpoints.

B.8.1.1.1 Aerobic degradation

The route of degradation of isoflucypram in soil under aerobic conditions in the laboratory was investigated using two radiolabel positions (phenyl- and pyrazole-label).

A summary of the route of degradation of isoflucypram in soil is given in section B.1.1.6 and Figure B.8-1 above.

B.8.1.1.1.1: Aerobic degradation study of isoflucypram

Previous evaluation:	None, new active substance.

Author: Hellpointner, E.; Junge, T.; 2014;
Title: [14C]BCS-CN88460: Aerobic metabolism/degradation in four soils
Report No.: EnSa-13-1043
Document No.: M-486690-01-1
Guideline(s): OECD Test Guideline No. 307, Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009. US EPA OCSPP, Test Guideline No. 835.4100. Japanese MAFF New Test Guidelines Annex No. 2-5-2
Guideline deviation(s): not specified

GLP/GEP: yes

Study Summary

The route and rate of degradation of pyrazole-labelled isoflucypram were studied in four soils under aerobic conditions in the dark in the laboratory for 120 days at 20.0°C and 53.1% of the maximum water holding capacity:

Table B.8.1.1.1.1- 1: Selected soils

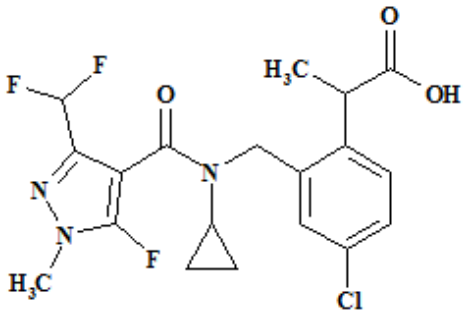
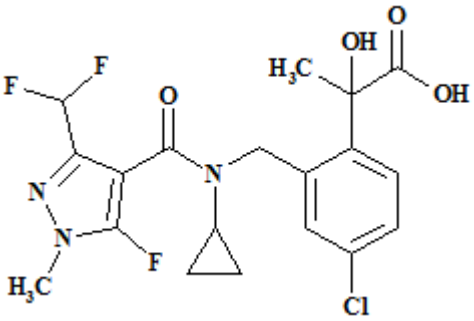
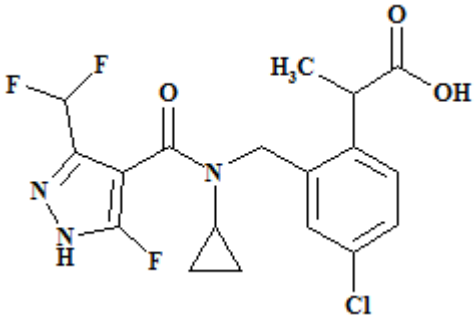
Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Hanscheider Hof	Burscheid, Germany	loam	5.7	2.9
Laacher Hof AXXa	Monheim, Germany	loamy sand	6.3	2.0
Hoefchen Am Hohenseh	Burscheid, Germany	silt loam	6.6	1.9
Dollendorf II	Blankenheim, Germany	loam	7.4	5.2

A study application rate of 200 µg per kg soil dry weight was applied based on a maximum single field application rate of isoflucypram of 75 g per hectare, assuming incorporation of 2.5cm depth.
The test was performed in static systems consisting of Erlenmeyer flasks each containing 100 g soil (dry weight equivalents) and equipped with traps for the collection of carbon dioxide and volatile organic compounds.
Duplicate samples were processed and analysed 0, 2, 6, 15, 28, 50, 62, 84, 104 and 120 days after treatment (DAT). At each sampling interval, the soil was extracted three times at ambient temperature using acetonitrile/water 1/1 (*v/v*). Furthermore, two microwave-accelerated extraction steps were performed using acetonitrile/water 1/1 (*v/v*) at 70°C and methanol/water 1/1 (*v/v*) at 50°C. The amounts of test item and degradation products in soil extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. Test item and degradation products were identified by HPLC-MS(/MS) including accurate mass determination and/or by co-chromatography with reference items.
Mean material balances were 100.3% AR (range from 99.5 to 101.3% AR) for soil Hanscheider Hof, 97.7% AR (range from 95.2 to 101.2% AR) for soil Laacher Hof AXXa, 98.7% AR (range from 97.1 to 100.2% AR) for soil Hoefchen Am Hohenseh and 98.5% AR (range from 95.9 to 100.1% AR) for soil Dollendorf II.
The maximum amount of carbon dioxide was 1.8, 2.5, 2.8 and 3.0% AR at study end (DAT-120) in soil Hanscheider Hof, Laacher Hof AXXa, Hoefchen Am Hohenseh and Dollendorf II, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for all soils.

Extractable residues decreased from DAT-0 to DAT-120 from 99.0 to 91.9% AR in soil Hanscheider Hof, from 100.4 to 88.3% AR in soil Laacher Hof AXXa, from 98.7 to 88.3% AR in soil Hoefchen Am Hohenseh and from 95.8 to 85.6% AR in soil Dollendorf II.
Non-extractable residues (NER) increased in soil Hanscheider Hof from DAT-0 to DAT 104 from 1.5 to 6.3% AR and slightly declined to 5.8% AR until DAT-120. In soil Laacher Hof AXXa NER increased from DAT-0 to DAT-120 from 0.8 to 5.8% AR. NER increased in soil Hoefchen Am Hohenseh from DAT-0 to DAT-104 from 1.3 to 8.0% AR and slightly declined to 7.7% AR until DAT-120. In soil Dollendorf II, NER increased from DAT-0 to DAT-104 from 3.2 to 11.6% AR and slightly declined to 10.7% AR until DAT-120.
The amount of isoflucypram in the soil extracts decreased from DAT-0 to DAT-120 from 98.2 to 82.6% AR in soil Hanscheider Hof, from 99.8 to 70.1% AR in soil Laacher Hof AXXa, from 98.2 to 77.2% AR in soil Hoefchen Am Hohenseh and from 95.3 to 72.2% AR in soil Dollendorf II.
Three degradation products were identified with the following maximum amounts: M12 with 5.8% AR at DAT-104 in soil Dollendorf II, M10 with 3.8% AR at DAT-104 in soil Dollendorf II and M11 with 1.1% AR at DAT 104 in soil Laacher Hof AXXa. The total unidentified residues amounted to a

maximum of 8.9% AR and no single component exceeded 3.6% AR at any sampling interval for all soils.

Table B.8.1.1.1.1- 2: Identified degradation products (maximum occurrence) in soils (in percent of applied radioactivity)

Compound	Chemical structure	Maximum occurrence in soil [%], mean of replicates
M12 *(*BCS-CN88460-carboxylic acid*)*		5.8
*M10 (*BCS-CN88460-lactic acid *)*		3.8
*M11 (*BCS-CN88460-desmethyl-carboxylic acid*)*		1.1
CO_2		3.0

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazole-labelled isoflucypram

Sample-ID:	KML 9427
Specific activity:	3.90 MBq/mg (105.34 µCi/mg)
Radiochemical purity:	> 99% (HPLC with radioactivity detector)
	> 99% (TLC, scan)
Chemical purity:	> 98% (HPLC with UV-detector, 210 nm)

Reference item

unlabelled isoflucypram

Sample-ID:	BCS-CN88460-01-02
Chemical purity:	98.4% (^{1}H-NMR)

2. Test soils

The study was carried out using four different soils (see Table B.8.1.1.1.1- 3). The soils were taken from agricultural use areas representing different geographical origin and different soil properties as required by the guidelines. The plant protection product use history of the soils for at least 5 years is known with no pesticide usage for a period of 5 years . The soils were sampled freshly from the fields (upper horizon of 0 to 20 cm) and sieved to a particle size of ≤ 2 mm.

Table B.8.1.1.1.1- 3: Physico-chemical properties of test soils

Parameter	**Results**	
Soil designation	Hanscheider Hof	Laacher Hof AXXA
Geographic location		
City	Burscheid	Monheim
State	North Rhine-Westphalia	North Rhine-Westphalia
Country	Germany	Germany
Soil series	no information available	no information available
Textural class (USDA)	loam	loamy sand
Sand [%] (50 µm – 2 mm)	33	77
Silt [%] (2 µm – 50 µm)	50	16
Clay [%] (< 2 µm)	17	7
pH - in 0.01 M $CaCl_2$ 1/2	5.7	6.3
- in water 1/1	6.0	6.5
- in saturated paste	5.9	6.5
- in soil/1 N KCl 1/1	5.3	6.1
Organic carbon (combustion) [% OC]	2.9	2.0
Organic matter [a] [% OM]	5.0	3.4
Cation exchange capacity [meq/100 g]	10.0	9.0
Water holding capacity		
maximum (MWHC) [g H_2O *ad* 100 g DW]	63.0	50.3
at 1/3 bar (pF 2.0) [%]	29.3	15.8
Bulk density (disturbed) [g/cm^3]	1.06	1.23
Soil microbial biomass [mg microbial C/kg soil DW]		
DAT-0[b]	806	1191
DAT-50	507	727
DAT-120	418	560

Table B.8.1.1.1.1- 3 *(cont.)*: Physico-chemical properties of test soils

Parameter	Results	
Soil designation	Hoefchen am Hohenseh	Dollendorf II
Geographic location City State Country	 Burscheid North Rhine-Westphalia Germany	 Blankenheim North Rhine-Westphalia Germany
Soil series	no information available	no information available
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	silt loam 25 58 17	loam 37 38 25
pH - in 0.01 M $CaCl_2$ 1/2 - in water 1/1 - in saturated paste - in soil/1 N KCl 1/1	6.6 6.8 6.8 6.2	7.4 7.5 7.5 7.0
Organic carbon (combustion) [% OC] Organic matter [a] [% OM]	1.9 3.3	5.2 9.0
Cation exchange capacity [meq/100 g]	11.7	17.8
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 1/3 bar (pF 2.0) [%]	 56.1 31.7	 84.5 43.1
Bulk density (disturbed) [g/cm^3]	1.10	1.00
Soil microbial biomass [mg microbial C/kg soil DW] DAT-0[b] DAT-50 DAT-120	 891 618 503	 2708 2186 2000

a) % organic matter = % organic carbon x 1.724
b) BIO sampes were applied with solvent of application solution (204 µL methanol)
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

The study was performed with static incubation test systems. Erlenmeyer flasks of 300 mL volume were used as incubation vessels and each flask was fitted with a trap attachment (permeable for oxygen) containing soda lime for absorption of carbon dioxide and a polyurethane (PU) foam plug for adsorption of volatile organic compounds (VOC).
For preparation of the test systems, 100 g dry weight equivalents of the sieved soils were weighed into each flask. Soil moisture was adjusted to 55% of the maximum water holding capacity (MWHC) for the individual test systems by addition of de-ionized water. The flasks were then fitted with trap attachments.
The untreated test systems were equilibrated to study conditions for 5 days prior to application.
The study application rate (SAR) was based on the maximum single field application rate of isoflucypram of 75 g per hectare, resulting in the targeted SAR of 20.0 µg per 100g soil dry weight.
The test item was applied dropwise onto the soil surface of the respective test systems using a pipette. After application, the test vessels (except DAT-0 samples) were fitted with trap and placed into a temperature-controlled walk-in climatic chamber for incubation in the dark. Soils were considered by the RMS to meet the OECD 307 recommendation to have a microbial biomass of at least 1% of total organic carbon.

2. Sampling

Ten sampling intervals were distributed over the entire incubation period of 120 days. Duplicate samples were processed and analysed 0, 2, 6, 15, 28, 50, 62, 84, 104 and 120 days after treatment (DAT).

3. Analytical Procedures

Carbon dioxide absorbed by soda lime was liberated with 18% aqueous hydrochloric acid and trapped. The liberated carbon dioxide was purged into the trapping vessels by a stream of nitrogen. The radioactivity contents of these vessels were determined by liquid scintillation counting (LSC) and summed up to determine the total radioactivity liberated from soda lime.

The PU foam plug was extracted with 30 mL ethyl acetate to desorb volatile organic compounds. The radioactivity content was determined by LSC.

The entire soil of each test vessel was transferred into a centrifuge beaker using the extraction solvent. The soil was extracted three times at ambient conditions using a mechanical shaker followed by two accelerated extractions using a microwave with a magnetic stirrer.

The extraction procedure is summarised in the following table:

Table B.8.1.1.1.1- 4: Extraction procedure

Solvent	Volume	Minimum duration	Temperature	Extracts
ACN/H_2O 1/1 (*v/v*)	80 mL	30 min, shaking	ambient	3
ACN/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 70°C	1
MeOH/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 50°C	1

The amounts of test item and degradation products in soil extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles were determined by LSC. Non-extractable residues were determined by LSC following combustion of soil pellets and collection of $^{14}CO_2$. Test item and degradation products were identified by HPLC-MS(/MS) including accurate mass determination and/or by co-chromatography with reference items.

II. RESULTS AND DISCUSSION

The test systems were incubated under aerobic conditions in the dark in a walk-in climatic chamber at 20.0°C for 120 days. The test was performed at a soil moisture of 53.1% of the maximum water holding capacity (approximately pF2). No significant loss of moisture was observed throughout the study.
Determinations of microbial biomass were performed on DAT-0, DAT-50 and DAT-120 and demonstrated that the used soils were microbially viable.

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean DAT-0 recovery for the test item was between 95.3 and 99.8% AR for all soils. The mean recovery of the concentration procedure for the combined soil extracts was between 97.6 and 99.7% for all soils. These results demonstrate that the sample processing method was well suited to recover the applied test item from the soil and that the test item was stable under these conditions.

2. Verification of Chromatographic Procedures

The primary chromatographic method (HPLC/radiodetection) was acceptable for the quantitative analysis of the samples of this study as demonstrated by a mean HPLC recovery between 99.1 and 99.3% and a good linear fit for injected amounts of pyrazole-labelled isoflucypram on HPLC column (R^2 > 0.9991). The LOD of the primary chromatographic method was determined as 2.3 Bq absolute on column or 0.4% AR.

B. MATERIAL BALANCE

Mean material balances were 100.3% AR (range from 99.5 to 101.3% AR) for soil Hanscheider Hof, 97.7% AR (range from 95.2 to 101.2% AR) for soil Laacher Hof AXXa, 98.7% AR (range from 97.1 to 100.2% AR) for soil Hoefchen Am Hohenseh and 98.5% AR (range from 95.9 to 100.1% AR) for soil Dollendorf II (Table B.8.1.1.1.1- 5 and Table B.8.1.1.1.1- 6).

The complete material balances found at all sampling intervals for all soils demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.1.1.1.1- 5: Material balance of radioactivity in soils under aerobic conditions from mean values (expressed as percentage of applied radioactivity of two replicates)

Soil	Material balance			
	min.	max.	mean	RSD
Hanscheider Hof	99.5	101.3	100.3	0.6
Laacher Hof AXXa	95.2	101.2	97.7	1.7
Hoefchen Am Hohenseh	97.1	100.2	98.7	0.9
Dollendorf II	95.9	100.1	98.5	1.4

RSD = relative standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.1.1.1.1- 6.

The route of degradation of isoflucypram in soil under aerobic conditions is summarised in Table B.8.1.1.1.1- 7 to Table B.8.1.1.1.1- 10. The proposed degradation of isoflucypram in soil is presented in Figure B.8.1.1.1.1- 1.

Table B.8.1.1.1.1- 6: Material balance of radioactivity in soils under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

		DAT									
		0	**2**	**6**	**15**	**28**	**50**	**62**	**84**	**104**	**120**
Hanscheider Hof											
Volatiles											
carbon dioxide	A	n.a	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.5	1.7
	B	n.a	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.7	1.8
	mean	n.a.	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.6	1.8
volatile organic compounds	A	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	A	n.a	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.5	1.7
	B	n.a	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.7	1.8
	mean	n.a.	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.6	1.8
Extractable residues											
combined extract	A	97.1	97.3	94.9	95.1	95.8	94.7	93.4	90.9	90.2	90.7
	B	98.0	94.9	97.2	95.6	96.0	94.8	94.6	91.6	90.6	89.4
	mean	97.6	96.1	96.1	95.3	95.9	94.7	94.0	91.3	90.4	90.0
microwave extract	A	1.5	1.5	1.3	2.4	1.4	1.6	1.7	1.9	1.9	1.9
	B	1.5	1.6	1.9	2.3	1.4	1.6	1.6	1.9	1.9	1.9
	mean	1.5	1.5	1.6	2.3	1.4	1.6	1.6	1.9	1.9	1.9
total extractable residues	A	98.6	98.8	96.2	97.4	97.2	96.3	95.1	92.8	92.1	92.6
	B	99.5	96.4	99.1	97.9	97.5	96.4	96.1	93.5	92.6	91.3
	mean	99.0	97.6	97.7	97.6	97.3	96.3	95.6	93.2	92.3	91.9
Non-extractable residues	A	1.6	2.3	2.3	2.7	3.0	4.2	5.1	5.6	6.0	5.9
	B	1.5	2.4	1.8	2.6	2.9	4.3	4.8	5.1	6.5	5.7
	mean	1.5	2.4	2.0	2.7	2.9	4.2	4.9	5.4	6.3	5.8
Material balance	A	100.2	101.2	98.5	100.1	100.4	101.1	100.9	99.6	99.7	100.2
	B	100.9	98.9	100.9	100.6	100.6	101.2	101.7	99.8	100.8	98.8
	mean	**100.6**	**100.0**	**99.7**	**100.4**	**100.5**	**101.1**	**101.3**	**99.7**	**100.2**	**99.5**
Laacher Hof AXXa											
Volatiles											
carbon dioxide	A	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.1	1.7	2.2	2.6
	B	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.3	1.8	2.1	2.4
	mean	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.2	1.7	2.2	2.5
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	A	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.1	1.7	2.2	2.7
	B	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.3	1.8	2.1	2.4
	mean	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.2	1.8	2.2	2.5
Extractable residues											
combined extract	A	99.1	96.9	94.1	94.5	93.7	91.9	90.0	86.9	86.0	85.6
	B	99.5	96.6	95.8	94.6	95.5	93.0	89.0	88.0	85.9	87.1
	mean	99.3	96.8	95.0	94.6	94.6	92.5	89.5	87.4	86.0	86.4
microwave extract	A	1.2	1.0	1.2	1.8	1.0	1.4	1.3	1.9	1.9	1.9
	B	1.0	1.1	1.1	1.9	1.0	1.3	1.7	1.8	1.5	1.9
	mean	1.1	1.1	1.2	1.8	1.0	1.4	1.5	1.8	1.7	1.9
total extractable residues	A	100.3	97.9	95.4	96.3	94.7	93.3	91.3	88.8	88.0	87.6
	B	100.5	97.7	97.0	96.5	96.5	94.3	90.6	89.7	87.5	89.1
	mean	100.4	97.8	96.2	96.4	95.6	93.8	91.0	89.3	87.7	88.3
Non-extractable residues	A	0.7	1.2	1.3	1.8	2.2	3.5	3.7	4.8	5.3	5.9
	B	0.8	1.1	1.4	1.8	2.3	3.3	4.5	5.5	5.5	5.8
	mean	0.8	1.2	1.3	1.8	2.3	3.4	4.1	5.2	5.4	5.8
Material balance	A	101.0	99.2	96.7	98.2	97.3	97.7	96.1	95.3	95.4	96.1
	B	101.3	98.9	98.3	98.4	99.2	98.5	96.5	97.0	95.1	97.2

	mean	**101.2**	**99.0**	**97.5**	**98.3**	**98.3**	**98.1**	**96.3**	**96.2**	**95.2**	**96.7**
Hoefchen am Hohenseh											
Volatiles											
carbon dioxide	A	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	0.7	1.8	2.2	2.6
	B	n.a.	< 0.1	< 0.1	0.1	0.3	0.9	1.3	1.7	2.3	2.9
	mean	n.a.	< 0.1	< 0.1	0.1	0.3	0.9	1.0	1.7	2.3	2.8
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	A	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	0.7	1.8	2.2	2.6
	B	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.3	1.7	2.3	2.9
	mean	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.0	1.7	2.3	2.8
Extractable residues											
combined extract	A	97.2	94.6	97.4	94.0	93.7	94.0	91.1	90.7	87.4	87.1
	B	98.1	93.2	95.1	93.7	95.7	94.3	90.9	87.0	85.3	86.5
	mean	97.7	93.9	96.3	93.8	94.7	94.2	91.0	88.8	86.3	86.8
microwave extract	A	1.1	1.1	1.3	2.1	1.3	1.2	1.5	1.8	1.6	1.5
	B	1.0	1.7	1.2	2.0	1.0	1.1	1.5	1.7	1.6	1.5
	mean	1.0	1.4	1.2	2.0	1.1	1.1	1.5	1.7	1.6	1.5
total extractable residues	A	98.3	95.7	98.7	96.0	95.0	95.3	92.6	92.6	92.5	88.5
	B	99.1	94.9	96.3	95.7	96.6	95.4	92.4	92.4	88.7	88.1
	mean	98.7	95.3	97.5	95.9	95.8	95.3	92.5	90.6	87.9	88.3
Non-extractable residues	A	1.3	1.6	1.8	2.2	2.3	4.0	4.4	5.5	8.0	7.5
	B	1.3	2.0	1.8	2.1	2.7	4.0	4.6	6.2	7.9	7.9
	mean	1.3	1.8	1.8	2.2	2.5	4.0	4.5	5.9	8.0	7.7
Material balance	A	99.6	97.3	100.5	98.4	97.7	100.2	97.7	99.7	99.2	98.7
	B	100.4	96.9	98.1	97.9	99.7	100.3	98.2	96.6	97.1	98.9
	mean	**100.0**	**97.1**	**99.3**	**98.1**	**98.7**	**100.2**	**98.0**	**98.2**	**98.2**	**98.8**
Dollendorf II											
Volatiles											
carbon dioxide	A	n.a.	< 0.1	< 0.1	0.1	0.3	0.8	1.1	1.8	2.5	3.1
	B	n.a.	< 0.1	< 0.1	0.1	0.3	0.5	1.1	1.7	2.6	2.8
	mean	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.8	2.5	3.0
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	A	n.a.	< 0.1	< 0.1	0.1	0.3	0.8	1.1	1.8	2.5	3.1
	B	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.7	2.6	2.8
	mean	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.8	2.5	3.0
Extractable residues											
combined extract	A	96.2	91.6	94.1	93.7	88.7	90.1	88.9	88.0	84.1	83.6
	B	92.0	95.0	94.9	93.8	92.8	91.9	89.8	83.5	84.3	84.2
	mean	94.1	93.3	94.5	93.8	90.7	91.0	89.3	85.8	84.2	83.9
microwave extract	A	1.3	2.8	1.6	1.9	1.3	1.3	1.2	1.6	1.9	1.8
	B	2.2	0.9	1.7	1.9	1.2	1.0	1.4	1.8	1.8	1.7
	mean	1.8	1.8	1.6	1.9	1.2	1.2	1.3	1.7	1.9	1.7
total extractable residues	A	97.5	94.4	95.6	95.6	90.0	91.4	90.1	89.7	86.0	85.4
	B	94.2	95.9	96.6	95.7	94.0	92.9	91.2	85.3	86.1	85.9
	mean	95.8	95.1	96.1	95.6	92.0	92.1	90.7	87.5	86.0	85.6
Non-extractable residues	A	2.6	5.6	2.7	3.9	4.1	4.8	6.1	7.3	11.4	11.1
	B	3.8	4.3	2.9	3.6	3.2	5.1	5.5	7.4	11.7	10.3
	mean	3.2	5.0	2.8	3.7	3.6	4.9	5.8	7.4	11.6	10.7
Material balance	A	100.1	100.0	98.4	99.6	94.3	96.9	97.4	98.9	99.8	99.6
	B	98.0	100.2	99.5	99.4	97.5	98.6	97.8	94.4	100.4	99.0
	mean	**99.1**	**100.1**	**99.0**	**99.5**	**95.9**	**97.7**	**97.6**	**96.6**	**100.1**	**99.3**

n.d.: not detected, n.a.: not analyzed, DAT: days after treatment

Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide was 1.8, 2.5, 2.8 and 3.0% AR at study end (DAT-120) in soil Hanscheider Hof, Laacher Hof AXXa, Hoefchen Am Hohenseh and Dollendorf II, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for all soils (Table B.8.1.1.1.1- 7 to Table B.8.1.1.1.1- 10).

Test item and degradation products in soil extracts

Extractable residues decreased from DAT-0 to DAT-120 from 99.0 to 91.9% AR in soil Hanscheider Hof, from 100.4 to 88.3% AR in soil Laacher Hof AXXa, from 98.7 to 88.3% AR in soil Hoefchen Am Hohenseh and from 95.8 to 85.6% AR in soil Dollendorf II.

The amount of isoflucypram in the soil extracts decreased from DAT-0 to DAT-120 from 98.2 to 82.6% AR in soil Hanscheider Hof, from 99.8 to 70.1% AR in soil Laacher Hof AXXa, from 98.2 to 77.2% AR in soil Hoefchen Am Hohenseh and from 95.3 to 72.2% AR in soil Dollendorf II.

Degradation of isoflucypram was accompanied by the formation of three degradation products, identified with the following maximum amounts in at least one soil: M12 with 5.8% AR at DAT-104 in soil Dollendorf II, M10 with 3.8% AR at DAT-104 in soil Dollendorf II and *M11* with 1.1% AR at DAT-104 in soil Laacher Hof AXXa. The total unidentified residues amounted to a maximum of 8.9% AR and no single component exceeded 3.6% AR at any sampling interval for all soils (Table B.8.1.1.1.1- 7 to Table B.8.1.1.1.1- 10).

Non-extractable residues

Non-extractable residues (NER) increased in soil Hanscheider Hof from DAT-0 to DAT 104 from 1.5 to 6.3% AR and slightly declined to 5.8% AR until DAT-120. In soil Laacher Hof AXXa NER increased from DAT-0 to DAT-120 from 0.8 to 5.8% AR. NER increased in soil Hoefchen Am Hohenseh from DAT-0 to DAT-104 from 1.3 to 8.0% AR and slightly declined to 7.7% AR until DAT-120. In soil Dollendorf II, NER increased from DAT-0 to DAT-104 from 3.2 to 11.6% AR and slightly declined to 10.7% AR until DAT-120 (Table B.8.1.1.1.1- 7 to Table B.8.1.1.1.1- 10).

Table B.8.1.1.1.1- 7: Degradation of isoflucypram in soil Hanscheider Hof under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		DAT									
		0	2	6	15	28	50	62	84	104	120
Isoflucypram	A	97.8	98.2	95.6	95.4	92.9	91.5	90.2	86.3	82.8	83.8
	B	98.5	96.4	99.0	96.2	94.5	93.0	89.8	86.2	81.6	81.3
	mean	98.2	97.3	97.3	95.8	93.7	92.3	90.0	86.3	82.2	82.6
M12	A	n.d.	n.d.	n.d.	1.0	1.5	1.6	1.4	2.1	2.4	2.3
	B	n.d.	n.d.	n.d.	0.6	1.2	1.7	2.1	2.3	2.9	2.6
	mean	n.d.	n.d.	n.d.	0.8	1.4	1.6	1.7	2.2	2.6	2.4
ROI 2	A	n.d.	n.d.	n.d.	0.6	1.0	1.4	1.7	1.6	0.9	1.9
	B	n.d.	n.d.	n.d.	0.8	0.8	1.2	1.4	1.2	1.3	1.7
	mean	n.d.	n.d.	n.d.	0.7	0.9	1.3	1.6	1.4	1.1	1.8
M10	A	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	0.6	0.7	0.8	0.7
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.7	0.9	0.8
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.7	0.7	0.8	0.8
ROI 4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	n.d.	0.5	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.7	0.7	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	< LOD	0.6	n.d.
M11	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.5
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD
ROI 6	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 7	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.8	0.7
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.7	0.4
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.8	0.6
ROI 8	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	n.d.
ROI 9	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD
Sum of unid./diff. residues	A	0.8	0.6	0.6	0.4	1.7	1.2	0.4	1.4	3.1	2.3
	B	0.9	< LOD	< LOD	< LOD	0.9	0.6	1.4	1.7	3.6	4.0
	mean	0.9	< LOD	< LOD	< LOD	1.3	0.9	0.9	1.5	3.3	3.2
Total extractable residues [a]	A	98.6	98.8	96.2	97.4	97.2	96.3	95.1	92.8	91.8	92.2
	B	99.5	96.4	99.0	97.6	97.5	96.4	96.1	93.5	92.3	91.3
	mean	99.0	97.6	97.6	97.5	97.3	96.3	95.6	93.2	92.0	91.8
Carbon dioxide [b]	A	n.a	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.5	1.7
	B	n.a	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.7	1.8
	mean	n.a.	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.6	1.8
Volatile organic compounds [b]	A	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [b]	A	1.6	2.3	2.3	2.7	3.0	4.2	5.1	5.6	6.0	5.9
	B	1.5	2.4	1.8	2.6	2.9	4.3	4.8	5.1	6.5	5.7
	mean	1.5	2.4	2.0	2.7	2.9	4.2	4.9	5.4	6.3	5.8
Total recovery [a]	A	100.2	101.1	98.5	100.1	100.4	101.0	100.9	99.6	99.3	99.9
	B	100.9	98.8	100.8	100.4	100.6	101.2	101.7	99.8	100.5	98.8
	mean	100.6	100.0	99.6	100.3	100.5	101.1	101.3	99.7	99.9	99.3

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, ROI: regions of interest

a) Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses

b) Values taken from Material Balance

Table B.8.1.1.1.1- 8: Degradation of isoflucypram in soil Laacher Hof AXXa under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		DAT									
		0	2	6	15	28	50	62	84	104	120
Isoflucypram	A	99.7	96.9	94.5	93.8	89.0	85.3	81.4	78.1	74.3	69.8
	B	99.8	96.9	96.8	94.1	91.8	84.3	80.6	75.3	70.7	70.4
	mean	99.8	96.9	95.6	93.9	90.4	84.8	81.0	76.7	72.5	70.1
M12	A	n.d.	n.d.	n.d.	1.3	1.8	3.1	3.2	3.8	4.8	4.8
	B	n.d.	n.d.	n.d.	1.3	2.1	3.3	3.3	4.3	5.0	5.9
	mean	n.d.	n.d.	n.d.	1.3	1.9	3.2	3.3	4.0	4.9	5.4
ROI 2	A	n.d.	n.d.	n.d.	0.9	1.6	2.3	2.3	2.1	2.1	3.3
	B	n.d.	n.d.	n.d.	0.9	1.4	2.4	2.9	2.9	2.9	3.6
	mean	n.d.	n.d.	n.d.	0.9	1.5	2.3	2.6	2.5	2.5	3.4
M10	A	n.d.	n.d.	n.d.	n.d.	0.6	1.3	1.5	1.9	2.2	2.5
	B	n.d.	n.d.	n.d.	n.d.	0.8	1.7	2.4	2.3	2.9	3.0
	mean	n.d.	n.d.	n.d.	n.d.	0.7	1.5	1.9	2.1	2.5	2.8
ROI 4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6
	B	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	0.6	0.6	0.8	0.6
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	< LOD	< LOD	< LOD	0.6
M11	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	n.d.	1.1	0.9
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	1.0	1.1
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.1	1.0
ROI 6	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.5	0.6	0.6
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.6	0.7
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.5	0.6	0.6
ROI 7	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.0	1.0	1.2
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.3	1.2	1.3
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.1	1.1	1.3
ROI 8	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD
ROI 9	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7
Sum of unid./diff. residues	A	0.6	1.0	0.8	< LOD	1.7	1.4	0.7	1.4	1.9	2.8
	B	0.7	0.8	< LOD	< LOD	< LOD	1.8	< LOD	1.9	2.4	1.8
	mean	0.6	0.9	0.4	< LOD	0.9	1.6	< LOD	1.7	2.1	2.3
Total extractable residues [a)]	A	100.3	97.9	95.4	96.0	94.7	93.3	91.3	88.2	88.0	87.3
	B	100.5	97.7	96.8	96.3	96.1	94.3	90.5	89.7	87.5	89.1
	mean	100.4	97.8	96.1	96.1	95.4	93.8	90.9	89.3	87.7	88.2
Carbon dioxide [b)]	A	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.1	1.7	2.2	2.6
	B	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.3	1.8	2.1	2.4
	mean	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.2	1.7	2.2	2.5
Volatile organic compounds [b)]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [b)]	A	0.7	1.2	1.3	1.8	2.2	3.5	3.7	4.8	5.3	5.9
	B	0.8	1.1	1.4	1.8	2.3	3.3	4.5	5.5	5.5	5.8
	mean	0.8	1.2	1.3	1.8	2.3	3.4	4.1	5.2	5.4	5.8
Total recovery [a)]	A	101.0	99.1	96.6	97.9	97.3	97.7	96.1	95.3	95.4	95.9
	B	101.3	98.9	98.1	98.2	98.8	98.5	96.3	97.0	95.1	97.2
	mean	101.2	99.0	97.4	98.0	98.1	98.1	96.2	96.2	95.2	96.5

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, ROI: regions of interest

a) Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses

b) Values taken from Material Balance

Table B.8.1.1.1.1- 9: Degradation of isoflucypram in soil Hoefchen Am Hohenseh under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		DAT									
		0	2	6	15	28	50	62	84	104	120
Isoflucypram	A	98.2	95.3	97.3	94.2	89.8	90.0	84.3	85.0	80.7	77.3
	B	98.3	94.3	95.6	93.7	92.4	89.1	85.9	79.9	76.5	77.0
	mean	98.2	94.8	96.4	93.9	91.1	89.5	85.1	82.4	78.6	77.2
M12	A	n.d.	n.d.	0.8	0.9	1.5	1.3	1.8	2.2	1.4	1.7
	B	n.d.	n.d.	0.4	0.9	1.1	1.4	1.2	1.4	1.4	1.5
	mean	n.d.	n.d.	0.6	0.9	1.3	1.3	1.5	1.8	1.4	1.6
ROI 2	A	n.d.	n.d.	n.d.	0.7	1.4	1.8	2.0	n.d.	2.4	2.8
	B	n.d.	n.d.	n.d.	0.8	1.2	2.1	1.7	1.7	2.6	2.5
	mean	n.d.	n.d.	n.d.	0.7	1.3	2.0	1.9	0.8	2.5	2.6
M10	A	n.d.	n.d.	n.d.	n.d.	0.7	0.9	1.7	1.5	1.5	1.5
	B	n.d.	n.d.	n.d.	n.d.	0.4	1.0	1.1	1.6	2.0	1.7
	mean	n.d.	n.d.	n.d.	n.d.	0.6	0.9	1.4	1.5	1.7	1.6
ROI 4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M11	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.6	0.9	0.6
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	0.6	0.5	0.5
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.6	0.7	0.6
ROI 6	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.	n.d
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.4	n.d.	0.4
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.5	n.d.	< LOD
ROI 7	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	1.1	0.5	1.0
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.9	1.0	0.8	1.0
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.1	0.7	1.0
ROI 8	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 9	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sum of unid./diff. residues [c)]	A	< LOD	0.5	0.5	< LOD	1.6	1.4	1.7	1.6	1.6	3.5
	B	0.8	0.6	< LOD	< LOD	1.5	1.8	< LOD	2.1	3.0	3.3
	mean	< LOD	0.5	< LOD	< LOD	1.6	1.6	0.9	1.8	2.3	3.4
Total extractable residues [a)]	A	98.2	95.7	98.7	95.8	95.0	95.3	92.2	92.5	89.0	88.5
	B	99.1	94.9	96.0	95.4	96.6	95.4	91.7	88.7	86.9	88.1
	mean	98.6	95.3	97.3	95.6	95.8	95.3	92.0	90.6	87.9	88.3
Carbon dioxide [b)]	A	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	0.7	1.8	2.2	2.6
	B	n.a.	< 0.1	< 0.1	0.1	0.3	0.9	1.3	1.7	2.3	2.9
	mean	n.a.	< 0.1	< 0.1	0.1	0.3	0.9	1.0	1.7	2.3	2.8
Volatile organic compounds [b)]	A	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [b)]	A	1.3	1.6	1.8	2.2	2.3	4.0	4.4	5.5	8.0	7.5
	B	1.3	2.0	1.8	2.1	2.7	4.0	4.6	6.2	7.9	7.9
	mean	1.3	1.8	1.8	2.2	2.5	4.0	4.5	5.9	8.0	7.7
Total recovery [a)]	A	99.5	97.3	100.5	98.1	97.7	100.2	97.3	99.7	99.2	98.7
	B	100.4	96.8	97.7	97.6	99.7	100.2	97.6	96.6	97.1	98.9
	mean	100.0	97.1	99.1	97.8	98.7	100.2	97.5	98.2	98.2	98.8

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, ROI: regions of interest

a) Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses

b) Values taken from Material Balance

c) No individual value above 3.6% of A.R.

Table B.8.1.1.1.1- 10: Degradation of isoflucypram in soil Dollendorf II under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		DAT									
		0	2	6	15	28	50	62	84	104	120
Isoflucypram	A	96.7	94.1	94.5	93.0	84.6	83.3	82.2	80.6	72.1	71.9
	B	94.0	95.8	94.7	93.2	89.6	87.0	82.5	75.1	66.5	72.4
	mean	95.3	95.0	94.6	93.1	87.1	85.1	82.4	77.9	69.3	72.2
M12	A	n.d.	n.d.	1.0	1.5	2.0	2.1	2.2	2.7	4.7	2.6
	B	n.d.	n.d.	1.1	1.1	1.9	2.8	2.5	3.1	7.0	2.4
	mean	n.d.	n.d.	1.1	1.3	1.9	2.5	2.4	2.9	5.8	2.5
ROI 2	A	n.d.	n.d.	n.d.	0.7	1.2	2.6	2.2	1.8	1.6	2.6
	B	n.d.	n.d.	n.d.	1.1	1.5	1.7	1.7	2.5	3.6	2.7
	mean	n.d.	n.d.	n.d.	0.9	1.4	2.1	2.0	2.1	2.6	2.6
M10	A	n.d.	n.d.	n.d.	n.d.	0.5	1.6	1.6	2.0	2.7	2.6
	B	n.d.	n.d.	n.d.	n.d.	0.6	1.0	1.5	1.7	4.9	3.1
	mean	n.d.	n.d.	n.d.	n.d.	0.6	1.3	1.5	1.8	3.8	2.9
ROI 4	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.4	n.d.
M11	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	< LOD	0.6	0.7
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	0.4	0.9	0.7
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	< LOD	0.8	0.7
ROI 6	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 7	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.5	2.0	1.0
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	0.8	1.8	1.3
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.1	1.9	1.1
ROI 8	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 9	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	mean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sum of unid./diff. residues	A	0.8	< LOD	< LOD	< LOD	1.7	1.7	0.4	< LOD	2.4	4.0
	B	< LOD	n.d.	0.8	< LOD	< LOD	0.4	1.5	1.5	0.6	3.2
	mean	< LOD	< LOD	0.4	< LOD	0.9	1.1	1.0	0.8	1.5	3.6
Total extractable residues [a]	A	97.5	94.1	95.5	95.2	90.0	91.4	90.1	88.6	86.0	85.4
	B	94.0	95.8	96.6	95.4	93.6	92.9	91.2	85.3	86.1	85.9
	mean	95.7	95.0	96.1	95.3	91.8	92.1	90.7	86.9	86.0	85.6
Carbon dioxide [b]	A	n.a.	< 0.1	< 0.1	0.1	0.3	0.8	1.1	1.8	2.5	3.1
	B	n.a.	< 0.1	< 0.1	0.1	0.3	0.5	1.1	1.7	2.6	2.8
	mean	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.8	2.5	3.0
Volatile organic compounds [b]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [b]	A	2.6	5.6	2.7	3.9	4.1	4.8	6.1	7.3	11.4	11.1
	B	3.8	4.3	2.9	3.6	3.2	5.1	5.5	7.4	11.7	10.3
	mean	3.2	5.0	2.8	3.7	3.6	4.9	5.8	7.4	11.6	10.7
Total recovery [a]	A	100.1	99.8	98.3	99.2	94.3	96.9	97.4	97.8	99.8	99.5
	B	97.8	100.1	99.5	99.0	97.1	98.6	97.8	94.4	100.4	99.0
	mean	98.9	99.9	98.9	99.1	95.7	97.7	97.6	96.1	100.1	99.3

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, ROI: regions of interest

a) Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses

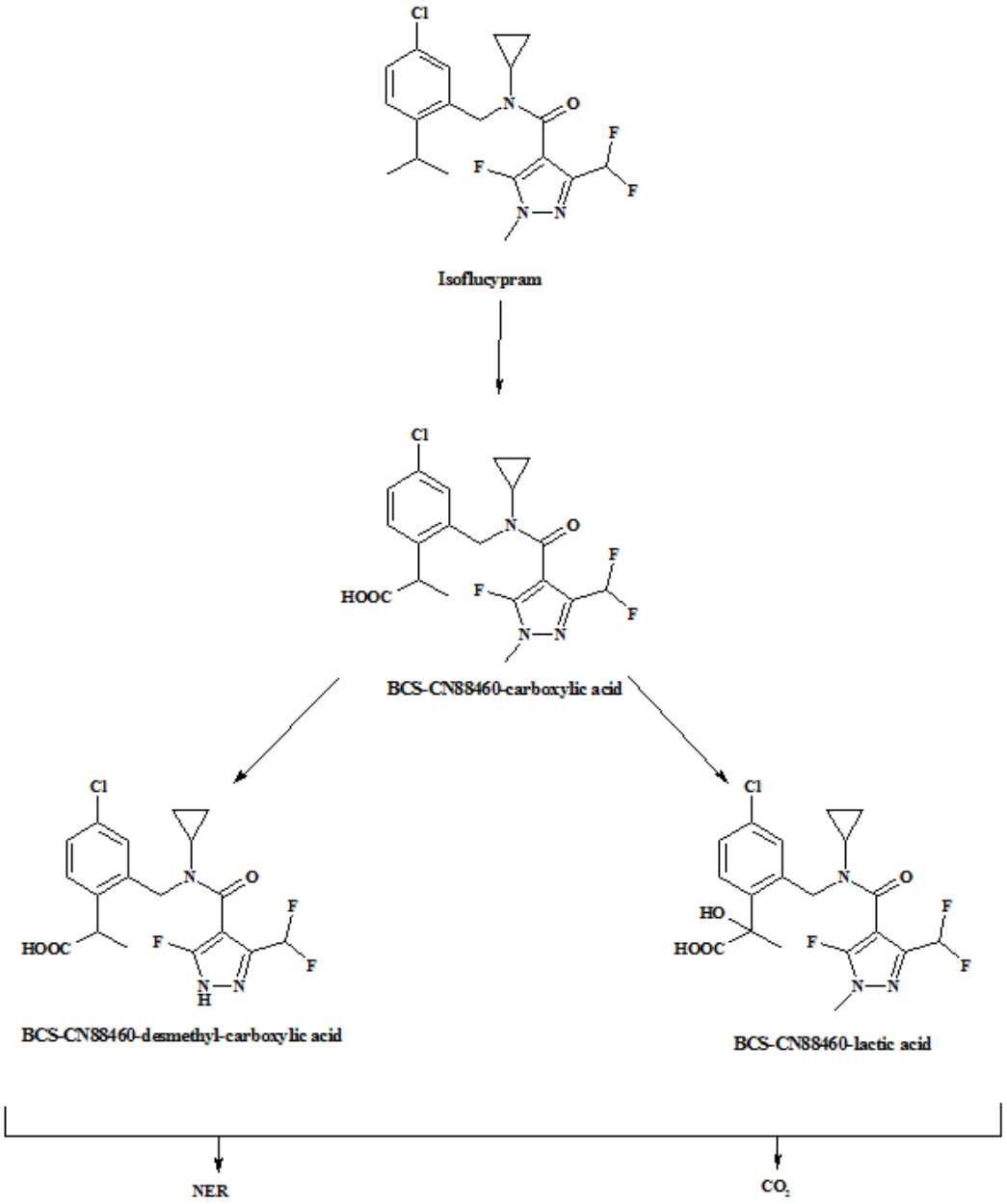
b) Values taken from Material Balance

D. DEGRADATION PATHWAY

Based on the results of the study, the following pathway for the degradation of pyrazole-labelled isoflucypram in soil under aerobic conditions is proposed (see Figure B.8.1.1.1.1- 1), with the following possible processes involved:

- carboxylation of isoflucypram to result in BCS-CN88460-carboxylic acid *(M12)*;
- hydroxylation of BCS-CN88460-carboxylic acid *(M12)* to result in BCS-CN88460-lactic acid *(M10)*;
- demethylation of BCS-CN88460-carboxylic acid *(M12)* to result in BCS-CN88460-desmethyl-carboxylic acid *(M11)*;
- mineralisation (carbon dioxide formation);
- formation of non-extractable residues (NER).

Figure B.8.1.1.1.1- 1: Proposed degradation pathway of pyrazole-labelled isoflucypram in soils under aerobic conditions



III. CONCLUSIONS

RMS found the study to be acceptable. An acceptable mass balance was achieved, chromatography was of an acceptable standard such that the endpoints can be relied upon and no deviations from the stated guideline were noted.

Isoflucypram was slowly degraded in soil under aerobic conditions in the dark in the laboratory. Three degradation products were identified with the following maximum amounts: M12 with 5.8% AR and was increasing at the end of the study; M10 with 3.8% AR and this metabolites was increasing at the end of the study; M11 with 1.1% AR.
Formation of non-extractable residues (NER) was up to 10.7% AR at study end (DAT-120), which may be an indication that the limited degradation of isoflucypram observed may have been microbially mediated. A kinetic assessment of the endpoints achieved has been evaluated in section B.8.1.1.4 below.

B.8.1.1.1.2: Aerobic degradation study of isoflucypram

Previous evaluation:	None, new active substance.

Author: Gabbert, D.; McConnell, L. L.; Arthur, E. L.; 2017;
Title: [Pyrazole-4-14C]BCS-CN88460: Aerobic soil metabolism in two US soils
Report No.: MELNN013
Document No.: M-588260-01-1
Guideline(s): OECD Test Guideline No. 307, Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009. US EPA OCSPP, Test Guideline No. 835.4100. PMRA: Daco No. 8.2.3.4.2 Biotransformation in Soil (TGAI), Aerobic Soil 20-30degrees C
Guideline deviation(s): none specified
GLP/GEP: yes

Study Summary
The route and rate of pyrazole-labelled isoflucypram was studied in two US soils under aerobic conditions in the dark in the laboratory for 123 days at 20.4°C and moisture content of between pF 2.0 and 2.5.

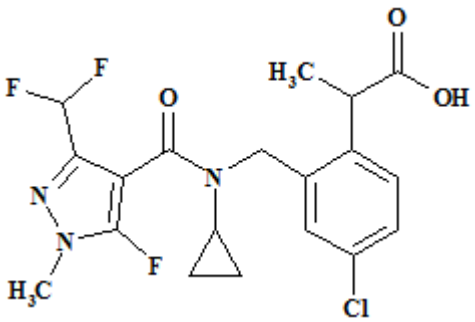
Table B.8.1.1.1.2-1: Selected soils

Soil Designation	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
CA soil	063014-S	Sanger, CA,	Sandy Loam	6.3	0.77
NE soil	062014-S	Louisville, NE	Silty Clay Loam	6.3	2.0

The study application rate was based on the anticipated maximum single field-use rate for isoflucypram of 75 g a.s. per hectare which corresponded to a concentration in soil of 0.2 µg of isoflucypram per g of soil as dry weight. In order to bridge to a higher rate, additional test systems were treated at 0.43 µg/g (equivalent to approximately 150 g a.s. per hectare). These test systems were also used for metabolite identification purposes and only 3 time points were selected: 76, 88 and 123 DAT.
The test was performed with a flow-through system consisting of cylindrical bottles each containing

75 g soil (dry weight equivalents) attached to a series of volatile traps for the collection of carbon dioxide and volatile organic compounds.
Replicate samples were processed and analysed at 0, 6, 14, 21, 28, 60, 88, and 123 days after treatment (DAT). At each sampling interval, the soil was extracted three times at ambient temperature: once using acetonitrile and additional two times using acetonitrile/water 4:1 (*v/v*). Furthermore, two microwave-accelerated extraction steps were performed using acetonitrile/water 4:1 (*v/v*) at 70°C and methanol/water 9:1 (*v/v*) at 50°C, respectively. The amounts of test substance and degradation products in soil extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. The test substance and degradation products were identified by LC/ESI-MS under positive and negative ion mode.
On the final interval at DAT-123, an additional ambient extraction step with two non-polar organic solvents was added after microwave-accelerated extraction. An ethyl acetate extraction was followed by extraction with hexane. Radioactivity of the combined extracts was determined by LSC and found to be ≤ 0.9% of applied radioactivity (AR) for the CA soil and ≤ 2.1% for NE soil. Therefore, the primary extraction method was effective at determining extractable residues.
Mean material balances were 97.0% AR (94.9% to 98.2% AR) for CA soil and 96.8% AR (95.1% to 97.8% AR) for NE soil. Extractable residues decreased from 94.7% AR at DAT-0 to 92.3% AR at DAT-123 in CA soil and from 94.8% at DAT-0 to 83.0% AR DAT-123 in NE soil. Non-extractable residues (NER) increased from 0.2% at DAT-0 to 3.4% AR at DAT-123 in CA soil and from 0.3% at DAT-0 to 10.7% AR at DAT-123 in NE soil. Formation of volatile compounds, primarily carbon dioxide was low as demonstrated by values of ≤ 3.3% AR, and shows slow mineralisation is occurring. The amount of isoflucypram in the soil extracts decreased from 94.7% at DAT-0 to 86.2% AR at DAT-123 in CA soil, and from 94.8% at DAT-0 to 64.4% AR at DAT-123 in NE soil. One soil metabolite *M12* – was isolated and identified from the NE soil extract. This metabolite was formed at a maximum of 1.3% and 9.6% AR in the CA and NE soil, respectively, at DAT-123. Unidentified minor degradates occurred, and individual components were ≤ 4.0% AR at any sampling interval.

Table B.8.1.1.1.2-2: Identified degradation products (maximum occurrence) in soils (in percent of applied radioactivity)

Compound	Chemical structure	Maximum occurrence in soil [%]
M12 (BCS-CN88460-carboxylic acid)		9.6
CO_2		3.2

Based on results of this laboratory study, isoflucypram degrades slowly under aerobic conditions to form M12 and other minor metabolites in addition to NER and CO_2.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item and Reference Substances

Test item

Pyrazole-labelled isoflucypram

Sample-ID:	C-1173
Specific activity:	4.22 MBq/mg (113.92 µCi/mg)
Radiochemical purity:	> 99% (HPLC with radioactivity detector)

Reference substances

Unlabelled isoflucypram

Sample-ID:	K-2124
Chemical purity:	98.4%

unlabelled BCS-CN88460-carboxylic acid *(M12)*

Sample-ID:	K-2176
Chemical purity:	98.8%

2. Test soils

The study was carried out using two different soils (see Table B.8.1.1.1.2- 3). The soils were taken from agricultural use areas representing different geographical origins and different soil properties as required by the guidelines. The plant protection product use history of the soils indicates no pesticides applied in the last 5 years at the Louisville, NE site and no pesticides applied in the last 4 years at the Sanger, CA site.

The soils were sampled freshly from the fields (upper horizon of 0 to 20 cm) and sieved to remove rocks and plant material. From collection to usage was less than 3 months (21 and 27 days respectively) and stored at 4°C.

Table B.8.1.1.1.2-3: Physico-chemical properties of test soils

Parameter	**Results**	
Soil designation	CA soil	Ne soil
Geographic location		
City	Sanger	Louisville
State	California	Nebraska
Country	USA	USA
Soil series	Hanford series	no information available
Textural class (USDA)	sandy loam	silty clay loam
Sand [%] (50 µm – 2 mm)	68.6	15.0
Silt [%] (2 µm – 50 µm)	27.3	50.1
Clay [%] (< 2 µm)	4.1	34.9
pH - in 0.01 M $CaCl_2$ 1/2	6.3	6.3
- in water 1/1	6.7	6.7
- in saturated paste	6.6	6.6
Organic carbon (combustion) [% OC]	0.77	2.0
Organic matter [a] [% OM]	1.3	3.5
Cation exchange capacity [meq/100 g]	6.7	17.2
Water holding capacity		
maximum (MWHC) [g H_2O *ad* 100 g DW]	29.1	52.4
at 1/10 bar (pF 2.0) [%]	21.8	43.0
at 1/3 bar (pF 2.5) [%]	9.8	32.7
at 15 bar [%]	4.2	14.6
Bulk density (disturbed) [g/cm^3]	1.22	1.02
Soil microbial biomass [CFU/g soil DW]		
DAT-0 biomass UT[b]		
- Actinomycetes	1,140,000	943,000
- Fungi	11,900	9,620
….-.Bacteria	2,150,000	1,510,000
DAT-123 biomass UT[b] / biomass SC[c]		
- Actinomycetes	42,700 / 79,500	378,000 / 407,000
- Fungi	9,590 / 10,400	14,100 / 8,140
….-.Bacteria	698,000 / 1,210,000	1,490,000 / 1,420,000

a) % organic matter = % organic carbon x 1.724
b) Biomass-UT test systems were left untreated.
c) Biomass-SC test systems were applied with solvent of application solution (200 µL methanol).
DW: dry weight DAT: days after treatment

B. STUDY DESIGN

1. Experimental Conditions

A flow-through test system for degradation in soil under aerobic conditions was used. The test system consisted of a salinized cylindrical glass flask connected to a flow-through system, containing an ethylene glycol trap for volatile organics followed by two 2 M potassium hydroxide traps, with tropaeolin-O to indicate saturation by colour change from orange to yellow, for collecting CO_2 and a 1 M sulfuric acid trap for volatile acids. The headspace of the test systems was continuously purged with humidified air throughout the study.

For preparation of the test systems, 75 g dry weight equivalents of the sieved soils were weighed into each flask. Additional metabolite identification (MID) test systems were treated at 2x the kinetics rate. These test systems were used to determine a degradation rate and for the purposes of isolating and identifying major degradates formed in the study. Soil moistures were adjusted to between pF 2.0 and pF 2.5 for individual test systems by addition of purified water, flasks were placed in the dark at 20.4 °C ±0.03 °C. The flasks were then connected to the flow-through traps. The untreated test systems were equilibrated at study conditions for 8 days prior to test substance application. Due to the method chosen to determine the microbial biomass, UKRMS cannot confirm that biomass meets the recommendation

for biomass to be a minimum of 1% of organic carbon as outlined in OECD307. However UKRMS is sufficiently satisfied that the soils are microbially active and the decline is as would be expected.
For the application of kinetic samples each test system received 16.7 µg of isoflucypram resulting in an application rate of 0.22 µg/g of the test substance which corresponds to single field use rate of 75 g isoflucypram per hectare
For the application of metabolite identification samples isoflucypram was applies with 0.43 µg/g (equivalent to approximately 150 g a.s. per hectare).

2. Sampling

Eight sampling intervals were distributed over the incubation period of 123 days. Replicate samples were processed and analysed 0, 6, 14, 21, 28, 60, 88, and 123 days after treatment (DAT).
In order to bridge to a higher application rate, a single higher rate MID test system per each soil was extracted and analysed by HPLC/radiodetection at three intervals – 76, 88, and 123 DAT.

3. Analytical Procedures

Sample preparation and processing:
Prior to opening an incubated test system for processing of soil, volatiles possibly still present in the head space of the test system were purged into the trap attachment by increasing the vacuum. The traps were then disconnected and the soil was transferred to a Teflon centrifuge bottle and extracted.

Processing of volatile traps:
The volume of the ethylene glycol and 1 M H_2SO_4 traps were recorded. The two 2 M KOH traps were combined and volume recorded. Three 0.5 mL aliquots of each were radioassayed by LSC to determine the total radioactivity trapped.

Processing of soil:
The entire soil content of each test system was transferred into a Teflon bottle using the extraction solvent. The soil was extracted three times at ambient conditions using a mechanical shaker followed by a two microwave extractions with a magnetic stirrer. The extraction procedure is summarised in the following table:

Table B.8.1.1.1.2-4: Extraction procedure

Solvent	Volume	Minimum duration	Temperature	Cycles
Acetonitrile	80 mL	30 min, shaking	ambient	1
Acetonitrile/Water (4:1, *v/v*)	80 mL	30 min, shaking	ambient	2
Acetonitrile/Water (4:1, *v/v*)	80 mL	10 min, stirring	microwave, 70°C	1
Methanol/Water (9:1, *v/v*)	80 mL	10 min, stirring	microwave, 50°C	1

After each extraction step, the extract and soil were separated by centrifugation and decanted and filtered into a glass graduated cylinder. The volumes of the combined ambient and microwave soil extracts were determined. The radioactivity content of these extracts was determined by LSC and concentrated extracts were characterized for parent and degradates by HPLC/radiodetection.
On the final interval at DAT-123, two additional extraction steps with non-polar solvents were included after the microwave extraction steps. The soil was extracted with 80 mL of ethyl acetate with shaking for 15 min followed by an extraction with 80 mL of hexane with shaking for 15 min. After each extraction step, the extract and soil were separated by centrifugation and decanted, filtered, and combined into a glass graduated cylinder. The volumes of the combined soil extracts were determined. The radioactivity content of these extracts was determined by radioassay of replicate aliquots. However, these extracts were not included in the HPLC analysis due to very low amounts extractable.

The extracted soils were air-dried, homogenised and non-extractable residues were determined by combustion/LSC.

II. RESULTS AND DISCUSSION

The test systems were incubated under aerobic conditions in the dark in a walk-in climatic chamber at 20.4°C for 123 days. The test was performed at a soil moisture level between pF 2.0 and pF 2.5 for each soil. Losses of moisture were observed throughout the study, so periodic moisture adjustments were made to specific test systems as necessary. Determinations of microbial biomass were performed on DAT-0and DAT-123 and demonstrated that the used soils were microbially viable, but the CA soil showed a significant decline in activity at the end of the study (Table B.8.1.1.1.2-3). Under the conditions of a laboratory experiment a decrease of microbial biological activity is inevitable due to the absence of any further amendment of nutrients.

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean DAT-0 recovery for the test substance was 94.9% and 95.1% AR for CA and NE soils.
The mean recovery of the concentration procedure for the combined soil extracts was between 90.2% to 105.0% AR for all soils. These results demonstrate that the sample processing method was well suited to recover the applied test substance from the soil and that the test substance was stable under these conditions.

2. Verification of Chromatographic Procedures

The purity of the treatment solution was checked by HPLC prior to test system treatments. The HPLC analysis showed a radiochemical purity of 100%.
The flow-through detector was found to have a linear response over a range of 240 dpm to 194,814 dpm with a coefficient of determination (r^2) of 0.9999. The limit of detection (LOD) was 322 dpm or 0.9% AR. The limit of quantitation (LOQ) was 1.4% of AR.

B. MATERIAL BALANCE

Mean material balances were 97.0% AR (range from 94.9 to 98.2% AR) for soil CA, and 96.8% AR (range from 95.1 to 97.8% AR) for soil NE (Table B.8.1.1.1.2-5 and Table B.8.1.1.1.2-6).
The complete material balances found at all sampling intervals for all soils demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.1.1.1-5: Material balance of radioactivity in soils under aerobic conditions from mean values (expressed as percentage of applied radioactivity of two replicates)

Soil	Material balance			
	min.	max.	mean	SD
CA	94.9	98.2	97.0	1.2
NE	95.1	97.8	96.8	1.0

SD = standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

Distribution of residues

In the CA test systems, the extractable radioactive residues in the soil increased from an average of 94.9% at DAT-0 to 98.2% AR at DAT-14, and then decreased to 95.6% at DAT-88, remaining approximately constant at 97.6% AR at DAT-123. The unextractable or bound radioactive residues in

the soil increased from an average of 0.2% on DAT-0 to 3.4% AR on DAT-123. Total volatiles were 0.1% at DAT-6 increasing to 2.0% AR at DAT-123.
In the NE test systems, the extractable radioactive residues in the soil increased from an average of 95.1% at DAT-0 to 97.7% AR on DAT-6, and then decreasing to 97.0% AR at DAT-123. The unextractable or bound radioactive residues in the soil increased from an average of 0.3% on DAT-0 to 10.7% AR on DAT-123. Total volatiles were 0.1% at DAT-6 increasing to 3.3% AR at DAT-123.

The detailed figures of the radioactivity distribution are presented in Table B.8.1.1.1.2-6.

Table B.8.1.1.1.2-6: Distribution of radioactivity in soils under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

		Sampling times [days]							
		0	**6**	**14**	**21**	**28**	**60[a)]**	**88**	**123**
CA soil									
Volatiles									
carbon dioxide	A	n.a.	0.1	0.1	0.2	0.6	0.4	0.9	1.3
	B	n.a.	0.1	0.1	0.2	0.5	0.5	0.9	2.5
	mean	n.a.	0.1	0.1	0.2	0.5	0.5	0.9	1.9
volatile organic compounds	A	n.a.	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
	B	n.a.	< LOD	0.1	0.1	0.1	< LOD	< LOD	0.1
	mean	n.a.	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
total volatiles	A	n.a.	0.1	0.2	0.2	0.6	0.5	0.9	1.4
	B	n.a.	0.1	0.2	0.2	0.6	0.5	1.0	2.6
	mean	n.a.	0.1	0.2	0.2	0.6	0.5	0.9	2.0
Extractable radioactivity									
ambient extract	A	92.8	95.3	93.7	93.1	92.2	90.7	86.3	86.8
	B	93.4	94.2	96.1	94.9	93.1	91.0	89.8	89.0
	mean	93.1	94.7	94.9	94.0	92.7	90.8	88.0	87.9
aggressive extract	A	1.6	2.3	2.4	3.1	3.1	3.1	3.6	3.7
	B	1.6	2.4	2.5	2.9	3.2	3.4	3.8	3.5
	mean	1.6	2.4	2.4	3.0	3.1	3.3	3.7	3.6
ethyl acetate/ hexane	A	-	-	-	-	-	-	-	0.7
	B	-	-	-	-	-	-	-	0.9
	mean	-	-	-	-	-	-	-	0.8
subtotal extractable	A	94.4	97.6	96.1	96.2	95.3	93.8	89.8	91.1
	B	95.0	96.6	98.6	97.8	96.2	94.4	93.6	93.4
	mean	94.7	97.1	97.4	97.0	95.8	94.1	91.7	92.3
Non-extractable residues	A	0.2	0.5	0.8	0.8	1.0	1.7	2.9	3.4
	B	0.1	0.5	0.6	0.9	0.9	2.0	3.1	3.3
	mean	0.2	0.5	0.7	0.9	1.0	1.9	3.0	3.4
Material balance	A	94.6	98.2	97.1	97.2	97.0	96.0	93.6	95.9
	B	95.2	97.2	99.4	98.9	97.7	97.0	97.7	99.4
	mean	94.9	97.7	98.2	98.1	97.4	96.5	95.6	97.6

a) Only one replicate was analysed for the day 60 interval for NE soil
n.a. not analysed.

Table B.8.1.1.1.2-6 cont

		Sampling times [days]							
		0	**6**	**14**	**21**	**28**	**60[a)]**	**88**	**123**
NE soil									
Volatiles									
carbon dioxide	A	n.a.	0.1	0.3	0.4	0.2	1.2	1.9	3.1
	B	n.a.	0.1	0.2	0.4	0.2	-	2.1	3.3
	mean	n.a.	0.1	0.3	0.4	0.2	1.2	2.0	3.2
volatile organic compounds	A	n.a.	< LOD	0.1	0.1	0.1	< LOD	< LOD	0.1
	B	n.a.	< LOD	0.1	< LOD	0.1	-	< LOD	0.1
	mean	n.a.	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
total volatiles	A	n.a.	0.1	0.4	0.4	0.3	1.3	1.9	3.2
	B	n.a.	0.1	0.3	0.4	0.3	-	2.1	3.3
	mean	n.a.	0.1	0.3	0.4	0.3	1.3	2.0	3.3
Extractable radioactivity									
ambient extract	A	94.5	86.2	90.2	88.5	88.6	83.5	78.1	74.6
	B	89.1	92.6	87.4	89.3	85.6	-	79.3	72.9
	mean	91.8	89.4	88.8	88.9	87.1	83.5	78.7	73.8
aggressive extract	A	2.4	8.3	4.9	6.6	5.8	7.0	6.6	7.8
	B	3.5	4.3	6.6	5.4	5.8	-	6.4	7.1
	mean	3.0	6.3	5.7	6.0	5.8	7.0	6.5	7.5
ethyl acetate/hexane	A	-	-	-	-	-	-	-	2.1
	B	-	-	-	-	-	-	-	1.5
	mean	-	-	-	-	-	-	-	1.8
subtotal extractable	A	96.9	94.5	95.1	95.1	94.4	90.5	84.7	84.5
	B	92.7	96.9	94.0	94.7	91.4	-	85.7	81.6
	mean	94.8	95.7	94.6	94.9	92.9	90.5	85.2	83.0
Non-extractable residues	A	0.3	2.8	1.7	2.6	3.3	6.0	7.9	9.8
	B	0.3	1.0	2.2	2.5	3.1	-	9.0	11.7
	mean	0.3	1.9	2.0	2.5	3.2	6.0	8.4	10.7
Material balance	A	97.2	97.4	97.2	98.1	98.0	97.8	94.5	97.5
	B	93.0	98.0	96.6	97.6	94.8	-	96.7	96.6
	mean	95.1	97.7	96.9	97.8	96.4	97.8	95.6	97.0

a) Only one replicate was analysed for the day 60 interval for NE soil

Composition of residues

The route of degradation of isoflucypram in soil under aerobic conditions is summarised in Table B.8.1.1.1.2-7 and Table B.8.1.1.1.2-8. The proposed degradation of isoflucypram in soil is presented in Figure B.8.1.1.1.2- 2.

Identification and characterisation of degradation products:

- Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide was 1.9 and 3.2% AR at study end (DAT-123) in the CAsoil and the NE soil, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for both soils (Table B.8.1.1.1.2-7 and Table B.8.1.1.1.2-8).

- Test item and degradation products in soil extracts

The amount of isoflucypram in the soil extracts decreased from DAT-0 to DAT-123 from 94.7 to 86.2% AR in CA soil and from 94.8 to 64.4% AR in NE soil.

No major degradation products were formed in the CA soil. However, one minor degradation product

was M12 and increased from 1.1% at DAT-60 to 1.3% AR at DAT-123. One additional unidentified minor degradate was also formed with the maximum amount for a single compound of ≤ 4.0% AR. Total unidentified radioactivity was ≤ 4.0% AR for any interval.
One major degradation product was formed in the NE soil was identified as M12 and it increased from 1.4% AR at DAT-21 to 9.6% AR at DAT-123. Two minor unidentified degradates were formed with the maximum amount for a single compound of ≤ 4.0% AR.
Total unidentified radioactivity was ≤ 7.2% AR for any interval.
The results are summarised in Table B.8.1.1.1.2-7 and Table B.8.1.1.1.2-8.

Table B.8.1.1.1.2-7: Degradation of isoflucypram in CA soil under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		Sampling times [days]							
		0	6	14	21	28	60	88	123
Isoflucypram	A	94.4	97.6	96.1	96.2	95.3	89.1	89.8	84.3
	B	95.0	96.6	98.6	97.8	96.2	91.8	89.7	88.2
	mean	94.7	97.1	97.4	97.0	95.8	90.4	89.8	86.2
M12	A	< LOD	< LOD	< LOD	< LOD	< LOD	2.2	< LOD	2.6
	B	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	1.1	< LOD	1.3
Unknown 1	A	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	B	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Unknown 2	A	< LOD	< LOD	< LOD	< LOD	< LOD	2.6	< LOD	3.6
	B	< LOD	< LOD	< LOD	< LOD	< LOD	2.6	3.9	4.4
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	2.6	1.9	4.0
Unknown 3	A	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	B	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Unidentified radioactivity	A	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.6
	B	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.8
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.7
Total extractable radioactivity	A	94.4	97.6	96.1	96.2	95.3	93.8	89.8	91.1
	B	95.0	96.6	98.6	97.8	96.2	94.4	93.6	93.4
	mean	94.7	97.1	97.4	97.0	95.8	94.1	91.7	92.3
Carbon dioxide	A	n.a.	0.1	0.1	0.2	0.6	0.4	0.9	1.3
	B	n.a.	0.1	0.1	0.2	0.5	0.5	0.9	2.5
	mean	n.a.	0.1	0.1	0.2	0.5	0.5	0.9	1.9
Volatile organics	A	n.a.	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
	B	n.a.	< LOD	0.1	0.1	0.1	< LOD	< LOD	0.1
	mean	n.a.	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
Total volatile	A	n.a.	0.1	0.2	0.2	0.6	0.5	0.9	1.4
	B	n.a.	0.1	0.2	0.2	0.6	0.5	1.0	2.6
	mean	n.a.	0.1	0.2	0.2	0.6	0.5	0.9	2.0
Bound residues	A	0.2	0.5	0.8	0.8	1.0	1.7	2.9	3.4
	B	0.1	0.5	0.6	0.9	0.9	2.0	3.1	3.3
	mean	0.2	0.5	0.7	0.9	1.0	1.9	3.0	3.4
Total recovery	A	94.6	98.2	97.1	97.2	97.0	96.0	93.6	95.9
	B	95.2	97.2	99.4	98.9	97.7	97.0	97.7	99.4
	mean	94.9	97.7	98.2	98.1	97.4	96.5	95.6	97.6

Table B.8.1.1.1.2-8: Degradation of isoflucypram in NE soil under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		Sampling times [days]							
		0	**6**	**14**	**21**	**28**	**60[a)]**	**88**	**123**
Isoflucypram	A	96.9	94.5	95.1	92.3	94.4	86.4	73.7	65.2
	B	92.7	96.9	94.0	94.7	91.4	-	76.3	63.6
	mean	94.8	95.7	94.6	93.5	92.9	86.4	75.0	64.4
M12	A	< LOD	< LOD	< LOD	2.8	< LOD	4.1	7.0	10.1
	B	< LOD	< LOD	< LOD	< LOD	< LOD	-	6.3	9.1
	mean	< LOD	< LOD	< LOD	1.4	< LOD	4.1	6.7	9.6
Unknown 2	A	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	4.0	4.1
	B	< LOD	< LOD	< LOD	< LOD	< LOD	-	3.1	4.0
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.6	4.0
Unknown 3	A	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.0
	B	< LOD	< LOD	< LOD	< LOD	< LOD	-	< LOD	3.4
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.2
Unidentified radioactivity	A	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	2.1
	B	< LOD	< LOD	< LOD	< LOD	< LOD	-	< LOD	1.5
	mean	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.8
Total extractable radioactivity	A	96.9	94.5	95.1	95.1	94.4	90.5	84.7	84.5
	B	92.7	96.9	94.0	94.7	91.4	-	85.7	81.6
	mean	94.8	95.7	94.6	94.9	92.9	90.5	85.2	83.0
Carbon dioxide	A	n.a.	0.1	0.3	0.4	0.2	1.2	1.9	3.1
	B	n.a.	0.1	0.2	0.4	0.2	-	2.1	3.3
	mean	n.a.	0.1	0.3	0.4	0.2	1.2	2.0	3.2
Volatile organics	A	n.a.	< LOD	0.1	0.1	0.1	< LOD	< LOD	0.1
	B	n.a.	< LOD	0.1	< LOD	0.1	-	< LOD	0.1
	mean	n.a.	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
Total volatile	A	n.a.	0.1	0.4	0.4	0.3	1.3	1.9	3.2
	B	n.a.	0.1	0.3	0.4	0.3	-	2.1	3.3
	mean	n.a.	0.1	0.3	0.4	0.3	1.3	2.0	3.3
Bound residues	A	0.3	2.8	1.7	2.6	3.3	6.0	7.9	9.8
	B	0.3	1.0	2.2	2.5	3.1	-	9.0	11.7
	mean	0.3	1.9	2.0	2.5	3.2	6.0	8.4	10.7
Total recovery	A	97.2	97.4	97.2	98.1	98.0	97.8	94.5	97.5
	B	93.0	98.0	96.6	97.6	94.8	-	96.7	96.6
	mean	95.1	97.7	96.9	97.8	96.4	97.8	95.6	97.0

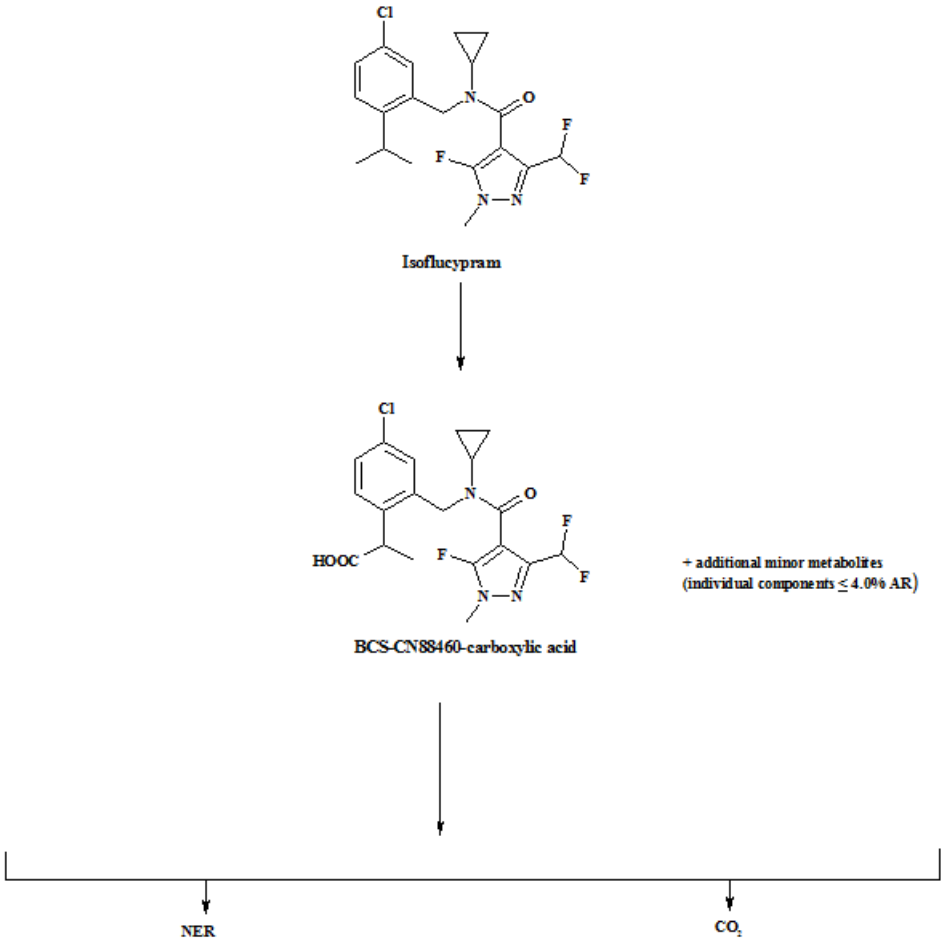
a) Only one replicate was analysed for the day 60 interval, due to miss application.

D. DEGRADATION PATHWAY

Based on the results of the study, the pathway for the degradation of pyrazole-labelled isoflucypram in soil under aerobic conditions is proposed in Figure B.8.1.1.1.2- 1.

Under aerobic soil conditions, *M12* was formed. Furthermore, CO_2 (max 3.3%) and non-extractable residue (max 10.7%) were also formed during this study.

Figure B.8.1.1.1.2-1: Proposed degradation pathway of pyrazole-labelled isoflucypram in soils under aerobic conditions



III. CONCLUSIONS

RMS found the study to be acceptable. An acceptable mass balance was achieved, chromatography was of an acceptable standard such that the endpoints can be relied upon and no deviations from the stated guideline were noted.

Isoflucypram degrades slowly under aerobic conditions in the laboratory. One soil metabolite, *M12*, was isolated and identified from the NE soil extract. This metabolite was formed at a maximum of 9.6% AR in the NE soil but only 1.3% AR in the CA soil. Formation of non-extractable residues (NER) was ≤ 10.7% AR and formation of volatiles was low (≤ 3.3% AR) in both soils, but shows further degradation and mineralisation of isoflucypram is occurring. Similar degradation rates and routes are seen at higher (2x) application rates. The RMS notes that the soils have the same pH; this is not anticipated to have a negative impact on the assessment as pH dependency can be considered alongside the soils in the Pyrazole labelled study (B.8.1.1.1.1), conducted on a wider pH range of soils. A kinetic assessment is provided below in section B.8.1.1.4.1

B.8.1.1.1.3: Aerobic degradation study of isoflucypram

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; Kasel, D.; 2017;
Title: [Phenyl-UL-14C]BCS-CN88460: Aerobic degradation / metabolism in one soil
Report No.: EnSa-16-0986
Document No.: M-599926-01-1
Guideline(s): OECD Test Guideline No. 307, Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009. US EPA OCSPP, Test Guideline No. 835.4100. Japanese MAFF New Test Guidelines Annex No. 2-5-2
Guideline deviation(s): none specified
GLP/GEP: yes

Study Summary
The route and rate of degradation of phenyl-labelled isoflucypram were studied in one soil under aerobic conditions in the dark in the laboratory in the dark at 20 ± 2 °C and 55 ± 5% of the maximum water holding capacity (approximately pF2) for 125 days:

Table B.8.1.1.1.3- 1: Selected soil

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Laacher Hof AXXa	Monheim, Germany	loamy sand	5.8	1.6

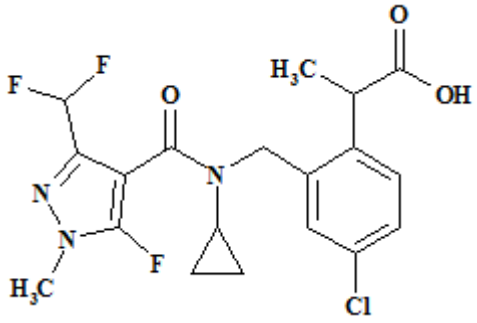
A target study application rate of 200 µg/kg soil dry weight equivalent was based on a maximum single field application rate of isoflucypram of 75 g/ha.

The test was performed in static systems consisting of Erlenmeyer flasks each containing 100 g soil (dry weight equivalents) and equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds. An actual study application rate of 18.8 µg/per unit soil dry weight equivalent was applied.

Duplicate samples were processed and analysed 0, 2, 6, 14, 28, 50, 65, 85, 100 and 125 days after treatment (DAT). At each sampling interval, the soil was extracted three times at ambient temperature using acetonitrile/water 1/1 (*v/v*) at 50°C. Furthermore, two microwave-assisted extraction steps were performed using acetonitrile/water 1/1 (*v/v*) at 70°C and methanol/water 1/1 (*v/v*) at 50°C. The amounts of test item and degradation products in soil extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. Test item identity was confirmed by HPLC-MS(/MS) including accurate mass determination and degradation products were identified by co-chromatography with reference items.
Mean material balances were 103% of the applied radioactivity (AR) (range from 101 to 105% AR).
The maximum amount of carbon dioxide was 5.2% AR at study end (DAT-125). Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals.
Extractable residues decreased from DAT-0 to DAT-125 from 100 to 92.0% AR.
Non-extractable residues (NER) increased from DAT-0 to DAT 125 from 0.7 to 6.4% AR.
The amount of isoflucypram in the soil extracts decreased from DAT-0 to DAT-125 from 100 to 75.5% AR.

Besides the formation of carbon dioxide, *M12* was the only degradation product identified. Its maximum occurrence was 6.2% AR at DAT-125. The total unidentified residues amounted to a maximum of 13.1% AR and no single component exceeded 5.9% AR at any sampling interval.

Table B.8.1.1.1.3- 2: Identified degradation products (maximum occurrence) in soil (in percent of applied radioactivity)

Compound	Chemical structure	Maximum occurrence in soil [%]
*M12 (*BCS-CN88460-carboxylic acid*)*		6.2
CO_2		5.2

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Phenyl-labelled isoflucypram
Sample-ID: KML 10238
Specific activity: 4.13 MBq/mg
Radiochemical purity: > 98% (HPLC with radioactivity detector)
Chemical purity: > 98% (HPLC with UV-detector, 210 nm)

Reference items

unlabelled isoflucypram
Batch-ID: BCS-CN88460-PU-01
Chemical purity: 99.1% (^{1}H-NMR)

unlabelled BCS-CN88460-carboxylic acid *(M12)*
Batch-ID: BCS-CY26497-01-04
Chemical purity: 98.8% (various methods)

2. Test soil

The study was carried out using one soil for the metabolism part and testing of the simplified extraction method (SEM) and one additions soil used for testing of the SEM, only (see Table B.8.1.1.1.3-3). The soils are well characterised and the plant protection product use history of the soils for at least 5 years is known and accepted by RMS with no pesticide usage for the previous 5 years. Soils were stored for 9 days in refrigerated conditions before equilibration.
The soils were collected fresh from the fields (upper horizon of 0 to 20 cm) and sieved to a particle size

of ≤ 2 mm. Soil collection and handling were in accordance to ISO 10381-6[1]. SEM samples were analysed at DAT 0, 65 and 125 only

Table B.8.1.1.1.3-3: Physico-chemical properties of test soils

Parameter	**Results**	
Soil designation	Laacher Hof AXXA[a)]	Dollendorf II[b)]
Geographic location City State Country	 Monheim North Rhine-Westphalia Germany	 Blankenheim North Rhine-Westphalia Germany
Soil series	no information available	no information available
Textural class (USDA) sand [%] (50 µm – 2 mm) silt [%] (2 µm – 50 µm) clay [%] (< 2 µm)	loamy sand 80 16 4	clay loam 28 40 32
pH - in soil/0.01 M $CaCl_2$ 1/2 - in soil/water 1/1 - in saturated paste - in soil/1 N KCl 1/1	5.8 6.1 6.0 5.6	7.1 7.3 7.2 6.9
Organic carbon (combustion) [% OC] Organic matter [c)] [% OM]	1.6 2.8	4.9 8.4
Cation exchange capacity [meq/100 g]	8.5	23.1
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 1/3 bar (pF 2.0) [%]	 49.6 17.7	 77.4 40.0
Bulk density (disturbed) [g/cm^3]	1.19	0.97
Soil microbial biomass [mg microbial C/kg soil DW] DAT-0 BIO- DAT-65 BIO- / BIO+ DAT-125 BIO- / BIO+	 690 500 / 493 401 / 381	 2287 1627 / 1592 1511 / 1400

a) Soil Laacher Hof AXXa was used for the metabolism study and testing of the SEM (simplfied extraction method)
b) Soil Dollendorf II was used for testing of the SEM, only
c) % organic matter = % organic carbon x 1.724
BIO- samples were left untreated
BIO+ samples were applied with solvent of application solution (400 µL methanol)
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

The study was performed with static incubation test systems. Erlenmeyer flasks of 300 mL volume were used as test vessels and each test vessel was fitted with a trap attachment (permeable for oxygen) containing soda lime for absorption of carbon dioxide and a polyurethane (PU) foam plug for adsorption of volatile organic compounds (VOC).

For preparation of the test systems, 100 g dry weight equivalents of the sieved soils were weighed into each test vessel. Soil moisture was adjusted to 55 ± 5% of the maximum water holding capacity (MWHC) for the individual test vessels by addition of de-ionized water, samples were maintained in the dark at 20°C ± 2°C. The test vessels were then fitted with trap attachments.

The untreated test systems were equilibrated to study conditions for 5 days prior to application. Soils were considered by the RMS to meet the OECD 307 recommendation to have a microbial biomass of at least 1% of total organic carbon.

[1] International Organization for Standardization (2009):
ISO 10381-6:2009(E): Soil quality – Sampling – Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory

For the application of the degradation samples the study application rate (SAR) was based on the maximum single field application rate of isoflucypram of 75 g per hectare, resulting in the targeted SAR of 18.8 µg per 100g soil dry weight (400 µl/test system).
The test item was applied dropwise onto the soil surface of the respective test systems using a pipette. After application, the test vessels (except DAT-0 samples) were fitted with trap attachments and placed into a walk-in climatic chamber for incubation.

The degradation product identification samples were prepared to generate larger amounts of degradation products for structure elucidation (2 samples). For this purpose a 3-fold SAR was applied. After application the samples were handled as described for the degradation samples.

2. Sampling

Ten sampling intervals were distributed over the entire incubation period of 125 days. Duplicate samples were processed and analysed 0, 2, 6, 14, 28, 50, 65, 85, 100 and 125 days after treatment (DAT).
Samples for testing of the simplified extraction method were processed and analysed 0, 65 and 125 days after treatment.
Microbial soil biomass was determined at start, middle and end of the study (DAT-0, DAT-65 and DAT-125).

3. Analytical Procedures

Carbon dioxide absorbed by soda lime was liberated with 18% aqueous hydrochloric acid and trapped. The liberated carbon dioxide was purged into the trapping vessels by a stream of nitrogen. The radioactivity contents of these vessels were determined by liquid scintillation counting (LSC) and summed up to determine the total radioactivity liberated from soda lime.

The PU foam plug was extracted with 50 mL ethyl acetate to desorb volatile organic compounds. The radioactivity content was determined by LSC.

The entire soil of each test vessel was transferred into a centrifuge beaker using the extraction solvent. The soil was extracted three times at ambient conditions using a mechanical shaker followed by two accelerated extractions using a microwave with a magnetic stirrer.

The extraction procedure is summarised in the following table:

Table B.8.1.1.1.3-4: Extraction procedure

Solvent	Volume	Minimum duration	Temperature	Cycles
ACN/H_2O 1/1 (*v/v*)	80 mL	30 min, shaking	ambient	3
ACN/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 70°C	1
MeOH/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 50°C	1

After each extraction step, extract and soil were separated by centrifugation and decantation. The volume of the first three ambient extracts was combined and made up to 250 mL while both microwave extracts were filled up to 100 mL each using the respective extraction solvent. The radioactivity content of these extracts was determined by LSC. The exhaustively extracted soils were lyophilised, homogenised by a mortar grinder and non-extractable residues (NER) were determined by combustion/LSC.

SEM samples were used for metabolite generation only. Soil sample received a double rate of test substance and a simplified extraction method was used to extract the metabolites. SEM samples were divided into 5 equal portions, 40 mL of acetonitrile/ water/ acetic acid 400/100/3 (v/v/v) was added and subjected to a microwave extraction, 15 minutes 70°C. Samples were centrifuged and supernatants pooled. Following LSC samples were concentrated and analysed by HPLC. Results of the SEM were

not used for evaluation route and rate calculations.

II. RESULTS AND DISCUSSION

The test systems were incubated under aerobic conditions in the dark in a walk-in climatic chamber at a temperature of 20°C ± 2 °C for 125 days. The test was performed at a soil moisture of 53.0% of the maximum water holding capacity (equivalent to pF2). No significant loss of moisture was observed throughout the study.
Determinations of microbial biomass were performed on DAT-0, DAT-65 and DAT-125 and demonstrated that the used soils were microbially viable.

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean DAT-0 recovery for the test item was 100%. The mean recovery of the concentration procedure for the combined soil extracts was between 98.3%. These results demonstrate that the sample processing method was well suited to recover the applied test item from the soil and that the test item was stable under these conditions.

2. Verification of Chromatographic Procedures

The primary chromatographic method (HPLC/radiodetection) was acceptable for the quantitative analysis of the samples of this study as demonstrated by a mean HPLC recovery 96.3% and a good linear fit for injected amounts of phenyl-labelled isoflucypram on column (R^2 = 0.9999). The LOD of the primary chromatographic method was determined as 7.5 Bq absolute on column or 0.7% AR.

B. MATERIAL BALANCE

Mean material balances were 103% AR (range from 101 to 105% AR) (see Table B.1.1.3-5).
The complete material balances found at all sampling intervals for all soils demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.1.1.1.3-5: Material balance of radioactivity in soils under aerobic conditions from mean values (expressed as percentage of applied radioactivity of two replicates)

Soil	Material balance			
	min.	max.	mean	RSD [%]
Laacher Hof AXXa	101.0	104.5	102.5	1.3

RSD = relative standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.1.1.1.3- 5.
The route of degradation of isoflucypram in soil under aerobic conditions is summarised in Table B.1.1.1.3-6 and B.8.1.1.1.3-7.
The proposed degradation of isoflucypram in soil is presented in Figure B.8.1.1.1.3- 1.

Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide was 5.2% AR at study end (DAT-125) (Table B.8.1.1.1.3-6).
Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals.

Test item and degradation products in soil extracts

Extractable residues decreased from DAT-0 to DAT-125 from 100 to 92.0% AR (Table B.8.1.1.1.3-5).
The amount of isoflucypram in the soil extracts decreased from DAT-0 to DAT-125 from 100 to

75.5% AR. Degradation of isoflucypram was accompanied by the formation of *M12* with 6.2% AR at DAT-125. The total unidentified residues amounted to a maximum of 13.1% AR and no single component exceeded 5.9% AR at any sampling interval.

Non-extractable residues

Non-extractable residues (NER) increased from DAT 0 to DAT 125 from 0.7 to 6.4% AR.

Table B.8.1.1.1.3-6: Material balance of radioactivity in soils under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

		Days after treatment									
		0	**2**	**6**	**14**	**28**	**50**	**65**	**85**	**100**	**125**
Volatiles											
carbon dioxide	A	n.a.	< 0.1	0.2	0.5	1.1	2.4	3.1	2.8	4.1	5.0
	B	n.a.	< 0.1	0.2	0.1	1.1	2.3	2.7	4.3	4.5	5.4
	mean	n.a.	< 0.1	0.2	0.3	1.1	2.3	2.9	3.5	4.3	5.2
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1
total volatiles	A	n.a.	< 0.1	0.3	0.5	1.1	2.4	3.1	2.8	4.3	5.0
	B	n.a.	< 0.1	0.2	0.2	1.2	2.3	2.7	4.3	4.6	5.4
	mean	n.a.	< 0.1	0.2	0.3	1.1	2.4	2.9	3.5	4.4	5.2
Extractable residues											
ambient extract	A	94.5	97.0	95.6	93.4	93.8	89.7	92.3	85.4	88.7	83.4
	B	96.8	97.7	95.9	94.0	92.1	90.1	90.7	85.0	86.6	83.6
	mean	95.6	97.3	95.7	93.7	93.0	89.9	91.5	85.2	87.7	83.5
microwave extract 1	A	3.8	4.4	4.1	4.6	4.4	4.4	4.5	4.8	5.0	6.8
	B	3.4	4.2	3.6	4.4	4.1	4.4	4.8	4.9	5.0	6.2
	mean	3.6	4.3	3.9	4.5	4.3	4.4	4.6	4.8	5.0	6.5
microwave extract 2	A	1.1	1.2	1.4	1.2	1.4	1.3	1.5	1.8	1.8	2.1
	B	1.1	1.1	1.4	1.3	1.3	1.3	1.7	1.9	1.6	2.0
	mean	1.1	1.2	1.4	1.3	1.4	1.3	1.6	1.8	1.7	2.0
total extractable residues	A	99.3	102.6	101.1	99.3	99.6	95.5	98.3	92.0	94.9	92.2
	B	101.3	103.0	100.8	99.7	97.6	95.8	97.2	91.8	93.2	91.8
	mean	100.3	102.8	101.0	99.5	98.6	95.6	97.7	91.9	94.4	92.0
Non-extractable residues	A	0.6	0.9	1.2	1.6	2.2	3.1	3.9	5.6	5.1	6.3
	B	0.7	0.9	1.2	1.5	2.3	3.2	3.8	5.5	5.5	6.6
	mean	0.7	0.9	1.2	1.5	2.2	3.2	3.8	5.6	5.3	6.4
Material balance	A	100.0	103.6	102.5	101.4	102.9	100.9	105.3	100.4	105.0	103.5
	B	102.0	103.9	102.3	101.4	101.0	101.3	103.6	101.6	103.3	103.8
	mean	101.0	103.7	102.4	101.4	102.0	101.1	104.5	101.0	104.1	103.7

n.a.: not analysed

Table B.8.1.1.1.3- 7: Degradation of isoflucypram in soil Laacher Hof AXXa under aerobic conditions (expressed as percentage of applied radioactivity, two replicates)

Compound		Days after treatment									
		0	2	6	14	28	50	65	85	100	125
Isoflucypram	A	99.3	102.6	101.1	97.2	95.4	88.3	88.4	72.6	83.4	75.3
	B	101.3	103.0	100.8	97.9	93.0	88.2	87.6	73.5	79.5	75.8
	mean	100.3	102.8	101.0	97.6	94.2	88.2	88.0	73.0	81.5	75.5
U1	A	n.d.	n.d.	n.d.	< LOD	1.4	2.1	3.2	5.9	4.0	2.9
	B	n.d.	n.d.	n.d.	0.7	1.5	2.1	3.1	6.0	4.1	3.2
	mean	n.d.	n.d.	n.d.	< LOD	1.5	2.1	3.2	5.9	4.0	3.0
M12	A	n.d.	n.d.	n.d.	1.5	2.2	2.5	3.0	6.3	4.0	6.6
	B	n.d.	n.d.	n.d.	1.1	2.2	2.9	3.0	5.3	4.4	5.9
	mean	n.d.	n.d.	n.d.	1.3	2.2	2.7	3.0	5.8	4.2	6.2
Sum of unid./diff. residues*	A	n.d.	n.d.	n.d.	n.d.	n.d.	2.6	3.8	7.2	3.5	7.5
	B	n.d.	n.d.	n.d.	n.d.	0.8	2.5	2.9	7	5.3	6.9
	mean	n.d.	n.d.	n.d.	n.d.	0.4	2.5	3.4	7.1	4.4	7.2
Total extractable residues [a)]	A	99.3	102.6	101.1	98.7	99.0	95.5	98.3	92.1	84.9	92.2
	B	101.3	103.0	100.8	99.7	97.6	95.8	96.6	91.8	93.2	91.8
	mean	100.3	102.8	101.0	99.2	98.3	95.6	97.5	91.9	94.1	92.0
Carbon dioxide [b)]	A	n.a.	< 0.1	0.2	0.5	1.1	2.4	3.1	2.8	4.1	5.0
	B	n.a.	< 0.1	0.2	0.1	1.1	2.3	2.7	4.3	4.5	5.4
	mean	n.a.	< 0.1	0.2	0.3	1.1	2.3	2.9	3.5	4.3	5.2
Volatile organic compounds [b)]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1
Non-extractable residues [b)]	A	0.6	0.9	1.2	1.6	2.2	3.1	3.9	5.6	5.1	6.3
	B	0.7	0.9	1.2	1.5	2.3	3.2	3.8	5.5	5.5	6.6
	mean	0.7	0.9	1.2	1.5	2.2	3.2	3.8	5.6	5.3	6.4
Total recovery [a)]	A	100.0	103.6	102.5	100.7	102.2	100.9	105.3	100.4	104.4	103.5
	B	102.0	103.9	102.2	101.4	101.0	101.3	103.1	101.6	103.2	103.8
	mean	101.0	103.7	102.4	101.1	101.6	101.1	104.2	101.0	103.8	103.7

n.d.: not detected, n.a.: not analysed

a) Difference to material balance values due to rounding errors as well as clean up and chromatographic losses

b) Values taken from material balance

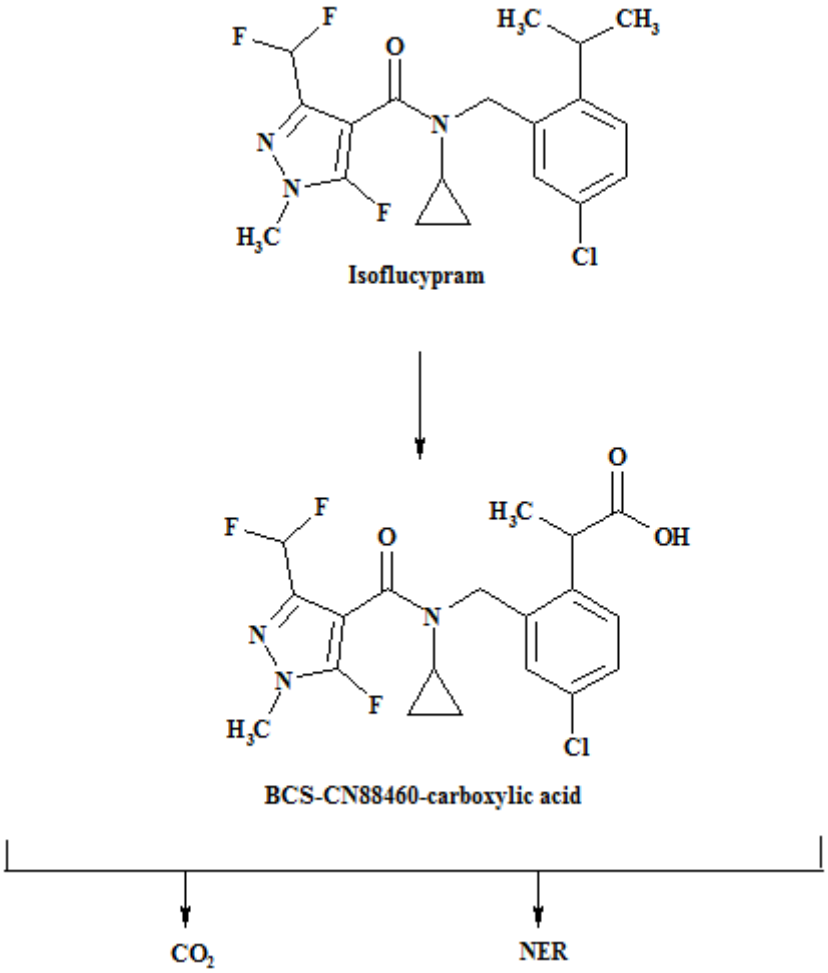
* No individual component above 5% A.R.

D. DEGRADATION PATHWAY

Based on the results of the study, the following pathway for the degradation of phenyl-labelled isoflucypram in soil under aerobic conditions is proposed (see Figure B.8.1.1.1.3- 1), with the following possible processes involved:

- oxidation of isoflucypram to result in M12;
- mineralisation (carbon dioxide formation);
- formation of non-extractable residues (NER).

Figure B.8.1.1.1.3-1: Proposed degradation pathway of phenyl-labelled isoflucypram in soil under aerobic conditions



III. CONCLUSIONS

RMS found the study to be acceptable. An acceptable mass balance was achieved, chromatography was of an acceptable standard such that the endpoints can be relied upon and no deviations from the stated guideline were noted.

Isoflucypram was slowly degraded in soil under aerobic conditions in the laboratory in the dark.

Formation of carbon dioxide was up to 5.2% AR at study end.
Besides the formation of carbon dioxide, M12 was the only degradation product identified. Its maximum occurrence was 6.2% AR at DAT-125.
Formation of non-extractable residues (NER) was up to 6.4% AR at study end, which may be an indication that the limited degradation of isoflucypram observed may have been microbially mediated..
A kinetic assessment of the data is provided in section B.8.1.1.4.1

B.8.1.1.2.: Anaerobic degradation of isoflucypram

The route of degradation of isoflucypram in soil under anaerobic conditions in the laboratory was investigated using the pyrazole-label.
A summary of the route of degradation of isoflucypram in soil is given in this section.

B.8.1.1.2.1: Anaerobic degradation study of isoflucypram

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; Kasel, D.; 2015;
Title: [Pyrazole-4-14C]BCS-CN88460: Anaerobic degradation / metabolism in one soil
Report No.: EnSa-14-0146
Document No.: M-513456-01-1
Guideline(s): OECD Test Guideline No. 307, Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009. US EPA OCSPP, Test Guideline No. 835.4100. Japanese MAFF New Test Guidelines Annex No. 2-5-2
Guideline deviation(s): none specified
GLP/GEP: yes

Study Summary
The route and rate of degradation of pyrazole-labelled isoflucypram were studied in one soil under anaerobic conditions for 120 days, after an aerobic incubation phase of 30 days (total study duration of of 150 days).

Table B.8.1.1.2.1- 1: Selected soil

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Laacher Hof AXXa	Monheim, Germany	loamy sand	6.7	1.6

A study application rate of 200 µg per kg soil dry weight was applied based on a maximum single field application rate of isoflucypram of 75 g per hectare.
The test was performed in static systems consisting of Erlenmeyer flasks each containing 100 g soil (dry weight equivalents) For the aerobic incubation phase the flasks were equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds. For the anaerobic incubation phase, the traps were replaced by gas sampling bags for the collection of volatiles.
After application of the test item, the test systems were incubated under aerobic conditions in the dark at 51.9% of the maximum water holding capacity for 30 days. Then, the soil of each test system was flooded with oxygen-depleted de-ionized water, mimicking a field flooding scenario, and set under an atmosphere of nitrogen to achieve anaerobic conditions.
Duplicate samples were processed and analysed 0 and 30 days after treatment (DAT) during the aerobic incubation phase and at DAT-30, -32, -37, -44, -60, -92, -120 and 150 of the anaerobic incubation phase. The sampling intervals of the anaerobic incubation phase correspond to 0, 2, 7, 14, 30, 62, 90 and 120 days after soil flooding (DASF).

At each sampling interval of the aerobic incubation phase, the soil was extracted three times at ambient temperature using acetonitrile/water 1/1 (*v/v*). Furthermore, two microwave-assisted extraction steps were performed using acetonitrile/water 1/1 (*v/v*) at 70°C and methanol at 50°C.

At each sampling interval of the anaerobic incubation phase (from DASF-0 onwards), the water was separated from the soil by decantation. Afterwards, the soil was extracted as described for the aerobic incubation phase.
The amounts of test item and degradation products in soil extracts and water were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. The test item was identified by HPLC-MS(/MS) including accurate mass determination.
Mean material balance was 101.3% AR (range from 98.6 to 104.5% AR).
The maximum amount of carbon dioxide formed was 0.2 % AR at the end of the aerobic incubation phase (DAT-30) and < 0.1% AR during the entire anaerobic incubation phase. Formation of volatile organic compounds (VOC) during the aerobic and anaerobic incubation phases was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals.
Extractable residues varied between 95.2 to 104.1% AR over the total study duration (150 days).
Non-extractable residues (NER) increased during the aerobic incubation phase from DAT-0 to DAT-30 from 0.4 to 1.5% AR. During the following anaerobic incubation phase, NER increased further until DASF-120 to 4.2% AR.
Within the aerobic incubation phase, the amount of isoflucypram decreased from DAT-0 to DAT-30 from 104.1 to 92.6% AR. During the following anaerobic incubation phase, the amount of isoflucypram varied between 90.3 and 97.6% AR.
Since no degradation products of isoflucypram > 5% AR were found, no identification attempts were made. The total unidentified residues amounted to a maximum of 4.7% AR and no single component exceeded 3.1% AR at any sampling interval.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazole-labelled isoflucypram

Sample-ID:	KML 9710
Specific activity:	4.22 MBq/mg (113.92 µCi/mg)
Radiochemical purity:	> 98% (HPLC with radioactivity detector)
	> 99% (TLC, scan)
Chemical purity:	> 99% (HPLC with UV-detector, 210 nm)

Reference item

Unlabelled isoflucypram

Sample-ID:	BCS-CN88460-01-02
Chemical purity:	> 98% (^{1}H-NMR)

2. Test soil

The study was carried out using one soil considered representative for agricultural soils (see Table B.8.1.1.2.1- 2). No plant protection products had been used on the soil for at least 5 years. The soils were sampled freshly from the field (upper horizon of 0 to 20 cm) and sieved to a particle size of ≤ 2 mm.
The soil microbial biomass was determined at start and end of the aerobic incubation phase. The soil microbial viability was determined at the end of the anaerobic incubation phase (see Table B.8.1.1.2.1- 3 and Table B.8.1.1.2.1- 4, respectively). Soils were considered by the RMS to meet the OECD 307 recommendation to have a microbial biomass of at least 1% of total organic carbon.

Table B.8.1.1.2.1-2: Physico-chemical properties of the test soil

Parameter	Results
Soil designation	Laacher Hof AXXA
Geographic location City State Country	 Monheim North Rhine-Westphalia Germany
Soil taxonomic classification (USDA)	Sandy, mixed, mesic Typic Cambudoll
Soil series	no information available
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	loamy sand 77 16 7
pH - in 0.01 M $CaCl_2$ 1/2 - in water 1/1 - in saturated paste - in soil/1 N KCl 1/1	6.7 7.0 6.9 6.5
Organic carbon (combustion) [% OC] Organic matter [a] [% OM]	1.6 2.8
Cation exchange capacity [meq/100 g]	8.7
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 1/3 bar (pF 2.0) [%]	 44.0 14.2
Bulk density (disturbed) [g/cm^3]	1.25

a) % organic matter = % organic carbon x 1.724

Table B.8.1.1.2.1-3: Determination of microbial biomass (Expressed as mg microbial carbon per kg soil dry weight)

Soil	Sampling date	
	DAT-0[a] BIO-	DAT-30[a] BIO- / BIO+
Laacher Hof AXXa	997	630 / 639

BIO- samples were left untreated
BIO+ samples were applied with solvent of application solution (420 µL methanol)
a) Actual measurement of microbial biomass was performed after 2 and 32 days using DAT-0 and DAT-30 samples, respectively.

Table B.8.1.1.2.1-4: Determination of soil microbial viability during the anaerobic incubation phase (Expressed as colony forming units (CFU) per g soil)

Soil	Dilution	Sampling date DASF-120[a)]	
		BIO- [CFU/g soil dry weight]	BIO+ [CFU/g soil dry weight]
Laacher Hof AXXa	10^{-1}	not countable	not countable
	10^{-2}	not countable	not countable
	10^{-3}	$1.90 * 10^4$	$2.55 * 10^4$
	10^{-4}	$7.33 * 10^4$	$5.00 * 10^4$
	10^{-5}	not countable	$6.70 * 10^4$

BIO- samples were left untreated
BIO+ samples were applied with solvent of application solution (420 µL methanol)
a) Actual measurement of microbial biomass was performed after 157 days using DASF-120 (DAT-150) samples.

B. STUDY DESIGN

1. Experimental Conditions

The study was performed with static incubation test systems. Erlenmeyer flasks of 300 mL volume were used as incubation vessels. For the aerobic incubation phase each test vessel was fitted with a trap attachment (permeable for oxygen) containing soda lime for absorption of carbon dioxide and a polyurethane (PU) foam plug for adsorption of volatile organic compounds (VOC).
For the anaerobic incubation phase the trap attachments were replaced by sealable two-valve glass stoppers connected with gas sampling bags for the collection of volatiles. Additionally, the test systems were placed into a nitrogen flooded box in a walk-in climatic chamber.
For preparation of the test systems, 100 g dry weight equivalents of the sieved soils were weighed into each test vessel. Soil moisture was adjusted to 55% of the maximum water holding capacity (MWHC) for the individual test systems by addition of de-ionized water.
The untreated test systems were equilibrated to study conditions for 3 days prior to application.
The study application rate (SAR) was based on the maximum single field application rate of isoflucypram of 75 g per hectare, resulting in the targeted SAR of 200 µg/kg soil dry weight.
The test item was applied dropwise onto the soil surface of the respective equilibrated test systems using a pipette. After application, the test vessels were fitted with trap attachments and placed into a temperature-controlled walk-in climatic chamber for incubation at 20°C ±2°C in the dark under aerobic conditions. At the end of aerobic phase 150mL of oxygen depleted de-ionised water was added. To remove residual oxygen units were flushed with Argon gas for 2 minutes using a glass tube. With the exception of Days after flooding 0 (DASF) samples, gas trapping bags were used to collect volatile gases. Samples were maintained in a nitrogen atmosphere to ensure anaerobic conditions.

2. Sampling

Two sampling intervals were distributed over the entire aerobic incubation phase of 30 days. Eight sampling intervals were distributed over the entire anaerobic incubation phase of 120 days.
Duplicate samples were processed and analysed 0 and 30 days after treatment (DAT) during the aerobic incubation phase and at DAT-30, -32, -37, -44, -60, -92, -120 and -150 of the anaerobic incubation phase. The sampling intervals of the anaerobic incubation phase correspond to 0, 2, 7, 14, 30, 62, 90 and 120 days after soil flooding (DASF).

3. Analytical Procedures

The amounts of test item and degradation products in soil extracts and water were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. The test item was identified by HPLC-MS(/MS) including accurate mass determination.

Carbon dioxide absorbed by soda lime was liberated with 18% aqueous hydrochloric acid and the liberated carbon dioxide was purged into the trapping vessels by a stream of nitrogen. The radioactivity contents of these vessels were determined by LSC and summed up to determine the total radioactivity liberated from soda lime.

The PU foam plug was extracted with 50 mL ethyl acetate to desorb volatile organic compounds (VOC). The radioactivity content was determined by LSC.
The test vessel with the gas sampling bag was connected to the volatiles. Volatiles present in the gas sampling bag were slowly purged using a stream of nitrogen over a soda lime trap for absorption of carbon dioxide. The radioactivity contents of these vessels were determined by LSC.

After determination of redox potential, pH value and oxygen content, the water was separated from the soil by decantation into a centrifuge beaker. Then, the water was centrifuged. The clear supernatant was decanted and the volume determined. The radioactivity content of the water was determined by LSC. The solids after centrifugation were combined with the soil.

The entire soil of each test vessel was transferred into a centrifuge beaker used for the processing of the water, containing already the solids for the water using the extraction solvent. The soil was extracted three times at ambient temperature using a mechanical shaker followed by two extraction steps using a microwave with a magnetic stirrer.

The extraction procedure is summarised in the following table:

Table B.8.1.1.2.1-5: Extraction procedure

Solvent	Volume	Minimum duration	Temperature	Cycles
ACN/H_2O 1/1 (*v/v*)	80 mL	30 min, shaking	ambient	3
ACN/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 70°C	1
MeOH	80 mL	10 min, stirring	microwave, 50°C	1

After each extraction step, extract and soil were separated by centrifugation and decantation.
Afterwards, the combined ambient extracts were filled up to volumes of 250 mL (aerob samples), 300 mL (anaerobic samples except DASF 62) and 350 mL (DASF 62) using the extraction solvent. The microwave soil extracts were filled up to a volume of 100 mL using the respective extraction solvent. The radioactivity content of these extracts was determined by LSC.
The exhaustively extracted soil was lyophilized, homogenized and NER were determined by combustion/LSC.

II. RESULTS AND DISCUSSION

The test systems were incubated in a walk-in climatic chamber in the dark at 20.4 °C for a total study period of 150 days. The aerobic incubation phase was maintained for 30 days. After forcing the test systems to anaerobic conditions, the anaerobic incubation phase was maintained for 120 days.
The aerobic incubation phase was performed at a soil moisture of 55% of the maximum water holding capacity. No significant loss of moisture was observed throughout the aerobic incubation phase.
The anaerobic incubation phase was performed under flooded conditions with oxygen-depleted de-ionized water.
Determinations of soil microbial biomass or viability were performed at start and end of the aerobic incubation phase and at the end of the anaerobic incubation phase. The results demonstrated that the used soil was microbially viable and that an anaerobic microflora was established in the test systems during the anaerobic incubation phase.
Oxygen contents in the water decreased from a maximum concentration of 8.9 mg/L at DASF-0 to < 1.5

mg/L from DASF-30 onwards. This demonstrated the shift from aerobic to anaerobic conditions.

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean recovery of the test item at DAT 0 was 104.1% AR. The overall mean recovery of the concentration procedures for water and combined soil extracts was 99.3%. These results demonstrate that the sample processing methods were well suited to recover the applied test item from the soil and that the test item was stable under these conditions.

2. Verification of Chromatographic Procedures

The primary chromatographic method (HPLC/radiodetection) was well suited for the quantitative analysis of the samples of this study as demonstrated by a mean HPLC recovery of 96.3% and a good linear fit for injected amounts of pyrazole-labelled isoflucypram on HPLC column ($R^2 > 0.9998$). The LOD of the primary chromatographic method was determined as 9.1 Bq absolute on column or 1.1% AR.

B. MATERIAL BALANCE

Mean material balances was 101.3% AR (range from 98.6 to 104.5% AR) (Table B.1.1.2.1-6).
The complete material balances found at all sampling intervals demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.1.1.2.1-6: Material balance of radioactivity under anaerobic conditions after an aerobic incubation phase (expressed as percentage of applied radioactivity)

Soil	Material balance			
	min.	max.	mean	RSD
Laacher Hof AXXa	98.6	104.5	101.3	2.3

RSD = relative standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.1.1.2.1- 7. The route of degradation of isoflucypram in soil under anaerobic conditions is summarised in Table B.8.1.1.2.1- 8.

Table B.8.1.1.2.1-7: Material balance of radioactivity under anaerobic conditions after an aerobic incubation phase (expressed as percentage of applied radioactivity, two replicates)

		Sampling intervals									
	DAT	0	30	30	32	37	44	60	92	120	150
	DASF	N/A		0	2	7	14	30	62	90	120
Volatiles											
- volatiles of aerobic incubation phase											
carbon dioxide [a)]	A	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	B	n.a.	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	mean	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
volatile organic compounds [a)]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles aerobic phase	A	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	B	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	mean	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

- volatiles of anaerobic incubation phase											
carbon dioxide	A	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	n.d.	< 0.1
	B	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
	mean	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
volatile organic compounds	A	N/A		n.a.	< 0.1	< 0.1	< 0.1	n.d.	< 0.1	< 0.1	< 0.1
	B	N/A		n.a.	< 0.1	n.d.	< 0.1	< 0.1	n.d.	< 0.1	< 0.1
	mean	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles anaerobic phase	A	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
- total carbon dioxide	A	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	B	n.a.	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	mean	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
- total volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
- total volatiles	A	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	B	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	mean	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Extractable residues											
- water	A	N/A		4.4	4.5	4.3	4.2	4.1	4.0	4.0	4.2
	B	N/A		4.4	4.4	4.4	4.2	4.0	4.1	3.7	4.2
	mean	N/A		4.4	4.5	4.4	4.2	4.1	4.1	3.9	4.2
- soil											
ambient extract	A	100.6	91.0	87.0	93.2	86.7	85.9	86.0	82.7	87.4	86.0
	B	99.0	91.6	87.7	93.0	87.0	88.3	85.7	84.2	88.6	86.9
	mean	99.8	91.3	87.3	93.1	86.8	87.1	85.9	83.5	88.0	86.5
microwave extract	A	3.4	4.2	5.1	3.5	4.1	4.6	5.6	6.0	6.3	6.5
	B	3.2	4.1	4.5	3.6	4.3	4.5	5.5	5.6	6.2	6.0
	mean	3.3	4.1	4.8	3.5	4.2	4.6	5.6	5.8	6.2	6.2
microwave extract 2	A	0.9	1.5	1.8	1.3	1.4	1.5	1.6	1.9	2.6	2.0
	B	0.9	1.4	1.7	1.2	1.5	1.8	1.7	2.0	1.8	1.8
	mean	0.9	1.4	1.8	1.2	1.4	1.7	1.7	1.9	2.2	1.9
total soil extractable residues	A	104.9	96.7	93.9	98.0	92.2	92.1	93.2	90.5	96.3	94.5
	B	103.2	97.1	93.8	97.8	92.7	94.6	93.0	91.8	96.5	94.7
	mean	104.1	96.9	93.9	97.9	92.5	93.3	93.1	91.2	96.4	94.6
- total extractable residues	A	104.9	96.7	98.3	102.5	95.5	96.3	97.3	94.5	100.3	98.7
	B	103.2	97.1	98.3	102.2	97.2	98.8	97.0	95.9	100.3	98.9
	mean	104.1	96.9	98.3	102.3	96.8	97.6	97.2	95.2	100.3	98.8
Non-extractable residues	A	0.5	1.6	1.8	1.5	1.7	2.2	2.9	3.8	4.1	4.6
	B	0.4	1.5	1.6	1.3	1.7	2.3	3.0	3.9	4.0	3.9
	mean	0.4	1.5	1.7	1.4	1.7	2.2	3.0	3.8	4.0	4.2
Material balance	A	105.4	98.4	100.2	104.1	98.3	98.7	100.4	98.5	104.6	103.4
	B	103.6	98.8	100.0	103.7	99.0	101.3	100.2	99.9	104.4	103.0
	mean	104.5	98.6	100.1	103.9	98.7	100.0	100.3	99.2	104.5	103.2

N/A: not applicable, n.d.: not detected, n.a.: not analysed, DAT: days after treatment, DASF: days after soil flooding

a) Formation of carbon dioxide and volatile organic compounds of the aerobic incubation phase were determined at DAT-30 and DASF-0 and the mean value was assumed for all other sampling intervals

Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide formed was 0.2% AR at the end of the aerobic incubation phase (DAT-30) and < 0.1% AR during the entire anaerobic incubation phase. Formation of volatile organic compounds (VOC) during the aerobic and anaerobic incubation phases was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals (Table B.1.1.2.1-7 and Table B.1.1.2.1-8).

Test item and degradation products in the entire system

Extractable residues varied between 95.2 to 104.1% AR over the total study duration (150 days).
Within the aerobic incubation phase, the amount of isoflucypram decreased from DAT-0 to DAT-30 from 104.1 to 92.6% AR. During the following anaerobic incubation phase, the amount of isoflucypram varied between 90.3 and 97.6% AR (Table B.1.1.2.1-8).
Since no degradation products of isoflucypram > 5% AR were found, no identification attempts were made. The total unidentified residues amounted to a maximum of 4.7% AR and no single component exceeded 3.1% AR at any sampling interval (Table B.8.1.1.2.1- 8).

Non-extractable residues

Non-extractable residues (NER) increased during the aerobic incubation phase from DAT-0 to DAT-30 from 0.4 to 1.5% AR. During the following anaerobic incubation phase, NER increased further until DASF-120 to 4.2% AR (Table B.1.1.2.1-8).

Table B.8.1.1.2.1-8: Degradation of isoflucypram under anaerobic conditions after an aerobic incubation phase (expressed as percentage of applied radioactivity, two replicates)

Compound		Sampling intervals									
	DAT	0	30	30	32	37	44	60	92	120	150
	DASF	N/A		0	2	7	14	30	62	90	120
Isoflucypram (Water)	A	N/A	N/A	1.9	2.3	1.8	2.2	1.9	1.6	1.4	2.0
	B	N/A	N/A	1.9	1.5	1.8	1.6	1.3	2.1	2.1	1.8
	mean	N/A	N/A	1.9	1.9	1.8	1.9	1.6	1.9	1.7	1.9
Isoflucypram (Soil)	A	104.9	92.9	90.2	95.7	89.7	89.9	90.9	88.0	93.9	92.2
	B	103.2	92.3	90.6	95.6	90.4	92.4	90.8	88.9	94.1	92.3
	mean	104.1	92.6	90.4	95.7	90.1	91.1	90.9	88.5	94.0	92.2
Isoflucypram (entire system)	A	104.9	92.9	92.1	98.0	91.5	92.0	92.9	89.6	95.4	94.2
	B	103.2	92.3	92.5	97.1	92.2	94.0	92.1	91.1	96.1	94.1
	mean	104.1	92.6	92.3	97.6	91.9	93.0	92.5	90.3	95.7	94.2
Sum of unid./diff. residues [a] (entire system)	A	n.d.	3.7	5.3	4.5	4.1	3.2	3.8	1.4	3.2	3.8
	B	n.d.	3.3	4	4.2	3.6	2.8	2.5	4.2	2.8	4.1
	mean	n.d.	3.5	4.7	4.3	3.8	3.0	3.1	2.8	3.0	4.0
Total extractable residues [b] (entire system)	A	104.9	96.7	97.5	102.5	95.7	95.3	96.6	91.0	98.5	98.0
	B	103.2	95.5	96.5	101.3	95.8	96.8	94.6	95.3	99.0	98.2
	mean	104.1	96.1	97.0	101.9	95.7	96.0	95.6	93.2	98.7	98.1
Carbon dioxide [c] (sum aerobic and anaerobic)	A	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	B	n.a.	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	mean	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volatile organic compounds [c] (sum aerobic and anaerobic)	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [c]	A	0.5	1.6	1.8	1.5	1.7	2.2	2.9	3.8	4.1	4.6
	B	0.4	1.5	1.6	1.3	1.7	2.3	3.0	3.9	4.0	3.9
	mean	0.4	1.5	1.7	1.4	1.7	2.2	3.0	3.8	4.0	4.2
Total recovery [b]	A	105.4	98.4	99.4	104.1	97.5	97.6	99.6	94.9	102.8	102.8
	B	103.6	97.2	98.2	102.8	97.6	99.3	97.8	99.4	103.1	102.3
	mean	104.5	97.8	98.8	103.5	97.5	98.5	98.7	97.2	102.9	102.5

n.d.: not detected, n.a.: not analyzed, DAT: days after treatment, DASF: days after soil flooding N/A: not applicable

a) Minor degradates are summed up to sum of unidentified / diffuse residues
b) Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses
c) Values taken from Material Balance

D. DEGRADATION PATHWAY

At all sampling intervals, the amount of isoflucypram extractable from soil was > 90% AR. Therefore, a pathway for the degradation of pyrazole-labelled isoflucypram in soil under anaerobic conditions after an aerobic incubation period cannot be proposed based on the results of the study.

III. CONCLUSIONS

RMS found the study to be acceptable. An acceptable mass balance was achieved, chromatography was of an acceptable standard such that the endpoints can be relied upon and no deviations from the stated guideline were noted.
Isoflucypram was not degraded in soil under anaerobic conditions in the laboratory in the dark. Formation of carbon dioxide during the aerobic and the anaerobic incubation phase was insignificant with values of 0.2% AR and < 0.1% AR, respectively.
Formation of non-extractable residues (NER) was up to 4.2% AR at study end.
No degradation products > 5% AR were formed and consequently the applicant did not identify any degradation products. A kinetic assessment is performed by the RMS is provided below in section B.8.1.1.4.1.

B.8.1.1.3.: Photolytic degradation of isoflucypram

The photolytic route of degradation of isoflucypram in soil under aerobic conditions in the laboratory was investigated using the pyrazole-radiolabel).
A summary of the route of degradation of isoflucypram in soil is given in the introductory section B.8. and Figure B.8.-1.

B.8.1.1.3.1: Photolysis degradation study of isoflucypram

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; 2013;
Title: [Pyrazole-4-14C]BCS-CN88460: Phototransformation on soil
Report No.: EnSa-13-0200
Document No.: M-467307-01-1
Guideline(s): European Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009; SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides; OECD Draft Test Guideline: Phototransformation of Chemicals on Soil Surfaces; US EPA OCSPP Test Guideline No. 835.2410; Canadian PMRA Environmental Chemistry and Fate Guideline DACO 8.2.3.3.1
Guideline deviation(s): none specified
GLP/GEP: yes

Study Summary

The photolytic route and rate of degradation of pyrazole-labelled isoflucypram was studied on one soil under exposure to simulated sunlight and aerobic conditions in the laboratory for 10 days at 20.0°C and a soil moisture of 51.7% of the maximum water holding capacity (53.3% for dark samples) in comparison to samples incubated in the dark.

Table B.8.1.1.3.1- 1: Selected soil

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Laacher Hof AXXa	Monheim, Germany	sandy loam	6.3	1.6

A nominal test concentration of 7.7 µg per test system was applied based on a single field use rate of isoflucypram of 75 g/ha.

The test was performed in static systems consisting of quartz glass vessels each containing 3 g soil (dry weight equivalents), resulting in a soil layer of approximately 3 mm in height, closed by quartz glass lids and equipped with traps for the collection of carbon dioxide and volatile organic compounds. The test systems were continuously exposed to artificial irradiation by a Xenon lamp with a < 290 nm cut-off filter (irradiance of 1307 W/m^2 for range from 300 to 2450 nm). In addition, samples were incubated in the dark.

Duplicate samples were processed and analysed 0, 1, 2, 3, 6, 8, and 10 days after treatment for both irradiated and dark samples. 10 Days of continuous irradiation corresponded to 36.4 solar summer days at Phoenix, Arizona, USA. At each sampling interval, the soil was extracted three times at ambient temperature using ACN / water (1/1, *v/v*), once by microwave-accelerated extraction at 70°C using ACN / water (1/1, *v/v*) and finally once by microwave-accelerated extraction at 50°C using methanol. The amounts of test item and possible degradation products in soil extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues (NER) were determined by LSC and combustion/LSC, respectively. Test item was identified by HPLC and TLC co-chromatography with reference items and by HPLC-MS(/MS) including accurate mass determination.

Mean material balances were 102.4% AR (range of 100.7 to 107.0% AR) for irradiated samples and 102.9% AR (range of 99.3 to 107.5% AR) for dark samples.

The maximum amount of carbon dioxide was 0.2 and < 0.1% AR at study end (DAT-10) in irradiated and dark samples, respectively.

Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for both irradiated and dark samples.

Extractable residues remained constant from DAT-0 to DAT-10 (range 99.3 to 106.7% AR in irradiated samples and 99.1 to 107.4% AR in dark samples. NER increased from < 0.1% AR at DAT-0 to 1.2 and 0.2% AR at DAT-10 in irradiated and dark samples, respectively.

The amount of isoflucypram also remained constant from DAT-0 to DAT-10 (range 96.5 to 106.1% AR in irradiated samples and 97.4 to 107.4% AR in dark samples), indicating neither photolytic degradation nor a significant difference in irradiated and dark samples.

Neither in the irradiated nor in the dark samples degradation products of pyrazole-labelled isoflucypram above the identification triggers were formed in this study. The total unidentified residues amounted to a maximum of 2.8% AR.

It is concluded that the degradation of isoflucypram is driven by microbial degradation under typical conditions in the environment and photodegradation plays no role in the overall fate of isoflucypram.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazole-labelled isoflucypram
Sample-ID: KML 9480
Specific activity: 3.90 MBq/mg (105.34 µCi/mg)
Radiochemical purity: > 99% (HPLC with radioactivity detector)
Chemical purity: > 98% (HPLC with UV-detector, 210 nm)

Reference item

unlabelled isoflucypram
Sample-ID: AZ 18080

2. Test soil

The soil was sampled freshly from the field and sieved to a particle size of ≤ 2 mm. The physico-chemical characteristics are shown in Table B.8.1.1.3.1- 2.

Table B.8.1.1.3.1- 2: Physico-chemical properties of the test soil

Parameter	Results
Soil designation	Laacher Hof AXXA
Geographic location City State Country	 Monheim North Rhine-Westphalia Germany
Soil series	no information available
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	sandy loam 71 18 11
pH - in 0.01 M $CaCl_2$ 1/2 - in water 1/1 - in saturated paste - in soil/1 N KCl 1/1	6.3 6.6 6.6 6.1
Organic carbon (combustion) [% OC] Organic matter [a] [% OM]	1.6 2.8
Cation exchange capacity [meq/100 g]	8.4
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 0.1 bar (pF 2.0) [%]	 50.5 17.1
Bulk density (disturbed) [g/cm^3]	1.22
Soil microbial biomass [mg microbial C/kg soil DW]	1339

a) % organic matter = % organic carbon x 1.724

B. STUDY DESIGN

1. Experimental Conditions

Quartz glass vessels (36 mm inner diameter, 35 mm height, inner surface area 10.2 cm^2) were used as incubation vessels. The upper edge of a vessel is beaded and provided with a ground joint and a glass neck with ground joint is attached to the side of the wall. The ground joint of the upper edge of each

vessel was covered with vacuum grease and each vessel was closed with round quartz glass covers being 3 mm thick (sealed with metallic clips). Additionally, the glass neck of each vessel was closed with a trap attachment (permeable for oxygen), containing soda lime for absorption of carbon dioxide and a polyurethane (PU) foam plug for adsorption of volatile organic compounds.
The photolysis test systems were placed in a Suntest® unit containing a xenon lamp simulating natural sunlight. The light emission was filtered with a 290 nm cut-off UV-filter, which eliminated all wavelengths < 290 nm. The temperature inside the Suntest® unit was maintained by a cooling plate connected to a cryostat unit. The intensity of the xenon lamp was determined at the beginning and the end of the overall test period.
For preparation of the test systems, 3 g dry weight equivalents of the sieved soil were weighed into each test vessel. Soil moisture was adjusted to 55% of the maximum water holding capacity (MWHC) for the individual test systems by addition of de-ionized water. The test vessels were then closed with quartz glass covers and fitted with trap attachments.

All test systems were incubated in the same Suntest® to guarantee the same light intensity for all samples.
50 µL of the application solutions were applied dropwise onto the soil surface of the respective test systems using a pipette to obtain the nominal test item concentration of 7.7 µg per test system (based on the single field use rate of isoflucypram of 75 g/ha. All test vessels were left open for 15 minutes to facilitate the evaporation of application solvent methanol.
After evaporation of the application solvent, the test vessels (except DAT-0 samples) were closed with quartz glass covers, weighed and fitted with trap attachments. The irradiated samples were placed into the Suntest® unit for irradiation and the dark samples in a temperature-controlled (irradiated: 20.0°C, dark: 19.6°C) walk-in climatic chamber.

2. Sampling

Seven sampling intervals were distributed over the entire incubation period of 10 days. Duplicate samples were processed and analysed after 0, 1, 2, 3, 6, 8, and 10 days of incubation for both irradiated and dark samples. Samples were processed and analysed within 1 day.

3. Analytical Procedures

Carbon dioxide absorbed by soda lime was liberated with 18% aqueous hydrochloric acid and trapped. The liberated carbon dioxide was purged into the trapping vessels by a stream of nitrogen. The radioactivity contents of these vessels were determined by liquid scintillation counting (LSC) and summed up to determine the total radioactivity liberated from soda lime.

The PU foam plug was extracted with 5 mL ethyl acetate to desorb volatile organic compounds. The radioactivity content was determined by LSC.

The entire soil of each test vessel was transferred into a centrifuge beaker using the first extraction solvent. The soil was extracted three times at ambient conditions using a mechanical shaker followed by two accelerated extraction steps using a microwave with a magnetic stirrer.

The extraction procedure is summarised in the following table B.8.1.1.3.1-3:

Table B.8.1.1.3.1- 3: Extraction procedure

Solvent	Volume	Minimum duration	Temperature	Cycles
ACN/H_2O 1/1 (*v/v*)	10 mL	30 min, shaking	ambient	3
ACN/H_2O 1/1 (*v/v*)	10 mL	10 min, stirring	microwave, 70°C	1
Methanol	10 mL	10 min, stirring	microwave, 50°C	1

After each extraction step, extract and soil were separated by centrifugation and decantation.
The volumes of the combined ambient soil extracts and the microwave soil extracts were determined

separately. The radioactivity content of these extracts was determined by LSC. The exhaustive extracted soils were air-dried and NER were determined by combustion/LSC.

The test item was identified by HPLC and TLC co-chromatography with reference items and by HPLC-MS(/MS) including accurate mass determination.

II. RESULTS AND DISCUSSION

The irradiated and dark test systems were incubated in a Suntest® unit exposed to simulated sunlight and in a walk-in climatic chamber in the dark, respectively, under aerobic conditions at 20.0°C (dark test systems: 19.6°C) for 10 days. The average irradiance of irradiated samples was 814 W/m^2).
The test was performed at soil moistures of 51.7% and 53.3% of the maximum water holding capacity in irradiated and dark samples, respectively (see Table B.8.1.1.3.1-4). No significant loss of moisture was observed throughout the study.

Table B.8.1.1.3.1-4: Soil moisture during study incubation

Samples	Soil moistures [% MWHC]		
	mean	min	max
Irradiated	51.7	48.4	55.0
Dark	53.3	48.4	55.0

Determination of the microbial biomass demonstrated that the used soil was microbial viable (see Table B.8.1.1.3.1- 2).

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean DAT-0 recovery for the test item was 100.7% AR. The concentration recovery for the combined soil extracts was 96.9% AR. These results demonstrate that the sample processing method was well suited to recover the applied test item from the soil and that the test item was stable under these conditions.

2. Verification of Chromatographic Procedures

The primary chromatographic method (HPLC/radiodetection) was well suited for the quantitative analysis of the samples of this study as demonstrated by a HPLC recovery of 99.2% and a good linear fit for injected amounts of pyrazole-labelled isoflucypram on HPLC column (R^2 > 0.9999). The LOD of the primary chromatographic method was determined as 15.6 Bq absolute on column or 0.6% AR.

B. MATERIAL BALANCE

Mean material balances were 102.4% AR (range of 100.7 to 107.0% AR) for irradiated samples and 102.9% AR (range of 99.3 to 107.5% AR) for dark samples (Table B.8.1.1.3.1-5).

The complete material balances found at all sampling intervals for both irradiated and dark samples demonstrated that no significant portion of radioactivity dissipated from the test systems or was lost during sample processing.

Table B.8.1.1.3.1- 5: Material balance of radioactivity in irradiated and dark samples (expressed as percentage of applied radioactivity, mean of two replicates)

Soil	Samples	Material balance				
		min.	max.	mean	SD	RSD
Laacher Hof AXXa	irradiated	100.7	107.0	102.4	2.1	2.1%
	dark	99.3	107.5	102.9	2.9	2.8%

SD: standard deviation, RSD: relative standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.1.1.3.1- 6.

The route of degradation of isoflucypram in soil under aerobic irradiated and dark conditions is summarised in Table B.8.1.1.3.1- 7.

Table B.8.1.1.3.1- 6: Material balance of radioactivity in irradiated and dark samples (expressed as percentage of applied radioactivity, two replicates)

		DAT 0	1	2	3	6	8	10
Irradiated samples								
Volatiles								
carbon dioxide	A	n.a.	< 0.1	< 0.1	0.1	0.2	0.1	0.2
	B	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.2	0.2
	mean	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.2
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	A	n.a.	< 0.1	< 0.1	0.1	0.2	0.1	0.2
	B	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.2	0.2
	mean	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.2
Extractable residues								
ambient extract	A	102.3	100.7	106.2	99.5	98.6	98.7	98.4
	B	98.4	102.4	105.3	102.9	98.7	96.5	98.2
	mean	100.3	101.5	105.8	101.2	98.6	97.6	98.3
microwave extract 1	A	0.2	0.6	0.7	1.1	1.5	1.4	1.1
	B	0.2	0.7	0.8	1.1	0.9	1.3	1.3
	mean	0.2	0.6	0.7	1.1	1.2	1.4	1.2
microwave extract 2	A	0.1	0.2	0.2	0.4	0.3	0.3	0.4
	B	0.1	0.2	0.2	0.3	0.2	0.3	0.5
	mean	0.1	0.2	0.2	0.3	0.3	0.3	0.5
total extractables	A	102.6	101.5	107.1	101.0	100.5	100.4	99.9
	B	98.7	103.2	106.3	104.2	99.7	98.2	100.0
	mean	100.6	102.4	106.7	102.6	100.1	99.3	99.9
Non-extractable residues	A	< 0.1	0.3	0.3	0.8	1.3	1.3	1.0
	B	< 0.1	0.3	0.3	0.6	0.6	1.2	1.4
	mean	< 0.1	0.3	0.3	0.7	0.9	1.2	1.2

Material balance	A	102.6	101.4	107.4	101.9	101.9	101.8	101.1
	B	98.7	103.1	106.1	104.2	100.4	99.5	101.6
	mean	100.6	102.3	106.8	103.1	101.2	100.7	101.3
Dark samples								
Volatiles								
carbon dioxide	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Extractable residues								
ambient extract	A	102.3	102.7	106.9	105.2	102.0	97.4	101.5
	B	98.4	101.8	106.4	106.2	97.0	99.0	100.7
	mean	100.3	102.2	106.6	105.7	99.5	98.2	101.1
microwave extract 1	A	0.2	0.4	0.5	0.5	0.9	0.8	0.7
	B	0.2	0.4	0.7	0.5	0.7	0.8	0.8
	mean	0.2	0.4	0.6	0.5	0.8	0.8	0.8
microwave extract 2	A	0.1	0.1	0.1	0.1	0.2	0.1	0.2
	B	0.1	0.1	0.1	0.2	0.2	0.1	0.2
	mean	0.1	0.1	0.1	0.2	0.2	0.1	0.2
total extractables	A	102.6	103.2	107.5	105.8	103.0	98.3	102.5
	B	98.7	102.3	107.3	106.9	97.9	99.9	101.8
	mean	100.6	102.7	107.4	106.4	100.5	99.1	102.1
Non-extractable residues	A	< 0.1	0.1	0.1	0.2	0.2	0.2	0.3
	B	< 0.1	0.1	0.1	0.2	0.2	0.2	0.2
	mean	< 0.1	0.1	0.1	0.2	0.2	0.2	0.2
Material balance	A	102.6	103.3	107.7	106.0	102.2	97.5	102.7
	B	98.7	102.4	107.4	107.1	97.2	99.1	102.0
	mean	100.6	102.8	107.5	106.6	99.7	98.3	102.4

n.d.: not detected; n.a.: nota analysed; DAT: days after treatment. Totals are subject to rounding errors.

Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide was 0.2 and < 0.1% AR at study end (DAT-10) in irradiated and dark samples, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for both irradiated and dark samples (Table B.8.1.1.3.1- 7).

Test item and degradation products in soil extracts

Extractable residues remained constant from DAT-0 to DAT-10 (range 99.3 to 106.7% AR in irradiated samples and 99.1 to 107.4% AR in dark samples.

The amount of isoflucypram also remained constant from DAT-0 to DAT-10 (range 96.5 to 106.1% AR in irradiated samples and 97.4 to 107.4% AR in dark samples), indicating neither photolytic degradation nor a significant difference in irradiated and dark samples.

Neither in the irradiated nor in the dark samples degradation products of pyrazole-labelled isoflucypram above the identification triggers were formed in this study. The total unidentified residues amounted to a maximum of 2.8% AR (Table B.8.1.1.3.1- 7).

Non-extractable residues

Non-extractable residues (NER) increased from < 0.1% AR at DAT-0 to 1.2 and 0.2% AR at DAT-10 in irradiated and dark samples, respectively (Table B.8.1.1.3.1- 7).

Table B.8.1.1.3.1-7: Degradation of isoflucypram in irradiated and dark samples (expressed as percentage of applied radioactivity, two replicates)

Compound	Samples	DAT						
		0	1	2	3	6	8	10
irradiated								
Isoflucypram	A	102.6	101.1	106.4	100.1	98.7	98.2	98.4
	B	98.7	102.8	105.8	103.6	98.8	94.8	97.5
	mean	100.6	102.0	106.1	101.8	98.8	96.5	97.9
Sum of unid./diff. residues [a]	A	n.d.	< LOD	0.7	0.9	1.8	2.2	1.5
	B	n.d.	< LOD	< LOD	< LOD	0.9	3.5	2.5
	mean	n.d.	< LOD	< LOD	< LOD	1.3	2.8	2.0
Total extractable residues [b]	A	102.6	101.1	107.1	101.0	100.5	100.4	99.9
	B	98.7	102.8	105.8	103.6	99.7	98.2	100.0
	mean	100.6	102.0	106.5	102.3	100.1	99.3	99.9
Carbon dioxide [c]	A	n.a.	< 0.1	< 0.1	0.1	0.2	0.1	0.2
	B	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.2	0.2
	mean	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.2
Volatile organic compounds [c]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [c]	A	< 0.1	0.3	0.3	0.8	1.3	1.3	1.0
	B	< 0.1	0.3	0.3	0.6	0.6	1.2	1.4
	mean	< 0.1	0.3	0.3	0.7	0.9	1.2	1.2
Total recovery [b]	A	102.6	101.4	107.4	101.9	101.9	101.8	101.1
	B	98.7	103.1	106.1	104.2	100.4	99.5	101.6
	mean	100.6	102.3	106.8	103.1	101.2	100.7	101.3
dark								
Isoflucypram	A	102.6	103.2	107.5	105.8	102.0	96.7	101.1
	B	98.7	102.3	107.3	106.9	97.0	98.2	100.2
	mean	100.6	102.7	107.4	106.4	99.5	97.4	100.7
Sum of unid./diff. residues [a]	A	n.d.	n.d.	n.d.	n.d.	< LOD	0.6	1.3
	B	n.d.	n.d.	n.d.	n.d.	< LOD	0.6	1.5
	mean	n.d.	n.d.	n.d.	n.d.	< LOD	0.6	1.5
Total extractable residues [b]	A	102.6	103.2	107.5	105.8	102.0	97.3	102.5
	B	98.7	102.3	107.3	106.9	97.0	98.8	101.8
	mean	100.6	102.7	107.4	106.4	99.5	98.1	102.1
Carbon dioxide [c]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Volatile organic compounds [c]	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues [c]	A	< 0.1	0.1	0.1	0.2	0.2	0.2	0.3
	B	< 0.1	0.1	0.1	0.2	0.2	0.2	0.2
	mean	< 0.1	0.1	0.1	0.2	0.2	0.2	0.2
Total recovery [b]	A	102.6	103.3	107.7	106.0	102.2	97.5	102.7
	B	98.7	102.4	107.4	107.1	97.2	99.1	102.0
	mean	100.6	102.8	107.5	106.6	99.7	98.3	102.4

n.d.: not detected, n.a.: not analysed, DAT: days after treatment

a) Minor degradates are summed up to unidentified residues

b) Difference to material balance values due to rounding errors as well as clean up and chromatographic losses

c) Values taken from material balance

D. KINETIC ANALYSIS OF DATA

The amount of isoflucypram effectively remained constant from DAT-0 to DAT-10 (range 96.5 to 106.1% AR in irradiated samples and 97.4 to 107.4% AR in dark samples), suggesting neither photolytic degradation nor a significant difference in irradiated and dark samples. Thus kinetic analysis was not performed.

III. CONCLUSIONS

RMS found the study to be acceptable. An acceptable mass balance was achieved, chromatography was of an acceptable standard such that the endpoints can be relied upon and no deviations from the stated guideline were noted.

The amount of isoflucypram effectively remained constant from DAT-0 to DAT-10 (range 96.5 to 106.1% AR in irradiated samples and 97.4 to 107.4% AR in dark samples), suggesting neither photolytic degradation nor a significant difference in irradiated and dark samples. A kinetic assessment performed by the RMS is provided in section B.8.1.1.4.1 below.
The study suggests that photodegradation will not play a significant role in the overall fate of isoflucypram in soil.

B.8.1.1.4.: Kinetic assessment of the laboratory degradation studies of isoflucypram

The degradation behaviour of isoflucypram in soil was investigated under aerobic and anaerobic conditions in the laboratory as well as under field conditions. The route and rate of degradation of isoflucypram under laboratory aerobic conditions was investigated in three different studies (Hellpointner, E.; Junge, T.; (2014), section B.8.1.1.1.1; Gabbert, D.; McConnell, L. L.; Arthur, E. L.; (2017) section B.8.1.1.1.2; Heinemann, O.; Kasel, D.; (2017) section B.8.1.1.1.3. The kinetic models and DT_{50} values in soil of isoflucypram and its major degradation product in soil under laboratory conditions are summarised in sections B.8.1.1.4.1.

Modelling input values for the calculation of predicted environmental concentrations of isoflucypram and its major soil and aquatic degradation product in soil (PEC_{soil}), groundwater (PEC_{gw}) and surface water (PEC_{sw}) were derived from studies and kinetic evaluations (according to FOCUS (2006[1]/2014[2]) and EFSA (2014[3]) summarised in the following section in Dossier.

The DT_{50} values and maximum occurrences / formation fractions in soil and aquatic systems of isoflucypram and its major degradation product are listed below.

B.8.1.1.4.1: A Kinetic assessment study for the laboratory degradation studies of isoflucypram

Previous evaluation:	None, new active substance.

[1] FOCUS kinetics (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 2.0, 434 pp.

[2] FOCUS, 2014: Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, Version: 1.1; Date: 18 December 2014

[3] EFSA, 2014: Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil, European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2014;12(5):3662

Aerobic Laboratory Degradation in soil

Kinetic assessments for the aerobic degradation of Isoflucypram were conducted within the individual studies supplied (Hellpointner, E.; Junge, T.; (2014), section B.8.1.1.1.1; Gabbert, D.; McConnell, L. L.; Arthur, E. L.; (2017) section B.8.1.1.1.2; Heinemann, O.; Kasel, D.; (2017). DT_{50} values have been summarised within this section and not under each final report. A summary of the percent Applied Radioactivity (A.R.) is listed in tables B.8.1.1.4.1-1 to B.8.1.1.4.1-1

Table B.8.1.1.4.1-1: Summary table for Percent of applied radioactivity from the Hellpointner, E.; Junge, T.; (2014), study. Isoflucypram and its major metabolite.

Soil	Soil Type	Compound		DAT 0*	2	6	15	28	50	62	84	104	120
Hanscheider Hof	Loam	Isoflucypram	A	100.2	98.2	95.6	95.4	92.9	91.5	90.2	86.3	82.8	83.8
			B	100.9	96.4	99.0	96.2	94.5	93.0	89.8	86.2	81.6	81.3
		M12	A	n.d.	n.d.	n.d.	1.0	1.5	1.6	1.4	2.1	2.4	2.3
			B	n.d.	n.d.	n.d.	0.6	1.2	1.7	2.1	2.3	2.9	2.6
Laacher Hof AXXa	Loamy sand	Isoflucypram	A	101.0	96.9	94.5	93.8	89.0	85.3	81.4	78.1	74.3	69.8
			B	101.3	96.9	96.8	94.1	91.8	84.3	80.6	75.3	70.7	70.4
		M12	A	n.d.	n.d.	n.d.	1.3	1.8	3.1	3.2	3.8	4.8	4.8
			B	n.d.	n.d.	n.d.	1.3	2.1	3.3	3.3	4.3	5.0	5.9
Hoefchen Am Hohenseh	Silt loam	Isoflucypram	A	99.5	95.3	97.3	94.2	89.8	90.0	84.3	85.0	80.7	77.3
			B	100.4	94.3	95.6	93.7	92.4	89.1	85.9	79.9	76.5	77.0
		M12	A	n.d.	n.d.	0.8	0.9	1.5	1.3	1.8	2.2	1.4	1.7
			B	n.d.	n.d.	0.4	0.9	1.1	1.4	1.2	1.4	1.4	1.5
Dollendorf II	Loam	Isoflucypram	A	100.1	94.1	94.5	93.0	84.6	83.3	82.2	80.6	72.1	71.9
			B	97.8	95.8	94.7	93.2	89.6	87.0	82.5	75.1	66.5	72.4
		M12	A	n.d.	n.d.	1.0	1.5	2.0	2.1	2.2	2.7	4.7	2.6
			B	n.d.	n.d.	1.1	1.1	1.9	2.8	2.5	3.1	7.0	2.4

* Total mass balance value

Table B.8.1.1.4.1-2: Summary table for Percent of applied radioactivity from the Gabbert, D.; McConnell, L. L.; Arthur, E. L.; (2017), study. Isoflucypram and its major metabolite.

Soil	Soil Type	Compound		DAT 0*	6	14	21	28	60	88	123	
CA	Sandy loam	Isoflucypram	A	94.6	97.6	96.1	96.2	95.3	89.1	89.8	84.3	
			B	95.2	96.6	98.6	97.8	96.2	91.8	89.7	88.2	
		M12	A	< LOD	< LOD	< LOD	< LOD	< LOD	2.2	< LOD	2.6	
			B	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	
NE	Silty clay loam	Isoflucypram	A	97.2	94.5	95.1	92.3	94.4	86.4	73.7	65.2	
			B	93.0	96.9	94.0	94.7	91.4	-	76.3	63.6	
		M12	A	< LOD	< LOD	< LOD	2.8	< LOD	4.1	7.0	10.1	
			B	< LOD	< LOD	< LOD	< LOD	< LOD	-	6.3	9.1	

* Total mass balance value

Table B.8.1.1.4.1-3: Summary table for Percent of applied radioactivity from the Heinemann, O.; Kasel, D.; (2017), study. Isoflucypram and its major metabolite.

Soil	Soil Type	Compound		DAT 0*	2	6	14	28	50	65	85	100	125
Laacher Hof AXXa	Loam sand	Isoflucypram	A	100.0	102.6	101.1	97.2	95.4	88.3	88.4	72.6	83.4	75.3
			B	102.0	103.0	100.8	97.9	93.0	88.2	87.6	73.5	79.5	75.8
		M12	A	n.d.	n.d.	n.d.	1.5	2.2	2.5	3.0	6.3	4.0	6.6
			B	n.d.	n.d.	n.d.	1.1	2.2	2.9	3.0	5.3	4.4	5.9

* Total mass balance value

RMS has evaluated the kinetic assessments within the individual study reports. The study authors' kinetic assessments were performed removing some data points as outliers. Sufficient justification had not been provided in either the final reports or in the applicant kinetic summary as to why these data are considered to be outliers and should be removed as either artefacts of experimental error or genuine outliers.

The RMS reperformed the kinetic calculations using the model CAKE v 3.2. SFO and FOMC models were run initially to check acceptability for modelling and persistence endpoints. UKRMS cannot see sufficient justification for the removal of any data points, therefore the data as provided in tables B.8.1.1.4.1-1 to B.8.1.1.4.1-3 have been used as the basis of the kinetic calculations. The RMS assessment is supplied below for the generation of modelling and persistence endpoints.

For the Hellpointner (2014) aerobic soil degradation study, a kinetic assessment was performed by the RMS according to FOCUS (2006). Isoflucypram and M12 were assessed together as sufficient M12 data points were available. No data points were removed by the RMS. Results of the SFO assessment are presented in table B.8.1.1.4.1-4 and the FOMC in table B.8.1.1.4.1-5. SFO fits were found to be acceptable by the RMS for modelling endpoints. Chi^2 values are low, visual fits are acceptable for both parent and metabolites and little difference between SFO and FOMC is observed. SFO fits are also accepted for persistence/ triggering assessments as FOMC fits are not a significant improvement on SFO. Copies of the SFO fit graphs and residual plots are provided in figures B.8.1.1.4.1-1 to B.8.1.1.4.1-4. Metabolite fits are provided individually on a different scale for clarity.

Table B.8.1.1.4.1-4: Kinetic assessment results from Hellpointner (2014), Single First Order.

		Soil name							
modelling endpoint assessment		Hanscheider Hof –		Laacher Hof AXXa		Hoefchen Am Hohenseh		Dollendorf II	
		Isoflucypram	M12	Isoflucypram	M12	Isoflucypram	M12	Isoflucypram	M12
Parameters	Model	SFO							
	Pini (%)	98.7	n/a	98.4	n/a	97.4	n/a	96.62	n/a
	K	0.002	0.015	0.003	0.006	0.002	0.045	0.003	0.014
	Formation fraction	n/a	0.32	n/a	0.26	n/a	0.42	n/a	0.31
Statistics	χ^2 (%)	0.699	9.4	0.999	8.06	0.851	9.55	1.72	32.5*
	t-test (P value)	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.06
	Dt_{50} (days)	438	48.1	236	108	347	15.5	256	48.5
	Dt_{90} (days)	1450	160	782	357	1150	51.3	849	161
	Visual Fit	Excellent	Good	Excellent	Excellent	Excellent	Excellent	Excellent	Good

* High Chi^2 may be due to low concentrations and associated variability

Table B.8.1.1.4.1-5: Kinetic assessment results from Hellpointner (2014), First Order Multi Compartment.

Persistence endpoint assessment		Soil name							
		Hanscheider Hof –		Laacher Hof AXXa		Hoefchen Am Hohenseh		Dollendorf II	
		Isoflucypram	M12	Isoflucypram	M12	Isoflucypram	M12	Isoflucypram	M12
Parameters	Model	FOMC isoflucypram , SFO M12							
	Pini (%)	98.7	n/a	98.68	n/a	97.57	n/a	96.96	n/a
	α	3.101	n/a	1.041	n/a	4.237	n/a	1.172	n/a
	β	1900	n/a	305.9	n/a	2070	n/a	380.2	n/a
	Formation fraction	n/a	0.34	n/a	0.5	n/a	0.35	n/a	0.26
Statistics	χ^2 (%)	0.931	12	0.498	7.99	1.21	10.3	1.79	32.5
	t-test (P value)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Dt_{50} (days)	477	51.6	289	198	368	16.5	307	65
	Dt_{90} (days)	2100	171	2490	657	1490	54.7	2330	216
	$Dt_{90/3.32}$ (days)	476	n/a	748	n/a	450	n/a	702	n/a
	Visual Fit	Excellent	Good	Excellent	Excellent	Excellent	Excellent	Good	Good

Figure B.8.1.1.4.1-1. Fit graph and residuals for parent and metabolite Hanscheider Hof – soil (SFO).

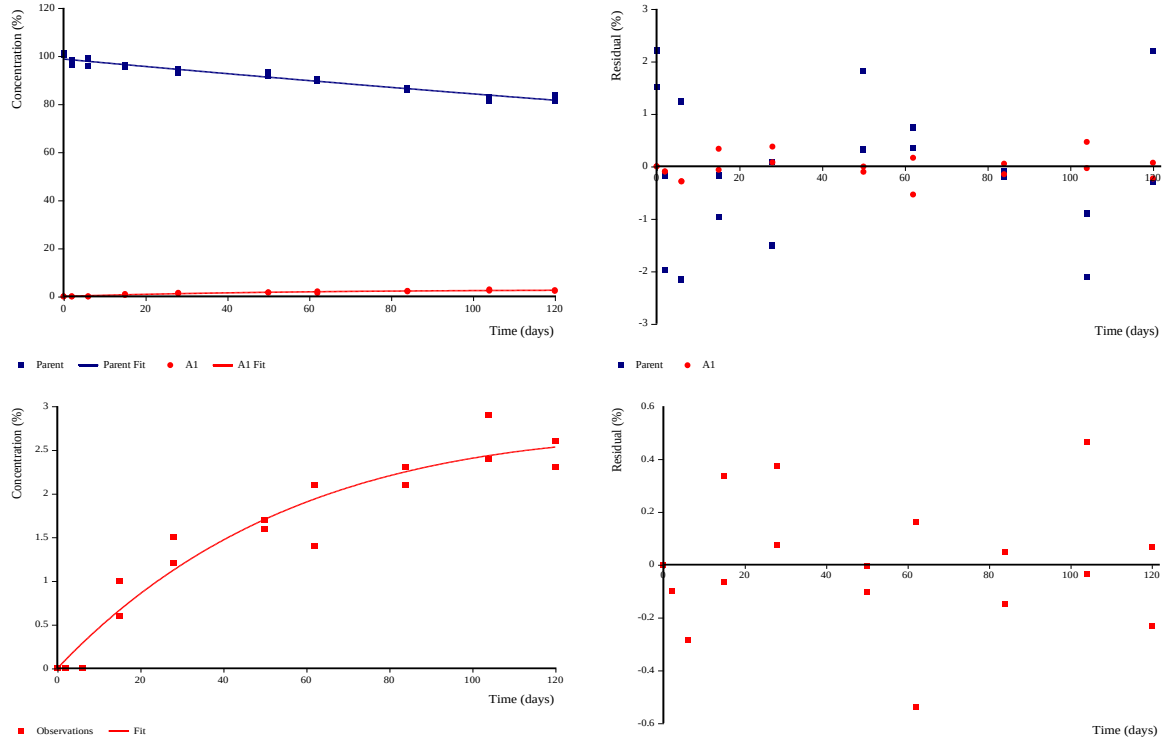


Figure B.8.1.1.4.1-2. Fit graph and residuals for parent and metabolite Laacher Hof AXXa – soil (SFO).

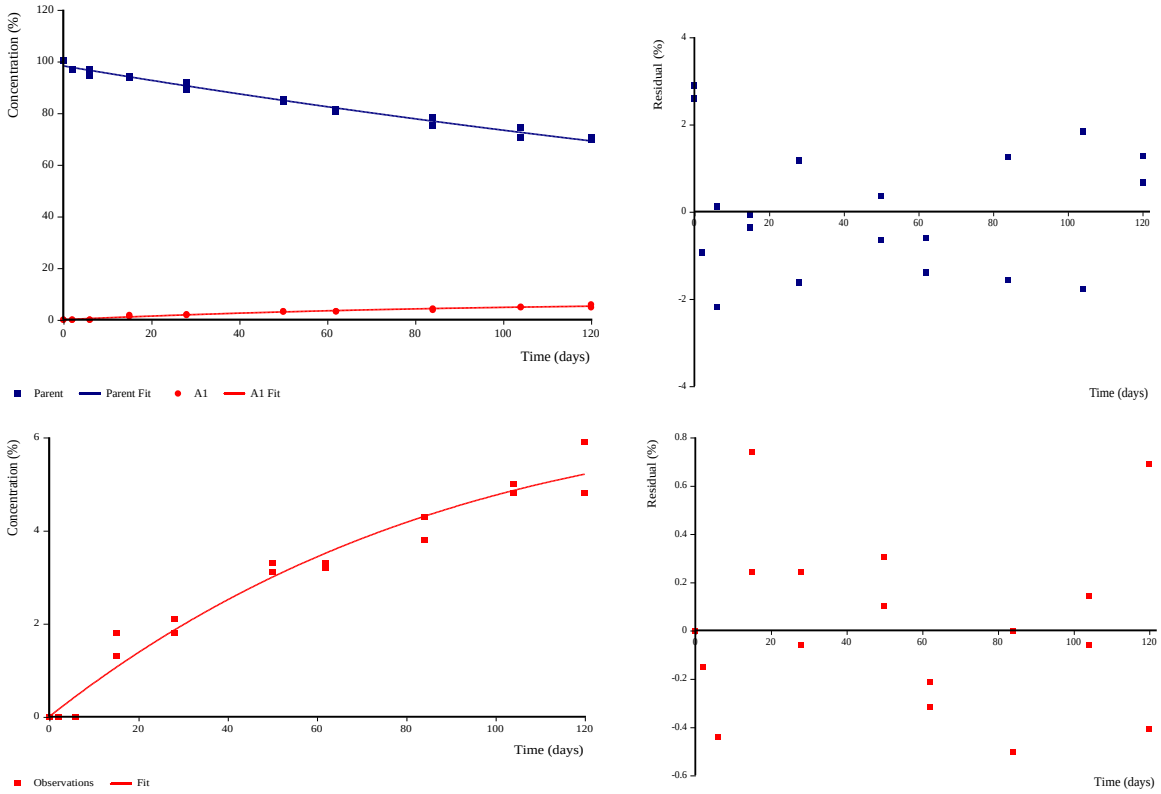


Figure B.8.1.1.4.1-3. Fit graph and residuals for parent and metabolite Hoefchen Am Hohenseh – soil (SFO).

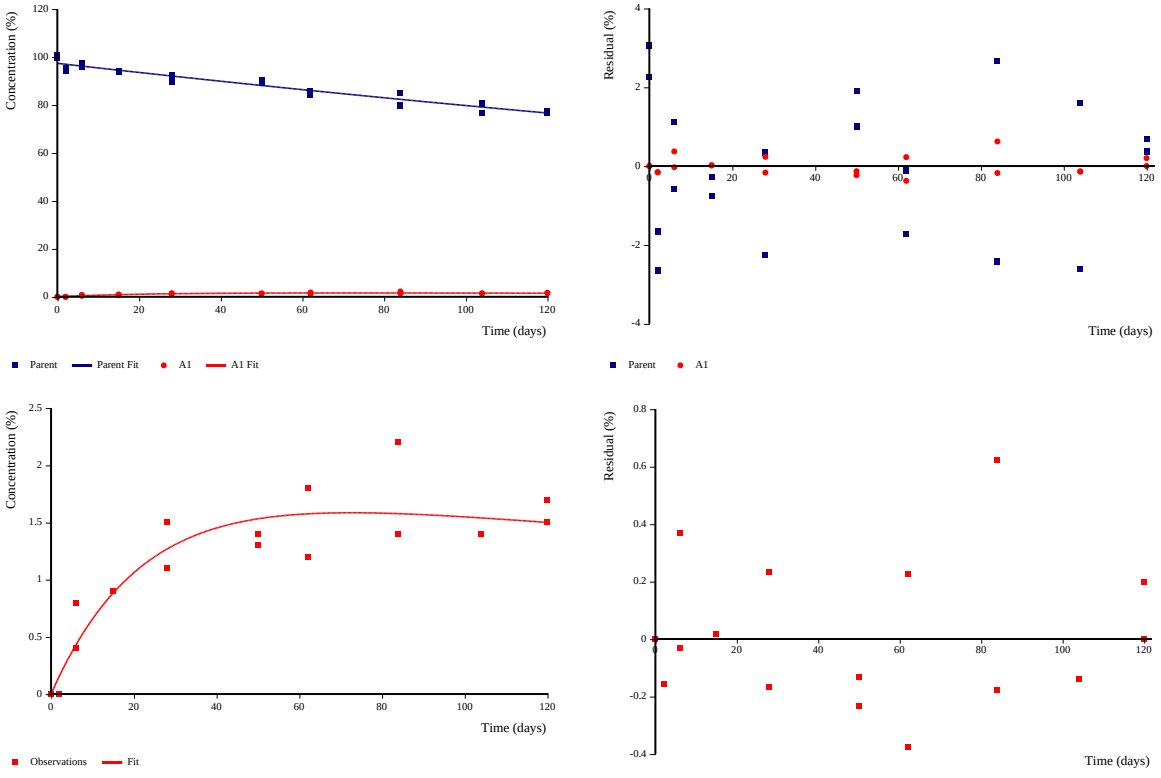
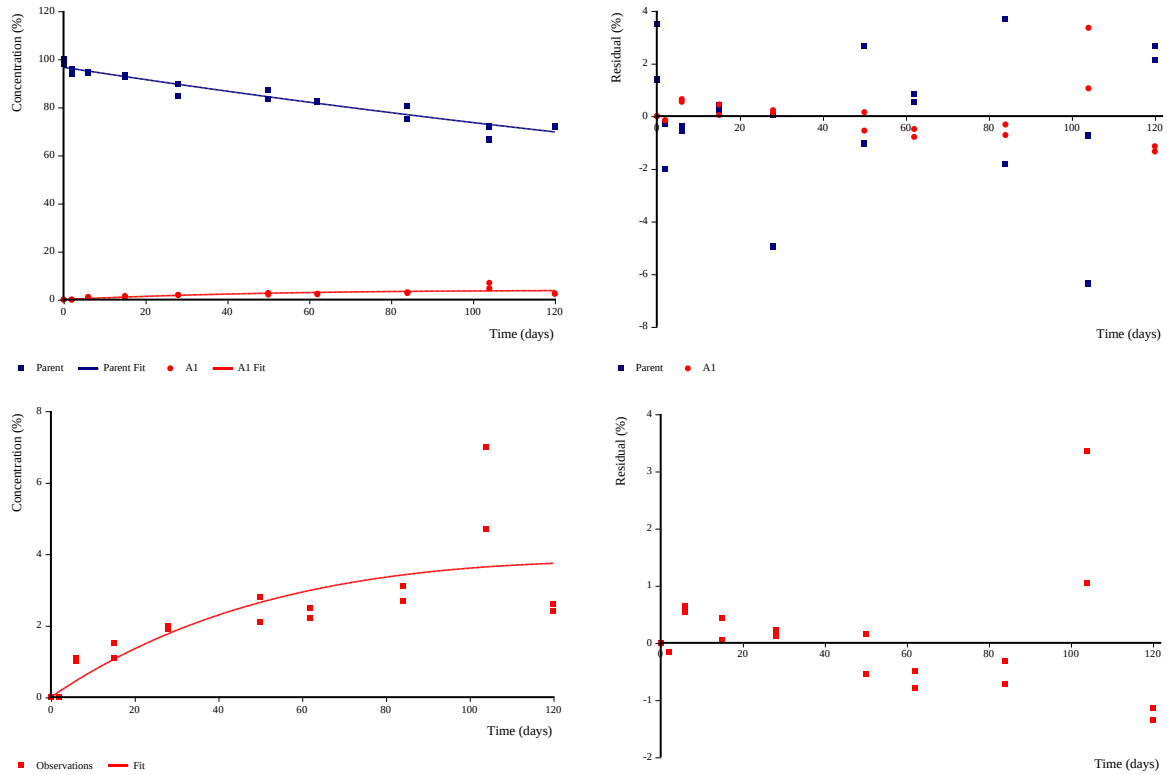


Figure B.8.1.1.4.1-4. Fit graph and residuals for parent and metabolite Dollendorf II – soil (SFO).



For the Gabbert et al (2017) study a kinetic assessment was performed according to FOCUS (2006) by the RMS. Only isoflucypram was assessed; M12 was not assessed as insufficient M12 data points were available. No outliers or data points were removed by the RMS. Results of the SFO assessment are presented in table B.8.1.1.4.1-6 and the FOMC results in table B.8.1.1.4.1-7. SFO fits were found to be acceptable by the RMS for modelling endpoints as chi^2 values are low, visual fits are acceptable for both parent and metabolites. For persistence/ triggering endpoints FOMC fits do not show a significant improvement over SFO, therefore SFO fits are used. Copies of the SFO fit graphs and residual plots are provided in figures B.8.1.1.4.1-5 to B.8.1.1.4.1-6.

Table B.8.1.1.4.1-6. Kinetic assessment results from Gabbert, D.; McConnell, L. L.; Arthur, E. L.; (2017), Single First Order.

		Soil name			
modelling endpoint assessment		CA		NE	
		Isoflucypram	M12	Isoflucypram	M12
Parameters	Model	SFO			
	Pini (%)	100	n/a	100	n/a
	K	0.00098	n/a	0.00312	n/a
	Formation fraction	n/a	n/a	n/a	n/a
Statistics	χ^2 (%)	1.11	n/a	2.31	n/a
	t-test (P value)	<0.001	n/a	<0.001	n/a
	Dt_{50} (days)	709	n/a	222	n/a
	Dt_{90} (days)	2350	n/a	738	n/a
	Visual Fit	Excellent	n/a	Good	n/a

Table B.8.1.1.4.1-7. Kinetic assessment results from Gabbert, D.; McConnell, L. L.; Arthur, E. L.; (2017), First Order Multi Compartment.

		Soil name			
Persistence endpoint assessment		CA		NE	
		Isoflucypram	M12	Isoflucypram	M12
Parameters	Model	FOMC			
	Pini (%)	100	n/a	100	n/a
	α	80.35	n/a	4.048	n/a
	β	0.00082	n/a	0.0012	n/a
	Formation fraction	n/a	n/a	n/a	n/a
Statistics	χ^2 (%)	1.18	n/a	2.46	n/a
	t-test (P value)	n/a	n/a	n/a	n/a
	Dt_{50} (days)	711	n/a	225	n/a
	Dt_{90} (days)	2390	n/a	921	n/a
	$Dt_{90/3.32}$ (days)	719	n/a	277	n/a
	Visual Fit	Excellent	n/a	Good	n/a

Figure B.8.1.1.4.1-5. Fit graph and residuals plot for parent CA – soil (SFO).

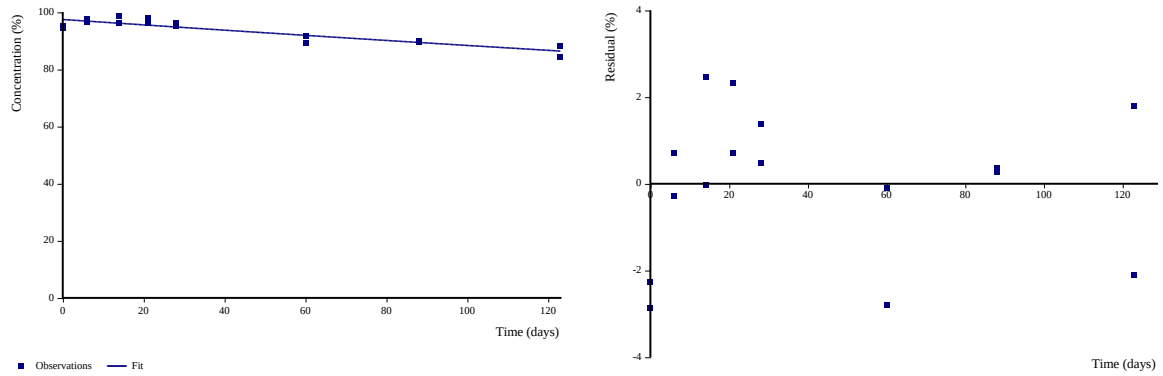
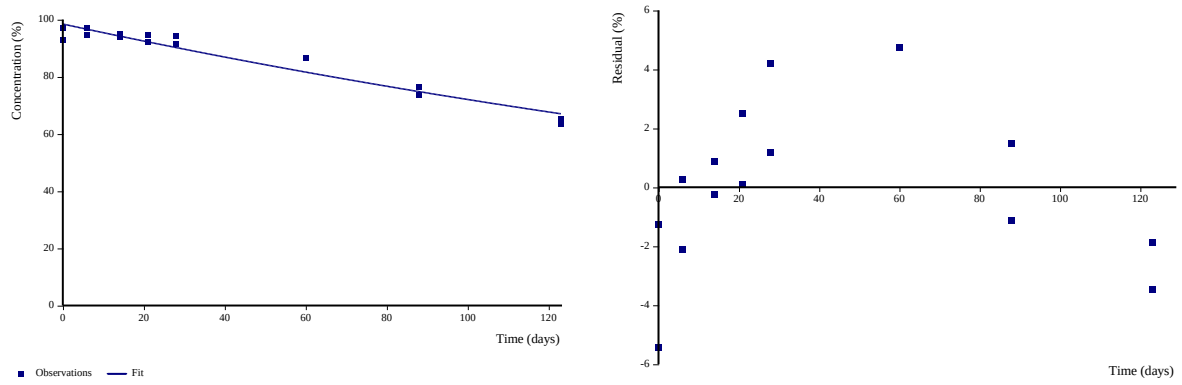


Figure B.8.1.1.4.1-6. Fit graph and residuals plot for parent NA – soil (SFO).



For the Heinemann, O.; Kasel, D.; (2017) study a kinetic assessment was performed according to FOCUS (2006) by the RMS. Isoflucypram and M12 were assessed together as sufficient M12 data points were available. No outliers or data points were removed by the RMS. Results of the SFO assessment are presented in table B.8.1.1.4.1-8 and the FOMC in table B.8.1.1.4.1-9. SFO fits were found to be acceptable by the RMS for modelling endpoints. For persistence/ triggering endpoints SFO was also accepted and no significant difference between SFO and FOMC was noted. Copies of the SFO fit graphs and residual plots are provided in figures B.8.1.1.4.1-1 to B.8.1.1.4.1-4. For metabolite M12 no degradation phase was seen (the metabolite concentrations were increasing at the final sample time). In light of the poor t-test the DT_{50} was fixed to 1000 days (K=0.0007) and the SFO fit re-run, kinetic results are presented in table B.8.1.1.4.1-9. The result for the fixed DT50 was accepted as the T-test result is acceptable and only a minor change in the formation fraction is noted.

Table B.8.1.1.4.1-8. Kinetic assessment results from Heinemann, O.; Kasel, D.; (2017). Single First Order.

		Soil name	
modelling endpoint assessment		Laacher Hof AXXa	
		Isoflucypram	M12
Parameters	Model	SFO isoflucypram, SFO M12	
	Pini (%)	101.7	n/a
	K	0.0026	0.0003
	Formation fraction	n/a	0.25
Statistics	χ^2 (%)	2.74	17.8
	t-test (P value)	<0.001	0.214
	Dt_{50} (days)	263	258
	Dt_{90} (days)	873	856
	Visual Fit	Excellent	Excellent

Table B.8.1.1.4.1-9. Kinetic assessment results from Heinemann, O.; Kasel, D.; (2017), First Order Multi Compartment.

		Soil	
persistence endpoint assessment		Laacher Hof AXXa	
		Isoflucypram	M12
Parameters	Model	FOMC isoflucypram, SFO M12	
	Pini (%)	102.6	n/a
	α	0.4898	K1 0.950
	β	136.3	n/a
	Formation fraction	n/a	0.21
Statistics	χ^2 (%)	2.76	17.7
	t-test (P value)	n/a	n/a
	Dt_{50} (days)	383	1000
	Dt_{90} (days)	7740	1000
	$Dt_{90/3.32}$ (days)	2330	1000
	Visual Fit	Excellent	Excellent

Figure B.8.1.1.4.1-7. Fit graph and residuals plot for parent Laacher Hof AXXa – soil (SFO) free fitted.

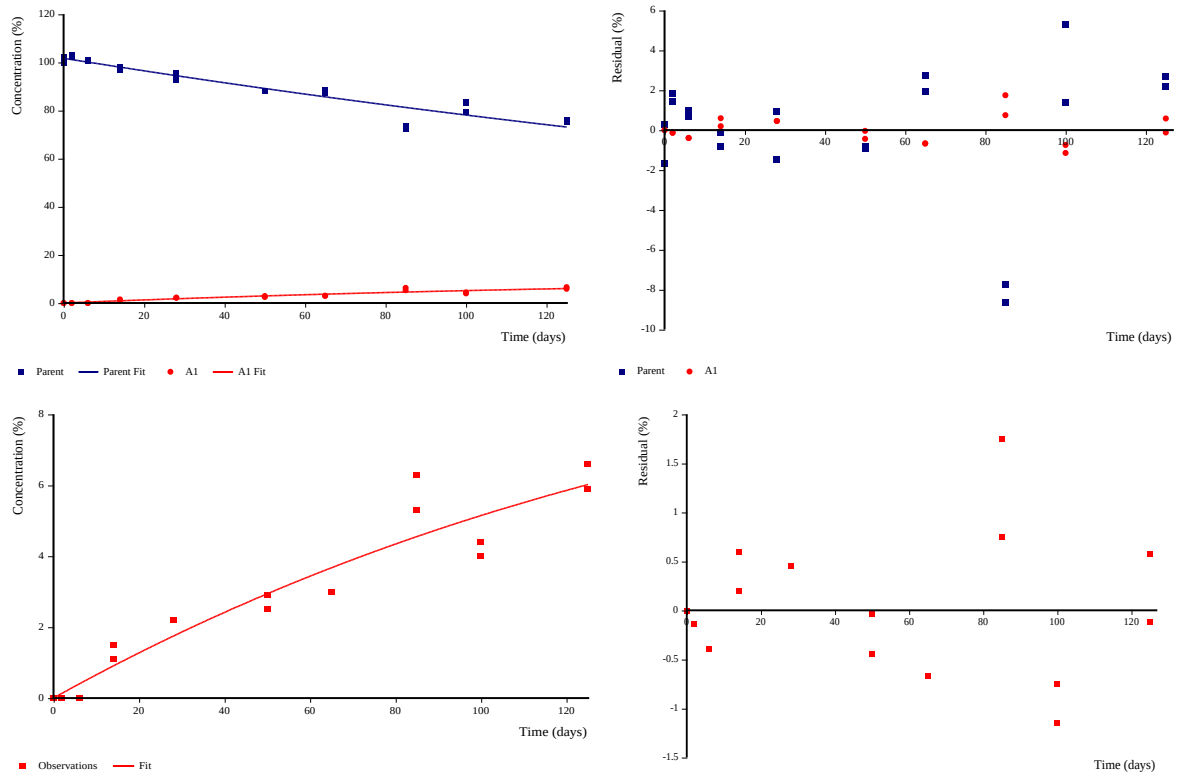
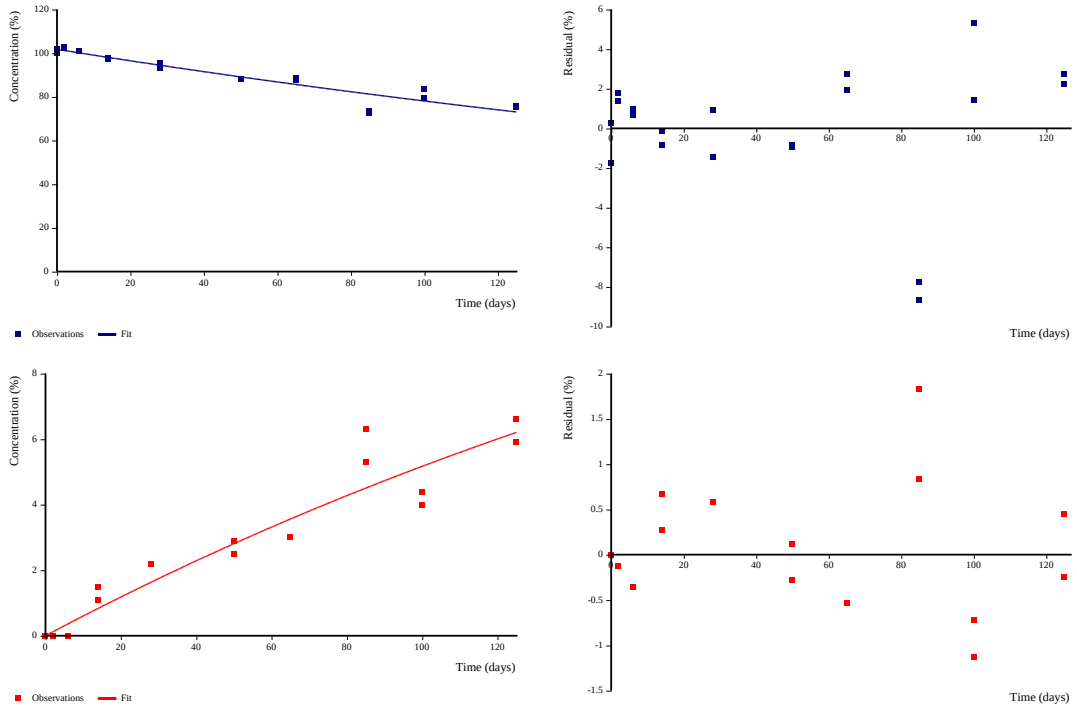


Table B.8.1.1.4.1-10. Kinetic assessment results from Heinemann, O.; Kasel, D.; (2017). Single First Order, DT50 metabolite fixed to 1000 days.

		Soil name	
modelling endpoint assessment		Laacher Hof AXXa	
		Isoflucypram	M12
Parameters	Model	SFO isoflucypram, SFO M12	
	Pini (%)	101.7	n/a
	K	0.0026	0.0007
	Formation fraction	n/a	0.23
Statistics	χ^2 (%)	2.74	17.1
	t-test (P value)	<0.001	n/a
	Dt_{50} (days)	262	1000
	Dt_{90} (days)	990	3290
	Visual Fit	Excellent	Excellent

Figure B.8.1.1.4.1-8. Fit graph and residuals plot for parent Laacher Hof AXXa – soil (SFO), DT50 M12 fixed 1000 days.



Summary of Laboratory aerobic degradation.

A summary of the kinetic assessment results is provided below in table B.8.1.1.4.1-11 for isoflucypram and table B.8.1.1.4.1-12 for the major metabolite M12. Given the DT50 and DT90 values in the laboratory aerobic soil studies, a field dissipation study was performed by the applicant in compliance with the data requirements under Regulation 283/2013. The field studies are described and assessed in the section below.

Table B.8.1.1.4.1-11. Kinetic assessment summary table for the laboratory aerobic degradation studies for modelling purposes - isoflucypram.

modelling endpoint assessment		Isoflucypram						
		Soil name						
		Hanscheider Hof –	**Laacher Hof AXXa**	**Hoefchen Am Hohenseh**	**Dollendorf II**	**CA**	**NE**	**Laacher Hof AXXa**
Soil parameters	Type	Loam	Loamy sand	Silt Loam	Loam	Sandy Loam	Silty clay loam	Loam Sand
	pH	5.7	6.3	6.6	7.4	6.3	6.3	5.8
	Model	SFO	SFO	SFO	SFO	SFO	SFO	SFO

	Temp °C	20	20	20	20	20.4	20.4	20
	MWHC %	53.1	53.1	53.1	53.1	64.9*	70.0^	55.0
Kinetic results	χ^2 (%)	0.699	0.999	0.851	1.72	1.11	2.31	2.74
	t-test (P value)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Visual Fit	Excellent	Excellent	Excellent	Excellent	Excellent	Good	Excellent
	Dt_{50} (days) (non-normalised)	438	236	347	256	709	222	263
	Dt_{90} (days) (non-normalised)	1450	782	1150	849	2350	738	873
	Dt_{50} (days) (normalised)	438	236	347	256	665.4	206.0	263
	Dt_{90} (days) (normalised)	1450	782	1150	849	2205.8	685.1	873

*18.9 g water/100g soil used, MWHC 29.1 g/100g. As confirmed from the final report

^36.7 g water/100g soil used, MWHC 52.4 g/100g. As confirmed from the final report.

Table B.8.1.1.4.1-12. Kinetic assessment summary table for the laboratory aerobic degradation studies for modelling purposes – metabolite M12

modelling endpoint assessment		M12						
		Soil name						
		Hanscheider Hof –	Laacher Hof AXXa	Hoefchen Am Hohenseh	Dollendorf II	CA	NE	Laacher Hof AXXa
Soil parameters	Type	Loam	Loamy sand	Silt Loam	Loam	Sandy Loam	Silty clay loam	Loam Sand
	pH	5.7	6.3	6.6	7.4	6.3	6.3	5.8
	Model	SFO- SFO	SFO- SFO	SFO-SFO	SFO-SFO	n.a.	n.a.	SFO-SFO
	Temp °C	20	20	20	20	20.4	20.4	20
	MWHC %	53.1	53.1	53.1	53.1	64.9	70.0	55.0
Kinetic results	χ^2 (%)	9.0	8.06	9.55	32.5	n.a.	n.a.	17.1
	t-test (P value)	<0.001	<0.001	<0.001	0.06	n.a.	n.a.	<0.001
	Visual Fit	Good	Excellent	Excellent	Good	n.a.	n.a.	Excellent
	Dt_{50} (days) (non-normalised)	48.1	107	15.5	48.5	n.a.	n.a.	1000
	Dt_{90} (days) (non-normalised)	160	356	51.3	161	n.a.	n.a.	3290
	Dt_{50} (days) (normalised)	48.1	107	15.5	48.5	n.a.	n.a.	1000
	Dt_{90} (days) (normalised)	160	356	51.3	161	n.a.	n.a.	3290
	Formation Fraction	0.32	0.26	0.42	0.31	n.a.	n.a.	0.23

Anaerobic laboratory degradation

A single study has been performed on the anaerobic degradation of isoflucypram (Heinemann and Kasel (2015). A summary of the endpoints is provided below in table B.8.1.1.4.1-13. No outliers were removed by the RMS, all concentrations were above the LOD. Insufficient data points were available to test the degradation of the major metabolite M12. SFO and FOMC tests were performed to assess if any biphasic nature to the degradation was present. CAKE v3.1 was used by the RMS. No clear decline phase was observed in the data for the anaerobic study, Kinetic results are presented in table B.8.1.1.4.1-14 and table B.8.1.1.4.1-15.

Table B.8.1.1.4.1-13: Summary table for Percent of applied radioactivity from the Heinemann.; (2015), study. Isoflucypram

Compound		Sampling intervals							
	DAT	30	32	37	44	60	92	120	150
	DASF	0	2	7	14	30	62	90	120
Isoflucypram (entire system)	A	92.1	98.0	91.5	92.0	92.9	89.6	95.4	94.2
	B	92.5	97.1	92.2	94.0	92.1	91.1	96.1	94.1
	mean	92.3	97.6	91.9	93.0	92.5	90.3	95.7	94.2

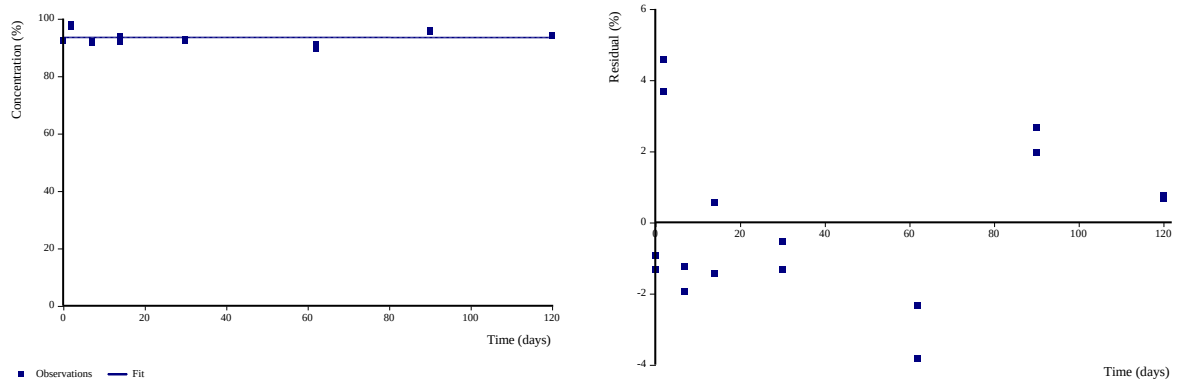
Table B.8.1.1.4.1-14: Kinetic assessment results from Heinemann (2015), Single First Order.

		Soil name
modelling endpoint assessment		Laacher Hof AXXa
		Isoflucypram
Parameters	Model	SFO
	Pini (%)	100
	K	<0.0001
	Formation fraction	n/a
Statistics	χ^2 (%)	1.84
	t-test (P value)	0.5
	Dt_{50} (days)	1000
	Dt_{90} (days)	1000
	Visual Fit	Good

Table B.8.1.1.4.1-15: Kinetic assessment results from Heinemann (2015), First Order Multi compartment.

		Soil
persistence endpoint assessment		Laacher Hof AXXa
		Isoflucypram
Parameters	Model	FOMC
	Pini (%)	100
	α	0.0012
	β	1.834
	Formation fraction	n/a
Statistics	χ^2 (%)	1.96
	t-test (P value)	n/a
	Dt_{50} (days)	1000
	Dt_{90} (days)	1000
	$Dt_{90/3.32}$ (days)	Not calculated
	Visual Fit	Good

Figure B.8.1.1.4.1-9: Fit graph and residuals plot for parent Laacher Hof AXXa – soil (SFO).



Photolytic degradation in soil

A single study has been performed on the photolytic degradation of isoflucypram in the presence of soil (Heinemann (2013). A summary of the endpoints is provided below in table B.8.1.1.4.1-16. No outliers were removed by the RMS, all concentrations were above the LOD. Insufficient endpoints were available to test the degradation of the major metabolite M12. SFO and FOMC tests were performed to assess if any biphasic nature to the degradation was present. CAKE v3.1 was used by the RMS. Fit graphs and plots of residuals are provided in tables B.8.1.1.4.1-10 and B.8.1.1.4.1-11. SFO fits were selected by the RMS. A marginally better χ^2 is noted in the FOMC model runs, however the DT_{50} values are virtually equal and FOMC DT_{50} values is only marginally more conservative.

Table B.8.1.1.4.1-16: Summary table for Percent of applied radioactivity from the Heinemann.; (2013), study. Isoflucypram

Compound	Samples	DAT 0	1	2	3	6	8	10
irradiated								
Isoflucypram	A	102.6	101.1	106.4	100.1	98.7	98.2	98.4
	B	98.7	102.8	105.8	103.6	98.8	94.8	97.5
	mean	100.6	102.0	106.1	101.8	98.8	96.5	97.9
dark								
Isoflucypram	A	102.6	103.2	107.5	105.8	102.0	96.7	101.1
	B	98.7	102.3	107.3	106.9	97.0	98.2	100.2
	mean	100.6	102.7	107.4	106.4	99.5	97.4	100.7

Table B.8.1.1.4.1-17: Kinetic assessment results from Heinemann (2013), Single First Order.

		Laacher Hof AXXa	
modelling endpoint assessment		Light	Dark
		Isoflucypram	Isoflucypram
Parameters	Model	SFO	
	Pini (%)	100	100
	K	0.006	0.005
	Formation fraction	n/a	n/a
Statistics	χ^2 (%)	1.61	2.23
	t-test (P value)	0.005	0.037
	Dt_{50} (days)	114	139
	Dt_{90} (days)	377	463
	Visual Fit	Acceptable	Acceptable

Table B.8.1.1.4.1-18: Kinetic assessment results from Heinemann (2013), First Order Multi compartment.

		Laacher Hof AXXa	
Persistence endpoint assessment		**Light**	**Dark**
		Isoflucypram	**Isoflucypram**
Parameters	**Model**	**FOMC**	
	Pini (%)	100	100
	K1	n/a	n/a
	Formation fraction	n/a	n/a
Statistics	**χ^2 (%)**	1.18	2.41
	t-test (P value)	n/a	n/a
	Dt_{50} (days)	114	183
	Dt_{90} (days)	384	2130
	$Dt_{90/3.32}$ (days)	116	642
	Visual Fit	Excellent	Excellent

Figure B.8.1.1.4.1-10: Fit graph and residuals plot for parent Laacher Hof AXXa soil, samples exposed to the light (SFO).

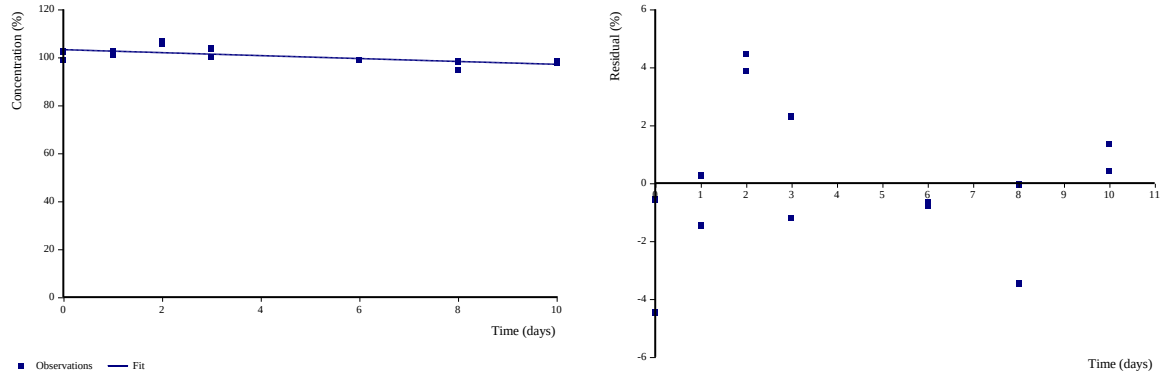
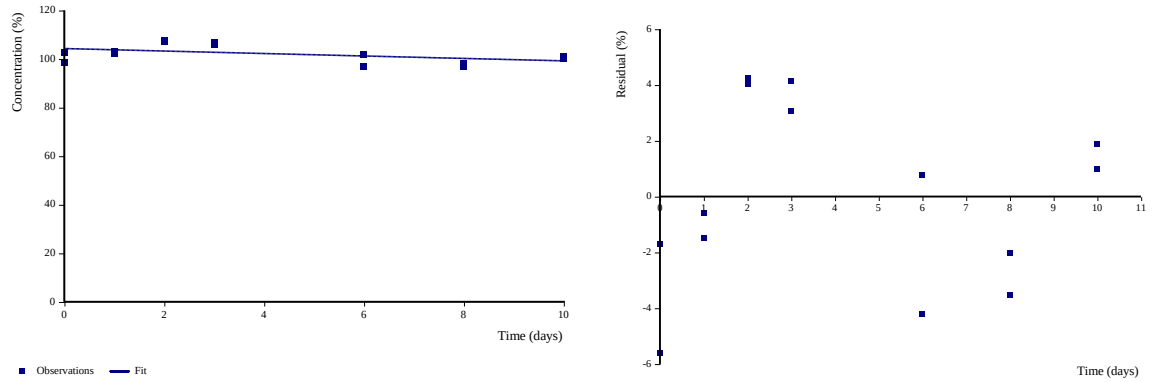


Figure B.8.1.1.4.1-11: Fit graph and residuals plot for parent Laacher Hof AXXa soil, samples not exposed to the light –(SFO).



B.8.1.1.5: Field dissipation studies

A field dissipation study was conducted with isoflucypram covering six European field sites, three in Northern Europe and three in Southern Europe using unlabelled Isoflucypram + Prothioconazole formulated as an emulsifiable concentrate.

B.8.1.1.5.1. Terrestrial field dissipation study

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; Junge, T.; 2017
Title: Terrestrial field dissipation study with BCS-CN88460 + prothioconazole EC 200 in Germany, United Kingdom, France (North), France (South), Italy and Spain
Report No.: 14-2750
Document No.: M-595964-01-1
Guideline(s): Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 including Data Requirements SANCO/11803/2010 Rev. 7 and Test Methods SANCO/11843/2010 Rev. 4
EPA Fate, Transport and Transformation Test Guidelines OPPTS 835.6100
Terrestrial Field Dissipation, October 2008
NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies, DIR2006-01, March 2006
Guideline deviation(s): none specified
GLP/GEP: yes

Study Summary

Soil dissipation of isoflucypram under European field conditions was investigated after application of Isoflucypram + Prothioconazole EC 200 on bare soil plots at six sites in Burscheid (Germany), Great Chishill (United Kingdom), Parcay Meslay (Northern France), St. Etienne du Gres (Southern France), Albaro di Ronco all Adige (Italy) and Vilobi d'Onyar (Spain). The sites are located in the ecoregions Northern and Southern Europe.
Isoflucypram + Prothioconazole EC 200 was sprayed once onto 256 to 920 m² plots at a rate of 2.00 L/ha, corresponding to nominal 100 g/ha isoflucypram. The plots received approximately 10 mm

water between DAT-0 and DAT-3, either by irrigation post application or by rainfall.. The control plots were at least 5 m away from the treated plots.
Soil samples were taken from day 0 before application up to 749 days post-application to a maximum depth of 60 cm, homogenised and analysed for isoflucypram and its degradation product M12.

Sub-samples of homogenised soil (5 or 20 g) were extracted in a microwave extractor with a mixture of acetonitrile/water/acetic acid (4000/1000/30, *v/v/v*). Potential matrix effects were eliminated by using an internal standard solution of isotopically labelled reference items added to sample extracts. Following separation of fine particles from soil extracts by centrifugation, identification and quantitation of the analytes was performed by high performance liquid chromatography using MS/MS detection in the multiple reaction monitoring mode. The analytical method was validated using three different soils. The limit of quantitation (LOQ) was 1.0 µg/kg and the limit of detection (LOD) was 0.3 µg/kg for each analyte.

The amount of isoflucypram decreased from DAT-0 to study end (DAT-713) from 98.2 to 28.7 g/ha at Burscheid (Germany), from DAT-0 to DAT-749 onwards from 96.8 g/ha to 20.3 g/ha at Great Chishill (United Kingdom), from DAT-0 to DAT-701 from 88.1 to 31.2 g/ha at Parcay Meslay (Northern France), from DAT-0 to DAT-205 from 90.9 g/ha to 4.20 g/ha at St. Etienne du Gres (Southern France), from DAT 0 to DAT-728 from 90.1 g/ha to 21.0 g/ha at Albaro di Ronco all Adige (Italy) and from DAT-0 to DAT-714 from 88.2 to 13.0 g/ha at Vilobi d'Onyar (Spain).
Residues of isoflucypram remained mainly in the top 0-40 cm of soil. Dissipation of isoflucypram from soil was moderately to fast with DT_{50} values ranging from 16.5 to 177 days for all test sites.
An overview of the results is given in the following table:

Table B.8.1.1.5.1-1 Applicant proposed Degradation of isoflucypram in soil

Soil	Soil type (USDA)	pH ($CaCl_2$)[a)]	Best fit kinetic model[b)]	DT_{50} [days]	DT_{90} [days]
Burscheid (Germany) 14-2750-01	silt loam (0-50 cm) loam (50-75 cm) sandy loam (75-100)	5.3	DFOP	143	> 1000
Great Chishill (United Kingdom) 14-2750-02	clay loam (0-30 cm) clay (30-100 cm)	7.0	DFOP	177	> 1000
Parcay Meslay (Northern France) 14-2750-03	loam (0-50 cm) clay loam (50-100 cm)	5.9	DFOP	147	> 1000
St. Etienne du Gres (Southern France) 14-2750-04	clay loam (0-30 cm) silty clay loam (30-50 cm) clay loam (50-75 cm) clay (75-100 cm)	7.5	DFOP	16.5	69.6
Albaro di Ronco all Adige (Italy) 14-2750-05	clay (0-75 cm) clay loam (75-100 cm)	7.0	DFOP	77.6	> 1000
Vilobi d'Onyar (Spain) 14-2750-06	loam (0-30 cm) sandy clay loam (30-100 cm)	5.8	DFOP	25.7	812

a) pH in 0-30 cm soil depth
b) DFOP: double first order in parallel

Dissipation of isoflucypram was accompanied by the formation of its degradation product M12.

The maximum amounts of M12 in the entire soil profiles were detected between DAT-30 and DAT-209 and ranged from 0.99 to 3.88 g/ha. However, the transient character of this metabolite is indicated by its decline to values below the LOD towards the end of the study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item:

Test item name:	Isoflucypram + Prothioconazole EC 200
Formulation type:	EC 200 (EC: emulsifiable concentrate
Analysis certificate no.:	FAR No.: 01785-00
Active substances (a.s.):	isoflucypram, prothioconazole
Batch no.:	2014-002032
Content a.s. nominal	50 g/L isoflucypram 150 g/L prothioconazole
Content a.s. actual	50.0 g/L isoflucypram 151.5 g/L prothioconazole

Reference items:

Name:	isoflucypram (BCS-CN88460)
Certificate of analysis:	AZ 18677; AZ 19352; AZ 20542
Name:	[d_5] BCS-CN88460 (BCN-CN88460 ISTD)
Certificate of analysis:	KML 9676
Name:	BCS-CN88460-carboxylic acid (BCS-CY26497) *(M12)*
Certificate of analysis:	MZ 00913; MZ 00984; MZ 01057; MZ 01102; MZ 01188
Name:	[d_5] BCS-CY26497 (BCN- CY26497 ISTD)
Certificate of analysis:	KML 9813

2. Test Sites

Six sites were selected (see Table B.8.1.1.5.1- 1), which are considered by the applicant to be typical for the ecoregions of Southern and Northern Europe. The sites were neither subjected to erosion, flooding nor run-off. The test plots had no significant slope and were largely free of stones. A field soil dissipation trial consisted of a treated and an untreated plot at each test site. The control plots were located at least 5 meters away from the treated plots. The selected sites had not been treated with chemicals which could influence the dissipation behaviour of isoflucypram or which could interfere with the analysis of the residues in soil. 2 sites (Burscheid and Great Chishill) were pesticide free. 1 site (St. Etienne du Gres) was treated with Glyphosate in the year of application. 1 site (Vilobi d'Onyar) was treated with flufenacet in the two years before application and trifloxystrobin three years before application. Parcay Meslay and Albaro di Ronco all Adige soils were treated with a variety of pesticides in the 3 years before application including a range of insecticides, herbicides and fungicides. RMS notes submitted chromatography in the final reports show no peaks that would indicate residues of significant quantities to impact on the reliability of the soil sites selected. Details are included in tables B.8.1.1.5.1-4 and B.8.1.1.5.1-5. The location, site descriptions, climatic data and properties of the soils are given in the following tables B.8.1.1.5.1-2 and B.8.1.1.5.1-3.

Table B.8.1.1.5.1- 2: Location, site description and climatic data of test sites

Site ID	**14-2750-01**	**14-2750-02**	**14-2750-03**
Site designation	Burscheid, Germany	Great Chishill, United Kingdom	Parcay Meslay, France
Geographic location: Latitude Longitude Country Ecoregion	 51°04.110'N 07°05.690'E Germany Northern EU	 52°03'17.07"N 0°08'33.78"E United Kingdom Northern EU	 47°27’47.0‘‘N 0°45’11.9‘‘E France Northern EU
Plot Size [m^2]	306	630	256
Distance from weather station used for climatic measurements	2 km distance from the plot	< 1 km away from the plot	At trial location
Meteorological conditions compared to long-term average within normal levels (yes/no)	yes	yes	yes
Site ID	**14-2750-04**	**14-2750-05**	**14-2750-05**
Site designation	St. Etienne du Gres, France	Albaro di Ronco all Adige, Italy	Vilobi d’Onyar, Spain
Geographic location: Latitude Longitude Country Ecoregion	 43°48’20.0‘‘N 004°43’12.0‘‘E France Southern EU	 45.345035 N 11.18979' E Italy Southern EU	 41°52`53,43"N 2°44`52,39" E Spain Southern EU
Plot Size [m2]	256	920	296
Distance from weather station used for climatic measurements	at trial location	0.1 km distance from the plot	0.4 km distance from the plot
Meteorological conditions compared to long-term average within normal levels (yes/no)	yes	yes	yes

Table B.8.1.1.5.1- 3: Properties of the soils from the test sites

Test Site and Trial No.	Soil depth [cm]	Soil type [USDA]	pH ($CaCl_2$)	pH (H_2O)	Organic carbon [%]
Burscheid (Germany) 14-2750-01	0-30	silt loam	5.3	5.4	1.0
	30-50	silt loam	5.5	5.9	0.2
	50-75	loam	5.7	6.0	0.1
	75-100	sandy loam	5.9	6.2	0.0
Great Chishill (United Kingdom) 14-2750-02	0-30	clay loam	7.0	7.0	2.0
	30-50	clay	7.5	7.7	0.2
	50-75	clay	7.6	7.9	1.5
	75-100	clay	7.6	7.8	1.9
Parcay Meslay (France) 14-2750-03	0-30	loam	5.9	6.3	1.2
	30-50	loam	6.1	6.5	0.5
	50-75	clay loam	6.5	6.9	0.2
	75-100	clay loam	6.4	6.8	0.2
St. Etienne du Gres (France) 14-2750-04	0-30	clay loam	7.5	7.8	2.3
	30-50	silty clay loam	7.6	7.8	2.3
	50-75	clay loam	7.6	7.9	1.8
	75-100	clay	7.7	8.0	2.2
Albaro di Ronco all Adige (Italy) 14-2750-05	0-30	clay	7.0	7.2	2.1
	30-50	clay	7.1	7.3	1.6
	50-75	clay	7.3	7.5	0.7
	75-100	clay loam	7.2	7.6	0.6
Vilobi d'Onyar (Spain) 14-2750-06	0-30	loam	5.8	6.1	0.7
	30-50	sandy clay loam	6.0	6.3	0.4
	50-75	sandy clay loam	6.5	6.9	0.1
	75-100	sandy clay loam	6.5	6.8	0.1

B.8.1.1.5.1-4: Pesticides used Parcay Meslay (France) soil prior to application

Year	Crop	Pesticide	Active substance(s)	Indication	Chemical group	Total amount of Product [kg/ha or L/ha]
2011	Rape	Cruiser OSR SC	Fludioxonil, Metalaxyl-M, Thiamethoxam	Fungicide Insecticide	PhenylPyrrole PhenylAmide Neonicotinoid	--
		Devin EC	Cycloxydime	Herbicide	Cyclohexanedione 'DIMS'	1.1 L/ha
2012		Caramba Star EC	Metconazole	Fungicide	Triazole	0.3 L/ha
		Karate Zeon CS	Lambda-Cyhalothrin	Insecticide	Pyrethoid	0.075 L/ha
		Talita EW	Tau-fluvalinate	Insecticide	Pyrethoid	0.2 L/ha
	Wheat	Gaucho 350 FS	Imidacloprid	Insecticide	Neonicotinoid	0.15 L/ha
		Redigo FS	Prothioconazole	Fungicide	Triazolinthiones	
2013	Wheat	Amistar Opti SC	Clorthalonil Azoxystrobin	Fungicide	Cloronitrile Methoxy-acrylate	1 L/ha
		Atlantis WG	Mesosulfuron-methyl Iodosulfuron-methyl-natrium Mefenpyr-diethyl	Herbicide	Sulfonyurea	0.2 kg/ha 0.1 kg/ha
		Harmony SX SG	Thifensulfuron Methyl	Herbicide	Sulfonyurea	
		Tanhao EC	Propiconazole	Fungicide	Triazole	1.1 L/ha

Year	Crop	Pesticide	Active substance(s)	Indication	Chemical group	Total amount of Product [kg/ha or L/ha]
			Prochloraz		Imidazole	
	Barley	Taspa EC	Propiconazole Difenoconazol	Fungicide	Triazoles	0.3 L/ha
		Armor GB	Cyromazine	Insecticide	Triazine	4 kg/ha
		Gaucho 350 FS	Imidacloprid	Insecticide	Neonicotinoid	0.15 L/ha
		Misol FS	Prothioconazole	Fungicide	Triazolinthiones	1.1 L/ha
		Quarz GT SC	Isoproturon Diflufenican	Herbicide	UREA Pyridinecarboxamide	2 L/ha
2014	Barley	Citadelle SC	Cyproconazole Clorthalonil	Fungicide	Triazole Chloronitrile	2 L/ha

B.8.1.1.5.1-4: Pesticides used Albaro di Ronco all Adige, (Italy) soil prior to application

Year	Crop	Pesticide	Active substance(s)	Indication	Chemical Group	Total amount of Product [kg/ha or L/ha]
2011	Soil	CGA 357261*	Z/E isomer of trifloxystrobin	Parent trifloxystrobin: Fungicide	Parent trifloxystrobin: Strobilurine	0.375 L/ha
		Noto 40 SC, Nicosulfuron	Nicosulfuron	Herbicide	Sulfonyurea	1.5 L/ha
2012	Soil	No pesticides				-
2013	Cabbage	Asystim				0.18 L/ha
		Coragen	Chlorantraniliprol	Insecticide	Diamide	0.93 L/ha
		Decis EVO	Deltamethrin	Insecticide	Pyrethroid	2.38 L/ha
		Goal 480 SC	Oxifluorfen	Herbicide	Diphenylether	0.125 L/ha
		Kohinor	Imidacloprid	Insecticide	Neonicotinoid	
		Lobby	Fluazifop butyl	Herbicide	Aryloxyphenoxy-Propionate	1.4 L/ha
		Priori	Azoxystrobin	Fungicide	Quinon-outside Inhibitors (Methoxy-acrylate)	0.95 L/ha
		Poltiglia Disperss	Copper	Fungicide	Inorganic	6.33 kg/ha
		Ridomil Gold R	Copper Oxychloride Metalaxyl-M	Fungicide	Inorganic PhenylAmide	5 kg/ha
		Rogor L 20	Dimethoate	Insecticide	Organophosphate	0.6 L/ha
		Silwet Velomex	Organomodified Trisiloxane	Coadjuvant		2.835 L/ha
		Sultan	Metazachlor	Herbicide	Chloroacetamide	1.6 L/ha
		Tomagan	Fluroxypyr	Herbicide	Synthetic auxin	0.25 L/ha

B. STUDY DESIGN

1. Experimental Conditions

For the spray application onto the soil surface the representative formulation Isoflucypram + Prothioconazole EC 200 was selected.

Isoflucypram + Prothioconazole EC 200 is an emulsifiable concentrate formulation, containing 50 g/L isoflucypram. The product was used once with an application rate of 2 L/ha and 400 L/ha water, corresponding to 100 g isoflucypram/ha.

The product was applied to bare soil with two applications from opposite directions with each applying 50% of the total test item rate at all test sites. First soil samples were taken immediately after each spraying.
The plots received approximately 10 mm water between DAT-0 and DAT-3, either by irrigation post application or by rainfall (see Table B.8.1.1.5.1- 5).

Table B.8.1.1.5.1- 5: Data for spray application

Trial no. and test site	**14-2750-01 Burscheid (Germany)**	**14-2750-02 Great Chishill (United Kingdom)**	**14-2750-03 Parcay Meslay (Northern France)**
Formulation	EC 200	EC 200	EC 200
Date of application	04-04-2014	06-06-2014	16-06-2014
Application rate of Isoflucypram & Prothioconazole EC 200 [kg/ha]	2.00 L/ha	2.00 L/ha	2.00 L/ha
Water rate [L/ha]	600.0	400.0	600.0
Concentration of Isoflucypram & Prothioconazole EC 200 in the spray liquid [%]	0.333	0.500	0.333
Concentration of isoflucypram a.s. in the spray liquid [%]	0.016666	0.0250	0.016666
Concentration of prothioconazole a.s. in the spray liquid [%]	0.05	0.0750	0.05
Application rate of isoflucypram a.s. [g/ha]	100	100	100
Application rate of prothioconazole a.s. [g/ha]	300	300	300
Air temperature at application [°C]	14	15	20
Wind speed [m/s] and direction	no wind	1.0 - 2.0 SE	2 S
Rainfall [mm] within 24 h after the application	no rain	1	no rain
Irrigation [mm] after application	10 (DAT-0)	9 (DAT-3)	9 (DAT-1)
Trial no. and test site	**14-2750-04 St. Etienne du Gres (Southern France)**	**14-2750-05 Albaro di Ronco all Adige (Italy)**	**14-2750-06 Vilobi d'Onyar (Spain)**
Formulation	EC 200	EC 200	EC 200
Date of application	12-05-2014	20-06-2014	12-05-2014
Application rate of Isoflucypram & Prothioconazole EC 200 [kg/ha]	2.00 L/ha	2.00 L/ha	2.00 L/ha
Water rate [L/ha]	600.0	600.0	600.0
Concentration of Isoflucypram & Prothioconazole EC 200 in the spray liquid [%]	0.333	0.333	0.333
Concentration of isoflucypram a.s. in the spray liquid [%]	0.016666	0.016666	0.016666
Concentration of prothioconazole a.s. in the spray liquid [%]	0.05	0.05	0.05
Application rate of isoflucypram a.s. [g/ha]	100	100	100
Application rate of prothioconazole a.s. [g/ha]	300	300	300
Air temperature at application [°C]	26	29	15
Wind speed [m/s] and direction	0.5 N-W	no wind	0.8 - 2.8 E
Rainfall [mm] within 24 h after the application	no rain	no rain	7.4
Irrigation [mm] after application	10 (DAT-0)	10 (DAT-0)	4 (DAT-2)

Table B.8.1.1.5.1-6: Rainfall and irrigation against long term averages Burscheid (Germany)

Period of time	Study period average 2014 rain-fall	2014 irri-gation [mm]	2014 sum	2015 rain-fall	2015 irri-gation [mm]	2015 sum	2016 rain-fall	2016 irri-gation [mm]	2016 sum	Long-term average Period of time	Long-term average rainfall [mm]
January	65		65	0		0	103		103	January	79
February	134		134	30		30	97		97	February	60
March	26		26	67		67	61		61	March	66
April	39	40	79	39	30	69	50		50	April	59
May	115		115	33	46	79	71		71	May	75
June	80	20	100	64	30	94				June	92
July	150		150	89		89				July	96
August	168		168	139		139				August	93
September	31	55	86	113		113				September	81
October	72	10	82	52	30	82				October	77
November	57		57	113		113				November	85
December	86		86	99		99				December	88
Jan-Dec	*1023*	*125*	*1148*	*838*	*136*	*974*					*951*
Apr-Dec	*798*	*125*	*923*								*746*
Jan-May							*382*		*382*		*339*

Table B.8.1.1.5.1-7: Rainfall and irrigation against long term averages Great Chishill (United Kingdom)

Period of time	Study period average 2014 rain-fall	2014 irri-gation [mm]	2014 sum	2015 rain-fall	2015 irri-gation [mm]	2015 sum	2016 rain-fall	2016 irri-gation [mm]	2016 sum	Long-term average Period of time	Long-term average rainfall [mm]
January				66		66	61		61	January	49
February				43		43	22		22	February	37
March				19		19	62		62	March	44
April				40	30	70	47		47	April	47
May				46		46	49	10	59	May	47
June	42	9	51	18		18	79		79	June	56
July	47	20	67	116		116				July	49
August	107		107	38		38				August	56
September	24		24	17		17				September	52
October	78		78	49		49				October	56
November	87		87	79		79				November	60
December	51		51	71		71				December	55
Jan-Dec				*602*	*30*	*632*					*608*
Jun-Dec	*436*	*29*	*465*								*384*
Jan-Jun							*320*	*10*	*330*		*280*

Table B.8.1.1.5.1-8: Rainfall and irrigation against long term averages Parcay Meslay (Northern France)

Study period average										Long-term average	
Period of time	2014			2015			2016			Period of time	rainfall [mm]
	rain-fall	irri-gation [mm]	sum	rain-fall	irri-gation [mm]	sum	rain-fall	irri-gation [mm]	sum		
January				58		58	83		83	January	66
February				59		59	22		22	February	56
March				39		39	82		82	March	50
April				53	7	60	46		46	April	56
May				4	9	49	98		98	May	62
June	55	9	64	28	9	37				June	46
July	78		78	15	18	33				July	53
August	95		95	73	10	83				August	43
September	94		94	106		106				September	53
October	94		94	47		47				October	71
November	58		58	55		55				November	70
December	46		46	30		30				December	71
Jan-Dec				*603*	*53*	*656*					*697*
Jun-Dec	*520*	*9*	*529*								*407*
Jan-May							*331*		*331*		*290*

Table B.8.1.1.5.1-9: Rainfall and irrigation against long term averages St. Etienne du Gres (Southern France)

Study period average										Long-term average	
Period of time	2014			2015			2016			Period of time	rainfall [mm]
	rain-fall	irri-gation [mm]	sum	rain-fall	irri-gation [mm]	sum	rain-fall	irri-gation [mm]	sum		
January				102		102				January	54
February				72		72				February	33
March				71		71				March	33
April				112		112				April	60
May	16	57.5	73.5	1		1				May	50
June	60	75	135	95		95				June	30
July	106	77.5	183.5							July	26
August	26	77.5	103.5							August	30
September	97	75	172							September	116
October	17	30	47							October	92
November	253	50	303							November	73
December	29		29							December	48
Jan-Dec											*645*
May-Dec	*604*	*442.5*	*1046.5*								*465*
Jan-Jun				*453*		*453*					*260*

Table B.8.1.1.5.1-10: Rainfall and irrigation against long term averages Albaro di Ronco all Adige (Italy)

Period of time	Study period average 2014 rainfall [mm]	2014 irrigation [mm]	2014 sum	2015 rainfall [mm]	2015 irrigation [mm]	2015 sum	2016 rainfall [mm]	2016 irrigation [mm]	2016 sum	Long-term average Period of time	Long-term average rainfall [mm]
January				22	15	37	48		48	January	40
February				93		93	135		135	February	40
March				47		47	43		43	March	44
April				36	45	81	52		52	April	75
May				56	15	71	120		120	May	74
June	82	10	92	56	15	71	112		112	June	63
July	124		124	25	43	68				July	64
August	112		112	31	53	84				August	75
September	39	30	69	46	40	86				September	79
October	67	30	97	119		119				October	86
November	131		131	19	65	84				November	79
December	65		65	4		4				December	62
Jan-Dec				*554*	*291*	*845*					*781*
Jun-Dec	*620*	*70*	*690*								*508*
Jan-Jun							*510*				*336*

Table B.8.1.1.5.1-11: Rainfall and irrigation against long term averages Vilobi d'Onyar (Spain)

Period of time	Study period average 2014 rainfall [mm]	2014 irrigation [mm]	2014 sum	2015 rainfall [mm]	2015 irrigation [mm]	2015 sum	2016 rainfall [mm]	2016 irrigation [mm]	2016 sum	Long-term average Period of time	Long-term average rainfall [mm]
January				16	30	46	5	60	65	January	29
February				31		31	30		30	February	39
March				74	30	104	21		21	March	66
April				18	40	58	96		96	April	65
May	94	14	108	27	20	47	100		100	May	74
June	50		50	72	10	82	28		28	June	51
July	92		92	6	30	36	6		5	July	32
August	74		74	114		114				August	43
September	252		252	80	115[a)]	195				September	68
October	11	80	91	35		35				October	67
November	169		169	38		38				November	83
December	48		48	1	60	61				December	27
Jan-Dec				*512*	*335*	*847*					*644*
May-Dec	*790*	*94*	*884*								*445*
Jan-Jul							*286*	*60*	*346*		*356*

a) error in irrigation programming

2. Sampling

The treated plot of the trial was divided into four sub-plots. From each sub-plot of the treated plot four soil cores were taken and combined to a sample at each sampling interval.
Before application four soil cores were taken from untreated control plot to a depth of 10 cm with a soil piercer (Ø 100 mm). Immediately after application two times 16 soil cores were taken from the treated plot to a depth of 10 cm with a soil piercer (Ø 100 mm), respectively.

All subsequent samplings were performed using a "Wacker Hammer" (Ø 48 to 100 mm). At each sampling interval 16 cores from the treated plot were taken. From control plots 16 soil cores were taken. The samples were taken to a maximum depth of 60 cm on the following occasions: 0 (post-application, 0-10 cm depth), 3-4, 7, 13-15, 27-30, 57-70, 88-111 (0-40 cm depth), 110-140, 143-168, 205-278, 345-402, 519-560, 701-749 (0-60 cm depth) days after treatment (DAT).
From the control plot samples were taken on the following occasions: 0 days before application and 345-402 days and 713-728 days after treatment.
In addition, for characterization soil samples were taken before application from the treated plots to a depth of 100 cm (10 soil cores).
The samples were deep-frozen within 24 hours. The frozen soil cores were cut into 10 cm segments and each horizon (laboratory samples) was milled separately in a hammer mill and carefully homogenized. An aliquot of each homogenized laboratory sample (analytical samples) was used for analysis. Soil cores and samples were stored at ≤ 18°C.

3. Analytical Procedures

The analytical method 01432[1] was developed for the determination of isoflucypram and its metabolite M12 in/on soil, UKRMS notes that this was not the same extraction procedure as in the laboratory studies the method has been validated by the RMS in the Chemistry section.
Soil samples of 20 or 5 g were extracted in a microwave extractor with a mixture of acetonitrile/water/acetic acid (4000/1000/30, *v/v/v*). The extracts were centrifuged to remove fine particles of the soil. Possible matrix effects of isoflucypram and the metabolite M12 are eliminated by using an internal standard solution of isotopically labelled reference items. Identification and quantitation of the active substance was done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode.
The limit of quantitation (LOQ) for each single analyte was 1.0 µg/kg in soil. The limit of determination (LOD) for each single analyte was 0.3 µg/kg.
During analysis of the samples concurrent recovery experiments were performed by spiking control samples with the analyte.
The mean recoveries were 98% (RSD 8.2%) for isoflucypram and 99% (RSD 8.7%) for M12.

4. Kinetic evaluation

The degradation kinetics of the test item was determined according to FOCUS kinetics (2006) using the software KinGUI 2 with three different kinetic models: Single first order (SFO), first order multi compartment (FOMC) and double first order in parallel (DFOP) was performed by the applicant. A full discussion of the kinetics is included in section B.8.1.1.5.2 below.

II. RESULTS AND DISCUSSION

A. ANALYTICAL RESULTS

Control samples
Residues of isoflucypram and M12 in control samples were < LOD for all samples taken.

Treated samples
The measured initial mean concentrations (n = 4) for isoflucypram were 98.2 g/ha (Burscheid, Germany), 96.8 g/ha (Great Chishill, United Kingdom), 88.1 g/ha (Parcay Meslay. Northern France), 90.9 g/ha (St. Etienne du Gres, Southern France), 90.1 g/ha (Albaro di Ronco all Adige, Italy) and 88.2 g/ha (Vilobi d'Onyar, Spain), representing 88 to 98% of the intended application rate.

[1] Koch, V.; 2014; Analytical method 01432 for the determination of BCS-CN88460 and the metabolite BCS-CN88460-carboxylic acid in soil and sediment by HPLC-MS/MS; M-499794-01-1 summarised in CA 4.1.2

Residues of isoflucypram remained mainly in the top 0-40 cm of soil. Dissipation of isoflucypram was accompanied by the formation of its degradation product M12.

The maximum amounts of M12*)* in the entire soil profiles were detected between DAT-30 and DAT-209 and ranged from 0.99 to 3.88 g/ha. However, the transient character of this metabolite is indicated by its decline to values below the LOQ towards the end of the study. Molar correction factor 1. 075 (mol mass metabolite /mol mass parent = 429.8 g/mol/ 399.84 g/mol)

Concentrations of isoflucypram and M12 are presented in tables B.8.1.1.5.1-12 to B.8.1.1.5.1-23 for each depth analysed.

Table B.8.1.1.5.1-12: Mean measured concentrations of isoflucypram for (Burscheid, Germany).

	Mean concentration from 4 replicates isoflucypram (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**13**	**28**	**70**	**91**	**110**	**160**	**209**	**370**	**538**	**713**
Plot 1													
0-10	74.5	68.4	74.3	66.4	65.5	46.1	37.6	41.5	31.6	25.2	20.4	20.8	18.0
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.57]	<LOD	<LOD	<LOD	<LOD	[0.82]	[0.84]	[0.53]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.45]	[0.62]	<LOD
30-40	-	<LOD	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
Plot 2													
0-10	77.1	76.3	71.5	69.7	52.6	61.6	41.9	38.0	42.1	30.5	25.5	20.4	14.7
10-20	--	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	--	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	--	<LOD	--	--	--	--	--	--	--	<LOD	<LOD	<LOD	<LOD
40-50	--	--	--	--	--	--	--	--	--	<LOD	<LOD	<LOD	<LOD
50-60	--	--	--	--	--	--	--	--	--	<LOD	<LOD	<LOD	<LOD
Plot 3													
0-10	73.4	66.5	64.4	59.9	59.7	36.4	30.9	35.0	30.4	28.9	26.6	18.4	18.9
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.98]	[1.16]	<LOD	[0.48]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.63]	<LOD	<LOD	<LOD	<LOD	<LOD	[0.59]
30-40	-	<LOD	-	-	-	-	<LOD	-	-	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
0-10	80.1	69.9	62.5	66.1	64.7	52.3	56.1	37.1	38.3	29.7	29.1	23.5	22.9
10-20	--	[0.95]	<LOD	<LOD	<LOD	<LOD	<LOD	[0.61]	<LOD	[0.51]	1.40	[0.71]	[0.92]
20-30	--	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	--	<LOD	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
40-50	--	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
50-60	--	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD

Table B.8.1.1.5.1-13: Mean measured concentrations of M12 for (Burscheid, Germany).

	Mean concentration from 4 replicates M12 (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**13**	**28**	**70**	**91**	**110**	**160**	**209**	**370**	**538**	**713**
Plot 1													
0-10	<LOD	<LOD	<LOD	<LOD	[0.58]	[0.91]	[0.65]	[0.99]	[0.72]	[0.55]	[0.44]	[0.66]	[0.44]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
Plot 2													
0-10	<LOD	<LOD	<LOD	<LOD	[0.45]	[0.96]	[0.77]	[0.62]	[1.05]	[0.83]	[0.62]	[0.66]	[0.43]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
Plot 3													
0-10	<LOD	<LOD	<LOD	<LOD	[0.43]	[0.64]	[0.74]	[0.53]	[0.92]	[0.83]	[0.68]	[0.60]	[0.50]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	<LOD	-	-	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD

50-60	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
Plot 4													
0-10	<LOD	<LOD	<LOD	<LOD	[0.52]	1.30	1.34	[0.54]	1.40	1.29	[1.08]	[1.06]	[0.95]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD

DAT = Days after Treatment
LOQ = 1.0 µg/kg LOD = 0.30 µg/kg [x] = LOD >< LOQ

Table B.8.1.1.5.1-14: Mean measured concentrations of isoflucypram for Great Chishill (United Kingdom)

	Mean concentration from 4 replicates isoflucypram (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**4**	**7**	**14**	**27**	**67**	**111**	**140**	**168**	**278**	**402**	**560**	**749**
Plot 1													
0-10	74.0	63.4	66.3	53.2	46.2	49.8	56.1	31.4	25.4	20.2	13.9	17.8	14.3
10-20	-	12.7	<LOD	2.52	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.91]	[0.54]	[0.31]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 2													
0-10	75.4	73.5	56.0	53.1	58.7	51.2	63.6	-	25.4	20.3	17.1	14.5	6.68
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.84]	<LOD	<LOD	[0.81]	[0.93]	<LOD	[0.55]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 3													
0-10	79.6	51.4	79.4	55.2	63.1	46.9	48.2	13.7	29.2	17.4	17.0	14.3	7.79
10-20	-	[0.31]	<LOD	<LOD	<LOD	<LOD	[0.79]	<LOD	1.23	[0.58]	1.28	[0.64]	[0.43]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 4													
0-10	81.9	56.9	70.5	60.0	51.7	31.1	53.2	28.2	24.2	26.4	21.6	24.4	17.4
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	1.03	<LOD	<LOD	[0.48]	<LOD	1.92	[0.86]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B.8.1.1.5.1-15: Mean measured concentrations of M12 for Great Chishill (United Kingdom)

	Mean concentration from 4 replicates M12 (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**4**	**7**	**14**	**27**	**67**	**111**	**140**	**168**	**278**	**402**	**560**	**749**
Plot 1													
0-10	<LOD	<LOD	<LOD	[0.35]	[0.39]	[0.77]	1.46	1.04	[0.75]	[0.42]	[0.39]	[0.39]	[0.33]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.34]	[0.32]	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 2													
0-10	<LOD	<LOD	<LOD	<LOD	[0.49]	[0.66]	1.39	-	[0.63]	[0.39]	[0.57]	<LOD	<LOD
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 3													
0-10	<LOD	<LOD	<LOD	<LOD	[0.55]	[0.77]	[0.90]	[0.54]	1.02	[0.44]	[0.49]	[0.32]	<LOD
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.33]	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 4													
0-10	<LOD	<LOD	<LOD	<LOD	[0.39]	[0.53]	1.59	[0.99]	[0.78]	[0.61]	[0.74]	[0.54]	[0.34]

10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.38]	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-

DAT = Days after Treatment
LOQ = 1.0 µg/kg LOD = 0.30 µg/kg [x] = LOD >< LOQ

Table B.8.1.1.5.1-16: Mean measured concentrations of isoflucypram for Parcay Meslay (Northern France)

	Mean concentration from 4 replicates isoflucypram (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**14**	**29**	**63**	**88**	**121**	**143**	**210**	**357**	**519**	**701**
Plot 1													
0-10	57.5	61.0	58.3	52.5	39.2	34.3	37.5	29.1	23.9	25.5	21.5	17.4	16.9
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.45]	1.15	4.05	<LOD	<LOD	<LOD	[0.39]	[0.30]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 2													
0-10	67.0	51.6	67.8	45.4	51.1	38.7	44.0	29.7	28.2	21.8	26.1	20.7	17.9
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.64]	<LOD	[0.31]	[0.43]	<LOD	[0.71]	1.09	[0.34]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 3													
0-10	71.1	58.4	71.7	59.2	43.7	41.5	35.1	24.4	30.6	24.5	22.3	19.8	16.9
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.79]	<LOD	[0.46]	[0.36]	[0.33]	<LOD	[0.47]	[0.33]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	<LOD	-
Plot 4													
0-10	83.0	87.9	64.2	59.3	43.0	39.0	38.1	31.2	28.8	29.6	20.0	20.0	15.8
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.37]	<LOD	<LOD	<LOD	<LOD	<LOD	[0.34]	[0.99]
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	<LOD	-

DAT = Days after Treatment
LOQ = 1.0 µg/kg LOD = 0.30 µg/kg [x] = LOD >< LOQ

Table B.8.1.1.5.1-17: Mean measured concentrations of M12 for Parcay Meslay (Northern France)

	Mean concentration from 4 replicates M12 (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**14**	**29**	**63**	**88**	**121**	**143**	**210**	**357**	**519**	**701**
Plot 1													
0-10	<LOD	<LOD	<LOD	<LOD	[0.40]	[0.66]	1.04	[0.85]	[0.80]	[0.61]	[0.64]	[0.64]	[0.54]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 2													
0-10	<LOD	<LOD	<LOD	<LOD	[0.52]	[0.57]	[0.60]	[0.56]	[0.45]	<LOD	[0.41]	[0.60]	[0.42]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	-	-
Plot 3													
0-10	<LOD	<LOD	<LOD	[0.32]	[0.48]	[0.92]	[0.77]	[0.80]	[1.00]	[0.68]	[0.74]	[0.79]	[0.71]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	<LOD	-
Plot 4													

0-10	<LOD	<LOD	<LOD	<LOD	[0.42]	[0.83]	[0.70]	[0.84]	[0.78]	[0.46]	[0.44]	[0.64]	[0.49]
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.42]	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	-	-	-	-	-
50-60	-	-	-	-	-	-	-	-	-	-	-	<LOD	-

DAT = Days after Treatment
LOQ = 1.0 µg/kg LOD = 0.30 µg/kg [x] = LOD >< LOQ

Table B.8.1.1.5.1-18: Mean measured concentrations of isoflucypram for St. Etienne du Gres (Southern France)

	Mean concentration from 4 replicates isoflucypram (µg/kg)									
MEAN	**DAT [days]**									
Layer [cm]	**0**	**3**	**7**	**14**	**30**	**58**	**92**	**116**	**151**	**205**
Plot 1										
0-10	64.9	62.5	40.8	40.9	15.7	11.3	5.66	7.03	7.81	3.75
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.38]	[0.42]	[0.39]	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-
Plot 2										
0-10	62.3	59.8	48.8	32.9	12.1	5.26	5.79	2.17	1.95	1.34
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.99]	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-
Plot 3										
0-10	61.8	57.9	55.5	36.6	23.5	8.29	3.44	4.51	4.62	1.81
10-20	-	<LOD	<LOD	<LOD	[0.38]	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-
Plot 4										
0-10	68.3	60.0	54.1	33.5	13.6	5.20	2.54	1.76	1.72	1.16
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-

Table B.8.1.1.5.1-19: Mean measured concentrations of M12 for St. Etienne du Gres (Southern France)

	Mean concentration from 4 replicates M12 (µg/kg)									
MEAN	**DAT [days]**									
Layer [cm]	**0**	**3**	**7**	**14**	**30**	**58**	**92**	**116**	**151**	**205**
Plot 1										
0-10	64.9	62.5	40.8	40.9	15.7	11.3	5.66	7.03	7.81	3.75
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.38]	[0.42]	[0.39]	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-
Plot 2										
0-10	62.3	59.8	48.8	32.9	12.1	5.26	5.79	2.17	1.95	1.34
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.99]	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-
Plot 3										
0-10	61.8	57.9	55.5	36.6	23.5	8.29	3.44	4.51	4.62	1.81
10-20	-	<LOD	<LOD	<LOD	[0.38]	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-
Plot 4										
0-10	68.3	60.0	54.1	33.5	13.6	5.20	2.54	1.76	1.72	1.16
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	-	-	-	-	-	-	-	-	-

DAT = Days after Treatment
LOQ = 1.0 µg/kg LOD = 0.30 µg/kg [x] = LOD >< LOQ

Table B.8.1.1.5.1-20: Mean measured concentrations of isoflucypram for Albaro di Ronco all Adige (Italy)

	Mean concentration from 4 replicates isoflucypram (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**14**	**28**	**62**	**89**	**122**	**157**	**209**	**369**	**531**	**728**
Plot 1													
0-10	65.8	54.2	55.4	47.5	45.6	35.8	28.3	32.5	20.5	17.5	20.8	15.7	9.22
10-20	-	<LOD	<LOD	<LOD	[0.55]	[0.56]	[0.35]	[0.46]	4.58	1.19	1.13	1.51	[0.80]
20-30	-	<LOD	<LOD	<LOD	[0.63]	1.77	[0.38]	[0.42]	[0.79]	1.18	[0.88]	1.17	[0.44]
30-40	-	-	-	-	<LOD	<LOD	[0.34]	<LOD	<LOD	[0.42]	[0.51]	[0.33]	[0.34]
40-50	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-
Plot 2													
0-10	65.4	51.2	56.7	38.0	48.2	32.0	20.7	18.8	25.3	16.1	22.7	15.4	11.1
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.47]	<LOD	1.39	[0.65]	[0.77]	3.47	[0.51]	1.35
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.65]	[0.96]	<LOD	1.10	1.04	1.31	[0.65]
30-40	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-
Plot 3													
0-10	63.9	51.9	51.9	47.7	57.8	19.1	22.5	23.4	18.3	12.6	18.0	17.7	10.3
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.45]	2.21	1.11	[0.39]	1.29	2.32	2.22	1.49
20-30	-	<LOD	<LOD	<LOD	<LOD	[0.60]	[0.45]	[0.75]	<LOD	1.08	3.54	1.01	2.21
30-40	-	-	-	-	-	[0.32]	<LOD	<LOD	<LOD	<LOD	[0.70]	<LOD	<LOD
40-50	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-
Plot 4													
0-10	67.1	54.3	49.4	78.8	27.1	30.2	19.6	16.0	16.8	17.2	28.7	16.8	11.4
10-20	-	<LOD	<LOD	<LOD	[0.71]	<LOD	[0.62]	1.18	[0.54]	[0.61]	1.09	1.16	[0.91]
20-30	-	<LOD	<LOD	[0.37]	<LOD	[0.63]	<LOD	<LOD	<LOD	[0.44]	[0.94]	[0.57]	[0.49]
30-40	-	-	-	<LOD	<LOD	<LOD	-	-	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-

Table B.8.1.1.5.1-21: Mean measured concentrations of M12 for Albaro di Ronco all Adige (Italy)

	Mean concentration from 4 replicates M12 (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**14**	**28**	**62**	**89**	**122**	**157**	**209**	**369**	**531**	**728**
Plot 1													
0-10	65.8	54.2	55.4	47.5	45.6	35.8	28.3	32.5	20.5	17.5	20.8	15.7	9.22
10-20	-	<LOD	<LOD	<LOD	[0.55]	[0.56]	[0.35]	[0.46]	4.58	1.19	1.13	1.51	[0.80]
20-30	-	<LOD	<LOD	<LOD	[0.63]	1.77	[0.38]	[0.42]	[0.79]	1.18	[0.88]	1.17	[0.44]
30-40	-	-	-	-	<LOD	<LOD	[0.34]	<LOD	<LOD	[0.42]	[0.51]	[0.33]	[0.34]
40-50	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-
Plot 2													
0-10	65.4	51.2	56.7	38.0	48.2	32.0	20.7	18.8	25.3	16.1	22.7	15.4	11.1
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.47]	<LOD	1.39	[0.65]	[0.77]	3.47	[0.51]	1.35
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	[0.65]	[0.96]	<LOD	1.10	1.04	1.31	[0.65]
30-40	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-
Plot 3													
0-10	63.9	51.9	51.9	47.7	57.8	19.1	22.5	23.4	18.3	12.6	18.0	17.7	10.3
10-20	-	<LOD	<LOD	<LOD	<LOD	[0.45]	2.21	1.11	[0.39]	1.29	2.32	2.22	1.49
20-30	-	<LOD	<LOD	<LOD	<LOD	[0.60]	[0.45]	[0.75]	<LOD	1.08	3.54	1.01	2.21
30-40	-	-	-	-	-	[0.32]	<LOD	<LOD	<LOD	<LOD	[0.70]	<LOD	<LOD
40-50	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-
Plot 4													
0-10	67.1	54.3	49.4	78.8	27.1	30.2	19.6	16.0	16.8	17.2	28.7	16.8	11.4
10-20	-	<LOD	<LOD	<LOD	[0.71]	<LOD	[0.62]	1.18	[0.54]	[0.61]	1.09	1.16	[0.91]
20-30	-	<LOD	<LOD	[0.37]	<LOD	[0.63]	<LOD	<LOD	<LOD	[0.44]	[0.94]	[0.57]	[0.49]
30-40	-	-	-	<LOD	<LOD	<LOD	-	-	<LOD	<LOD	<LOD	<LOD	<LOD
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD
50-60	-	-	-	-	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	-

Table B.8.1.1.5.1-22: Mean measured concentrations of isoflucypram for Vilobi d'Onyar (Spain)

	Mean concentration from 4 replicates isoflucypram (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**15**	**30**	**57**	**99**	**120**	**158**	**224**	**345**	**533**	**714**
Plot 1													
0-10	59.2	62.6	53.2	22.0	31.8	21.7	24.5	24.9	21.2	17.0	13.2	7.96	8.21

10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
Plot 2													
0-10	76.9	85.3	83.6	37.0	44.8	22.3	29.0	32.6	17.9	12.7	11.5	8.01	7.15
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.44]	<LOD	1.02	1.60
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
Plot 3													
0-10	68.9	67.7	68.9	30.7	34.2	26.6	26.5	28.1	24.9	11.9	12.0	8.90	6.35
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	[0.32]	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
Plot 4													
0-10	83.2	75.8	67.2	32.6	34.9	22.4	31.2	32.6	19.5	17.7	16.4	9.01	7.65
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-

Table B.8.1.1.5.1-23: Mean measured concentrations of M12 for Vilobi d'Onyar (Spain)

	Mean concentration from 4 replicates M12 (µg/kg)												
MEAN	**DAT [days]**												
Layer [cm]	**0**	**3**	**7**	**15**	**30**	**57**	**99**	**120**	**158**	**224**	**345**	**533**	**714**
Plot 1													
0-10	<LOD	<LOD	<LOD	[0.33]	[0.39]	<LOD	[0.44]	[0.42]	[0.46]	[0.32]	[0.39]	<LOD	<LOD
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
Plot 2													
0-10	<LOD	<LOD	<LOD	[0.49]	[0.53]	[0.33]	[0.50]	[0.57]	[0.41]	<LOD	<LOD	<LOD	<LOD
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
Plot 3													
0-10	<LOD	<LOD	<LOD	[0.42]	[0.39]	[0.41]	[0.47]	[0.60]	[0.70]	<LOD	<LOD	<LOD	<LOD
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
Plot 4													
0-10	<LOD	<LOD	<LOD	[0.49]	[0.47]	[0.36]	[0.55]	[0.49]	[0.41]	<LOD	[0.35]	<LOD	<LOD
10-20	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20-30	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
30-40	-	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
40-50	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-
50-60	-	-	-	-	-	-	-	-	<LOD	<LOD	-	-	-

DAT = Days after Treatment
LOQ = 1.0 µg/kg LOD = 0.30 µg/kg [x] = LOD >< LOQ

Analytical results of treated samples

Taking into account the bulk density of the wet soil samples (soil layers) the concentrations of isoflucypram (BCS-CN88460) in wet soil were transformed from µg/kg into amount/ area (g/ha) values by multiplication of the concentration of the test item in a wet soil layer with the corresponding bulk density of that wet soil layer and the height of that wet soil layer. The height of each soil layer is 0.1m. The following transfer calculation was used by the applicant.

$$\frac{Amount\ of\ analyte}{area}\left[\left[\frac{g}{ha}\right]\right] = c_{analyte,wet\ soil}\left[\frac{\mu g}{kg}\right] * \rho_{soil\ layer,wet\ soil}\left[\frac{kg}{L}\right] * h_{soil\ layer}[m] * 10^{-6}\left[\frac{g}{\mu g}\right] * 10^{3}\left[\frac{L}{m^3}\right] * 10^{4}\left[\frac{m^2}{ha}\right]$$

No mass balance was calculated by the applicant as the study design does not allow for it.

Table B.8.1.1.5.1- 24: Analytical results of isoflucypram preprocessed values in entire soil profiles (0-100 cm) [g/ha]

Subplot	Burscheid, Germany (14-2750-01) Days after application												
	0	**3**	**7**	**13**	**28**	**70**	**91**	**110**	**160**	**209**	**370**	**538**	**713**
T1	97.3	88.9	103	97.4	91.8	61.3	49.6	60.0	38.4	39.1	32.2	33.3	27.1
T2	101	106	97.7	97.6	73.5	83.7	55.5	55.1	55.1	47.3	34.2	28.7	22.2
T3	92.6	88.2	86.5	86.5	84.3	50.3	43.1	50.4	37.5	47.6	38.8	27.9	30.2
T4	102	93.3	85.2	90.4	88.3	67.3	74.2	53.4	51.2	44.1	42.1	33.8	35.2
Mean	**98.2**	**94.1**	**93.1**	**93.0**	**84.5**	**65.7**	**55.6**	**54.7**	**45.6**	**44.5**	**36.8**	**30.9**	**28.7**
	Great Chishill, United Kingdom (14-2750-02) Days after application												
	0	**4**	**7**	**14**	**27**	**67**	**111**	**140**	**168**	**278**	**402**	**560**	**749**
T1	97.2	115	93.1	79.0	73.8	77.0	81.6	49.9	40.4	31.8	26.9	28.4	22.9
T2	90.4	100	82.0	78.9	79.0	77.2	86.4	0.28[a)]	41.4	37.3	32.2	23.8	12.7
T3	100	85.6	114	88.1	93.1	77.2	71.1	22.9	51.3	32.4	33.4	25.2	14.6
T4	99.6	89.0	98.4	90.3	77.9	52.9	79.7	45.7	38.8	46.3	39.3	43.4	30.8
Mean	**96.8**	**97.4**	**96.9**	**84.1**	**81.0**	**71.1**	**79.7**	**39.5**	**43.0**	**37.0**	**33.0**	**30.2**	**20.3**
	Parcay Meslay, Northern France (14-2750-03) Days after application												
	0	**3**	**7**	**14**	**29**	**63**	**88**	**121**	**143**	**210**	**357**	**519**	**701**
T1	75.7	82.4	77.5	78.7	57.2	46.9	52.0	49.3	37.7	44.6	33.4	29.3	28.9
T2	86.4	71.3	86.8	70.3	79.1	53.9	55.4	46.4	47.4	36.6	40.1	36.7	31.4
T3	86.4	74.8	86.8	81.6	65.0	57.6	46.0	41.3	53.1	39.0	35.6	33.8	32.2
T4	104	112	79.0	86.0	57.1	53.9	51.1	49.5	44.3	47.4	31.4	34.8	32.1
Mean	**88.1**	**85.1**	**82.5**	**79.2**	**64.6**	**53.1**	**51.1**	**46.6**	**45.6**	**41.9**	**35.1**	**33.7**	**31.2**
	St. Etienne du Gres, Southern France (14-2750-04) Days after application												
	0	**3**	**7**	**14**	**30**	**58**	**92**	**116**	**151**	**205**			
T1	93.1	82.8	58.2	59.6	22.3	15.2	9.36	12.1	14.0	7.33			
T2	88.1	73.9	67.2	50.3	18.6	8.12	10.0	3.46	3.40	2.87			
T3	85.9	80.2	69.6	55.2	35.1	13.5	5.59	7.00	8.99	3.79			
T4	96.4	87.3	73.3	53.3	19.8	8.16	4.59	2.95	3.37	2.82			
Mean	**90.9**	**81.1**	**67.1**	**54.6**	**24.0**	**11.2**	**7.39**	**6.38**	**7.44**	**4.20**			
	Albaro di Ronco all Adige, Italy (14-2750-05) Days after application												
	0	**3**	**7**	**14**	**28**	**62**	**89**	**122**	**157**	**209**	**369**	**531**	**728**
T1	91.5	87	88.9	73.7	72.2	63.4	44.9	53.2	45.6	34.2	33.4	30.2	17.9
T2	90.2	83.8	87	56.6	68.8	52.3	35.3	35.6	49.2	29.2	40.5	28.3	22.7
T3	88.2	87.5	85.4	70.9	87.5	33.6	41.6	43.2	35.2	26.7	37.7	35.4	23.1
T4	90.3	90.9	82.1	119	40.5	52	32.9	27.6	30.5	29.7	40.6	29.8	20.2
Mean	**90.1**	**87.3**	**85.9**	**80.1**	**67.3**	**50.3**	**38.7**	**39.9**	**40.1**	**30.0**	**38.1**	**30.9**	**21.0**
	Vilobi d'Onyar, Spain (14-2750-06) Days after application												
	0	**3**	**7**	**15**	**30**	**57**	**99**	**120**	**158**	**224**	**345**	**533**	**714**
T1	75.6	79.2	67.4	35.3	44.5	31.9	31.9	30.9	30.1	27.2	22.5	12.4	13.6
T2	96.4	96.7	104	54.0	56.3	34.1	35.4	40.0	24.1	21.7	18.1	15.7	15.6
T3	86.3	86.9	76.9	44.8	43.1	41.7	34.7	31.2	33.1	21.3	19.8	15.1	10.2
T4	94.3	92.8	78.9	48.3	47.8	36.1	40.8	37.8	26.6	28.6	25.8	14.8	12.5
Mean	**88.2**	**88.9**	**81.8**	**45.6**	**47.9**	**36.0**	**35.7**	**35.0**	**28.5**	**24.7**	**21.6**	**14.5**	**13.0**

a) outlier, value not used for calculation

Table B.8.1.1.5.1- 25: Analytical results of M12 pre-processed mean values [g/ha]

Subplot	Burscheid, Germany (14-2750-01) Days after application												
	0	**3**	**7**	**13**	**28**	**70**	**91**	**110**	**160**	**209**	**370**	**538**	**713**
T1	0.00	0.00	0.00	0.26	1.12	1.49	1.17	1.74	1.19	1.13	0.95	1.26	0.93
T2	0.00	0.00	0.00	0.25	0.94	1.63	1.34	1.19	1.66	1.58	1.12	1.23	0.94
T3	0.00	0.00	0.00	0.26	0.91	1.17	1.30	1.05	1.44	1.63	1.23	1.18	1.04
T4	0.00	0.00	0.00	0.25	1.02	1.96	2.08	1.06	2.16	2.17	1.76	1.77	1.66
Mean	**0.00**	**0.00**	**0.00**	**0.26**	**1.00**	**1.56**	**1.47**	**1.26**	**1.61**	**1.63**	**1.27**	**1.36**	**1.14**
	Great Chishill, United Kingdom (14-2750-02) Days after application												
	0	**4**	**7**	**14**	**27**	**67**	**111**	**140**	**168**	**278**	**402**	**560**	**749**
T1	0.00	0.00	0.21	0.72	0.88	1.44	2.38	1.91	2.05	1.51	0.98	0.89	0.59
T2	0.00	0.00	0.00	0.22	0.91	1.26	2.11	0.00[a)]	1.30	0.97	1.29	0.24	0.00
T3	0.00	0.00	0.00	0.24	1.06	1.51	1.55	1.16	2.56	1.09	1.15	0.81	0.00
T4	0.00	0.00	0.00	0.22	0.85	1.18	2.58	2.60	1.49	1.31	1.60	1.18	0.86
Mean	**0.00**	**0.00**	**0.05**	**0.35**	**0.93**	**1.35**	**2.16**	**1.89**	**1.85**	**1.22**	**1.26**	**0.78**	**0.36**
	Parcay Meslay, Northern France (14-2750-03) Days after application												
	0	**3**	**7**	**14**	**29**	**63**	**88**	**121**	**143**	**210**	**357**	**519**	**701**
T1	0.00	0.00	0.00	0.22	0.88	1.19	1.67	1.50	1.55	1.35	1.30	1.38	1.19
T2	0.00	0.00	0.00	0.23	1.11	1.08	1.06	1.18	1.05	0.25	0.93	1.30	1.04
T3	0.00	0.00	0.18	0.75	1.01	1.54	1.32	1.61	2.01	1.35	1.49	1.62	1.64
T4	0.00	0.00	0.00	0.22	0.87	1.43	1.25	1.62	1.49	1.83	0.99	1.39	1.25
Mean	**0.00**	**0.00**	**0.05**	**0.36**	**0.97**	**1.31**	**1.33**	**1.48**	**1.53**	**1.20**	**1.18**	**1.42**	**1.28**
	St. Etienne du Gres, Southern France (14-2750-04) Days after application												
	0	**3**	**7**	**14**	**30**	**58**	**92**	**116**	**151**	**205**			
T1	0.00	0.20	1.45	2.50	3.91	3.43	2.50	2.67	1.57	1.25			
T2	0.00	0.18	1.61	2.60	3.65	3.73	2.76	2.83	1.71	0.29			
T3	0.00	0.21	1.41	2.43	4.53	4.83	2.87	2.69	1.71	1.02			
T4	0.00	0.22	1.04	2.24	3.43	3.29	3.75	2.86	2.01	0.33			
Mean	**0.00**	**0.20**	**1.38**	**2.44**	**3.88**	**3.82**	**2.97**	**2.76**	**1.75**	**0.72**			
	Albaro di Ronco all Adige, Italy (14-2750-05) Days after application												
	0	**3**	**7**	**14**	**28**	**62**	**89**	**122**	**157**	**209**	**369**	**531**	**728**
T1	< LOD	< LOD	0.24	0.27	1.20	1.81	2.23	3.37	1.92	1.21	1.23	1.06	1.05
T2	< LOD	< LOD	0.23	0.84	1.20	1.70	2.04	2.76	2.40	1.25	1.39	1.08	1.20
T3	< LOD	< LOD	0.25	0.91	1.26	1.64	2.14	2.51	1.37	1.03	1.04	1.60	1.41
T4	< LOD	0.25	0.67	1.09	1.19	1.57	1.96	1.98	1.27	0.24	1.30	0.82	1.20
Mean	**< LOD**	**0.06**	**0.35**	**0.78**	**1.21**	**1.68**	**2.09**	**2.66**	**1.74**	**0.93**	**1.24**	**1.14**	**1.22**
	Vilobi d'Onyar, Spain (14-2750-06) Days after application												
	0	**3**	**7**	**15**	**30**	**57**	**99**	**120**	**158**	**224**	**345**	**533**	**714**
T1	0.00	0.00	0.19	0.83	0.85	0.22	0.88	0.85	0.97	0.81	0.97	0.23	0.00
T2	0.00	0.00	0.19	1.01	0.96	0.78	0.92	1.02	0.85	0.24	0.00	0.00	0.00
T3	0.00	0.00	0.17	0.92	0.78	0.92	0.90	0.97	1.24	0.27	0.00	0.00	0.00
T4	0.00	0.00	0.18	1.04	0.96	0.86	1.04	0.89	0.88	0.24	0.84	0.24	0.00
Mean	**0.00**	**0.00**	**0.18**	**0.95**	**0.89**	**0.70**	**0.94**	**0.93**	**0.99**	**0.39**	**0.45**	**0.12**	**0.00**

a) outlier, value not used for calculation

B. KINETIC ANALYSIS

The data for isoflucypram were evaluated. The measured initial concentration at day 0 was included in the parameter optimization procedure. Based on criterion for chi^2 error to be minimal and visual assessment the best fit kinetic model was chosen for the evaluation of the dissipation time. The calculation considered the quantifiable residues for the whole soil profiles expressed in g/ha. The results proposed by the applicant are summarised in Table B.8.1.1.5.1- 26 with best fits highlighted in bold letters UKRMS has performed a separate validation below. For the six test sites Burscheid (Germany), Great Chishill (United Kingdom), Parcay Meslay (Northern France), St. Etienne du Gres (Southern France), Albaro di Ronco all Adige (Italy) and Vilobi d'Onyar (Spain) the dissipation of isoflucypram could be described using biphasic kinetics, a full and detailed assessment is provided in section B.8.1.1.5.2, a normalisation calculation is also performed for modelling endpoints.

Table B.8.1.1.5.1- 26: M12 maximum percentage formation of applied amount

Location	Maximum mean recovery Isoflucypram (g/ha)	Maximum mean recovery M12 (g/ha)	Maximum percentage formation of M12 converted from applied isoflucypram	Maximum percentage formation of M12 as mw equivalent from the parent applied rate.
Burscheid	98.2	1.63	1.66	1.72
Great Chishill	97.4	2.16	2.22	2.26
Parcay Meslay	88.1	1.53	1.74	1.45
St. Etienne du Gres	90.9	3.88	4.27	3.79
Albaro di Ronco all Adige	90.1	2.66	2.95	2.58
Vilobi d'Onyar	88.9	0.99	1.11	0.95

Table B.8.1.1.5.1- 27: Isoflucypram: calculation of dissipation times proposed by the applicant, but not relied upon.

Location and trial no.	**Kinetic Model** [a),b)]	**DT_{50} [d]**	**DT_{90} [d]**	**Visual Assessment** [c)]	**Chi^2 error [%]**
Burscheid	SFO	241	801	-	11.98
(Germany)	FOMC	155	> 1000	+	3.502
14-2750-01	**DFOP**	**143**	**> 1000**	**+**	**2.532**
Great Chishill	SFO	239	795	-	12.9
(United Kingdom)	FOMC	181	> 1000	+	10.41
14-2750-02	**DFOP**	**177**	**> 1000**	**+**	**10.68**
Parcay Meslay	SFO	309	> 1000	-	14.04
(Northern France)	FOMC	153	> 1000	+	2.814
14-2750-03	**DFOP**	**147**	**> 1000**	**+**	**2.236**
St. Etienne du Gres	SFO	18.0	59.7	-	8.801
(Southern France)	FOMC	16.1	77.8	+	6.861
14-2750-04	**DFOP**	**16.5**	**69.6**	**+**	**4.537**
Albaro di Ronco all Adige	SFO	198	659	-	18.41
(Italy)	FOMC	91.5	> 1000	o	7.388
14-2750-05	**DFOP**	**77.6**	**> 1000**	**o**	**5.523**
Vilobi d'Onyar	SFO	107	354	-	23.39
(Spain)	FOMC	35.3	> 1000	+	11.59
14-2750-06	**DFOP**	**25.7**	**812**	**+**	**10.29**

a) SFO: single first order, FOMC: first order multi compartment, DFOP: double first order in parallel
b) Best fits highlighted in **bold** letters
c) Visual assessment: + = good, o = moderate, - = poor

III. CONCLUSIONS

Application, collection and analytical methods are considered by the RMS to be acceptable. Soils were sufficiently irrigated to minimise soil surface processes within 3 days of application for 5 of the 6 soils for Parcay Meslay 9 mm of irrigation was performed. Significant irrigation was noted in the St. Etienne du Gres soil, this is considered acceptable by the RMS since wheat in not commonly grown during the summer in the south of France without significant irrigation. There is also no evidence that irrigation has carried either isoflucypram or its metabolite M12 through the soil profile (indicating that soils were saturated) and that irrigation was not lost either by evaporation or plant uptake.

Isoflucypram was dissipated slowly in soil under the conditions of field testing at six trial sites in Northern and Southern Europe. The dissipation of isoflucypram proposed by the applicant could be described by biphasic kinetic models with calculated best fit DT_{50} values between 16.5 and 177 days and DT90 of 69.6 -1000 days in the tested soils.
Residues of isoflucypram were shown to remain mainly in the top 0-40 cm soil layer with residue levels within the range of 4.2 to 31.2 g/ha at study end.
Dissipation of isoflucypram was accompanied by the formation of its degradation product M12 with maximum amounts in the entire soil profiles detected between DAT-30 and DAT-209 and ranged from 0.99 to 3.88 g/ha (molar correction factor 1.075) and 1.1% to 4.3% of parent applied. M12 was found in the top 0-20 cm only. A kinetic assessment is included in section B.8.1.1.5.2 below

B.8.1.1.5.2: Kinetic Evaluation of the Terrestrial Field Dissipation Study

Previous evaluation:	None, new active substance.

The field dissipation study Heinemann, O.; Junge, T.; (2017) has been assessed in section B.8.1.1.5.1 above. All applications were made to bare soil.
Weather data were not included as part of the final report; weather data were requested from the applicant and the appropriate data files were provided.
Model input datasets comprised of the residual amounts found in each replicate test system at each sampling interval.

Non-normalised assessment for persistence and PECsoil evaluations

The applicant has provided an assessment (Reinken, G.; Mikolasch, B.; (2017) using KinGUI v2.1. A full presentation of the statistical values K1, K2 and g was not presented by the applicant. Consequently the RMS performed separate calculations. The RMS could not reproduce all of the applicant's kinetic endpoints, particularly the RMS calculations resulted in longer DT_{90} values.

The UK-RMS has performed their assessment using CAKE v3.1. For the non-normalised assessment all data points have been placed into the model with the exception of the obvious outlier for the Great Chishill soil, T2, 140 day as per figure 7-1 of the FOCUS kinetics guidance (2006). Data is taken from table B.8.1.1.5.1-5 and B.8.1.1.5.1-6 above; no outliers were removed by the RMS. All four kinetic models have been tested, as the result is to be used for persistence and PECsoil evaluation. Therefore the RMS results are presented below. The rejected SFO fit graphs and residual plots are presented in figures B.8.1.1.5.2-1 to B.8.1.1.5.2-6. The accepted fit and residual graphs are presented in table B.8.1.1.5.2-7 to B.8.1.1.5.2-12. Fitting for metabolite M12 was also performed, fit and residual graphs are presented in table B.8.1.1.5.2-13 to B.8.1.1.5.2-18. An overview table of the kinetic assessment for isoflucypram, is presented in table B.8.1.1.5.2-1, with a more detailed summary table of the RMS accepted results in table B.8.1.1.5.2-2.

Table B.8.1.1.5.2-1: Non-normalised Field Dissipation Results for isoflucypram performed by the RMS.

Location and trial no.	Kinetic model[a),b)]	DT_{50} [d]	DT_{90} [d]	Visual assessment	Chi^2 error [%]	T test
Burscheid (Germany) 14-2750-01	SFO	241	801	Poor	12	<0.001
	FOMC	155	5960	Good	3.5	n/a
	DFOP	**143**	**2250**	**Good**	**2.53**	*
	HS	123	1830	Good	2.04	<0.001/ <0.001
Great Chishill (United Kingdom) 14-2750-02	SFO	239	795	Poor	12.9	<0.001
	FOMC	182	2270	Good	10.4	n/a
	DFOP	**177**	**1810**	**Good**	**10.7**	*
	HS	163	1500	Good	10.3	<0.001/ 0.060
Parcay Meslay (Northern France) 14-2750-03	SFO	309	1030	Poor	14.0	<0.001
	FOMC	153	>10000	Good	2.81	n/a
	DFOP	**147**	**2530**	**Good**	**2.24**	*
	HS	204	1990	Poor	2.94	<0.001/ <0.001
St. Etienne du Gres (Southern France) 14-2750-04	SFO	18	59.7	Poor	8.8	<0.001
	FOMC	16.1	77.8	Good	6.86	n/a
	DFOP	**16.5**	**69.6**	**Good**	**4.54**	*
	HS	16.7	80	Good	3.96	<0.001/ <0.001
Albaro di Ronco all Adige (Italy) 14-2750-05	SFO	169	562	Poor	19.7	<0.001
	FOMC	83.4	6130	Acceptable	8.55	n/a
	DFOP	**72.4**	**3090**	**Good**	**6.06**	*
	HS	71.2	2470	Poor	5.49	<0.001/ 0.009
Vilobi d'Onyar (Spain) 14-2750-06	SFO	106	352	Poor	23.2	<0.001
	FOMC	35.3	2140	Good	11.8	n/a
	DFOP	**25.9**	**762**	**Good**	**10.3**	*
	HS	25.5	>10000	Very poor	18.2	<0.001/ 0.5

a) SFO: single first order, FOMC: first order multi compartment, DFOP: double first order in parallel
b) Best fits highlighted in **bold** letters
* Results presented in table B.8.1.1.5.2-2

Table B.8.1.1.5.2-2: Summary of best fit Non-normalised Field Dissipation Results for isoflucypram from RMS modelling for use in PECsoil and persistence evaluations.

Location and trial no.	Kinetic Model a),b)	DT_{50} [d]	DT_{90} [d]	Visual Assessment	Chi^2 error [%]	T test	k1 (DT_{50})	k2 (DT_{50})	g
Burscheid (Germany) 14-2750-01	DFOP	143	2250	Good	2.53	K1= <0.001 K2= 0.076	0.0126 (55.1)	0.0006 (1170)	0.557
Great Chishill (United Kingdom) 14-2750-02	**DFOP**	**177**	**1810**	**Good**	**10.7**	**K1= 0.039 K2= 0.286**	**0.0078 (88.9)**	**0.0007 (1020)**	**0.610**
Parcay Meslay (Northern France) 14-2750-03	DFOP	147	2530	Good	2.24	K1= <0.001 K2= 0.003	0.0246 (28.1)	0.0007 (1040)	0.462
St. Etienne du Gres (Southern France) 14-2750-04	DFOP	16.5	69.6	Good	4.54	K1= <0.001 K2= 0.472	0.0464 (14.9)	0.0003 (2010)	0.934
Albaro di Ronco all Adige (Italy) 14-2750-05	**DFOP**	**72.4**	**3090**	**Good**	**6.06**	**K1= <0.001 K2= 0.1851**	**0.0210 (33.1)**	**0.0004 (1810)**	**0.627**
Vilobi d'Onyar (Spain) 14-2750-06	DFOP	25.9	762	Good	10.3	K1= <0.001 K2= <0.001	0.0718 (9.65)	0.0019 (361)	0.568

a) SFO: single first order, FOMC: first order multi compartment, DFOP: double first order in parallel
b) Worst case fits highlighted in **bold** letters, to address PECsoil and PECsoil accumulation

UK RMS notes the T-test values for some fits are high for the K2 value, visual fits and chi^2 results were considered to be more important for the best fit selection. This is in line with experience from the E.U. peer review procedure.

Table B.8.1.1.5.2-3: Summary Non-normalised Field Dissipation Results performed by the RMS for use in PECsoil and persistence evaluations for metabolite M12.

Location and trial no.	Kinetic Model a),b)	DT_{50} [d]	DT_{90} [d]	Visual Assessment	Chi^2 error [%]	T test	ff
Burscheid (Germany) 14-2750-01	DFOP-SFO	397	1320	Acceptable	13.1	<0.001	0.039
Great Chishill (United Kingdom) 14-2750-02	DFOP-SFO	120	397	Acceptable	13.7	<0.001	0.071
Parcay Meslay (Northern France) 14-2750-03	**DFOP-SFO**	**714**	**2370**	**Acceptable**	**11.2**	**<0.001**	**0.0361**
St. Etienne du Gres (Southern France) 14-2750-04	DFOP-SFO	71.4	237	Acceptable	9	<0.001	0.068
Albaro di Ronco all Adige (Italy) 14-2750-05	DFOP-SFO	333	1110	Poor *	22.3	<0.001	0.043
Vilobi d'Onyar (Spain) 14-2750-06	DFOP-SFO	142	473	Poor *	27.6	<0.001	0.020

a) SFO: single first order, FOMC: first order multi compartment, DFOP: double first order in parallel
b) Worst case highlighted in **bold** letters
* Visual assessments and chi^2 values are considered to be sufficiently poor that the values are no used further in the risk assessment.

SFO fitting - isoflucypram

Figure B.8.1.1.5.2-1: RMS rejected SFO fit and residual Burscheid soil for isoflucypram

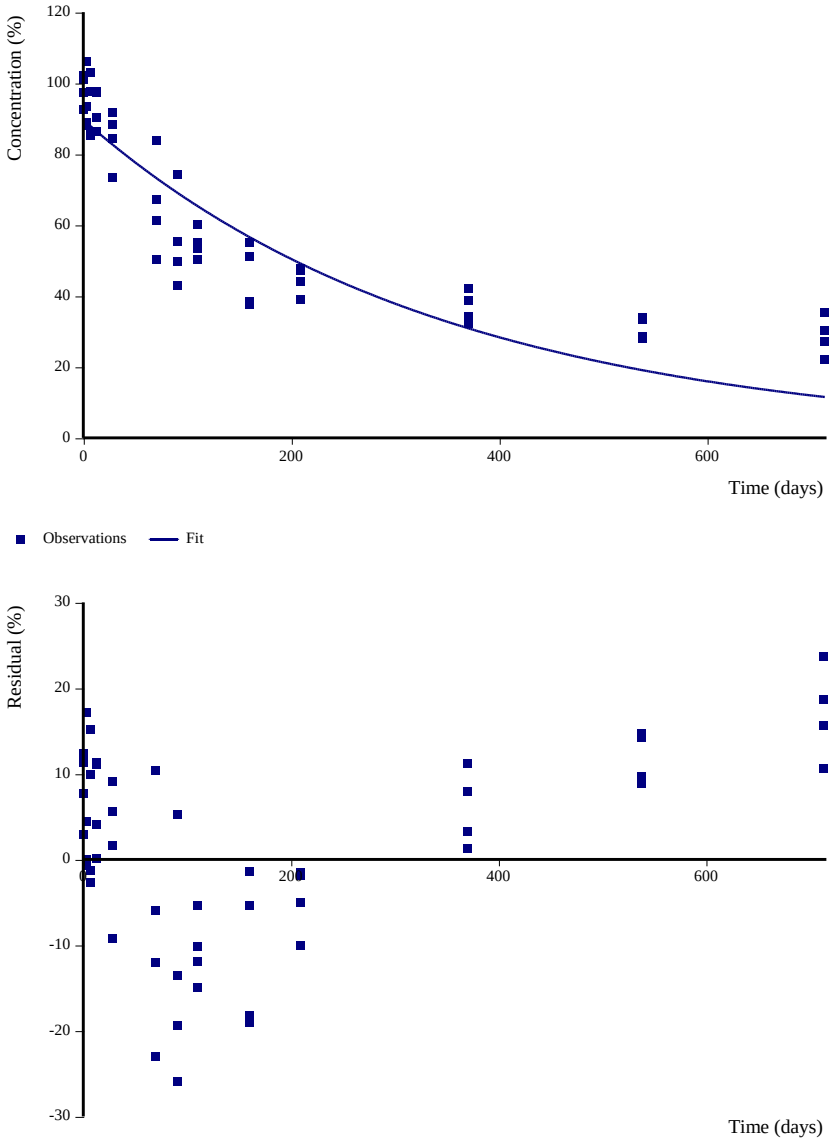


Figure B.8.1.1.5.2-2: RMS rejected SFO fit and residual Great Chishill soil for isoflucypram

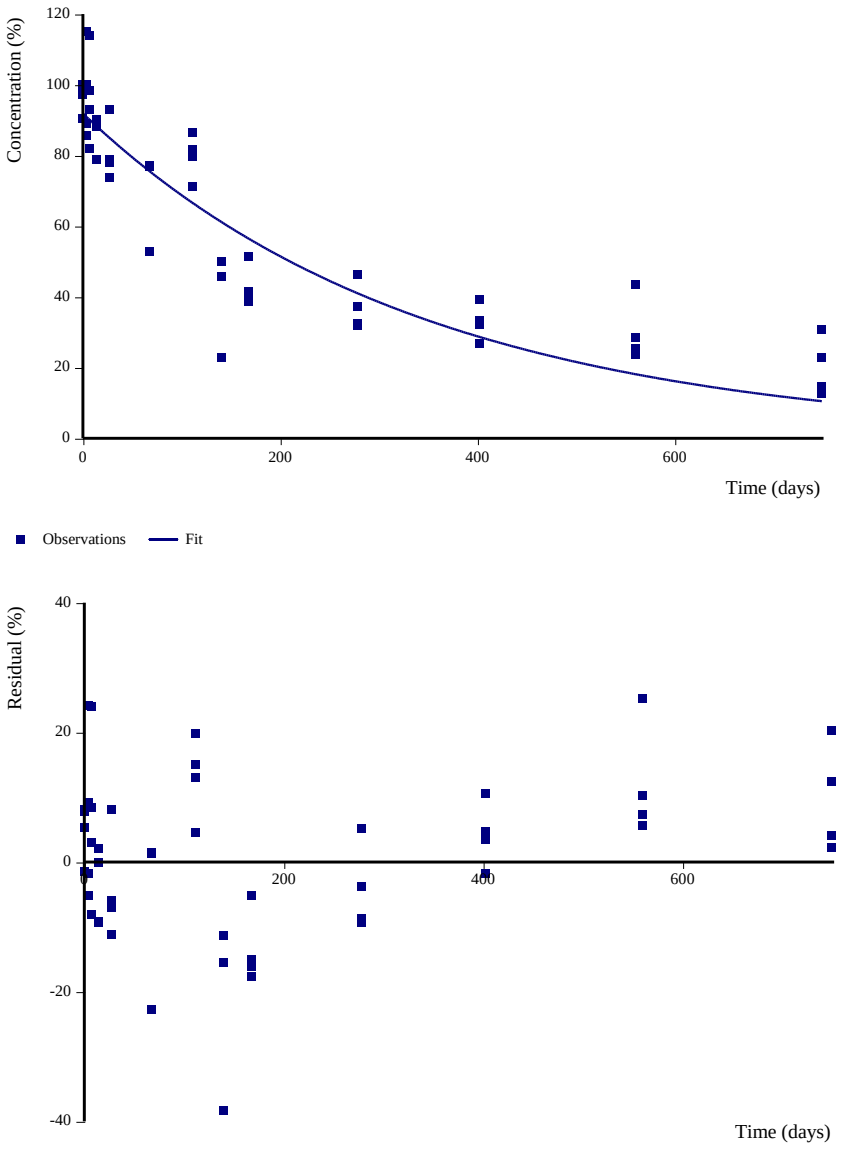


Figure B.8.1.1.5.2-3: RMS rejected SFO fit and residual Parcay Meslay soil for isoflucypram

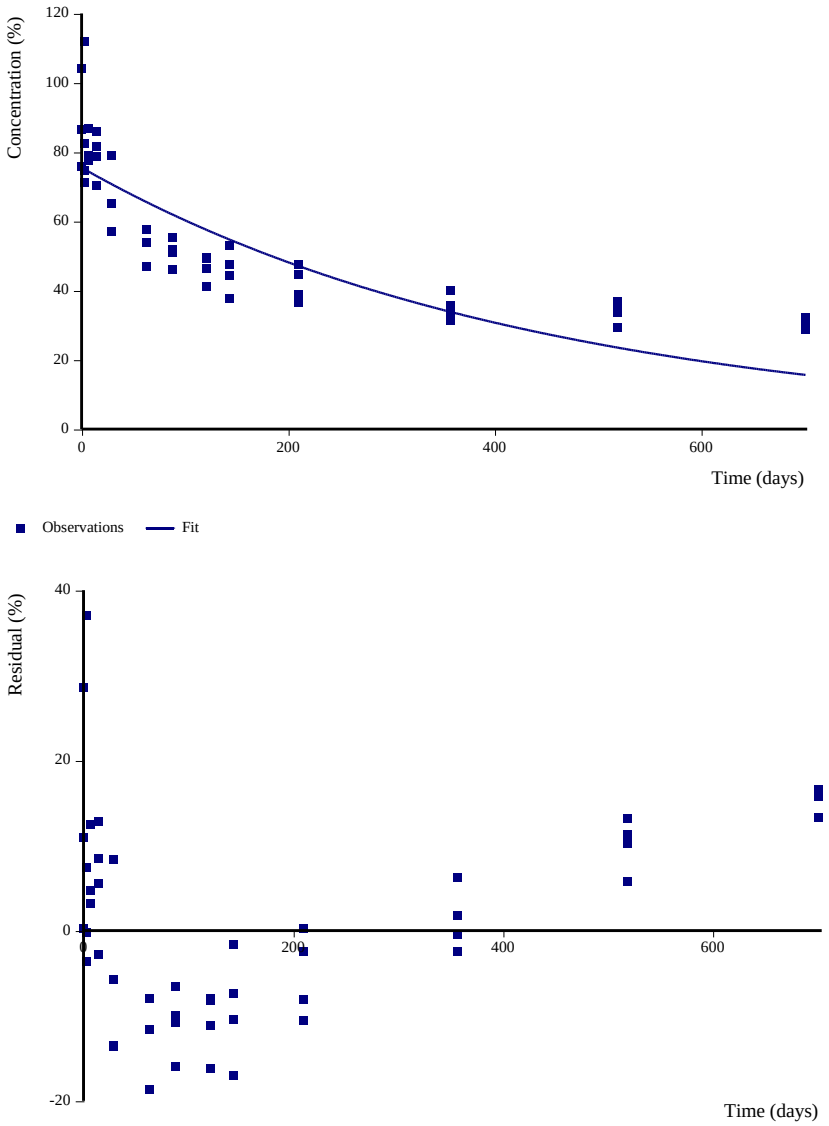


Figure B.8.1.1.5.2-4: RMS rejected SFO fit and residual St. Etienne du Gres soil for isoflucypram

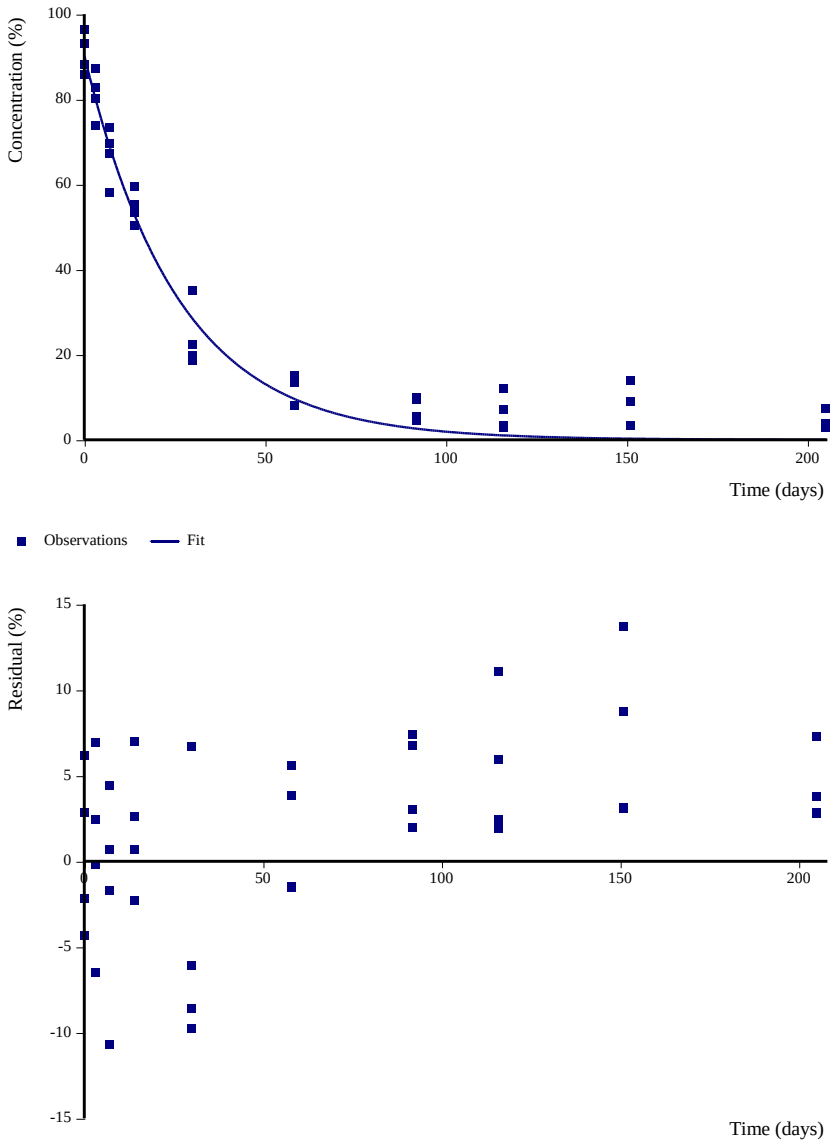


Figure B.8.1.1.5.2-5: RMS rejected SFO fit and residual Albaro di Ronco all Adige soil for isoflucypram

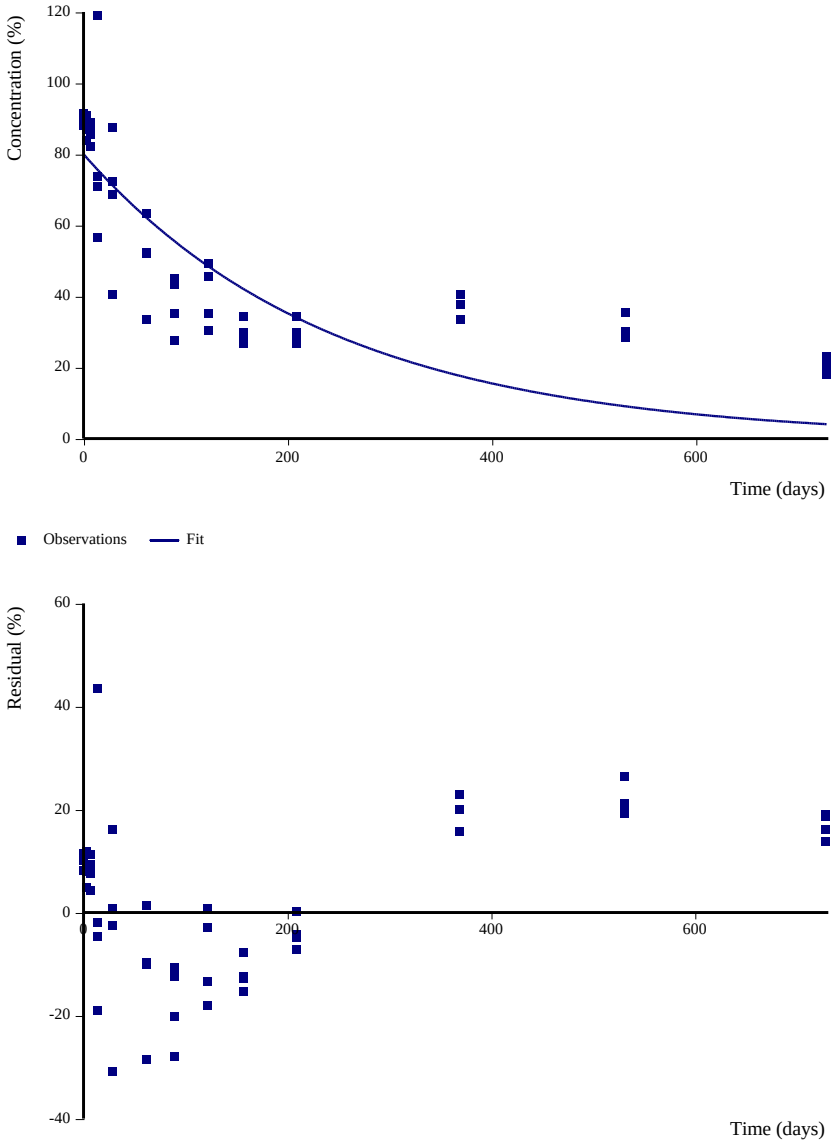
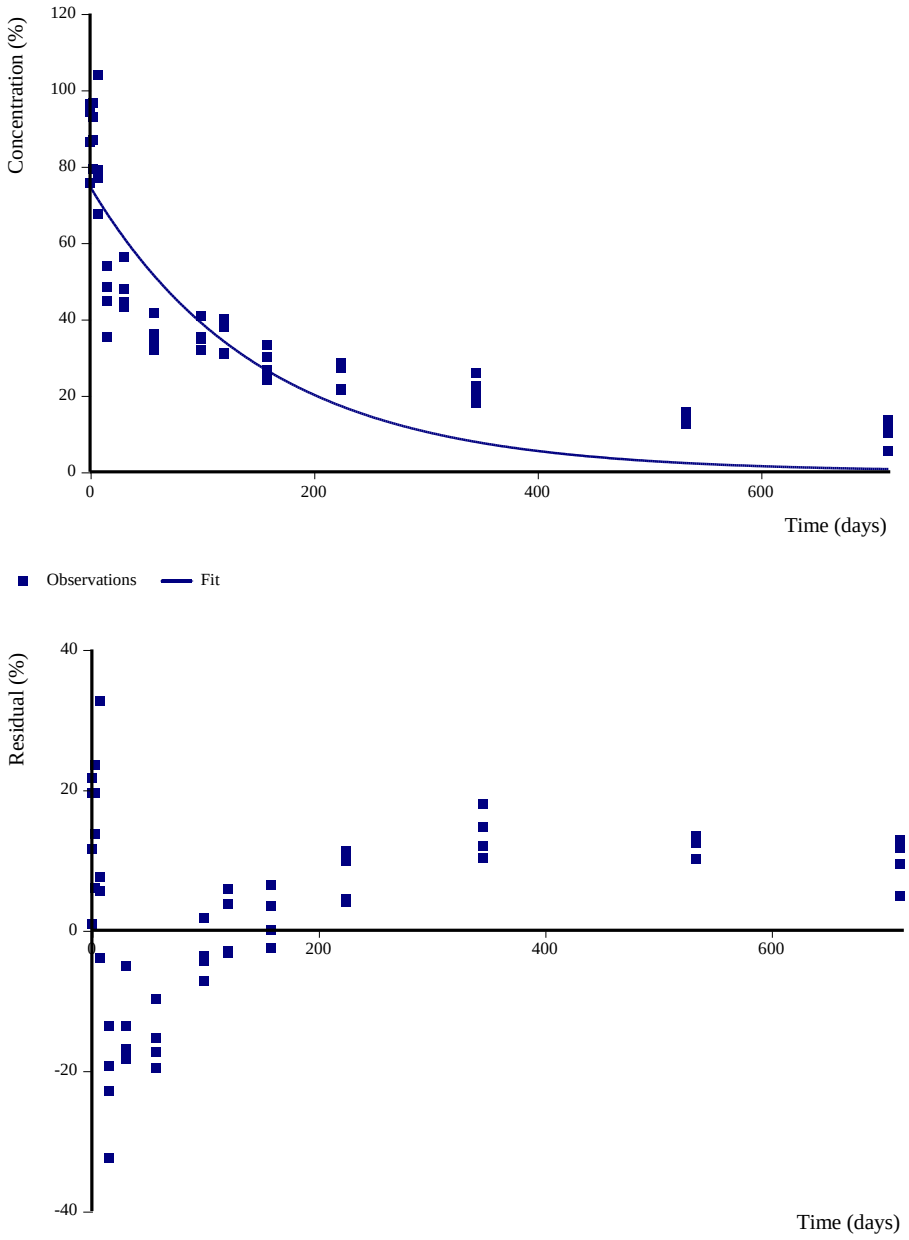


Figure B.8.1.1.5.2-6: RMS rejected SFO fit and residual Vilobi d'Onyar soil for isoflucypram



DFOP fitting - isoflucypram

Figure B.8.1.1.5.2-7: RMS accepted DFOP fit and residual Burscheid soil for isoflucypram

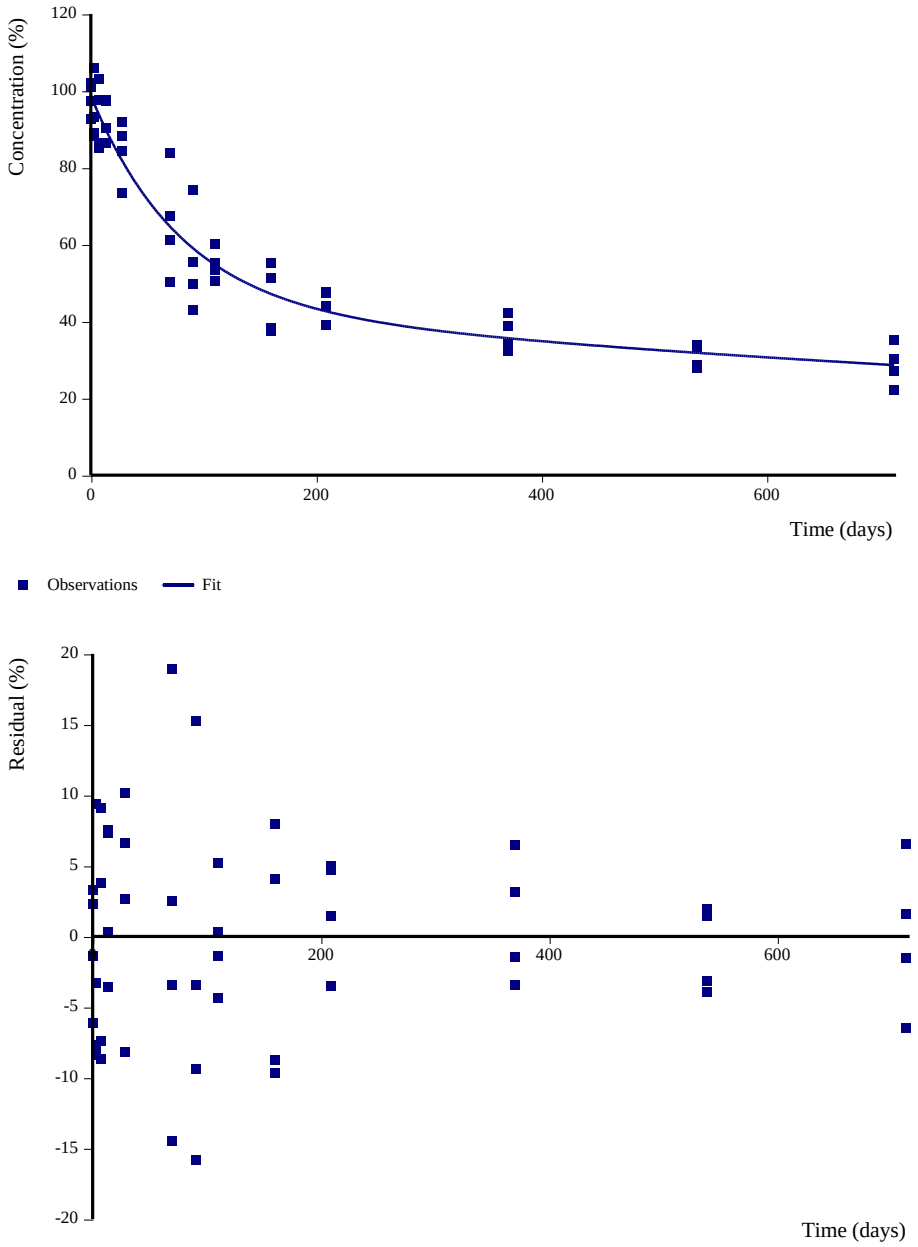


Figure B.8.1.1.5.2-8: RMS accepted DFOP fit and residual Great Chishill soil for isoflucypram

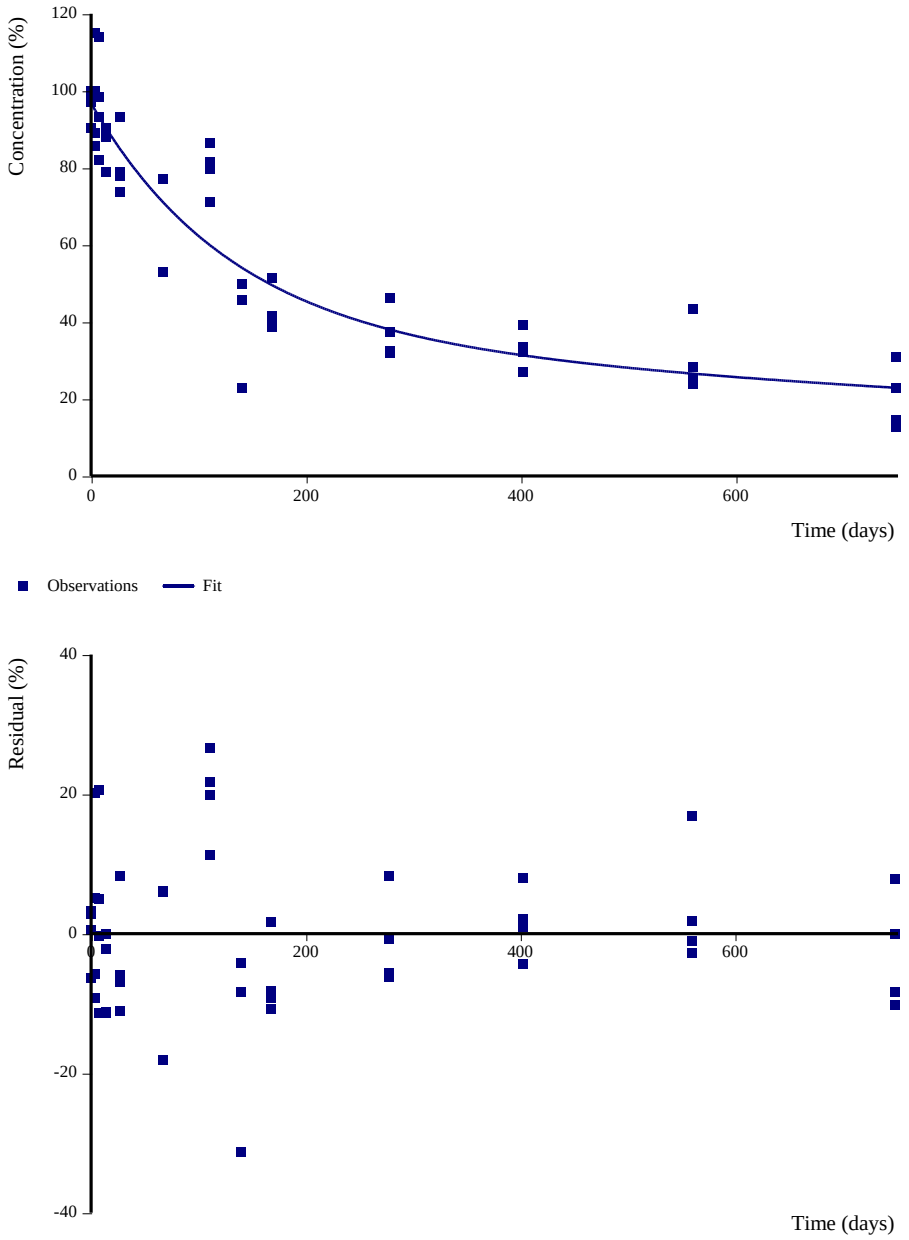


Figure B.8.1.1.5.2-9: RMS accepted DFOP fit and residual Parcay Meslay soil for isoflucypram

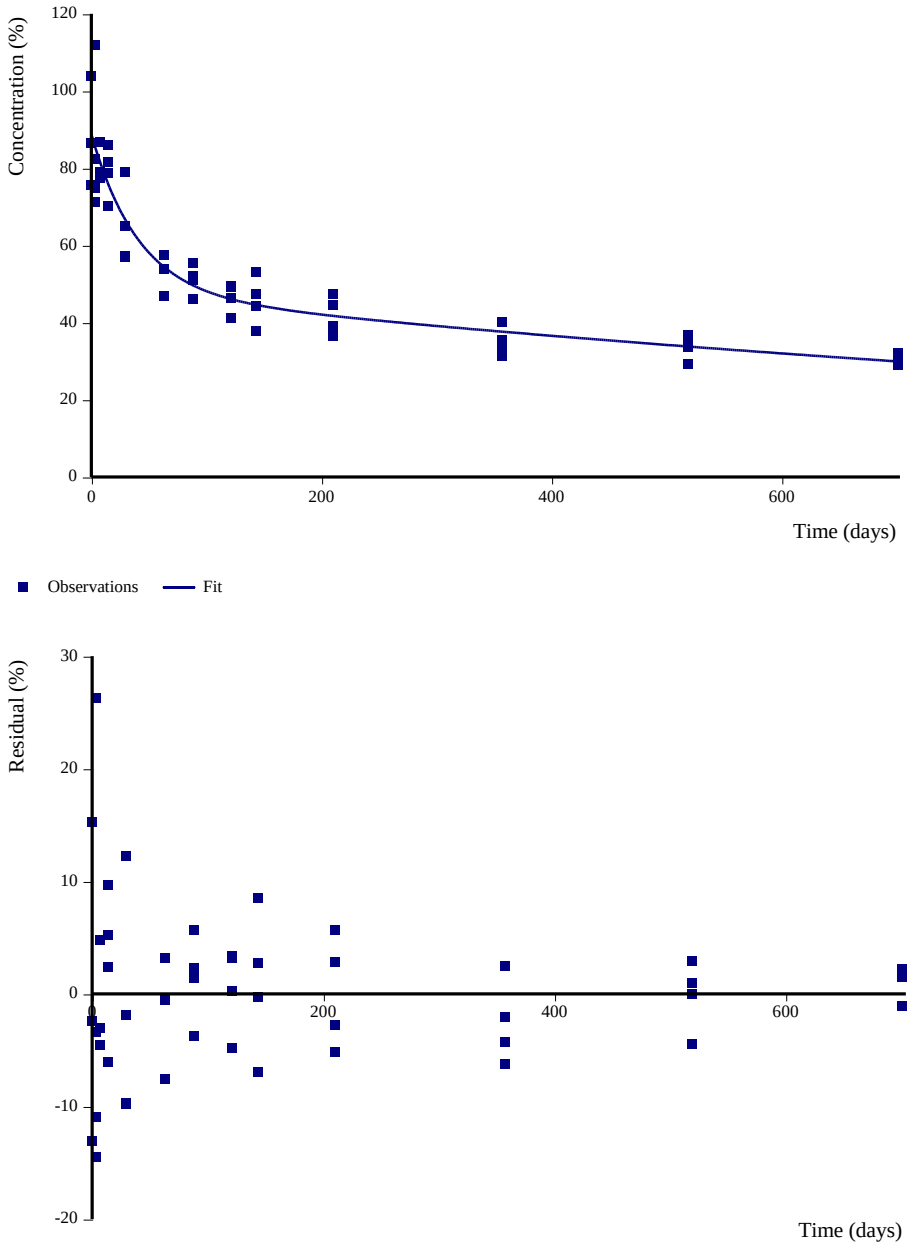


Figure B.8.1.1.5.2-10: RMS accepted DFOP fit and residual St. Etienne du Gres soil for isoflucypram

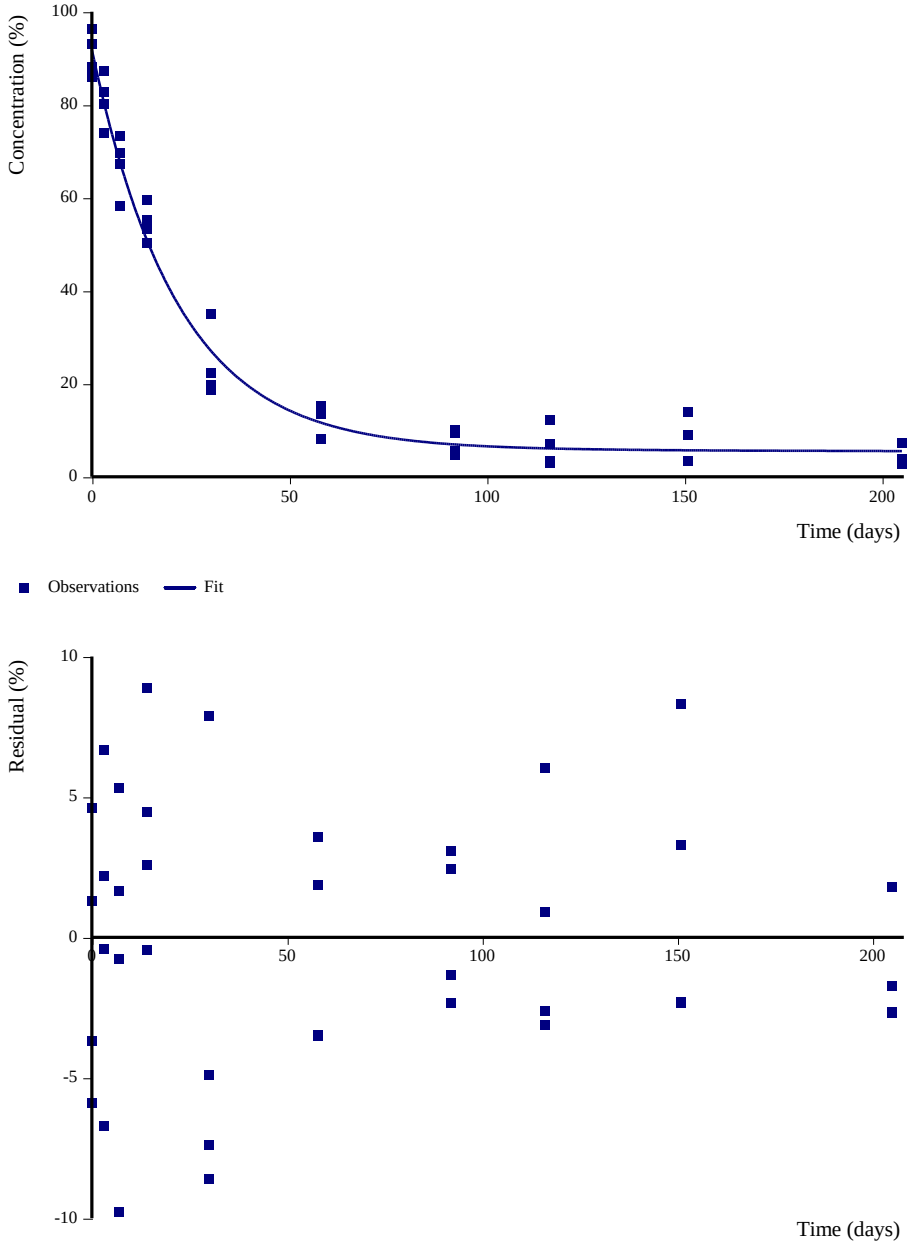


Figure B.8.1.1.5.2-11: RMS accepted DFOP fit and residual Albaro di Ronco all Adige soil for isoflucypram

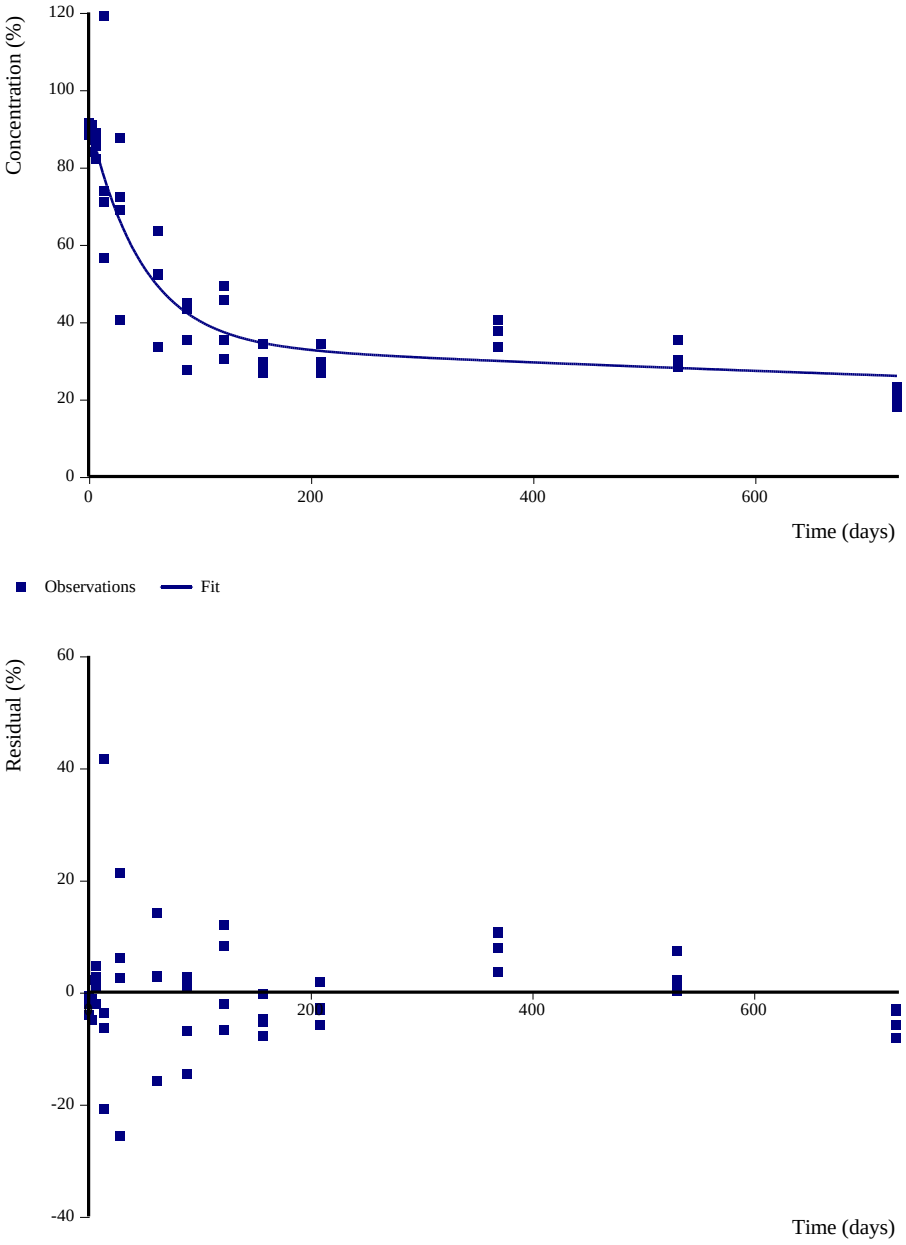
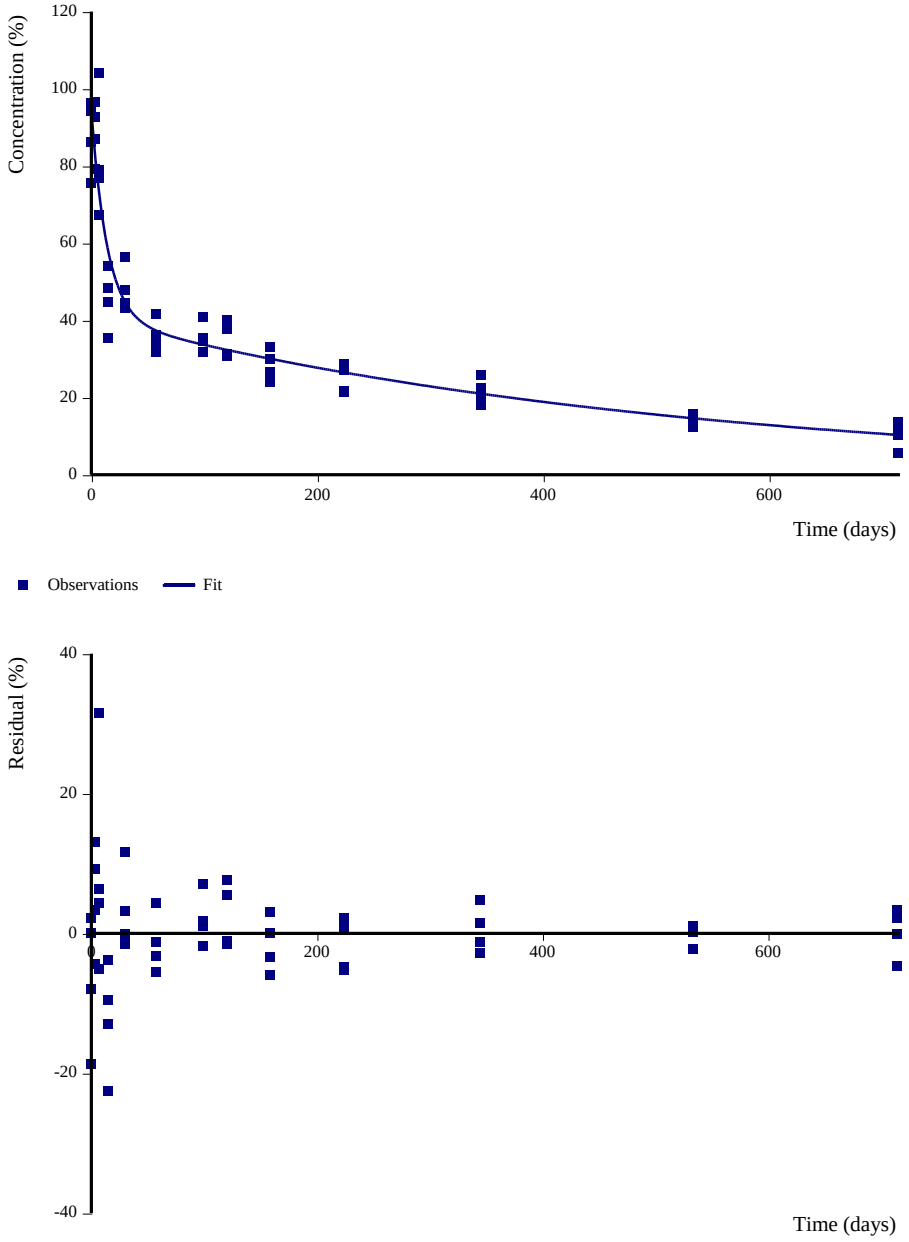


Figure B.8.1.1.5.2-12: RMS accepted DFOP fit and residual Vilobi d'Onyar soil for isoflucypram



Fitting for metabolite M12

Figure B.8.1.1.5.2-13: RMS accepted DFOP-SFO fit and residual Burscheid soil, metabolite M12

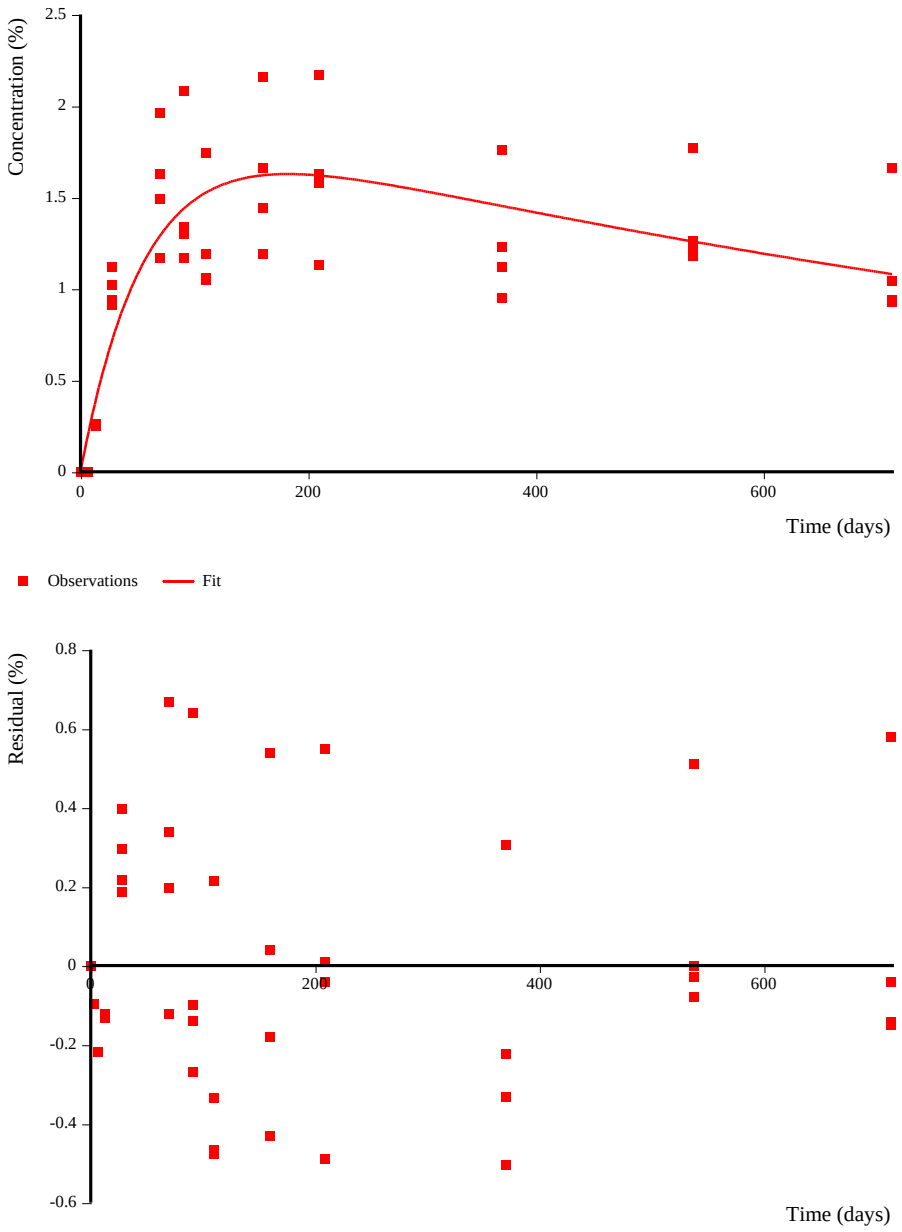


Figure B.8.1.1.5.2-14: RMS accepted DFOP-SFO fit and residual Great Chishill soil, metabolite M12

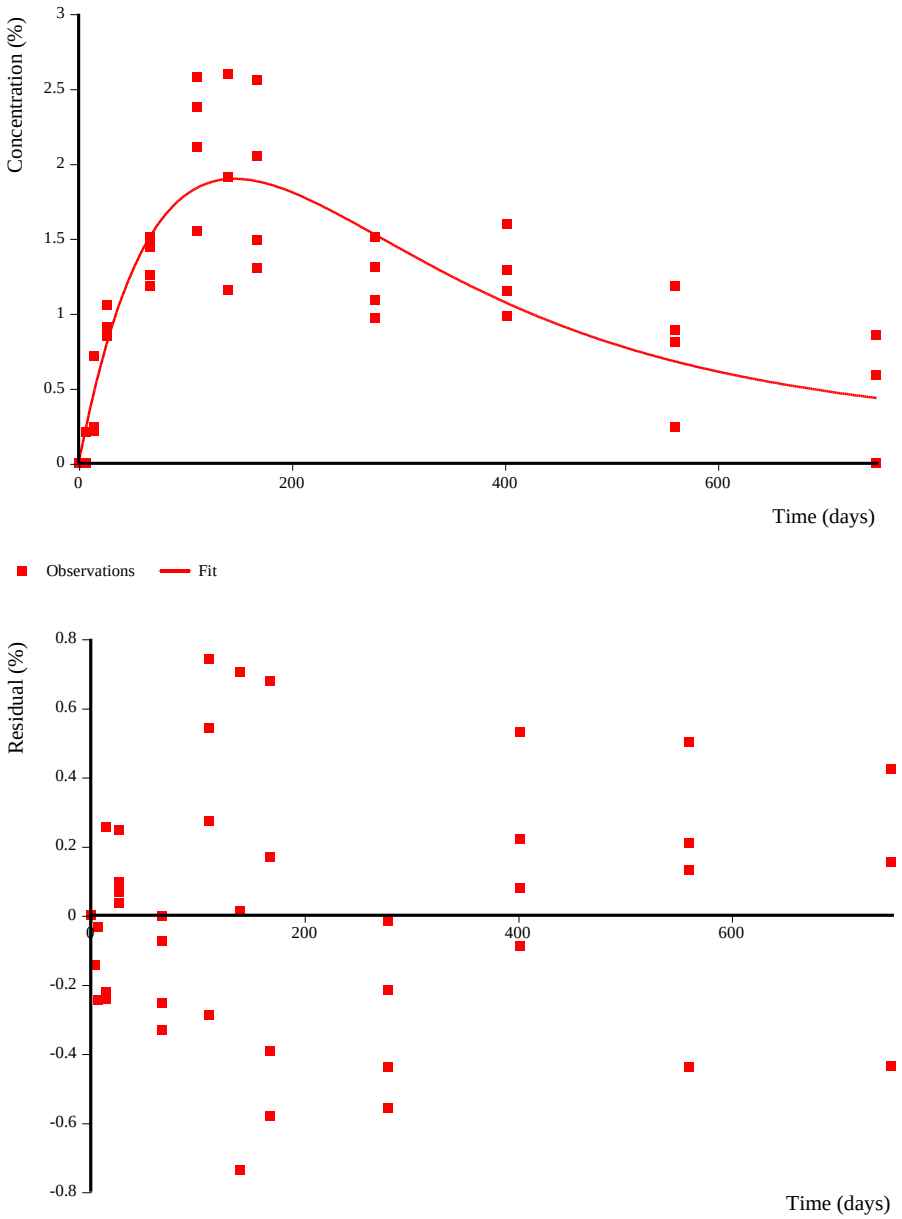


Figure B.8.1.1.5.2-15: RMS accepted DFOP-SFO fit and residual Parcay Meslay soil, metabolite M12

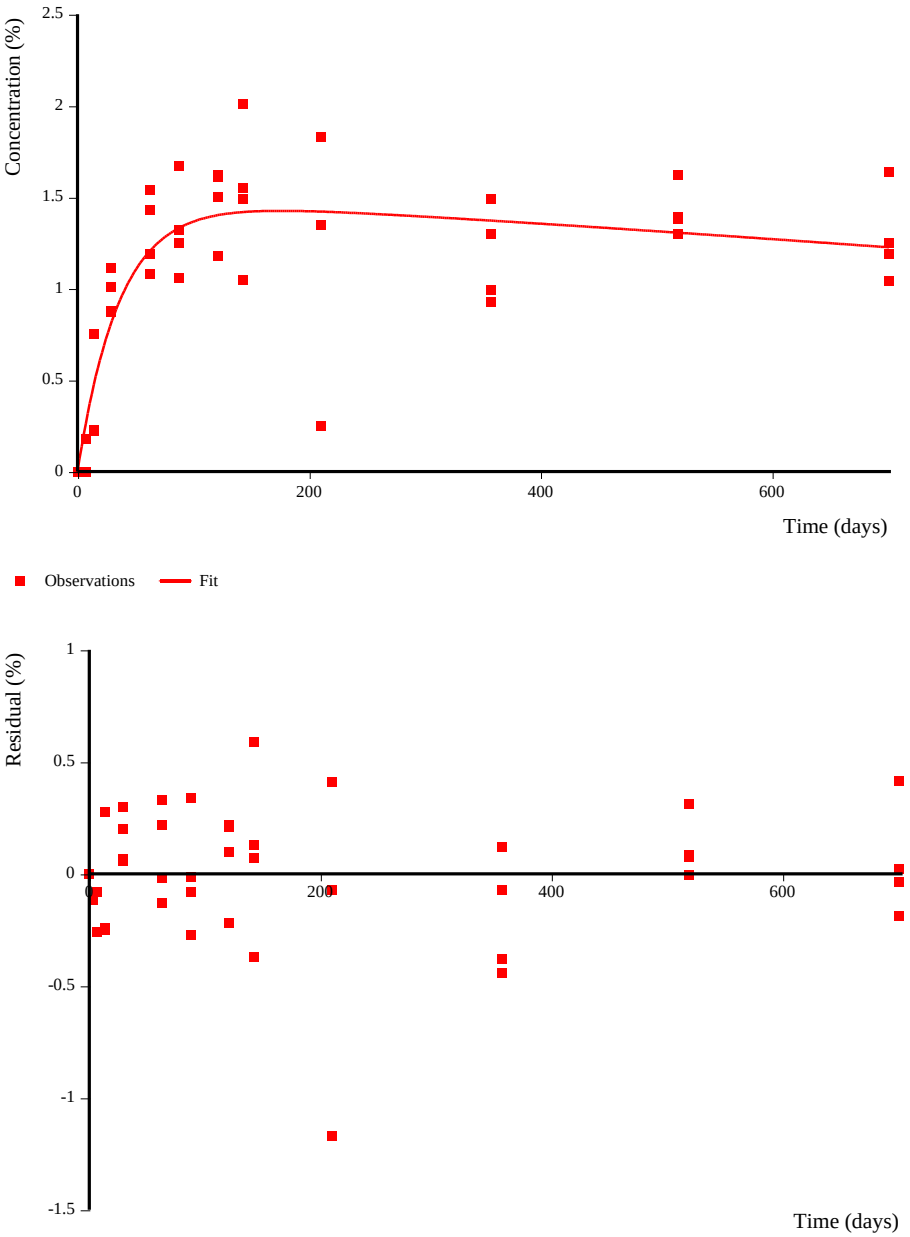


Figure B.8.1.1.5.2-16: RMS accepted DFOP-SFO fit and residual St. Etienne du Gres soil, metabolite M12

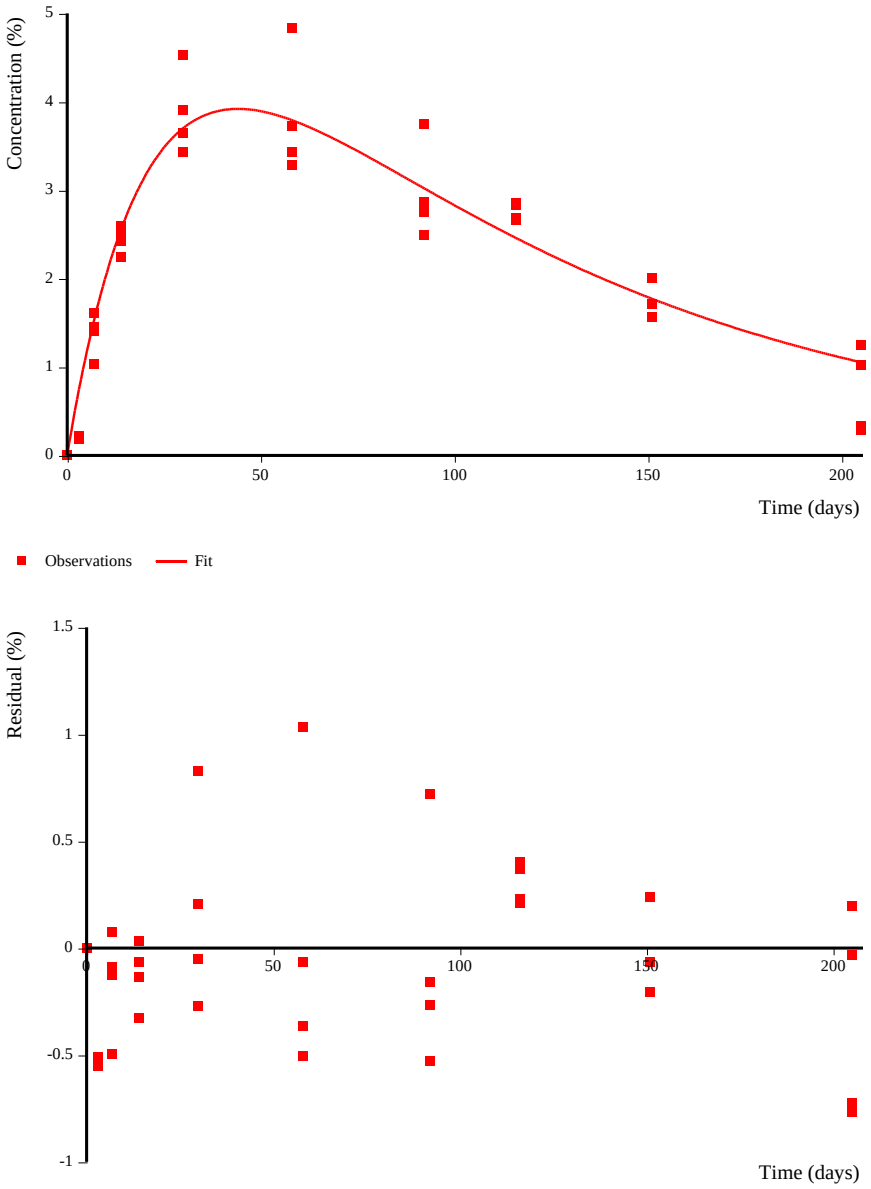


Figure B.8.1.1.5.2-17: RMS rejected DFOP-SFO fit and residual Albaro di Ronco all Adige soil, metabolite M12

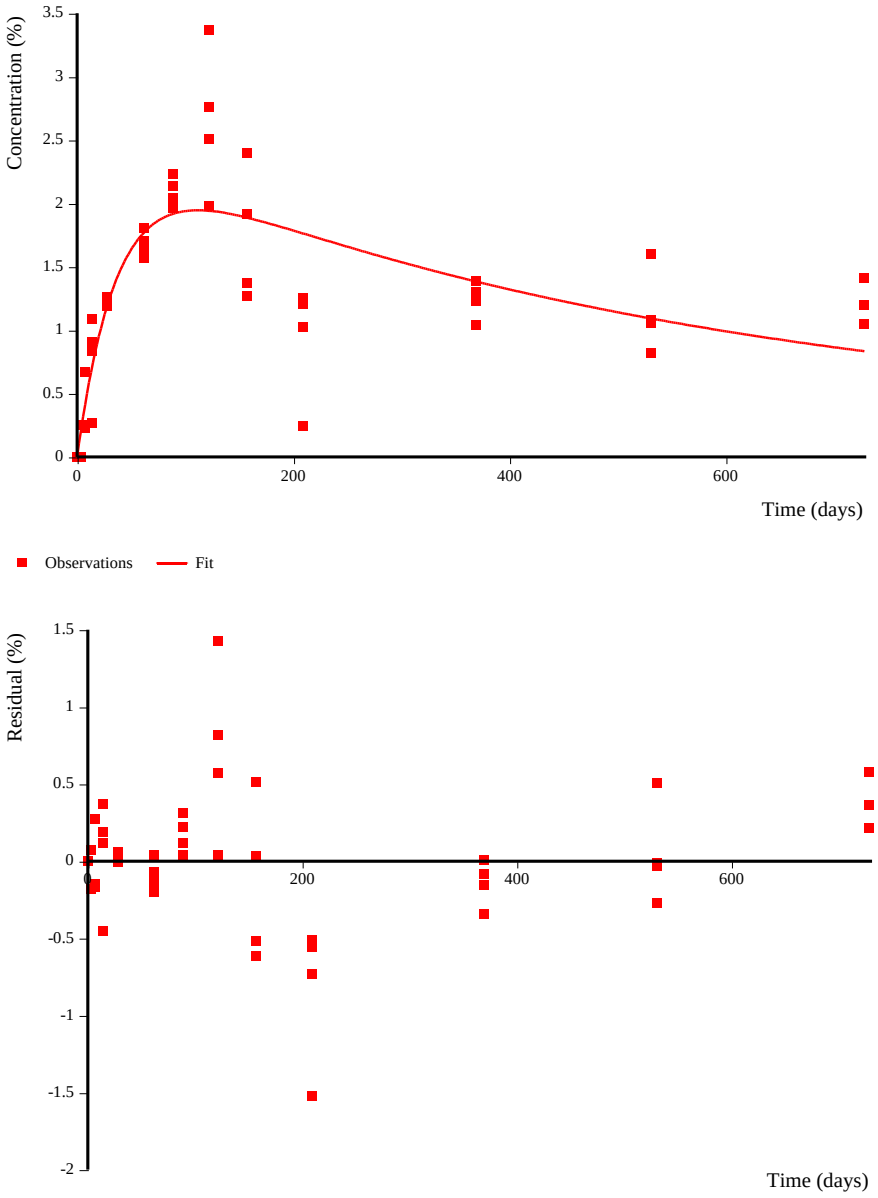
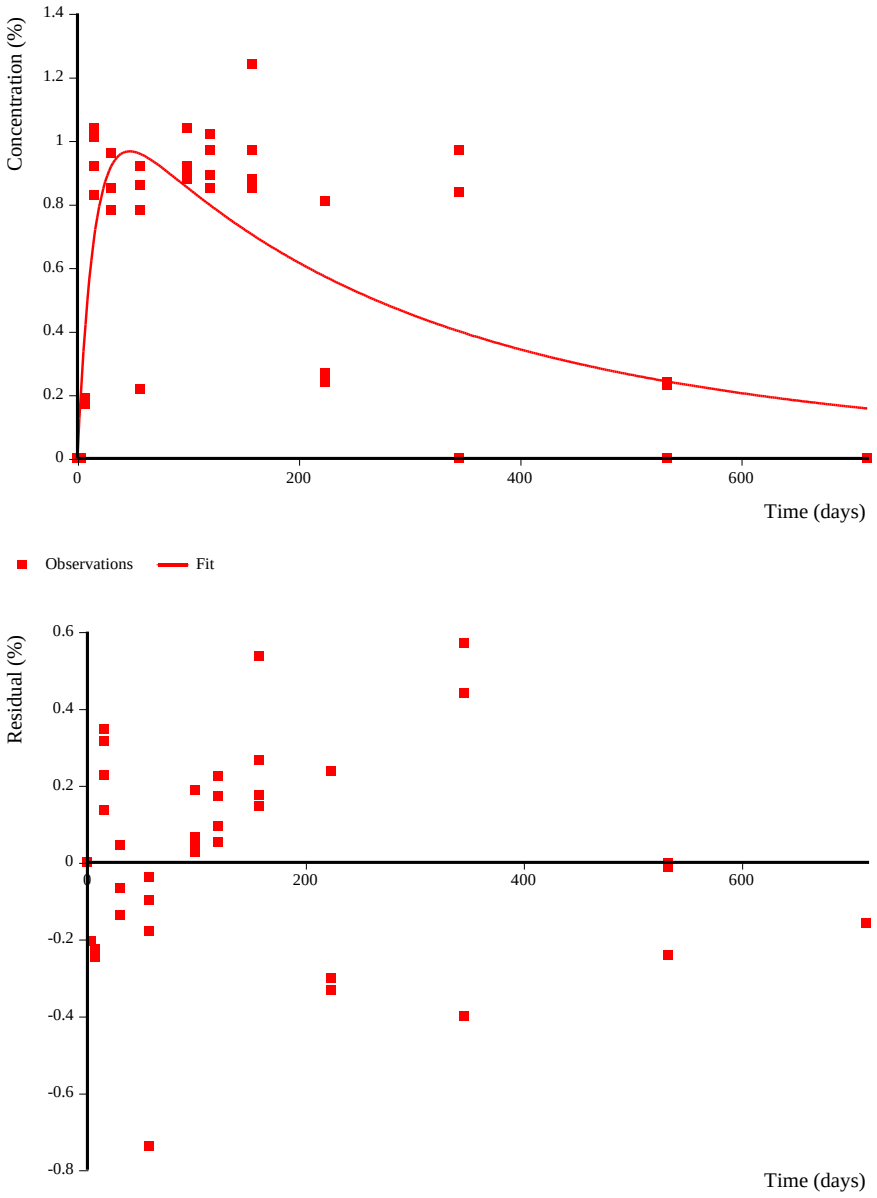


Figure B.8.1.1.5.2-18: RMS rejected DFOP-SFO fit and residual Vilobi d'Onyar soil for metabolite M12



Normalised assessment for PEC groundwater and PEC surface water evaluations

The field dissipation study (Heinemann, O.; Junge, T.; (2017)) is evaluated in section B.8.1.1.5.1 above. Normalised (20°C, 100% field capacity) degradation DT_{50} soil values for isoflucypram and its metabolite M12 under European field conditions were derived for modelling purposes according to FOCUS kinetics (FOCUS 2006[1], 2014[2]) and EFSA guidance on the evaluation of field dissipation studies (EFSA 2014[3]). Processes potentially occurring at the soil surface during the field study, *e.g.* photo-degradation and volatilisation should be eliminated to result finally in a $DegT_{50\ matrix}$ representing the degradation in the soil. This was considered following the EFSA framework for evaluation of existing field studies not tailored for $DegT_{50\ matrix}$ (EFSA 2014, Section 2.3.2).
In the experimental field studies assessed above, the active substance has been applied onto bare soil at a nominal rate of 100 g/ha isoflucypram in spring (April to June) 2014. Throughout the study period of approximately 2 years, irrigation activities were carried out.
The applicant simulated (with PEARL) daily soil temperatures and moisture contents to normalise the evaluated parameters to reference conditions according to FOCUS groundwater assumptions (Arrhenius equation, Q_{10} = 2.58; Walker equation, pF2) (FOCUS[4,5]). The residue data together with the transformed incubation times (transformed time approach, time step normalisation) were kinetically and statistically evaluated, based on the procedure explained by FOCUS kinetics, using the software tool KinGUI 2.1. This evaluation was further checked using PEARL v4.4.4 and CAKE v3.1 by the RMS. Details of the weather stations used to derive the climatic and soil data are presented in table B.8.1.1.5.2-4.

[1] FOCUS, 2006: Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics. EC Document Reference SANCO/10058/2005 version 2.0, 434 pp.

[2] FOCUS, 2014: Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1.

[3] EFSA, 2014: EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. 23.7.2014. EFSA Journal 2014; 12(5):3662. www.efsa.europa.eu/efsajournal. European Food Safety Authority EFSA, Parma, Italy

[4] FOCUS, 2014: Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU: The Final Report of the Ground Water Work Group of FOCUS: EC Document Reference: Sanco/13144/2010 version 3, 613 pp.

[5] FOCUS, 2014: Generic Guidance for Tier 1 FOCUS Groundwater Assessments, Version 2.2

Table B.8.1.1.5.2-4: Weather station and basic climate details.

Site	Weather station or data source	Distance to the site (km)	Total water incl. irrigation during study (mm)^	Mean Temperature during study °C	Reference Soil moisture at field capacity ~
Burscheid (Germany)	BCS weather station	2*	2112	11.1	39.9
Great Chishill (United Kingdom)	BCS weather station	<1	1361	10.4	34.4
Parcay Meslay (Northern France)	BCS weather station	At trial location	1389	12.3	32.5
St. Etienne du Gres (Southern France)	BCS weather station	At trial location	1050	17.1	34.4
Albaro di Ronco all Adige (Italy)	BCS weather station	0.1	1909	14.4	39.4
Vilobi d'Onyar (Spain)	BCS weather station	0.4	2290	14.6	32.5

* Site is greater than 1km in distance, from the weather station. No significant geographical features are noted around the test site that would invalidate the records taken from the test site.
^ Irrigation performed at all sites
~ as calculated by Rosetta

In the attempt to separate soil surface degradation processes, such as photo-degradation and volatilisation, from bulk soil degradation, an important threshold for a kinetic evaluation might be the time point, when the sum of precipitation and irrigation equals or exceeds 10 mm (EFSA 2014, Section 2.3.2). Details on the irrigation volumes applied at each site before the 10mm criteria was reached are detailed in table B.8.1.1.5.2-5. Metabolites values have been corrected with the mol mass correction factor of 1.074 (MM metabolite /MM parent = 429.8 g/mol/ 399.84g/mol) to form an equivalent g/ha.

Table B.8.1.1.2.5.2-5: Irrigation details for the field sites used.

Site	10 mm rain reached at normalised day	10 mm rain reached at natural day	Rain amount until this day (mm)	Normalised day of next experimental sample	Deleted day
Burscheid (Germany)	0	0	10.7	1.5	d0
Great Chishill (United Kingdom)	2.4	3	12.2	3.1	d0
Parcay Meslay (Northern France)	7.8	10	10.4	10.5	d0, d3, d7
St. Etienne du Gres (Southern France)	0	0	10.0	1.8	d0
Albaro di Ronco all Adige (Italy)	0	0	10.0	4.0	d0
Vilobi d'Onyar (Spain)	1.0	2	11.6	1.6	d0

Inputs used by the applicant and UK RMS are presented in tables B.8.1.1.2.5.2-6 to B.8.1.1.2.5.2-11. Samples <LOD were set to 0.5 LOD according to the guidance.

Table B.8.1.1.2.5.2-6: Normalised data table for Burscheid (Germany)

Days after application	Time step application date	Total residue isoflucypram	Total residue M12	Total residue isoflucypram	Total residue M12
		Reported		Entered into model	
(d)	(d)	g/ha	Eq g/ha	g/ha	Eq g/ha
0	0	97.3	0	-	-
0	0	101	0	-	-
0	0	92.6	0	-	-
0	0	102	0	-	-
3	1.5	88.9	0	88.9	0
3	1.5	106	0	106	0
3	1.5	88.2	0	88.2	0
3	1.5	93.3	0	93.3	0
7	3	103	0	103	0
7	3	97.7	0	97.7	0
7	3	86.5	0	86.5	0
7	3	85.2	0	85.2	0
13	4.8	97.4	0.24	97.4	0.24
13	4.8	97.6	0.23	97.6	0.23
13	4.8	86.5	0.24	86.5	0.24
13	4.8	90.4	0.23	90.4	0.23
28	12.4	91.8	1.04	91.8	1.04
28	12.4	73.5	0.87	73.5	0.87
28	12.4	84.3	0.85	84.3	0.85
28	12.4	88.3	0.95	88.3	0.95
70	38.4	61.3	1.39	61.3	1.39
70	38.4	83.7	1.52	83.7	1.52
70	38.4	50.3	1.09	50.3	1.09
70	38.4	67.3	1.82	67.3	1.82
91	51.7	49.6	1.09	49.6	1.09
91	51.7	55.5	1.25	55.5	1.25
91	51.7	43.1	1.21	43.1	1.21
91	51.7	74.2	1.93	74.2	1.93
110	70.8	60	1.62	60	1.62
110	70.8	55.1	1.11	55.1	1.11
110	70.8	50.4	0.98	50.4	0.98
110	70.8	53.4	0.99	53.4	0.99
160	109.2	38.4	1.11	38.4	1.11
160	109.2	55.1	1.54	55.1	1.54

160	109.2	37.5	1.34	37.5	1.34
160	109.2	51.2	2.01	51.2	2.01
209	137.1	39.1	1.05	39.1	1.05
209	137.1	47.3	1.47	47.3	1.47
209	137.1	47.6	1.52	47.6	1.52
209	137.1	44.1	2.02	44.1	2.02
370	176.8	32.2	0.88	32.2	0.88
370	176.8	34.2	1.04	34.2	1.04
370	176.8	38.8	1.14	38.8	1.14
370	176.8	42.1	1.64	42.1	1.64
538	294.2	33.3	1.17	33.3	1.17
538	294.2	28.7	1.14	28.7	1.14
538	294.2	27.9	1.1	27.9	1.1
538	294.2	33.8	1.65	33.8	1.65
713	347.5	27.1	0.87	27.1	0.87
713	347.5	22.2	0.87	22.2	0.87
713	347.5	30.2	0.97	30.2	0.97
713	347.5	35.2	1.54	35.2	1.54

Table B.8.1.1.2.5.2-7: Normalised data table Great Chishill (United Kingdom)

Days after application	Time step application date	Total residue isoflucypram	Total residue M12	Total residue isoflucypram	Total residue M12
		Reported		Entered into model	
(d)	(d)	g/ha	Eq g/ha		
0	0	97.2	0	-	-
0	0	90.4	0	-	-
0	0	100	0	-	-
0	0	99.6	0	-	-
4	3.1	115	0	115	0
4	3.1	100	0	100	0
4	3.1	85.6	0	85.6	0
4	3.1	89	0	89	0
7	5.3	93.1	0.21	93.1	0.21
7	5.3	82	0	82	0
7	5.3	114	0	114	0
7	5.3	98.4	0	98.4	0
14	9.3	79	0.67	79	0.67
14	9.3	78.9	0.2	78.9	0.2
14	9.3	88.1	0.22	88.1	0.22
14	9.3	90.3	0.2	90.3	0.2
27	17.8	73.8	0.82	73.8	0.82
27	17.8	79	0.85	79	0.85

27	17.8	93.1	0.99	93.1	0.99
27	17.8	77.9	0.79	77.9	0.79
67	51.3	77	1.34	77	1.34
67	51.3	77.2	1.17	77.2	1.17
67	51.3	77.2	1.4	77.2	1.4
67	51.3	52.9	1.1	52.9	1.1
111	79.1	-	-	-	-
111	79.1	-	-	-	-
111	79.1	-	-	-	-
111	79.1	-	-	-	-
140	95	49.9	1.78	49.9	1.78
140	95	-	-	-	-
140	95	22.9	1.08	22.9	1.08
140	95	45.7	2.42	45.7	2.42
168	106.3	40.4	1.91	40.4	1.91
168	106.3	41.4	1.21	41.4	1.21
168	106.3	51.3	2.38	51.3	2.38
168	106.3	38.8	1.39	38.8	1.39
278	132.9	31.8	1.4	31.8	1.4
278	132.9	37.3	0.9	37.3	0.9
278	132.9	32.4	1.01	32.4	1.01
278	132.9	46.3	1.22	46.3	1.22
402	190.6	26.9	0.91	26.9	0.91
402	190.6	32.2	1.2	32.2	1.2
402	190.6	33.4	1.07	33.4	1.07
402	190.6	39.3	1.49	39.3	1.49
560	272.1	28.4	0.83	28.4	0.83
560	272.1	23.8	0.22	23.8	0.22
560	272.1	25.2	0.75	25.2	0.75
560	272.1	43.4	1.1	43.4	1.1
749	338.5	22.9	0.55	22.9	0.55
749	338.5	12.7	-	12.7	-
749	338.5	14.6	-	14.6	-
749	338.5	30.8	-	30.8	-

Table B.8.1.1.2.5.2-8: Normalised data table Parcay Meslay (Northern France).

Days after application	Time step application date	Total residue isoflucypram	Total residue M12	Total residue isoflucypram	Total residue M12
		Reported		Entered into the model	
(d)	(d)	g/ha	Eq g/ha	g/ha	Eq g/ha
0	0	75.7	0	-	-
0	0	86.4	0	-	-

0	0	86.4	0	-	-
0	0	104	0	-	-
3	2.1	82.4	0	-	-
3	2.1	71.3	0	-	-
3	2.1	74.8	0	-	-
3	2.1	112	0	-	-
7	5.3	77.5	0	-	-
7	5.3	86.8	0	-	-
7	5.3	86.8	0.18	-	-
7	5.3	79	0	-	-
14	10.5	78.7	0.2	78.7	0.2
14	10.5	70.3	0.21	70.3	0.21
14	10.5	81.6	0.7	81.6	0.7
14	10.5	86	0.2	86	0.2
29	22.6	57.2	0.82	57.2	0.82
29	22.6	79.1	1.03	79.1	1.03
29	22.6	65	0.94	65	0.94
29	22.6	57.1	0.81	57.1	0.81
63	54.7	46.9	1.11	46.9	1.11
63	54.7	53.9	1	53.9	1
63	54.7	57.6	1.43	57.6	1.43
63	54.7	53.9	1.33	53.9	1.33
88	73.4	52	1.55	52	1.55
88	73.4	55.4	0.99	55.4	0.99
88	73.4	46	1.23	46	1.23
88	73.4	51.1	1.16	51.1	1.16
121	96.2	49.3	1.4	49.3	1.4
121	96.2	46.4	1.1	46.4	1.1
121	96.2	41.3	1.5	41.3	1.5
121	96.2	49.5	1.51	49.5	1.51
143	108.1	37.7	1.44	37.7	1.44
143	108.1	47.4	0.98	47.4	0.98
143	108.1	53.1	1.87	53.1	1.87
143	108.1	44.3	1.39	44.3	1.39
210	128.1	44.6	1.26	44.6	1.26
210	128.1	36.6	0.23	36.6	0.23
210	128.1	39	1.26	39	1.26
210	128.1	47.4	1.7	47.4	1.7
357	185.1	33.4	1.21	33.4	1.21
357	185.1	40.1	0.87	40.1	0.87
357	185.1	35.6	1.39	35.6	1.39
357	185.1	31.4	0.92	31.4	0.92
519	302.2	29.3	1.28	29.3	1.28
519	302.2	36.7	1.21	36.7	1.21

519	302.2	33.8	1.51	33.8	1.51
519	302.2	34.8	1.29	34.8	1.29
701	360.3	34.8	1.11	34.8	1.11
701	360.3	28.9	0.97	28.9	0.97
701	360.3	31.4	1.53	31.4	1.53
701	360.3	32.2	1.16	32.2	1.16

Table B.8.1.1.2.5.2-9: Normalised data table for St. Etienne du Gres (Southern France)

Days after application	Time step application date	Total residue isoflucypram	Total residue M12	Total residue isoflucypram	Total residue M12
		Reported		Entered into the model	
(d)	(d)	g/ha	Eq g/ha	g/ha	Eq g/ha
0	0	93.1	0	-	-
0	0	88.1	0	-	-
0	0	85.9	0	-	-
0	0	96.4	0	-	-
3	1.8	82.8	0.2	82.8	0.2
3	1.8	73.9	0.18	73.9	0.18
3	1.8	80.2	0.21	80.2	0.21
3	1.8	87.3	0.22	87.3	0.22
7	4.4	58.2	1.45	58.2	1.45
7	4.4	67.2	1.61	67.2	1.61
7	4.4	69.6	1.41	69.6	1.41
7	4.4	73.3	1.04	73.3	1.04
14	9.6	59.6	2.33	59.6	2.33
14	9.6	50.3	2.42	50.3	2.42
14	9.6	55.2	2.26	55.2	2.26
14	9.6	53.3	2.08	53.3	2.08
30	24.1	22.3	3.64	22.3	3.64
30	24.1	18.6	3.4	18.6	3.4
30	24.1	35.1	4.21	35.1	4.21
30	24.1	19.8	3.19	19.8	3.19
58	56.1	15.2	3.19	15.2	3.19
58	56.1	8.12	3.47	8.12	3.47
58	56.1	13.5	4.49	13.5	4.49
58	56.1	8.16	3.06	8.16	3.06
92	100.1	9.36	2.33	9.36	2.33
92	100.1	10	2.57	10	2.57
92	100.1	5.59	2.67	5.59	2.67
92	100.1	4.59	3.49	4.59	3.49
116	128.2	12.1	2.48	12.1	2.48
116	128.2	3.46	2.63	3.46	2.63

116	128.2	7	2.5	7	2.5
116	128.2	2.95	2.66	2.95	2.66
151	165.9	14	1.46	14	1.46
151	165.9	3.4	1.59	3.4	1.59
151	165.9	8.99	1.59	8.99	1.59
151	165.9	3.37	1.87	3.37	1.87
205	200.3	7.33	1.16	7.33	1.16
205	200.3	2.87	0.27	2.87	0.27
205	200.3	3.79	0.95	3.79	0.95
205	200.3	2.82	0.31	2.82	0.31

Table B.8.1.1.2.5.2-10: Normalised data table for Albaro di Ronco all Adige (Italy)

Days after application	Time step application date	Total residue isoflucypram	Total residue M12	Total residue isoflucypram	Total residue M12
		Reported		Entered into the model	
(d)	(d)	g/ha	Eq g/ha	g/ha	Eq g/ha
0	0	91.5	0	-	-
0	0	90.2	0	-	-
0	0	88.2	0	-	-
0	0	90.3	0	-	-
3	4	87	0	87	0
3	4	83.8	0	83.8	0
53	4	87.5	0	87.5	0
3	4	90.9	0.25	90.9	0.25
7	8.8	88.9	0.24	88.9	0.24
7	8.8	87	0.23	87	0.23
7	8.8	85.4	0.25	85.4	0.25
7	8.8	82.1	0.67	82.1	0.67
14	16.9	73.7	0.25	73.7	0.25
14	16.9	56.6	0.78	56.6	0.78
14	16.9	70.9	0.85	70.9	0.85
14	16.9	119	1.01	119	1.01
28	35	72.2	1.12	72.2	1.12
28	35	68.8	1.12	68.8	1.12
28	35	87.5	1.17	87.5	1.17
28	35	40.5	1.11	40.5	1.11
62	79.9	63.4	1.68	63.4	1.68
62	79.9	52.3	1.58	52.3	1.58
62	79.9	33.6	1.53	33.6	1.53

62	79.9	52	1.46	52	1.46
89	109.4	44.9	2.07	44.9	2.07
89	109.4	65.3	1.9	65.3	1.9
89	109.4	41.6	1.99	41.6	1.99
89	109.4	32.9	1.82	32.9	1.82
122	138.8	53.2	3.13	53.2	3.13
122	138.8	35.6	2.57	35.6	2.57
122	138.8	43.2	2.33	43.2	2.33
122	138.8	27.6	1.84	27.6	1.84
157	157	45.6	1.79	45.6	1.79
157	157	49.2	2.23	49.2	2.23
157	157	35.2	1.27	35.2	1.27
157	157	30.5	1.18	30.5	1.18
209	171.3	34.2	1.13	34.2	1.13
209	171.3	29.2	1.16	29.2	1.16
209	171.3	26.7	0.96	26.7	0.96
209	171.3	29.7	0.22	29.7	0.22
369	270	33.4	1.14	33.4	1.14
369	270	40.5	1.29	40.5	1.29
369	270	37.7	0.97	37.7	0.97
369	270	40.6	1.21	40.6	1.21
531	435.7	30.2	0.99	30.2	0.99
531	435.7	28.3	1	28.3	1
531	435.7	35.4	1.49	35.4	1.49
531	435.7	29.8	0.76	29.8	0.76
728	527.4	17.9	0.98	17.9	0.98
728	527.4	22.7	1.12	22.7	1.12
728	527.4	23.1	1.31	23.1	1.31
728	527.4	20.2	1.12	20.2	1.12

Table B.8.1.1.2.5.2-11: Normalised data table for Vilobi d'Onyar (Spain)

Days after application	Time step application date	Total residue isoflucypram	Total residue M12	Total residue isoflucypram	Total residue M12
		Reported		Entered into the model	
(d)	(d)	g/ha	Eq g/ha		
0	0	75.6	0	-	-
0	0	96.4	0	-	-
0	0	86.3	0	-	-
0	0	94.3	0	-	-
3	1.6	79.2	0	79.2	0
3	1.6	96.7	0	96.7	0
3	1.6	86.9	0	86.9	0

3	1.6	92.8	0.19	92.8	0.19
7	3.9	67.4	0.19	67.4	0.19
7	3.9	104	0.17	104	0.17
7	3.9	76.9	0.18	76.9	0.18
7	3.9	78.9	0.18	78.9	0.18
15	8.8	35.3	0.77	35.3	0.77
15	8.8	54	0.94	54	0.94
15	8.8	44.8	0.79	44.8	0.79
15	8.8	48.3	0.97	48.3	0.97
30	21.6	44.5	0.79	44.5	0.79
30	21.6	56.3	0.89	56.3	0.89
30	21.6	43.1	0.73	43.1	0.73
30	21.6	47.8	0.89	47.8	0.89
57	50.2	31.9	0.2	31.9	0.2
57	50.2	34.1	0.73	34.1	0.73
57	50.2	41.7	0.86	41.7	0.86
57	50.2	36.1	0.8	36.1	0.8
99	97.8	31.9	0.82	31.9	0.82
99	97.8	35.4	0.86	35.4	0.86
99	97.8	34.7	0.84	34.7	0.84
99	97.8	40.8	0.97	40.8	0.97
120	121.8	30.9	0.79	30.9	0.79
120	121.8	40	0.95	40	0.95
120	121.8	31.2	0.9	31.2	0.9
120	121.8	37.8	0.83	37.8	0.83
158	159.9	30.1	0.9	30.1	0.9
158	159.9	24.1	0.79	24.1	0.79
158	159.9	33.1	1.15	33.1	1.15
158	159.9	26.6	0.82	26.6	0.82
224	193.9	27.2	0.75	27.2	0.75
224	193.9	21.7	0.22	21.7	0.22
224	193.9	21.3	0.25	21.3	0.25
224	193.9	28.6	0.22	28.6	0.22
345	236.1	22.5	0.9	22.5	0.9
345	236.1	18.1	0	18.1	0
345	236.1	19.8	0	19.8	0
345	236.1	25.8	0.78	25.8	0.78
533	421.4	12.4	0.21	12.4	0.21
533	421.4	15.7	0	15.7	0
533	421.4	15.1	0	15.1	0
533	421.4	14.8	0.22	14.8	0.22
714	492.5	13.6	-	13.6	-
714	492.5	15.6	-	15.6	-
714	492.5	10.2	-	10.2	-

714	492.5	12.5	-	12.5	-

UK RMS has evaluated the applicants supplied modelling. UK RMS could not match the results supplied by the applicant. From inspection of the supplied modelling report (Reinken and Milkolasch (2017) UK RMS has concluded that the kinetic assessment has been performed using the data set excluding time points before 10mm of rainfall for SFO only. For all other fits time points before 10mm were used in the model runs and therefore the fits are not considered to be acceptable by the UK RMS because this procedure is not in accordance with the EFSA DEGT50 guidance.

UK RMS has excluded the appropriate time points for each soil and each kinetic fit. UK RMS results are provided in table B.8.1.1.2.5.2-12 for isoflucypram and table B.8.1.1.2.5.2-13 for M12. SFO fits were rejected by the UKRMS. Whilst Chi2 values were less than 15 for 5 of the 6 sites the curves were considered to give relatively poor visual fitting, especially with regards to the fitting of the DT90 (see visual fits in figures B.8.1.1.5.2-1 to B.8.1.1.5.2-12). Following the EFSA decision tree on field dissipation studies (EFSA 2014), an appropriate description of soil matrix degradation of isoflucypram could be derived using a DFOP model fit for 5 trials and HS model fit for one trial (DFOP g value = < 0.75 therefore DFOP was not considered to be the appropriate kinetic). The corresponding DT_{50} and formation fractions (f.f.) for the metabolite M12 have also been assessed. Kinetic fit graphs and residual fit graphs are presented in figures B.8.1.1.5.2-1 to B.8.1.1.2.5.2-6 for the parent, due to the low formation of metabolite; metabolite graphs and figures are presented to demonstrate the availability of formation and decline phases, see figures B.8.1.1.5.2-7 to B.8.1.1.5.2-12. Fits for M12 in the Albaro and Vilobi soils were rejected by the UKRMS to both poor visual fit and poor Chi^2 results. A summary table of the normalised field data is presented in table B.8.1.1.5.2-14 for isoflucypram and B.8.1.1.5.2-15 for M12.

Table B.8.1.1.2.5.2-12: Kinetic assessment results for the normalisation of isoflucypram performed by the RMS.

Site	Kinetic model[a)]	k_{fast} [1/d]	k_{slow} [1/d]	t-test, k_{fast} / k_{slow}	t_b [d]	g_{fast} = F_{field}	$DT_{50\ matrix}$ [d]	$DT_{90\ matrix}$ [d]	St. (χ^2err) [%]	Visual fit[b)]
Burscheid, Germany	SFO	-	0.004477	< 0.001	-	-	155	514	10.4	o
	DFOP	**0.04612**	**0.00240**	**< 0.001 / < 0.001**	**-**	**0.3819**	**92.8**	**759**	**3.21**	**+**
	HS	0.01256	0.00272	< 0.001 / < 0.001	39.8	-	111	702	3.68	+
Great Chishill, UK	SFO	-	0.005951	< 0.001	-	-	116	387	10.1	o
	DFOP	**0.01619**	**0.00166**	**0.0157/0.1894**		**0.5981**	**85**	**838**	**6.73**	**+**
	HS	0.0111	0.00464	<0.001/<0.001	36.5	-	98.6	446	9.04	+
Parcay Meslay, Northern France	SFO	-	0.00266	<0.001	-	-	261	867	10.6	o
	DFOP	**0.03694**	**0.00143**	**<0.001/<0.001**	**-**	**0.443**	**94.7**	**1200**	**3.5**	**+**
	HS	0.00963	0.00170	<0.001/<0.001	55.6	-	148	1090	4.19	+
St. Etienne du Gres, Southern France	SFO	-	0.0494	<0.001	-	-	14	46.7	13.1	o
	DFOP	0.0607	0.00125	< 0.001/ 0.3544	-	0.9135	13	63	4.81	+
	HS	**0.0522**	**0.00540**	**<0.001/ 0.0293**	**39.37**	**-**	**13.3**	**85.2**	**4.19**	**+**
Albaro, Italy	SFO	-	0.01741	< 0.001	-	-	214	710	14.4	o
	DFOP	**0.2294**	**0.00118**	**<0.001/ 0.0143**	**-**	**0.5152**	**90.7**	**1340**	**5.82**	**+**
	HS	0.00774	0.00130	<0.001/ 0.0030	97.54	-	89.6	1290	5.9	+
Vilobi, Spain	SFO	-	0.00436	<0.001	-	-	159	528	24.5	=
	DFOP	**0.1801**	**0.00265**	**< 0.001/ <0.001**	**-**	**0.5895**	**9.84**	**532**	**9.4**	**+**
	HS	0.08427	0.00301	<0.001/ <0.001	9.88	-	8.23	499	7.03	+

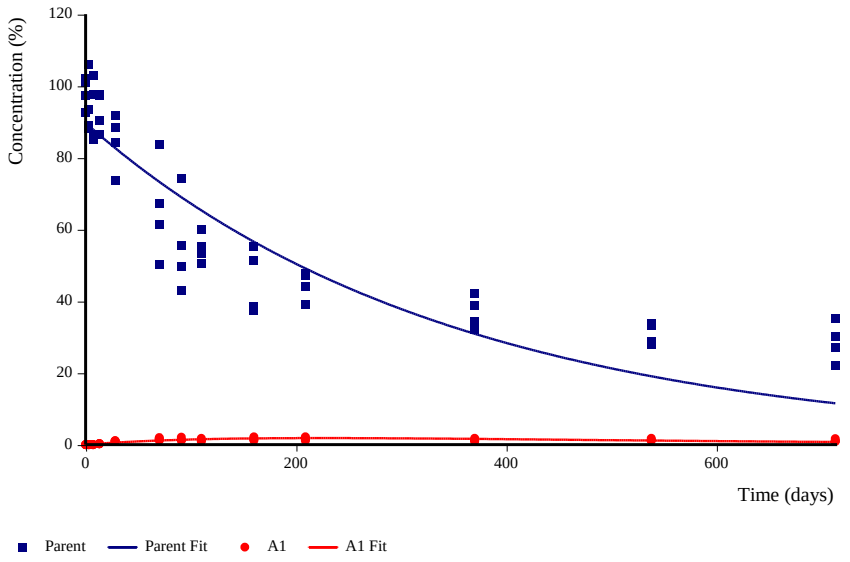
$DT_{50\ matrix}$ half-lives for modelling: DFOP, HS: DT_{50} of slow phase
a) SFO: single first order, DFOP: double first order in parallel, HS: Hockey stick
b) Visual fit: + = good, o = moderate, - = poor

B.8.1.1.2.5.2-13: Kinetic assessment results for the normalisation of M12 performed by the RMS.

Site	Kinetic model[a)]	k_{fast} [1/d]	t-test, k_{fast} / k_{slow}	$DT_{50\ matrix}$ [d]	$DT_{90\ matrix}$ [d]	St. (χ^2err) [%]	Formation fraction	Visual fit[b)]
Burscheid, Germany	SFO-SFO	-	< 0.001	41	136	20.7	0.1137	o
	DFOP-SFO	**0.0039**	**< 0.001**	**178**	**591**	**12.8**	**0.0378**	**+**
	HS-SFO	0.00428	< 0.001	162	539	13.1	0.0406	+
Great Chishill, UK	SFO-SFO	-	< 0.001	33.9	113	15	0.0963	o
	DFOP-SFO	**0.0093**	**<0.001**	**74**	**246**	**14.9**	**0.0503**	**+**
	HS-SFO	0.0100	0.0024	69	229	17.8	0.0535	+
Parcay Meslay, Northern France	SFO-SFO	-	< 0.001	43.3	144	16.6	0.185	o
	DFOP-SFO	**0.00175**	**0.003**	**396**	**1320**	**11.4**	**0.0314**	**+**
	HS-SFO	0.00234	<0.001	296	984	13.1	0.0389	+
St. Etienne du Gres, Southern France	SFO-SFO	-	< 0.001	72.7	242	10.1	0.0685	o
	DFOP-SFO	0.0085	<0.001	81.7	271	10.1	0.0664	+
	HS-SFO	**0.00862**	**<0.001**	**80.5**	**267**	**10.9**	**0.0675**	**+**
Albaro, Italy	SFO-SFO	-	< 0.001	29.8	132	25.5	0.1823	=
	DFOP-SFO	**0.0031**	**<0.001**	**222**	**738**	**23.5**	**0.0429**	**=**
	HS-SFO	0.00354	<0.001	196	650	23.1	0.0483	=
Vilobi, Spain	SFO-SFO	-	<0.001	8.52	28.3	29	0.3463	=
	DFOP-SFO	**0.0031**	**<0.001**	**223**	**742**	**26.2**	**0.0136**	**=**
	HS-SFO	0.0032	<0.001	214	709	24.8	0.0142	=

a) SFO: single first order, DFOP: double first order in parallel, HS: Hockey stick
b) Visual fit: + = good, o = moderate, - = poor

Figure B.8.1.1.2.5.2-1: Normalised SFO Kinetic fit and residual graphs rejected by UK RMS for isoflucypram and its metabolite M12 in the Burscheid soil.



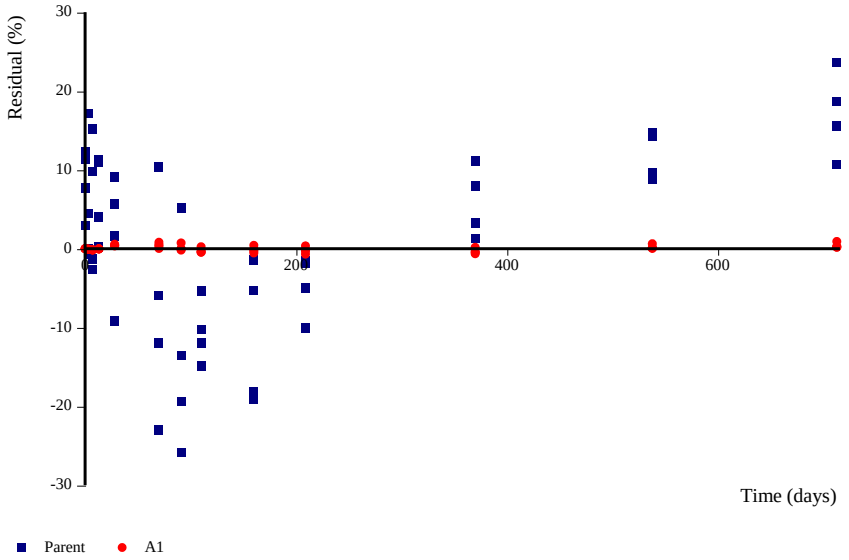
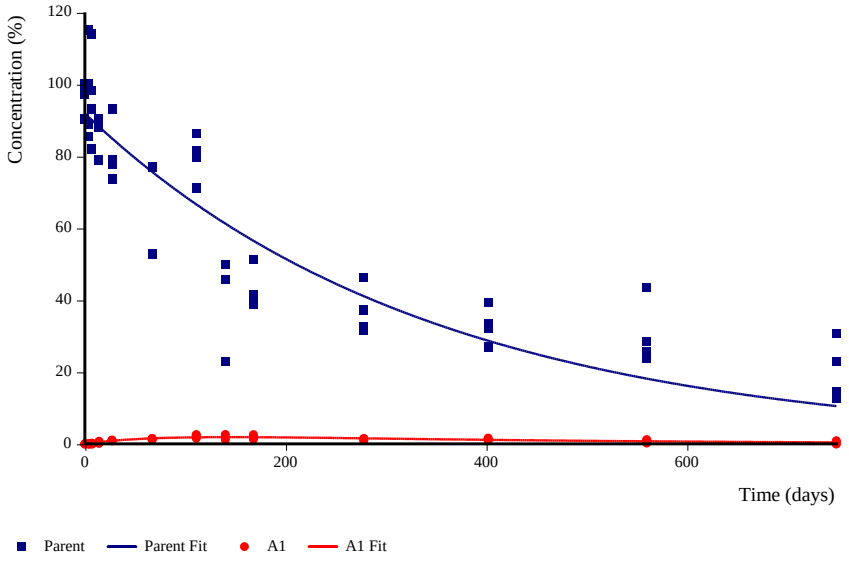


Figure B.8.1.1.2.5.2-2: Normalised SFO Kinetic fit and residual graphs rejected by UK RMS for isoflucypram and its metabolite M12 in the Great Chishill soil.



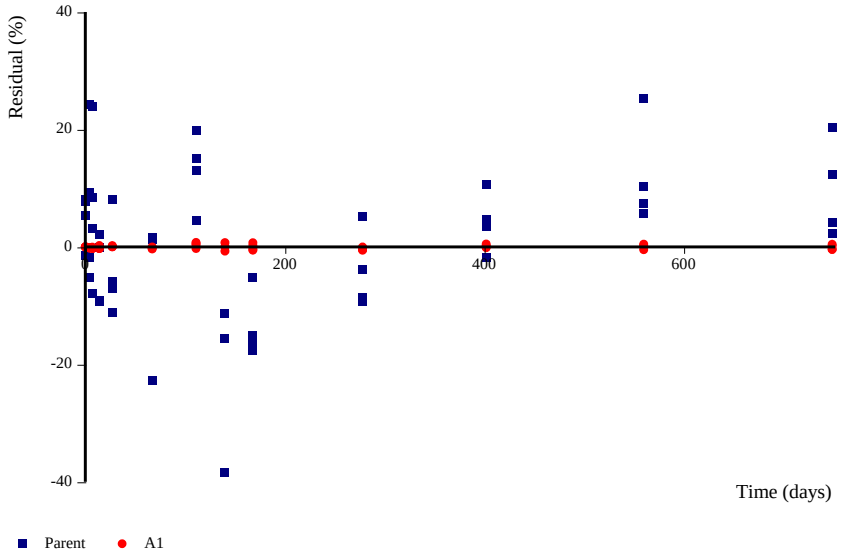
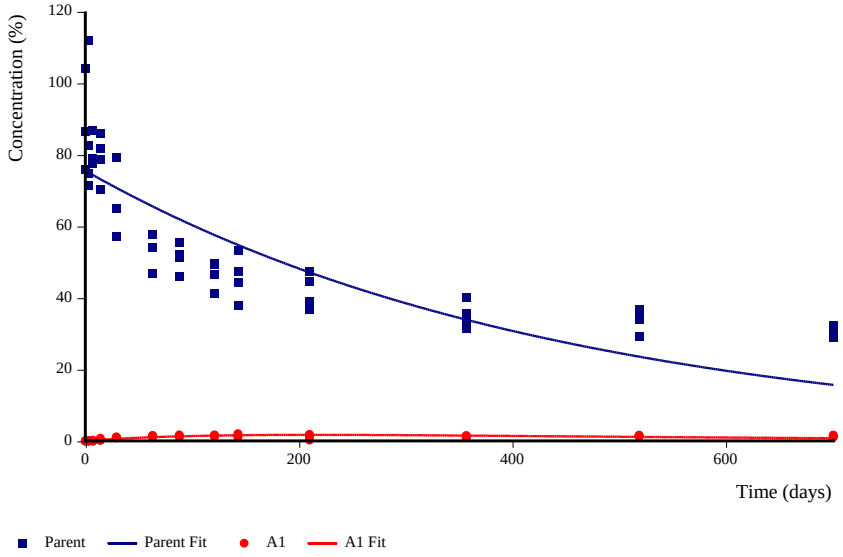


Figure B.8.1.1.2.5.2-3: Normalised SFO Kinetic fit and residual graphs arejected by UK RMS for isoflucypram and its metabolite M12 in the Parcay Meslay soil.



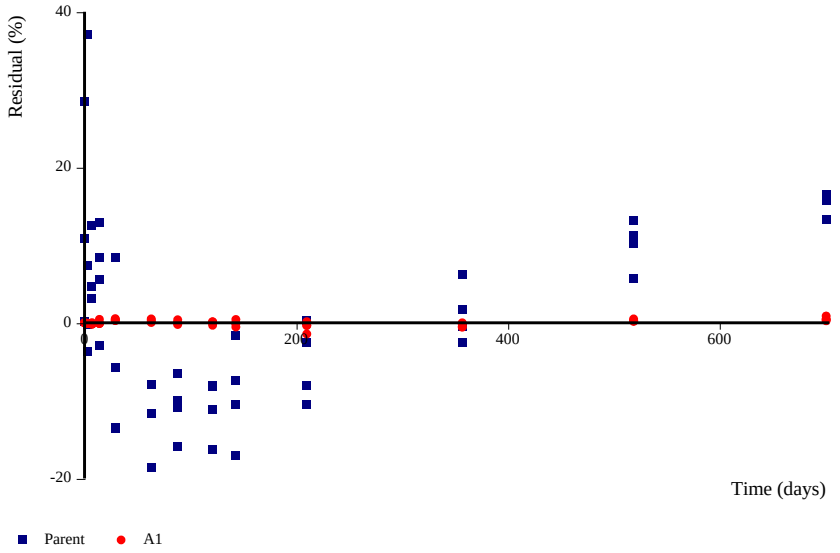
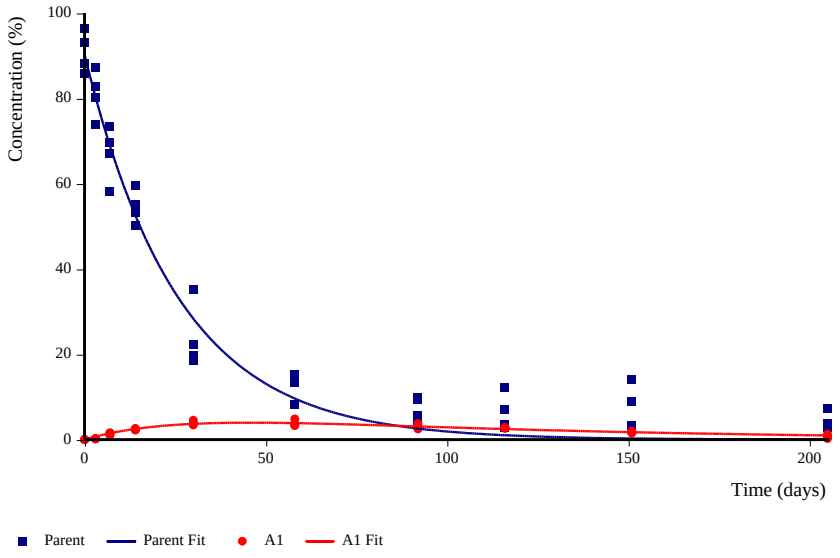


Figure B.8.1.1.2.5.2-4: Normalised SFO Kinetic fit and residual graphs rejected by UK RMS for isoflucypram and its metabolite M12 in the St. Etienne soil.



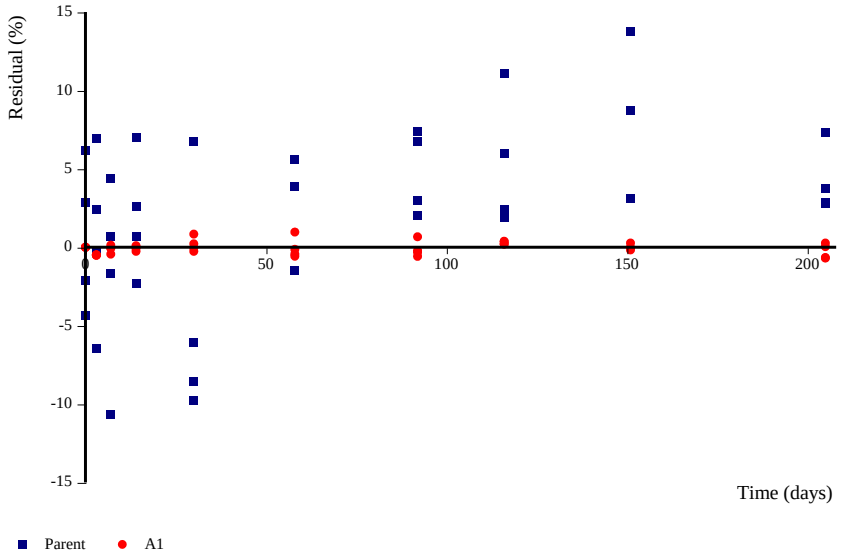
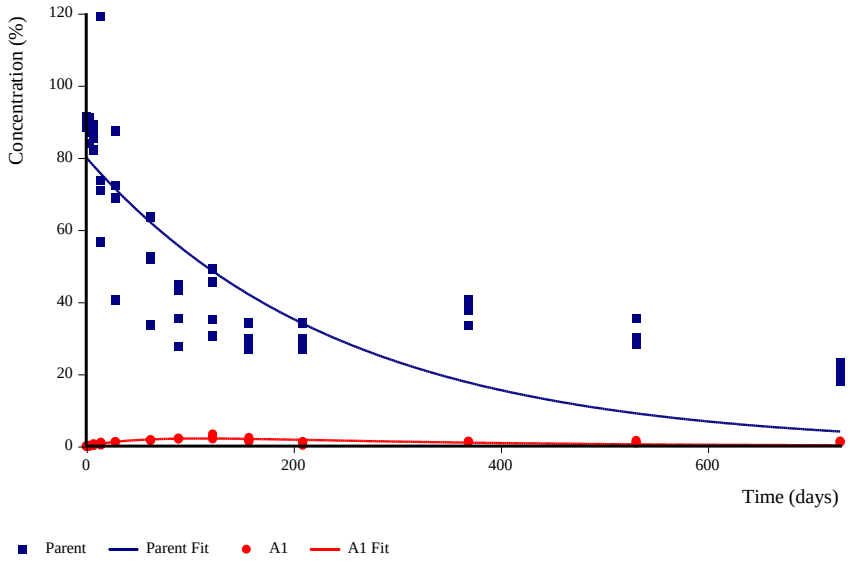


Figure B.8.1.1.2.5.2-5: Normalised SFO Kinetic fit and residual graphs rejected by UK RMS for isoflucypram and its metabolite M12 in the Albaro soil.



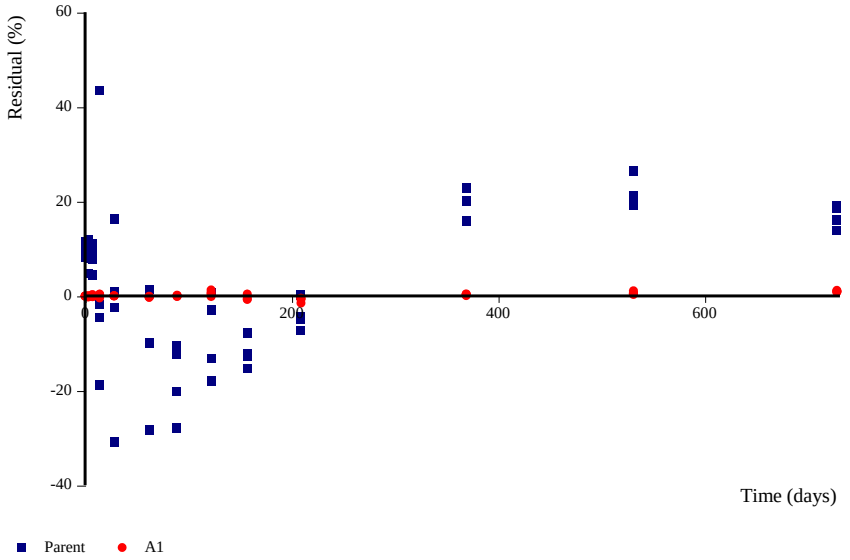
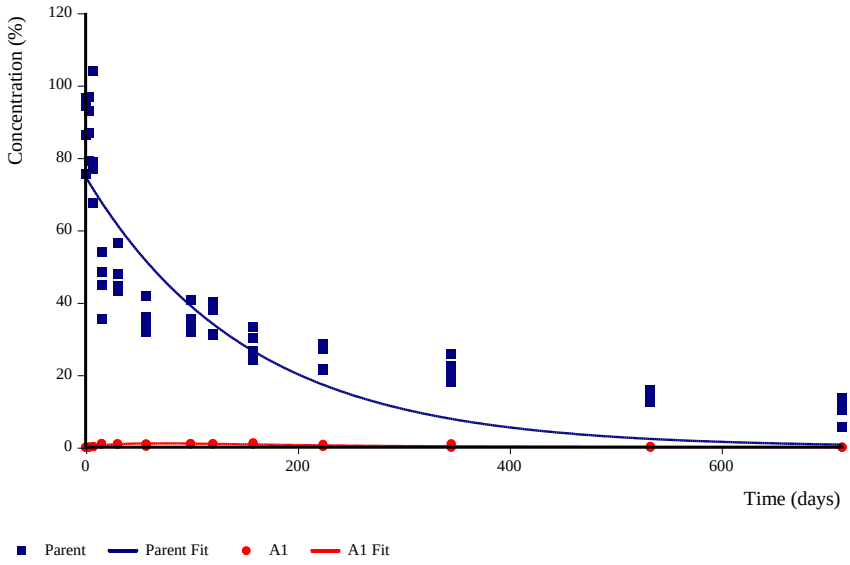


Figure B.8.1.1.2.5.2-6: Normalised SFO Kinetic fit and residual graphs rejected by UK RMS for isoflucypram and its metabolite M12 in the Vilobi soil.



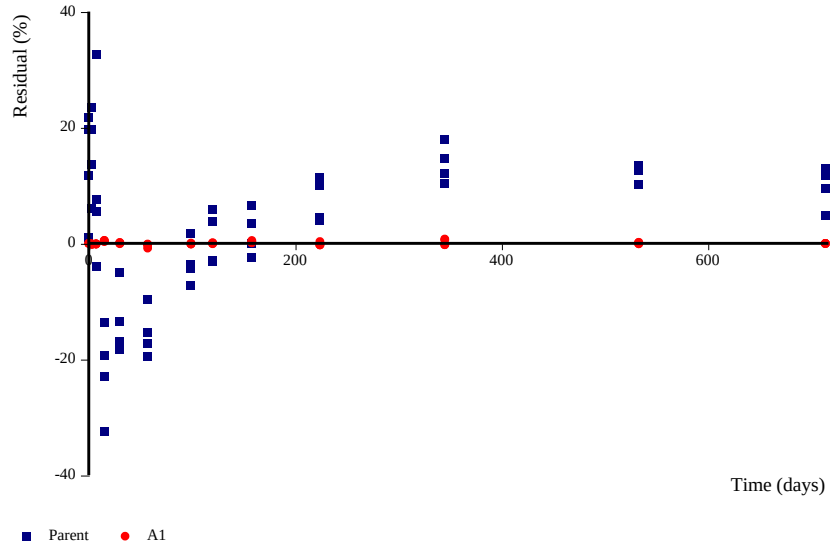


Figure B.8.1.1.2.5.2-7: Normalised SFO-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Burscheid soil.

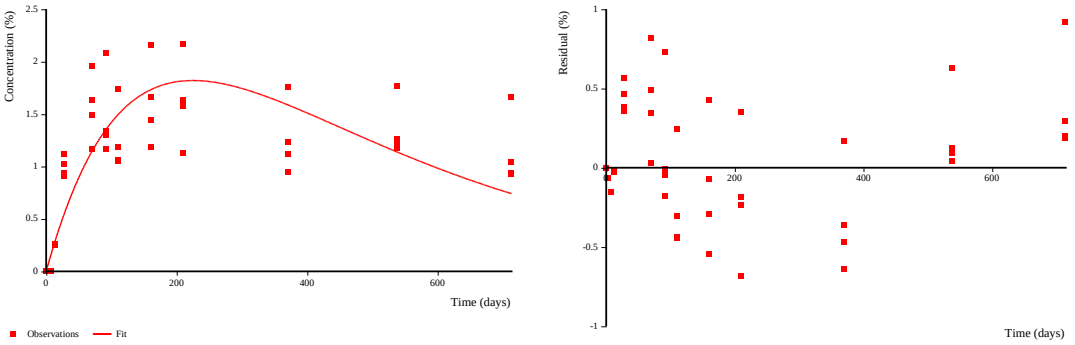


Figure B.8.1.1.2.5.2-8: Normalised SFO-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Great Chishill soil.

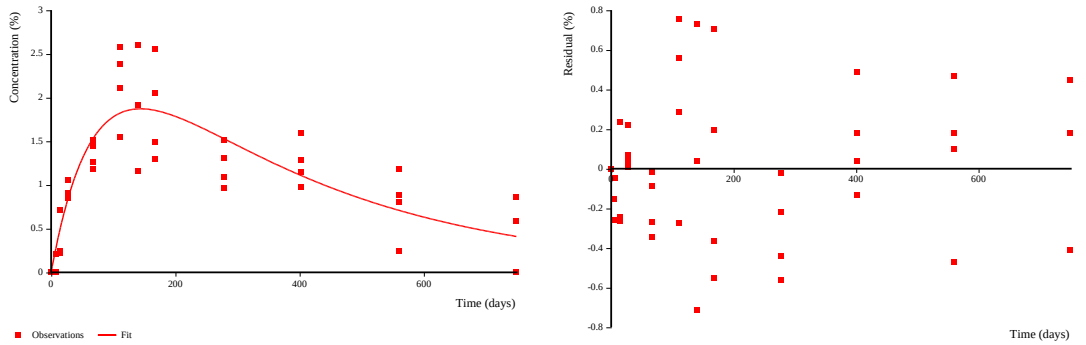


Figure B.8.1.1.2.5.2-9: Normalised SFO-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Parcay Meslay soil.

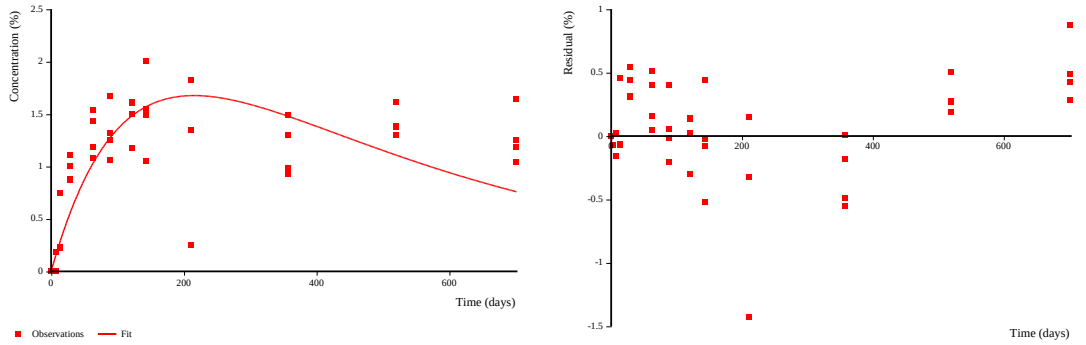


Figure B.8.1.1.2.5.2-10: Normalised SFO-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the St. Etienne soil.

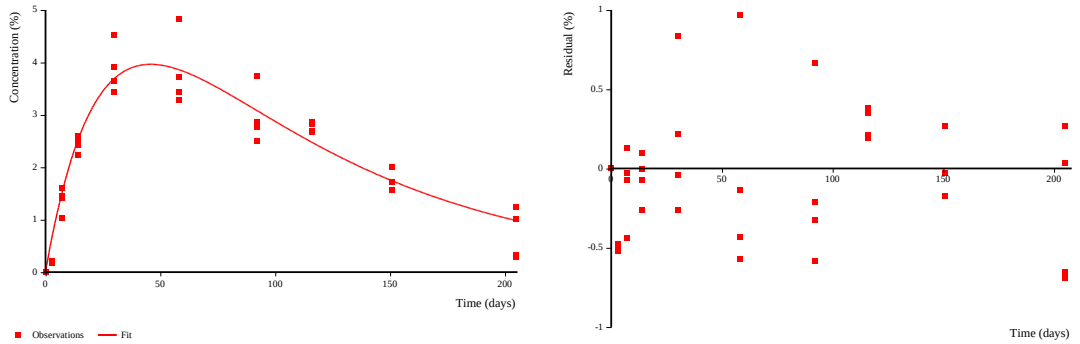


Figure B.8.1.1.2.5.2-11: Normalised SFO-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Albaro soil.

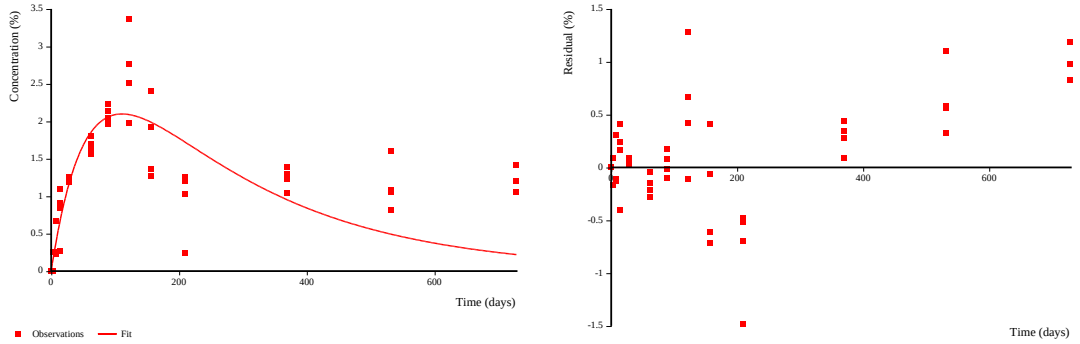


Figure B.8.1.1.2.5.2-12: Normalised SFO-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Vilobi soil

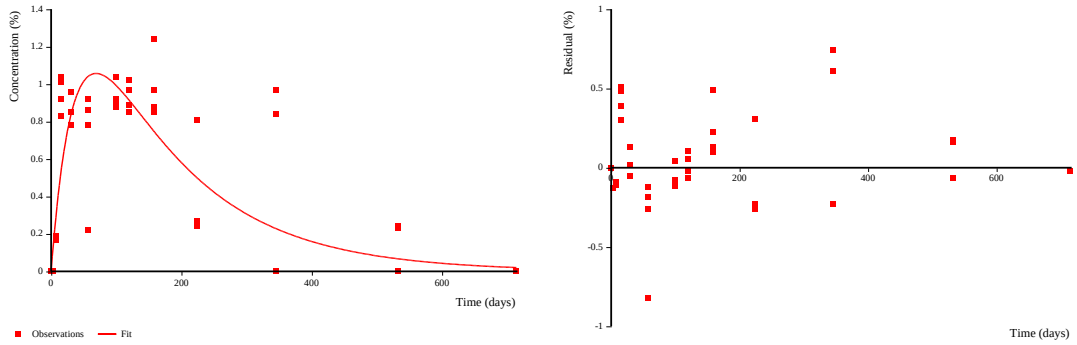
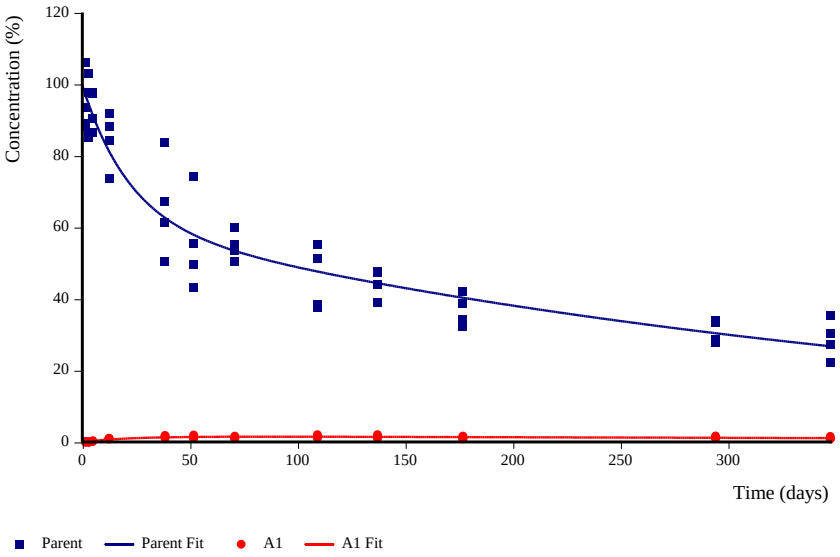


Figure B.8.1.1.2.5.2-13: Normalised DFOP Kinetic fit and residual graphs accepted by UK RMS for isoflucypram and its metabolite M12 in the Burscheid soil.



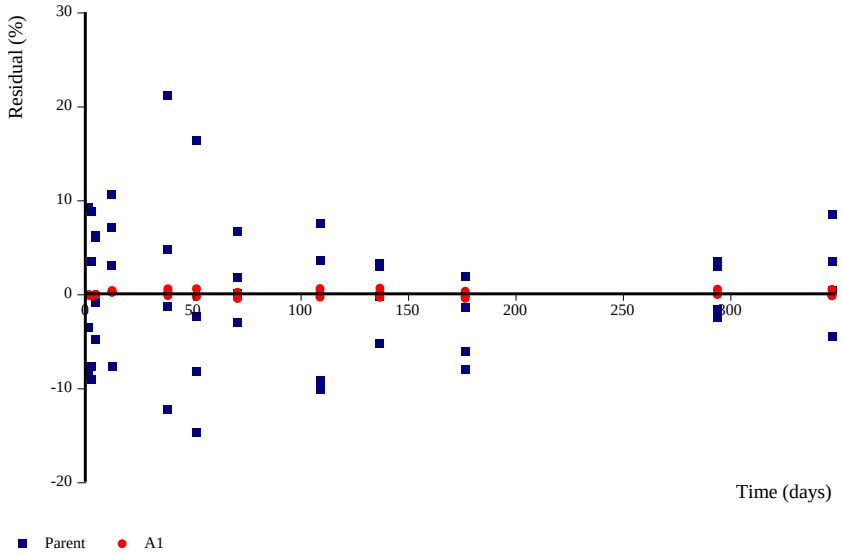
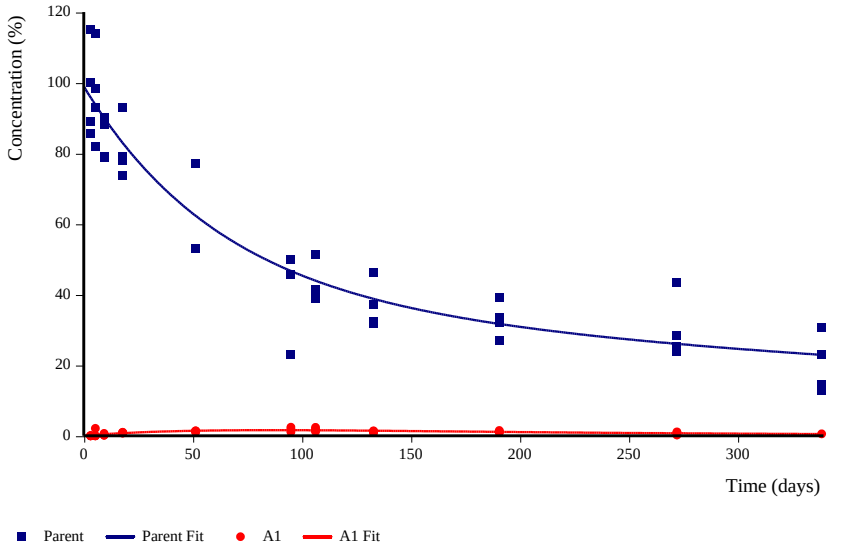


Figure B.8.1.1.2.5.2-14: Normalised DFOP Kinetic fit and residual graphs accepted by UK RMS for isoflucypram and its metabolite M12 in the Great Chishill soil.



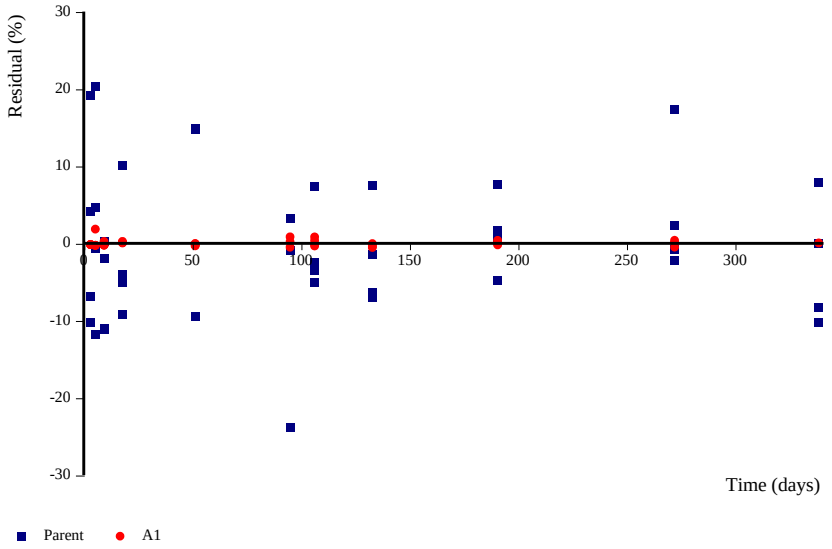


Figure B.8.1.1.2.5.2-15: Normalised DFOP Kinetic fit and residual graphs accepted by UK RMS for isoflucypram and its metabolite M12 in the Parcay Meslay soil.

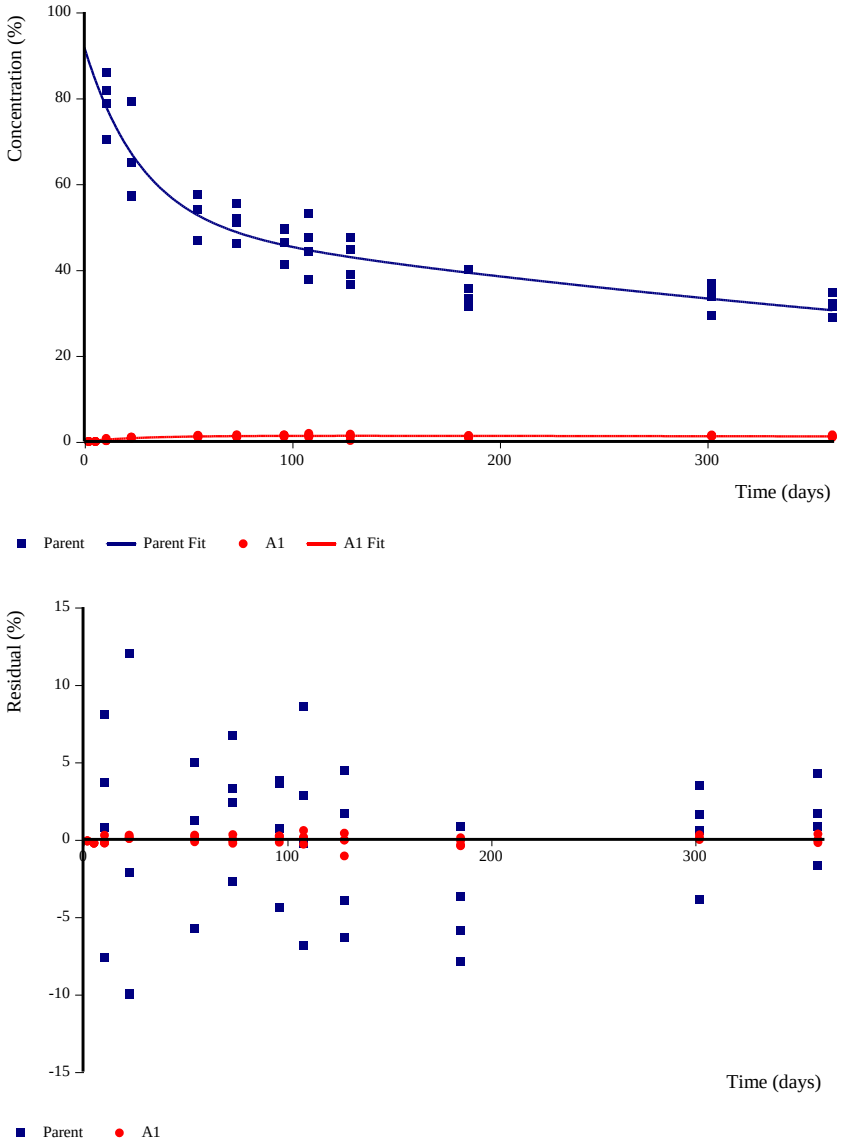


Figure B.8.1.1.2.5.2-16: Normalised HS Kinetic fit and residual graphs accepted by UK RMS for isoflucypram and its metabolite M12 in the St. Etienne soil.

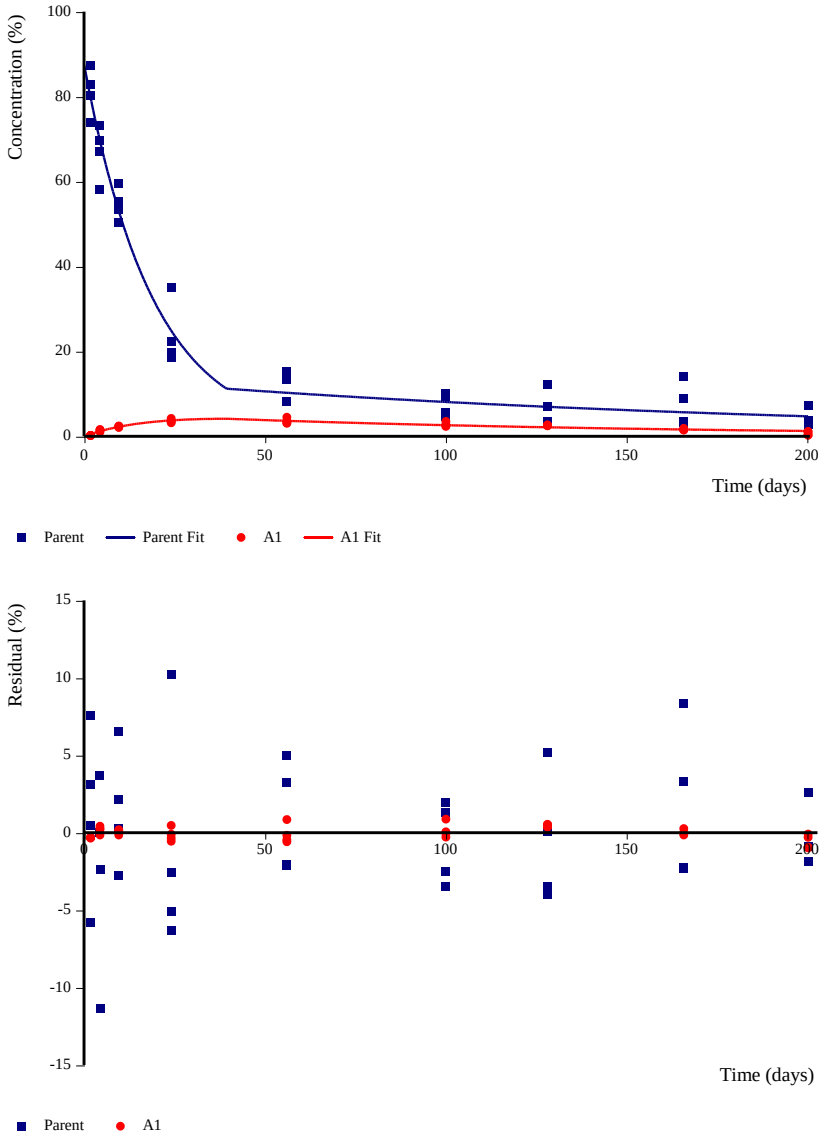


Figure B.8.1.1.2.5.2-17: Normalised DFOP Kinetic fit and residual graphs accepted by UK RMS for isoflucypram and its metabolite M12 in the Albaro soil.

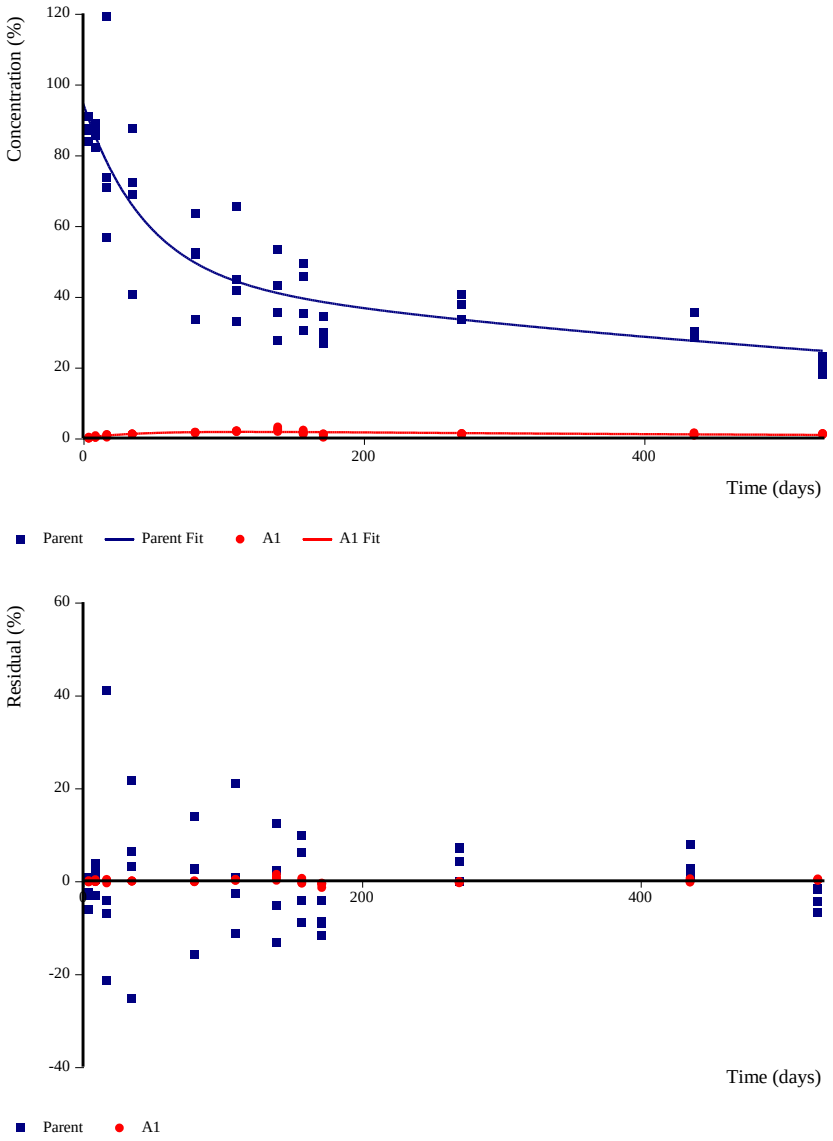


Figure B.8.1.1.2.5.2-18: Normalised DFOP Kinetic fit and residual graphs accepted by UK RMS for isoflucypram and its metabolite M12 in the Vilobi soil.

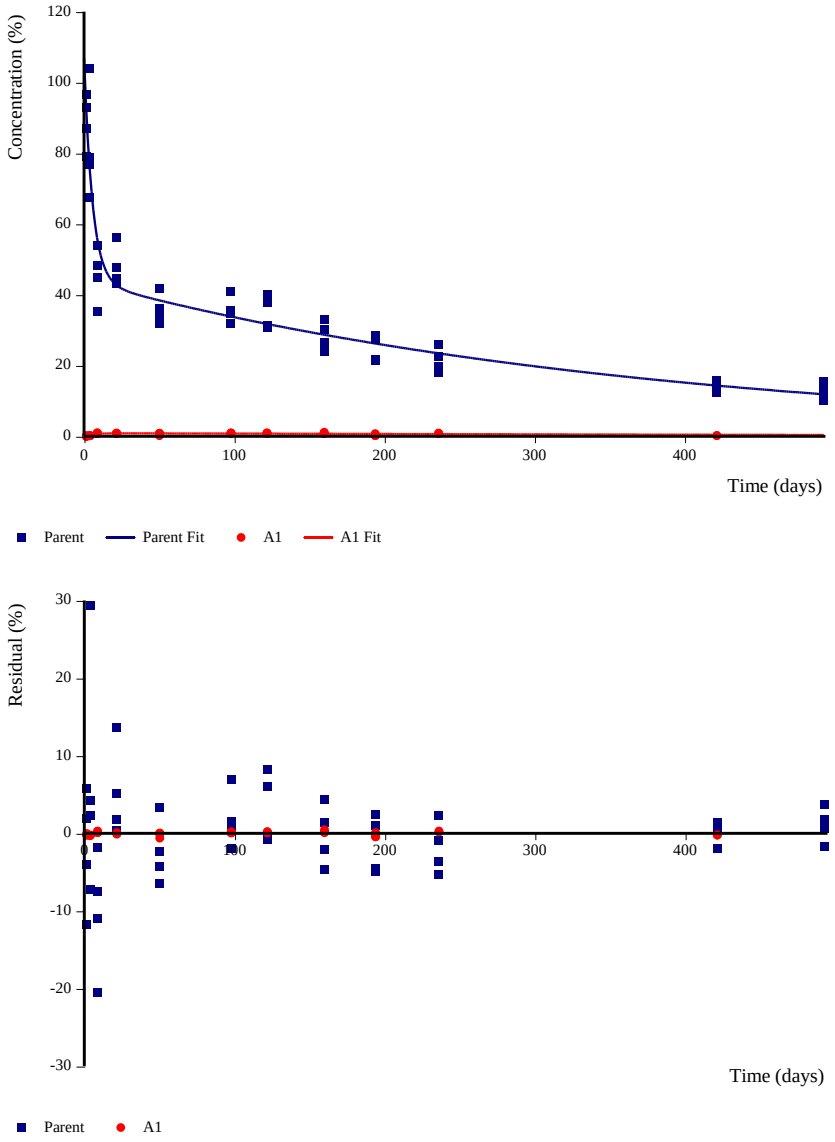


Figure B.8.1.1.2.5.2-19: Normalised DFOP-SFO Kinetic fit and residual graphs accepted by UK RMS for metabolite M12 in the Burscheid soil.

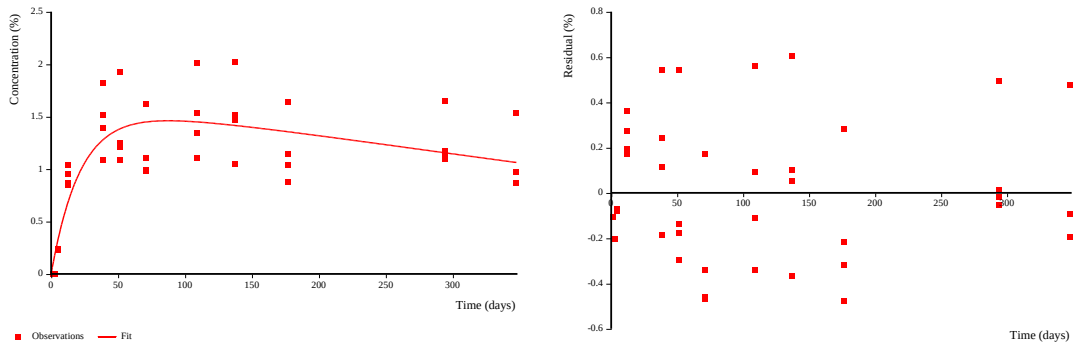


Figure B.8.1.1.2.5.2-20: Normalised DFOP-SFO Kinetic fit and residual graphs accepted by UK RMS for metabolite M12 in the Great Chishill soil.

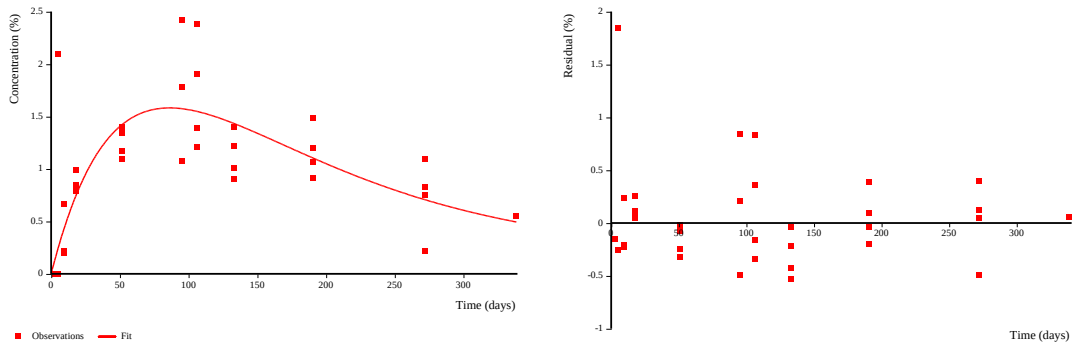


Figure B.8.1.1.2.5.2-21: Normalised DFOP-SFO Kinetic fit and residual graphs accepted by UK RMS for metabolite M12 in the Parcay Meslay soil.

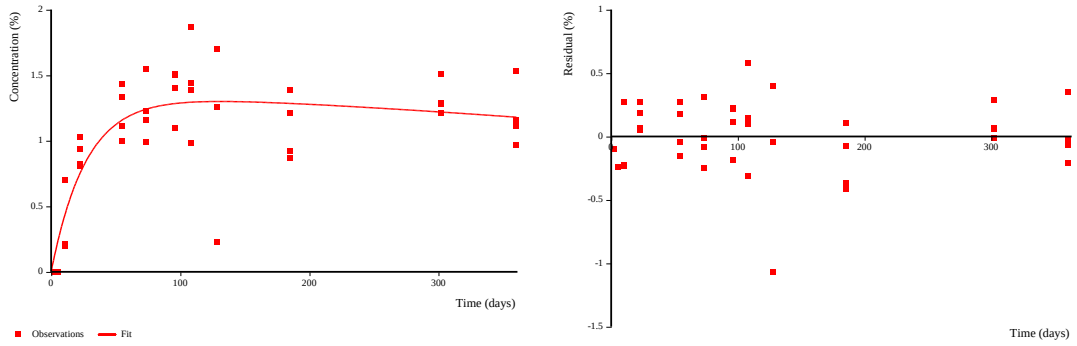


Figure B.8.1.1.2.5.2-22: Normalised HS-SFO Kinetic fit and residual graphs accepted by UK RMS for metabolite M12 in the St. Etienne soil.

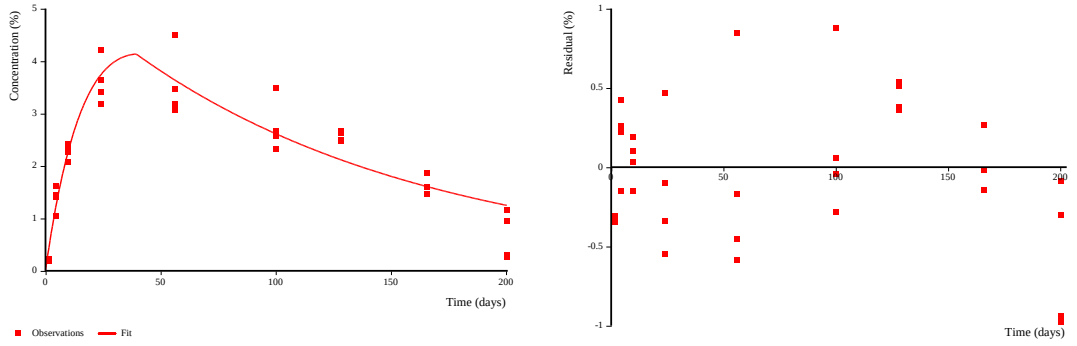


Figure B.8.1.1.2.5.2-23: Normalised DFOP-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Albaro soil.

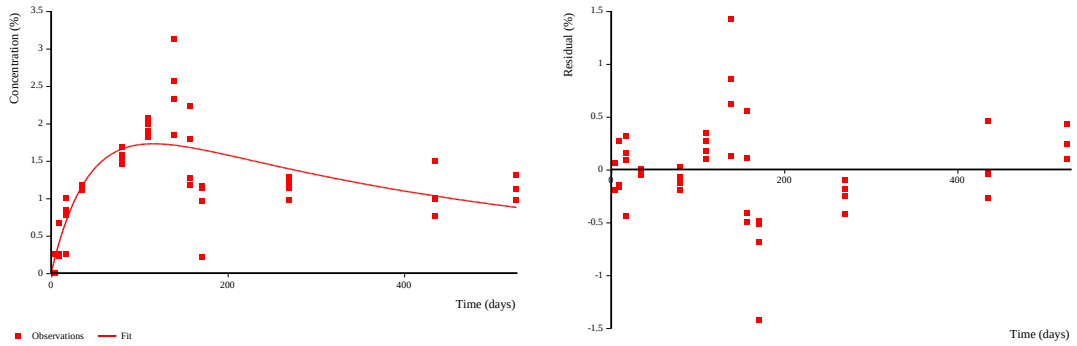


Figure B.8.1.1.2.5.2-24: Normalised DFOP-SFO Kinetic fit and residual graphs rejected by UK RMS for metabolite M12 in the Vilobi soil

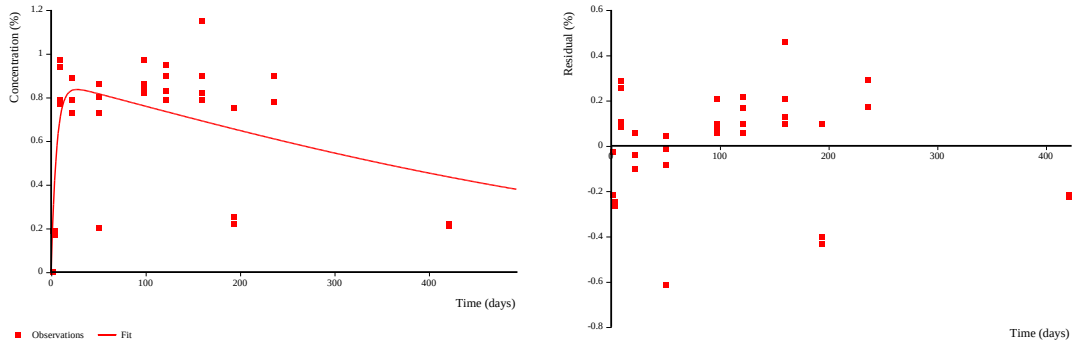


Table B.8.1.1.2.5.2-14: Summary table for the normalised field dissipation results 20°C 100% field capacity for isoflucypram.

Site	Soil	Timepoints removed	Kinetic Fit	DT50 (overall)	DT90 (overall)	Fast phase DT 50 (d)*	Slow phase DT 50 (d)	**St. (χ^2err) [%]**	**g/ tb**
Burscheid, Germany	Bare	d0	DFOP	92.8	759	15.03	289	3.21	0.3819
Great Chishill, UK	Bare	d0	DFOP	85	838	42.81	418	6.73	0.5981
Parcay Meslay, Northern France	Bare	d0, d3, d7	DFOP	94.7	1200	18.76	486	3.5	0.433
St. Etienne du Gres, Southern France	Bare	d0	HS	13.3	85.2	12.28	129	4.19	39.37
Albaro, Italy	Bare	d0	DFOP	90.7	1340	3.022	589	5.82	0.5152
Vilobi, Spain	Bare	d0	DFOP	9.84	532	3.849	261	9.4	0.5895

Geomean	45.5		11.1	324		

[a)] Normalised with a Q10 of 2.58 and a walker coefficient of 0.7

* Logn (2)/ k fast, see table B.8.1.1.2.5.2-12.

Table B.8.1.1.2.5.2-15: Summary table for the normalised field dissipation results 20°C 100% field capacity for M12 .

Site	Kinetic Fit	DT 50 (d) [a)]	f.f.	St. (χ^2err) [%]
Burscheid, Germany	DFOP-SFO	178	0.0378	12.8
Great Chishill, UK	DFOP-SFO	74	0.0503	14.9
Parcay Meslay, Northern France	DFOP-SFO	396	0.0314	11.4
St. Etienne du Gres, Southern France	HS-SFO	80.5	0.0675	10.9
Geomean		143.1	-	-
Arithmetic mean		-	0.045	

[a)] Normalised with a Q10 of 2.58 and a walker coefficient of 0.7

The overall geometric mean $DT_{50\ matrix}$ of isoflucypram, for modelling purposes according to FOCUS kinetics and EFSA (2014) based on these 6 values, can be given with 324 days and for the metabolite with 143.1 days (normalised to 20°C, 100% field capacity) using the 4 acceptable soils. The arithmetic mean formation fraction of the metabolite M12 in soil under field conditions is 0.045.

B.8.1.1.2.5.3: Combined laboratory and field dissipation kinetic assessment.

UK RMS has performed an assessment according to the EFSA (2014) guidance to address if the laboratory and field DegT50 values should be combined or whether the field values alone should be used to calculate a geometric mean degradation parameter for environmental exposure modelling. The geomean DT50 of the normalised laboratory data is 318.5 days. Following the EFSA flow chart Figure 3 in EFSA (2014), the DT50 is greater than 240 days and more than four field DegT50 matrix values are available. Therefore outcome of the assessment is that the data sets should not be combined for isoflucypram and the field data relied upon for calculation of the geometric mean DT50 for environmental exposure modelling. The normalised field data table is supplied below in table B.8.1.1.2.5.3-1 for Isoflucypram and B.8.1.1.2.3-2 for the metabolite M12. As described in the EFSA flow chart the slow phase DT50 value should be used and is presented. This results in a geomean DT50 for PECgw and PECsw of 324.3 days for isoflucypram.

The EFSA (2014) guidance is not clear if a separate metabolite assessment should be performed. Due to the low levels of metabolite formed in the field study RMS has performed an assessment to address if the combined laboratory and field assessment will produce more reliable values. The provided EFSA calculator was used and the result was that the laboratory and field data should be combined.

Table B.8.1.1.2.5.3-1: Field normalised DT50 assessment for use in PECgw and PECsw modelling for isoflucypram following the EFSA decision tree.

Soil	Experiment	Type	Kinetic Fit	Normalised long phase DT 50 (d)	Fast phase DT50 norm (d)	**St. (χ^2err) [%]**
Burscheid, Germany	Field	silt loam	DFOP	289	15.03	3.21
Great Chishill, UK		clay loam	DFOP	418	42.81	6.73
Parcay Meslay, Northern France		loam	DFOP	486	18.76	3.5
St. Etienne du Gres, Southern France		clay loam	HS	129	12.28	4.19
Albaro, Italy		clay	DFOP	589	3.022	5.82
Vilobi, Spain		loam	DFOP	261	3.849	9.4
Geomean				324	11.1	

Table B.8.1.1.2.5.3-2: Combined laboratory and field normalised DT50 assessment for use in PECgw and PECsw modelling for M12.

Soil	Experiment	Type	Kinetic Fit	Normalised DT 50 (d)	**St. (χ^2err) [%]**	**f.f.**
Hanscheider Hof –	Laboratory	Loam	SFO-SFO	48.1	9.0	0.32
Laacher Hof AXXa		Loamy sand	SFO-SFO	107	8.06	0.26
Hoefchen Am Hohenseh		Silt loam	SFO-SFO	15.5	9.55	0.42
Dollendorf II		Loam	SFO-SFO	48.5	32.5	0.31
CA		Sandy loam	SFO-SFO	-	-	-
NE		Silty loam	SFO-SFO	-	-	-
Laacher Hof AXXa		Loam sand	SFO-SFO	1000	17	0.23
Burscheid, Germany	Field	silt loam	DFOP-SFO	178	12.8	0.0378
Great Chishill, UK		clay loam	DFOP-SFO	74	14.9	0.0503
Parcay Meslay, Northern France		loam	DFOP-SFO	396	11.4	0.0314
St. Etienne du Gres, Southern France		clay loam	HS-SFO	80.5	10.9	0.0675
Geomean				105.5		-
Arithmetic mean				-		0.192

B.8.1.1.6: Summary of the degradation is soil

From the studies on the route of degradation in soil it can be concluded that isoflucypram was slowly degraded in soil under aerobic conditions to the final degradation product carbon dioxide. Parallel to mineralisation, bound residues were formed. A total of three metabolites were identified in the soil extracts along with the parent compound and carbon dioxide. Two of the metabolites (M10 (BCS-CN88460-lactic acid) and M11 (BCS-CN88460-desmethyl-carboxylic acid)) were found only in amounts < 5% of the applied radioactivity (AR). The highest concentrations were found for the major metabolite M12, with a maximum of 9.6% AR (123 DAT). Carbon dioxide was formed at a maximum of 5.2% (125 DAT). Under anaerobic conditions no degradation products > 5% were found. Maximum non extracted residues values of 11.6% AR were seen at 104 DAT. Photodegradation does not play a role in the overall fate of isoflucypram. It is noted that aerobic degradation studies were conducted up to a maximum of 123 days, where at study termination some metabolites (M10, unknown 2 and unknown 3, maximum 3.8%, 4%, and 3.2% A.R. respectively) were still increasing. Studies were not extended to assess if these metabolites would breach the 5% at 2 timepoints trigger, therefore this information remain unknown. These metabolites do not breech the 10%, 5% at 2 timepoints assessment trigger and insufficient information is available to perform any analysis.

A Normalised field geomean DT50 of 324.3 days has been calculated by the RMS using the K2 (slow phase) DT_{50} values for isoflucypram following EFSA (2014) guidance for use in PEC sw and PECgw calculations. For PECsoil and persistence calculations a worst case non-normalised field DT_{50} of 177 days has been calculated.

A combined laboratory and field geomean DT_{50} of 105.5 days has been calculated by the RMS for M12 with an arithmetic mean formation fraction of 0.192. A summary of maximum occurrences of the major metabolite M12, CO_2 and non-extractable residues in soil is given in Table B.8.1.1.6-1.

Table B.8.1.1.6-1: Summary of maximum occurrences of the major metabolite M12, carbon dioxide and non-extractable residues in soil (in percent of applied radioactivity)

Compound	Soil metabolism, aerobic [%]	Field	Soil metabolism, anaerobic [%]	Soil photolysis [%]
M12	9.6	4.3	-	-
Carbon dioxide	5.2	-	0.2	0.2
Non-extractable residues	11.6	-	0.2	1.2

The degradation of isoflucypram and its major metabolites under aerobic conditions has been addressed in both laboratory and field conditions. Non-normalised and normalised assessments were performed on all studies, three laboratory and 1 field dissipation. A summary of the results is presented in table B.8.1.1.6-2

Table B.8.1.1.6-2: Summary of DT50 values of isoflucypram and the major metabolite M12 .

Compound	Soil metabolism, aerobic non normalised worst case field [%]	Soil metabolism, aerobic normalised, field Geomean [%]	Soil photolysis [%]	Soil metabolism anaerobic	Formation fraction
Isoflucypram	177 (DFOP)*	324.3 ^	1000	1000	-
M12	397 (DFOP-SFO)	105.5~	-	-	0.192

*K1=0.078 (88.9 days DT_{50}), K2=0.0007 (1020 DT_{50}) g =0.610.
^ field values only following EFSA (2014 guidance)
~ Combined laboratory and field data

B.8.1.2. Adsorption and desorption in soil

The mobility of isoflucypram and the major soil metabolite M12 have been assessed in batch-equilibrium adsorption/desorption studies. The adsorption of isoflucypram to soil was investigated in two batch equilibrium studies, in a total of seven soils in studies by Stupp, H. P.; Junge, T.; (2014); and Herczog, K. J. S.; (2015). The adsorption of M12 to soil was investigated in two batch equilibrium studies, one with five soils by D'Ambrosio, A.; (2014) and one with four soils by Shrestha, S.; (2017). Summaries of the studies are provided below.

B.8.1.2.1.1: Adsorption of Isoflucypram

Previous evaluation:	None, new active substance.

Author: Stupp, H. P.; Junge, T.; 2014
Title: [Pyrazole-14C]BCS-CN88460: Adsorption/desorption on four European soils
Report No.: EnSa-13-0521
Document No.: M-499024-01-1
Guideline(s): OECD Test Guideline No. 106; US EPA OCSPP Test Guideline No. 835.1230; Canada PMRA Guideline DACO No. .2.4.2
Guideline deviation(s): none specified
GLP/GEP: yes

Study Summary
The adsorption behaviour of isoflucypram was studied in four soils in batch equilibrium experiments in the dark at 20.2°C:

Table B.8.1.2.1.1-1: Information on Selected soils

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Laacher Hof AXXa	Monheim, Germany	loamy sand	6.0	2.1
Hoefchen am Hohenseh 4a	Burscheid, Germany	silt loam	6.3	1.9
Hanscheider Hof	Burscheid, Germany	loam	5.4	2.3
Dollendorf II	Blankenheim, Germany	loam	7.2	5.1

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1/20 for all soils. Isoflucypram was applied at nominal concentrations of 1.0, 0.3, 0.1, 0.03 and 0.01 mg/L in aqueous 0.01 M $CaCl_2$ solution. Due to the low water solubility the test item was dissolved in 20 µL methanol and added to 20 mL aqueous solution (0.1% organic solvent). The desorption phase was performed by supplying pre adsorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution for one desorption cycle. Adsorption and the first desorption cycle took place for 24 hours. For the highest concentration, two additional desorption cycles were performed with 24 hours equilibration time each.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and the test item amounts in the supernatants were analysed by liquid scintillation counting (LSC). After the last desorption step, the samples were extracted with acetone. The organic extracts were separated by centrifugation and the test item amounts in the supernatants were analysed by LSC. After the extraction, the soil was dried and combusted. The trapped carbon dioxide after combustion was measured by LSC. The adsorption parameters were calculated using the Freundlich adsorption isotherm.

The test item was sufficient stable throughout the study. The mean parental mass balances calculated as recovery of isoflucypram from aqueous supernatant and soil extract in a pretest were 110.0, 107.5, 103.0 and 104.8% of the applied radioactivity (AR) for soil Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II, respectively.
Mean material balances were 104.7, 106.7, 107.0 and 106.1% AR for soil Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II, respectively.
In the definitive adsorption test 59.4 – 71.8% AR were adsorbed in soil Laacher Hof AXXa, 61.0 – 73.9% AR in soil Hoefchen am Hohenseh 4a, 63.1 – 74.6% AR in soil Hanscheider Hof and 76.8 – 87.1% AR in soil Dollendorf II.

The calculated adsorption constants $K_{f(ads)}$ of the Freundlich isotherms for the four test soils ranged from 29.184 to 58.711 mL/g (mean: 37.534 mL/g). The Freundlich exponents 1/n were in the range of 0.8690 to 0.8972 (mean: 0.8839), indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

In general the organic matter in soil, determined as organic carbon content, is the most important component responsible for binding organic chemicals. Therefore, the adsorption coefficients $K_{f(ads)}$ were correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For isoflucypram the calculated $K_{oc(ads)}$ values varied between 1151.2 and 1569.1 mL/g (mean: 1380.0 mL/g).

At the end of the adsorption and first desorption phase, 23.2 – 34.7%, 21.0 – 34.2%, 20.1 – 29.1% and 10.3 – 18.5% of the initially adsorbed amount were desorbed in soil Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II, respectively.

The desorption $K_{f(des)}$ and the normalised $K_{oc(des)}$ values were slightly higher (mean 1720.9 mL/g) than those obtained for the adsorption phase (mean 1380.0 mL/g).
There is no significant correlation between pH and adsorption for the investigated soils.
The following table summarises the key data of this study:

Table B.8.1.2.1.1- 2: Summary of the adsorption data of isoflucypram

Soil	Texture (USDA)	pH ($CaCl_2$)	OC [%]	Clay [%]	$K_{f(ads)}$ [mL/g]	1/n	$K_{oc(ads)}$ [mL/g]
Laacher Hof AXXa	loamy sand	6.0	2.1	9	29.184	0.8904	1389.7
Hoefchen am Hohenseh 4a	silt loam	6.3	1.9	19	29.812	0.8788	1569.1
Hanscheider Hof	loam	5.4	2.3	21	32.430	0.8972	1410.0
Dollendorf II	loam	7.2	5.1	23	58.711	0.8690	1151.2
arith. mean					**37.534**	**0.8839**	**1380.0**

According to Briggs[1], isoflucypram can be classified as immobile.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazole-labelled isoflucypram
Sample-ID: KML 9427
Specific activity: 3.90 MBq/mg (105.34 µCi/mg)
Radiochemical purity: > 99% (HPLC with radioactivity detector)
> 99% (TLC, scan)
Chemical purity: > 98% (HPLC with UV-detector, 210 nm)

Reference item

Reference items were not used. Test substance confirmed in the stock solution by H-NMR and HPLC/MS

[1] Briggs, G. G. (1973) A Simple Relationship Between Soil Adsorption of Organic Chemicals and their Octanol/Water Partition Coefficients *Proc. 7th British Insecticide and Fungicide Conference, Nottingham/UK.*

2. Test Soils

The study was carried out using four different soils (see Table B.8.1.2.1.1- 3). The soils were taken from agricultural use areas representing different geographical origin and different soil properties as required by the guidelines. The soils were sampled freshly from the fields (upper horizon of 0 to 20 cm) and sieved to a particle size of ≤ 2 mm.

Table B.8.1.2.1.1- 3: Physico-chemical properties of test soils

Parameter	**Results**	
Soil designation	Laacher Hof AXXA	Hoefchen am Hohenseh 4a
Geographic location City State Country	 Monheim North Rhine-Westphalia Germany	 Burscheid North Rhine-Westphalia Germany
Soil taxonomic classification (USDA)	Sandy, mixed, mesic Typic Cambudoll	Loamy, mixed, mesic Typic Argudalf
Soil series	no information available	no information available
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	loamy sand 83 8 9	silt loam 25 56 19
pH - in 0.01 M $CaCl_2$ 1/2 - in water 1/1 - in saturated paste - in soil/1 N KCl 1/1	6.0 6.3 6.3 5.8	6.3 6.6 6.6 6.0
Organic carbon (combustion) [% OC] Organic matter [a] [% OM]	2.1 3.6	1.9 3.3
Cation exchange capacity [meq/100 g]	9.5	10.8
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 1/3 bar (pF 2.0) [%]	 52.5 15.6	 60.4 26.4
Bulk density (disturbed) [g/cm^3]	1.22	1.09

a) % organic matter = % organic carbon x 1.724
DW: dry weight *cont.*

Table B.8.1.2.1.1- 3 *(cont.)*: Physico-chemical properties of test soils

Parameter	Results	
Soil designation	Hanscheider Hof	Dollendorf II
Geographic location City State Country	 Burscheid North Rhine-Westphalia Germany	 Blankenheim North Rhine-Westphalia Germany
Soil taxonomic classification (USDA)	Loamy skeletal, mixed, semiactive, mesic Dystric Eutrudept	fine-loamy, mixed, active, frigid Typic Eutrudept
Soil series	no information available	no information available
Textural class (USDA) Sand [%] 33 Silt [%] 50 Clay [%] 17	loam 41 38 21	loam 45 32 23
pH - in 0.01 M $CaCl_2$ 1/2 - in water 1/1 - in saturated paste - in soil/1 N KCl 1/1	5.4 5.7 5.7 5.0	7.2 7.4 7.4 7.0
Organic carbon (combustion) [% OC] Organic matter [a] [% OM]	2.3 4.0	5.1 8.8
Cation exchange capacity [meq/100 g]	9.7	20.6
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 1/3 bar (pF 2.0) [%]	 60.7 27.7	 79.3 41.4
Bulk density (disturbed) [g/cm^3]	1.04	0.98

a) % organic matter = % organic carbon x 1.724
DW: dry weight

B. STUDY DESIGN

1. Experimental Conditions

For the preliminary tests and for the definitive test the same equipment and experimental set-up was used. Important parameters for the test e.g. stability of the test item, adsorption to vessel surface, soil-to-solution ratio and equilibration time for adsorption were determined prior to the definitive test in preliminary tests.
In the definitive test, soil-to-solution ratios of 1/20 were used. The corresponding amounts were 1 g soil (dry weight) and 20 mL solution (corrected for soil moisture). The equilibration time was 24 hours for adsorption and each desorption step.

Centrifuge tubes with screw caps (material Teflon®, volume 42 mL) were used as test vessels. They were shaken by a mechanical overhead shaker in a walk-in climatic chamber at controlled temperature.

For the definitive test, the soil-to-solution ratio was 1 g soil (dry weight) and 20 mL aqueous 0.01 M $CaCl_2$ solution (corrected for soil moisture) for all soils. Soils were pre-equilibrated for 72 hours. The nominal concentrations of the test item were 0.01, 0.03, 0.1, 0.3 and 1.0 mg/L. 20 µL of the respective application solutions were pipetted into the suspensions consisting of 1 g soil (dry weight) and 20 mL aqueous 0.01 M $CaCl_2$ solution (corrected for soil moisture).

Due to the stability of the test item, the partition of the test item was determined based on the amount of radioactivity in the supernatant. The experiments were performed in duplicate.

2. Analytical Procedures

In the definitive test, 1 g soil (dry weight) was weighed into the centrifuge tubes and 20 mL aqueous 0.01 M $CaCl_2$ solution (corrected for soil moisture) was added. After equilibration by shaking for 72 hours, 20 µL of the respective application solution was added. The adsorption measurements were performed with five test item concentrations (0.01 to 1.0 mg/L) covering two orders of magnitude. The tubes were closed and the suspensions were agitated for a defined period of time (24 hours) using an overhead shaker at a constant temperature in the dark. The suspensions were centrifuged (10 min. at 4550 x g) and the supernatants were analysed by LSC. Additionally, the pH values of the adsorption supernatants were determined.
For the desorption experiment the supernatants were centrifuged and decanted, weighed and replaced by a corresponding volume of aqueous 0.01 M $CaCl_2$ solution.
After agitation for a defined period of time and centrifugation (10 min. at 4550 x g) the supernatant was decanted, weighed and analysed as described above.
In case of the highest concentration, two additional desorption steps were performed.
For the calculation of the mass balance, the remaining soil of the first replicates was extracted after the last desorption step with 10 mL acetone at ambient temperature by shaking for 30 minutes, centrifuged (10 min. at 4550 x g) and the supernatant was analysed by LSC. Afterwards, the soil was dried, combusted and analysed by LSC.

3. Calculations

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

The recovery of radioactivity for all soils is presented in Table B.8.1.2.1.1- 4.
Material balances (total radioactivity) were 104.7, 106.7, 107.0 and 106.1% AR for soil Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II. The test item was stable under the test conditions (parental mass balance: > 103% AR), with no metabolites observed in either the supernatant or soil extracts in the chromatography. Therefore, the sorption behaviour was calculated based on the radioactivity measured in the supernatant only.

The complete material balance values found for all samples demonstrated that no significant portion of radioactivity dissipated from the test systems or was lost during sample processing.

Table B.8.1.2.1.1- 4: Recovery of radioactivity after adsorption, desorption and extraction (as percentage of applied radioactivity, one replicate)

Conc. ID	Soil			
	Laacher Hof AXXa	Hoefchen am Hohenseh 4a	Hanscheider Hof	Dollendorf II
A	100.7	109.5	101.8	98.2
B	104.2	102.5	108.0	105.8
C	103.9	109.9	107.4	108.8
D	106.8	107.9	108.5	108.6
E	107.9	103.4	109.1	108.9
Mean[a)]	**104.7**	**106.7**	**107.0**	**106.1**
SD	**± 2.5**	**± 3.1**	**± 2.6**	**± 4.1**

a) mean for all soils: 106.1%

B. ADSORPTION RESULTS

The test was performed using soil-to-solution ratios of 1/20 (1 g soil dry weight and 20 mL solution). The equilibration time for adsorption was 24 hours.

In the definitive adsorption test 59.4 – 71.8% AR were adsorbed in soil Laacher Hof AXXa, 61.0 - 73.9% AR in soil Hoefchen am Hohenseh 4a, 63.1 – 74.6% AR in soil Hanscheider Hof and 76.8 – 87.1% AR in soil Dollendorf II. The respective concentrations in solution and in soil and the percentage of adsorbed test item are summarised in Table B.8.1.2.1.1- 5. Concentrations in soil are calculated by difference as a desorption step was performed using the same centrifuge tubes.

Table B.8.1.2.1.1- 5: Concentration of isoflucypram in the solid and liquid phases at the end of adsorption equilibrium

Concentration of isoflucypram	Soil [mg/kg]	Solution [mg/L]	Percentage adsorbed mean SD
Laacher Hof AXXA			
Control	N/A	N/A	
0.012 mg/L	0.170	0.003	71.8 ± 0.3
0.031 mg/L	0.437	0.009	70.7 ± 1.6
0.09 mg/L	1.279	0.027	70.3 ± 1.7
0.29 mg/L	3.883	0.094	67.3 ± 1.5
1.05 mg/L	12.480	0.427	59.4 ± 0.5
Hoefchen am Hohenseh 4a			
Control	N/A	N/A	
0.012 mg/L	0.175	0.003	73.9 ± 0.4
0.031 mg/L	0.450	0.008	72.7 ± 1.7
0.09 mg/L	1.302	0.026	71.5 ± 0.9
0.29 mg/L	3.888	0.094	67.4 ± 0.5
1.05 mg/L	12.818	0.410	61.0 ± 0.8
Hanscheider Hof			
0.012 mg/L	N/A	N/A	
0.031 mg/L	0.176	0.003	74.6 ± 1.9
0.09 mg/L	0.442	0.009	71.4 ± 0.6
0.29 mg/L	1.295	0.026	71.1 ± 0.7
1.05 mg/L	3.964	0.090	68.7 ± 1.1
0.012 mg/L	13.264	0.388	63.1 ± 1.9
Dollendorf II			
Control	N/A	N/A	
0.012 mg/L	0.206	0.002	87.1 ± 0.6
0.031 mg/L	0.524	0.005	84.7 ± 0.8
0.09 mg/L	1.544	0.014	84.8 ± 0.4
0.29 mg/L	4.741	0.051	82.2 ± 0.4
1.05 mg/L	16.148	0.243	76.8 ± 1.1

The adsorption behaviour of isoflucypram in the concentration range of two orders of magnitude (i.e. from 0.01 to 1.0 mg/L) was accurately described for all soils with the Freundlich equation. The correlation coefficients of the individual isotherms were 0.9963 to 0.9979 (mean: 0.9973).

The calculated adsorption constants $K_{f(ads)}$ of the Freundlich isotherms for the four test soils ranged from 29.184 to 58.711 mL/g (mean: 37.534 mL/g). The Freundlich exponents 1/n were in the range of 0.8690 to 0.8972 (mean: 0.8839), indicating that the concentration of the test item affected the adsorption behaviour in the examined concentration range.

In general the organic matter in soil, determined as organic carbon content, is the most important component responsible for binding organic chemicals. Therefore, the adsorption coefficients $K_{f(ads)}$ were correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behaviour in different soils. For isoflucypram the calculated $K_{oc(ads)}$ values varied between 1151.2 and 1569.1 mL/g (mean: 1380.0 mL/g).
An overview of the applicants results according to the Freundlich equation is presented in Table B.8.1.2.1.1- 6.

Table B.8.1.2.1.1- 6: Adsorption constants of isoflucypram in soils

Soil	**$K_{f(ads)}$ [mL/g]**	**1/n**	**$K_{oc(ads)}$ [mL/g]**	**r^2**
Laacher Hof AXXa	29.184	0.8904	1389.7	0.9963
Hoefchen am Hohenseh 4a	29.812	0.8788	1569.1	0.9979
Hanscheider Hof	32.430	0.8972	1410.0	0.9976
Dollendorf II	58.711	0.8690	1151.2	0.9976
Arith. mean	**37.534**	**0.8839**	**1380.0**	**0.9973**
Geo. mean			**1371.6**	

C. DESORPTION RESULTS

One desorption step was performed for each concentration. At the end of the first desorption phase, 23.2 – 34.7%, 21.0 – 34.2%, 20.1 – 29.1% and 10.3 – 18.5% of the initially adsorbed amount were desorbed in soil Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II, respectively. For the highest concentration, two additional desorption steps were performed. At the end of the second desorption phase, 31.4, 31.8, 27.0 and 17.9% of the adsorbed amounts after the first desorption phase were desorbed in soils Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II, respectively. At the end of the third desorption phase, 28.6, 30.6, 24.7 and 16.8% of the adsorbed amounts after the second desorption phase were desorbed in soils Laacher Hof AXXa, Hoefchen am Hohenseh 4a, Hanscheider Hof and Dollendorf II, respectively. The respective concentrations in solution and in soil and the percentage of desorbed test item are calculated in Table B.8.1.2.1.1- 7.

Table B.8.1.2.1.1- 7: Concentration of isoflucypram in the solid and liquid phases at the end of desorption equilibrium

Concentration of isoflucypram	Soil [mg/kg]	Solution [mg/L]	Percentage adsorbed mean SD
Laacher Hof AXXA			
Control	N/A	N/A	
0.012 mg/L	0.130	0.002	23.2 ± 0.4
0.031 mg/L	0.329	0.005	24.9 ± 0.7
0.09 mg/L	0.968	0.016	24.4 ± 1.6
0.29 mg/L	2.866	0.051	26.2 ± 1.1
1.05 mg/L	8.151	0.216	34.7 ± 0.5
Hoefchen am Hohenseh 4a			
Control	N/A	N/A	
0.012 mg/L	0.138	0.002	21.0 ± 0.3
0.031 mg/L	0.350	0.005	22.2 ± 1.2
0.09 mg/L	1.005	0.015	22.8 ± 1.0
0.29 mg/L	2.894	0.050	25.6 ± 0.2
1.05 mg/L	8.432	0.219	34.2 ± 0.7
Hanscheider Hof			
0.012 mg/L	N/A	N/A	
0.031 mg/L	0.141	0.002	20.1 ± 1.6
0.09 mg/L	0.340	0.005	23.1 ± 0.7
0.29 mg/L	0.998	0.015	22.9 ± 0.4
1.05 mg/L	2.986	0.049	24.7 ± 0.7
0.012 mg/L	9.417	0.192	29.1 ± 1.6
Dollendorf II			
Control	N/A	N/A	
0.012 mg/L	0.185	0.001	10.3 ± 0.6
0.031 mg/L	0.460	0.003	12.2 ± 0.5
0.09 mg/L	1.355	0.009	12.3 ± 0.5
0.29 mg/L	4.056	0.034	14.5 ± 0.5
1.05 mg/L	13.158	0.150	18.5 ± 0.9

The correlation coefficients of the individual isotherms were 0.9947 to 0.9978 (mean: 0.9964).
The calculated desorption constants $K_{f(des)}$ of the Freundlich isotherms for the four test soils ranged from 35.114 to 72.535 mL/g (mean: 46.831 mL/g), the exponents 1/n were in the range of 0.8669 to 0.9069 (mean: 0.8842). The $K_{oc(des)}$ values of the soils ranged from 1422.3 to 1897.3 mL/g (mean: 1720.9 mL/g). The $K_{oc(des)}$ values were slightly higher than the $K_{oc(ads)}$values (mean 1380.0 mL/g).
An overview about the results according to the Freundlich equation is presented in Table B.8.1.2.1.1- 8.

Table B.1.2.1.1- 8: Desorption constants of isoflucypram in soils

Soil	$K_{f(des)}$ [mL/g]	1/n	$K_{oc(des)}$ [mL/g]	r^2
Laacher Hof AXXa	36.038	0.8926	0.9947	**1716.1**
Hoefchen am Hohenseh 4a	35.114	0.8669	0.9956	**1848.1**
Hanscheider Hof	43.637	0.9069	0.9975	**1897.3**
Dollendorf II	72.535	0.8703	0.9978	**1422.3**
Arith. mean	**46.831**	**0.8842**	**0.9964**	**1720.9**

III. CONCLUSIONS

The adsorption constants $K_{f(ads)}$ of isoflucypram for the four test soils calculated based on the Freundlich isotherms ranged from 29.184 to 58.711 mL/g (mean: 37.534 mL/g). The respective $K_{oc(ads)}$ values were in the range of 1151.2 to 1569.1 mL/g (mean: 1380.0 mL/g).
The desorption constants $K_{f(des)}$ of isoflucypram were slightly higher than the respective adsorption constants.
There was no significant correlation between pH and adsorption for the investigated soils.
isoflucypram was stable in the course of the study. The parental mass balance for the adsorption / desorption phase was ≥ 103.0% AR. No major degradation product was observed.

Using the Briggs classification for the estimation of the mobility of crop protection agents in soil based on K_f and/or K_{oc} values, isoflucypram can be classified as immobile.

UK RMS has validated the study using the OECD106 evaluators checklist and associated Excel calculator. Inputs used by the RMS are presented in table B.8.1.2.1.1-9. A soil/ solution ratio of 1:20 (g-ml) was used, insufficient information is present to determine the f% loss as adsorption recovery values are above 100% and chromatography shows 100% parent at 96 hours in the stability test. F loss was entered as 0%. UKRMS notes the high adsorption values in the Dollendorf II soils, however the ratio used is 1:20 and accepts that soil weights less then 1g are difficult to use experimentally. The observed Koc is not greatly dissimilar to the values achieved for the other soils; the soil is therefore included in the assessment.

With regards to the assessment criteria the LOD was determined to be 0.5% AR (0.000006 mg/L for the lowest concentration), with the LOQ 1.5%AR (0.00018 mg/L for the lowest concentration) for chromatographic methods and for LSC the LOD was set to twice background (18- 24 cpm). The lowest concentration is significantly above the LOD in all aliquots. Kd x soil solution ratio is acceptable is all soils, with values between 1.51 and 10.35. Kfe/Kf also passes the assessment with a value of 1.0 for all soils. HSE evaluator achieves similar values to the applicant, therefore accepts the applicants values for Koc and 1/n for use in the regulatory assessment. Confidence intervals are presented in table B.8.1.2.1.1-10 and are considered by the UK RMS to be acceptable. A relationship between K_{FOC} and pH or Organic carbon and Kf was not noted. Log plot graphs and residual fit graphs are provided for each soil in figures B.8.1.2.1.1-1 to B.8.1.2.1.1-4.

B.8.1.2.1.1-9: Individual replicate values.

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)	Concentration in soil (µg/g)
Laacher Hof AXXa	1.05	0.421	12.9540
	1.05	0.433	12.3650
	0.29	0.099	3.7960
	0.29	0.09	3.9700
	0.09	0.029	1.2480
	0.09	0.026	1.3110
	0.031	0.01	0.4280
	0.031	0.009	0.4470
	0.012	0.003	0.1700
	0.012	0.003	0.1690
Hoefchen am Hohenseh 4a	1.05	0.402	12.9850
	1.05	0.418	12.6510
	0.29	0.096	3.8600
	0.29	0.093	3.9160
	0.09	0.027	1.2860
	0.09	0.025	1.3180
	0.031	0.008	0.4600
	0.031	0.009	0.4390
	0.012	0.003	0.1760
	0.012	0.003	0.1740
Hanscheider Hof	1.05	0.408	12.8570
	1.05	0.367	13.6710
	0.29	0.087	4.0290
	0.29	0.094	3.9000
	0.09	0.026	1.3070
	0.09	0.027	1.2820
	0.031	0.009	0.4450
	0.031	0.009	0.4380
	0.012	0.003	0.1720
	0.012	0.003	0.1810
Dollendorf II	1.05	0.255	15.9140
	1.05	0.232	16.3820
	0.29	0.05	4.7650
	0.29	0.053	4.7180
	0.09	0.013	1.5530
	0.09	0.014	1.5360
	0.031	0.005	0.5190
	0.031	0.004	0.5290
	0.012	0.002	0.2040
	0.012	0.001	0.2070

Table B.8.1.2.1.1-10: Confidence intervals

Soil	$K_{F,ads}$	$K_{F,ads}$ (lower 95%)	$K_{F,ads}$ (upper 95%)	1/n	1/n (lower 95%)	1/n (upper 95%)
Laacher Hof AXXa	28.654	24.555	33.436	0.882	0.842	0.922
Hoefchen am Hohenseh 4a	29.532	26.234	33.245	0.875	0.845	0.906
Hanscheider Hof	32.499	28.802	36.670	0.899	0.868	0.930
Dollendorf II	55.483	40.377	76.240	0.849	0.778	0.919

Figure B.8.1.2.1.1-1: Adsorption log plot and residuals graph for Laacher Hof AXXa soil

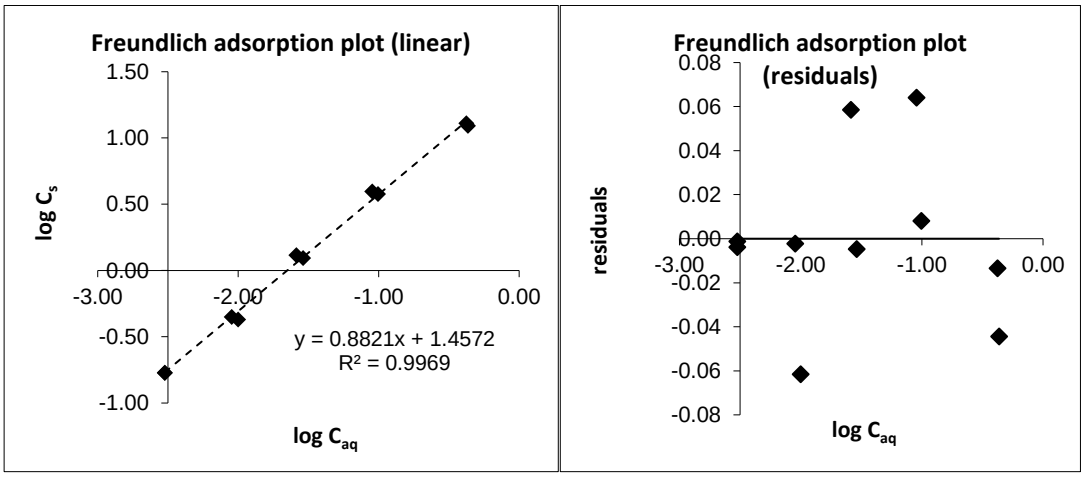


Figure B.8.1.2.1.1-2: Adsorption plot and residuals graph for Hoefchen am Hohenseh 4a

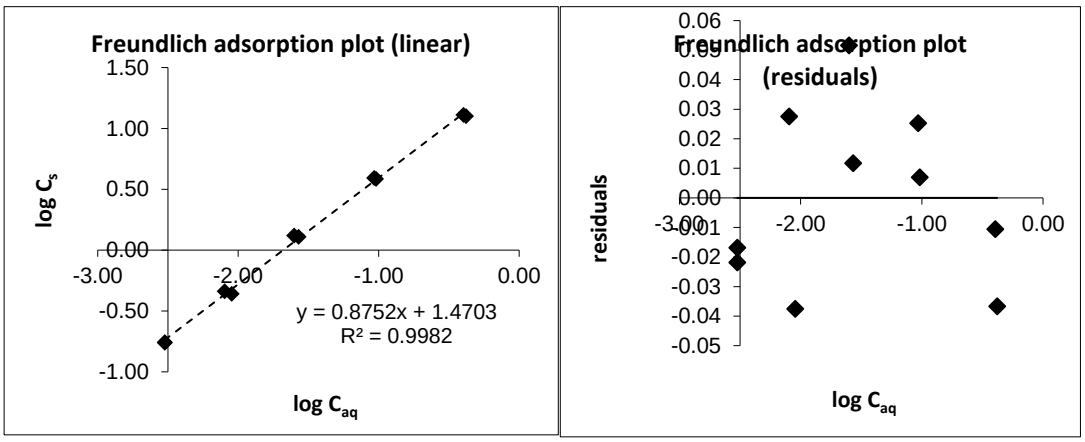


Figure B.8.1.2.1.1-3: Adsorption plot and residuals graph for Hanscheider Hof

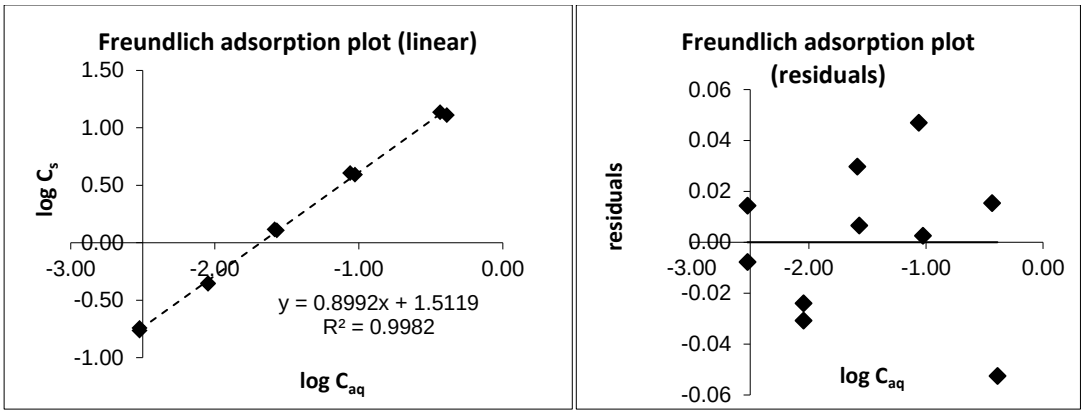
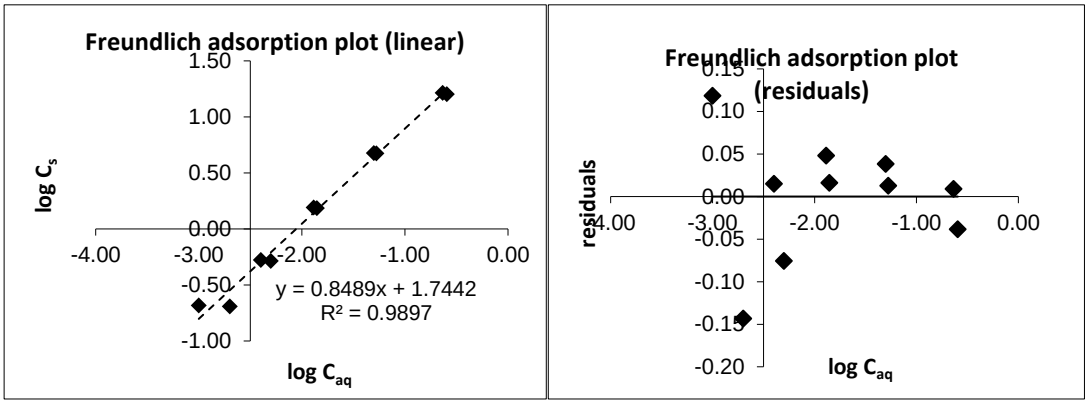


Figure B.8.1.2.1.1-4: Adsorption plot and residuals graph for Dollendorf II



B.8.1.2.1.2: Adsorption of Isoflucypram

Previous evaluation:	None, new active substance.

Author: Herczog, K. J. S.; 2015,
Title: [Pyrazole-4-14C]BCS-CN88460: Adsorption/Desorption on Two US Soils and One Sediment
Report No.: 032774-1
Document No.: M-518345-01-1
Guideline(s): OECD Guideline for the Testing of Chemicals, No. 106, Adsorption/Desorption, 2000
Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
US EPA Fate, Transport and Transformation Test Guidelines, OCSPP 835.1230, Adsorption/Desorption (Batch Equilibrium), 2008
Guideline deviation(s): 0.125% organic solvent was used for the application solutions as opposed to the 0.1% stated in section 30 of the guidance. This is not expected to have a significant effect on the result.
GLP/GEP: yes

Study Summary

The adsorption/desorption characteristics of pyrazole-labelled isoflucypram was examined using two North American soils and one North American sediment: UK RMS has considered the non-European soils selected for assessment and considers that the 2 soils selected are applicable for use, but the accepted convention in the EU pesticide regulatory process is that Koc values from aquatic sediments are not necessarily representative of sorption in soil. Consequently the result for the sediment is excluded from selection of soil adsorption parameters for environmental exposure modelling. The details and applicant results for the EFS-511 sediment have been retained for clarity in this assessment, but the result are not used in the further risk assessment.

Table B.8.1.2.1.2- 1: Selected soils and sediment

Soil/sediment	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
EFS-487 (soil)	Sanger, CA, USA	sandy loam	6.2	0.9
EFS-488 (soil)	Louisville, NE, USA	silt loam	6.5	1.8
EFS-511 (sediment)	Lawrence, KS, USA	silty clay loam	7.5	0.34

Preliminary experiments determined that isoflucypram (test material) had slight adsorption to the glass test vessel surfaces. However, < 3% adhered to glass in the presence of soil. The preliminary soil-to-solution ratio experiment was conducted with all three soil/sediment types (active soil) at 1:10, 1:4, and 1:2.5 soil-to-solution ratios (2 g soil, 20 mL $CaCl_2$, 5 g soil 20 mL $CaCl_2$ and 8g soil 20 mL CaCl2 respectively). Samples were centrifuged at 652 g, the guideline states that 3000g is preferable in section17(c), no calculation was provided that ensures particles less than 0.2 µm were separated. Sufficient adsorption to soil was noted, such that any small particles still remaining in the adsorption supernatant are not expected to greatly affect the result.
HPLC analysis of the adsorption aqueous fraction showed no degradation in all test systems after 24 hours of shaking. A 1:10 soil-to-solution ratio with two soils and one sediment was determined to be appropriate for the definitive study.

The equilibration time experiment conducted at 0.5 µg/L showed that isoflucypram was stable at the 24-hour time point. Soils were extracted using acetonitrile: water 8:2 v/v from the 48 hour timepoint. Supernatant and soil extracts were analysed by HPLC using appropriate methods. Supernatants were analysed up to 96 hours. Post extracted soils (PES) were examined using a Harvey OX-500 oxidiser, with sufficient machine background analysis.

One adsorption experiment and one desorption experiment were performed using the batch equilibration method with two soils and one sediment at five concentrations covering three orders of magnitude (0.005, 0.015, 0.05, 0.15 and 0.5 µg/mL) of the test substance in 0.01 M calcium chloride. The percent of isoflucypram adsorbed during the adsorption cycle to each matrix was calculated at all five test solution concentrations. The average percentage sorbed to soil or sediment at the end of the adsorption test ranged from 54.9 to 79.3% of the applied radioactivity. At the end of the desorption period, 15.9 to 33.4% of the applied radioactivity was sorbed to soil or sediment. The following tables summarise key data of this study.

Table B.1.2.1.2- 2: Freundlich adsorption isotherms of isoflucypram

Soil/sediment	Texture (USDA)	pH ($CaCl_2$)	OC [%]	$K_{f(ads)}$ [mL/g]	1/n	$K_{oc(ads)}$ [mL/g]
EFS-487, Sanger (soil)	sandy loam	6.2	0.9	13	1.0150	1394
EFS-488, Louisville (soil)	silt loam	6.5	1.8	25	0.9101	1384
EFS-511, Lawrence (sediment)	silty clay loam	7.5	0.34	12	0.9387	3594
Arith. mean				**16.7**	**0.9546**	**2124**

Table B.8.1.2.1.2- 3: Freundlich desorption isotherms of isoflucypram

Soil/sediment	Texture (USDA)	pH ($CaCl_2$)	OC [%]	$K_{f(des)}$ [mL/g]	1/n	$K_{oc(des)}$ [mL/g]
EFS-487, Sanger (soil)	sandy loam	6.2	0.9	23	1.0442	2591
EFS-488, Louisville (soil)	silt loam	6.5	1.8	33	0.9116	1810
EFS-511, Lawrence (sediment)	silty clay loam	7.5	0.34	19	0.9538	5479

Based on the results isoflucypram can be classified in the "immobile" mobility class in all soils/sediment according to the Briggs[1] classification system.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazole-labelled isoflucypram
Standard-ID: C-1173
Specific activity: ~45.55 µCi/µMole; ~252,900 (dpm/µg)
Radiochemical purity: > 99%

Reference item

Non-labelled isoflucypram
Standard-ID: K-2124
Chemical purity: 98.4

[1] Briggs, G. G. (1973)
A Simple Relationship Between Soil Adsorption of Organic Chemicals and their Octanol/Water Partition Coefficients
Proc. 7th British Insecticide and Fungicide Conference, Nottingham/UK.

2. Test Soils and Sediment

The two soils and one sediment selected for the study are typical of agricultural growing regions in North America and provide a variety of soil characteristics and geographic diversity.

Table B.8.1.2.1.2-4: Physico-chemical properties of test soils and sediment

Parameter	Results		
Soil	EFS-487 (soil)	EFS-488 (soil)	EFS-511 (sediment)
Geographic location			
City	Sanger	Louisville	Lawrence
State	California	Nebraska	Kansas
Country	USA	USA	USA
Latitude and longitude	N 36.70232 W 119.46355	N 41.03651 W 096.14983	39.0471331, -95.1965618
Soil taxonomic classification (USDA)	no information available		
Soil series	no information available		
Textural class (USDA)	sandy loam	silt loam	silty clay loam
Sand [%]	68.5	15.5	6.8
Silt [%]	28.4	63.0	63.5
Clay [%]	3.1	21.5	29.7
pH - in 0.01 M $CaCl_2$ (1:1)	6.2	6.5	7.5
- 1:1 soil:water ration	6.7	7.0	7.9
- in saturated paste	6.6	6.8	7.7
Organic carbon [% OC]	0.90	1.8	0.34
Organic matter [% OM]	1.5	3.2	0.58
Cation exchange capacity [meq/100 g]	6.7	16.6	20.1
Water holding capacity (gm/100 gm)	27.6	64.4	51.7
% moisture at 1/10 bar	23.4	38.6	44.1
% moisture at 2.0 pF units		38.6	
% moisture at 1/3 bar	10.9	27.8	34.9
% moisture at 2.5 pF units		27.8	
% moisture at 15 bar	4.1	17.7	15.7
Bulk density (disturbed) [gm/cc]	1.26	0.96	1.02

B. STUDY DESIGN

1. Experimental Conditions

The test system consisted of individually capped glass test vessels (50-mL glass test tubes with Teflon-lined caps) containing the soil and test solution. The soil-to-solution ratio was determined in preliminary experiments. During equilibration, the test system was continuously agitated (horizontally) using a mechanical device to keep the soil in suspension. All experiments were conducted in duplicate (unless otherwise noted) at a temperature of 20°C in the dark.

The test substance was dissolved in aqueous solution (0.01 M $CaCl_2$) at concentrations of 0.005, 0.015, 0.05, 0.15 and 0.5 µg/mL. All samples containing analytes (with or without soil) were studied in duplicate unless otherwise noted. Preliminary experiments were conducted to determine suitability of the test method and to establish test conditions.

The Freundlich coefficients, $K_{f(ads)}$ and $K_{f(des)}$, and the exponential constants, $1/n_{(ads)}$ and $1/n_{(des)}$, were calculated from adsorption and desorption isotherm experiments in each soil. K_f as a function of organic matter content ($K_{fom(ads)}$ and $K_{fom(des)}$) and organic carbon content ($K_{foc(ads)}$ and $K_{foc(des)}$) were also determined.

2. Analytical Procedures

Liquid scintillation counting analysis was performed on samples to quantify radioactivity. Mass balances of 79.3 to 102.9% were noted for EFS-488 soil and 93.4 to 111.2% for EFS-487 soil. Measurement was by the indirect method. No chemical mass balance is available.

Combustion of soil samples: Air-dried soil samples were combusted to determine ^{14}C residues remaining in soil after the relevant tests. Approximately 0.1 g sub-samples of each extracted soil pellet were weighed in triplicate into ceramic combustion boats and combusted for 2 minutes. The generated $^{14}CO_2$ was collected directly into Harvey™ scintillation cocktail and assayed by LSC.
The efficiency of the oxidizer was determined daily by combusting soil blanks and spiking the generated scintillation cocktail vials with a known volume of a ^{14}C-uracil standard (vial spikes). Aliquots of the same standard were then spiked onto soil replicates that were then combusted (soils spikes). The oxidizer efficiency was calculated by dividing the recovered radioactivity from the soil spikes by the mean radioactivity in the vial spikes. This correction factor was applied to each sample combusted on that instrument that day. Acceptable oxidizer recoveries were between 95 and 102%.

HPLC analyses were conducted on all samples and the radioactivity detected using an in-line radioactivity detector. Radioactivity was detected in the effluent using a liquid scintillation cell. The effluent was passed through the UV detector and then through the radioactivity flow detector. Radioactivity in the effluent was detected and quantified with a radioactive flow detector. Percentages of radioactivity in the separated components were quantified by integrating the peaks.

3. Calculations

Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

The material balance for each soil was determined on each sample at each test concentration. The material balance for each sample in the adsorption/desorption experiment was determined as the sum of the amount of radioactivity in the aqueous phase of the adsorption and desorption phases and the amount of radioactivity in the soil phase (sum of extract and post extraction solids combustion) divided by the amount of radioactivity applied to the sample. The average material balances for each test concentration ranged from 93.4 - 111.2% for the Sanger, CA (EFS-487) soil, 79.3 - 102.9% for the Louisville, NE (EFS-488) soil and 93.7 - 96.8% for the Lawrence, KS (EFS-511) sediment. The soil extracts, one replicate from each soil/sediment type from the highest test concentration following desorption, were analysed by HPLC. The radioactivity associated with the extract was 100% pyrazole-labelled isoflucypram. Since the values in soil contain both the extracted and post extracted solids (PES) values, a chemical mass balance in soil cannot be verified by the RMS for the soil component, as the chemical componentry of the PES cannot be verified. UK RMS notes that the OECD guideline states that chemical mass balance must be >90%, this cannot be achieved in the EFS-488 soil at 0.015, 0.05 and 0.15 µg/mL concentrations.

Table B.8.1.2.1.2- 5: Determination of the mass balance for adsorption/desorption experiments (as percentage of applied radioactivity, mean of two replicates)

Concentration [µg/mL]	Soil/sediment		
	EFS-487, Sanger, CA, sandy loam (soil)	EFS-488, Louisville, NE, silt loam (soil)	EFS-511, Lawrence, KS, silty clay loam (sediment)
0.005	107.4	102.9	96.8
0.015	111.2	89.8	94.7
0.05	97.7	89.0	94.3
0.15	101.3	79.3	93.7
0.5	93.4	93.3	94.0

B. ADSORPTION RESULTS

The percent of isoflucypram adsorbed during the adsorption cycle was calculated for both soils and one sediment at all five test solution concentrations. A summary of the average percent adsorbed on each soil can be found in the table below.

Table B.8.1.2.1.2- 6: Average percent adsorbed of isoflucypram on each soil/sediment

Soil	Percentage adsorbed (average[a)])
EFS-487, Sanger, CA, sandy loam (soil)	54.9
EFS-488, Louisville, NE, silt loam (soil)	79.3
EFS-511, Lawrence, KS, silty clay loam (sediment)	61.2

a) Calculations performed using the data from all concentrations 0.005-0.5 µg/mL

The values for the Freundlich adsorption and desorption isotherms, K_f, were derived from the linear form of the Freundlich equation.

A summary of the adsorption isotherms proposed by the applicant for each soil can be found in the table B.8.1.2.1.2-7. The values for the Freundlich adsorption isotherms, $K_{f(ads)}$, ranged from 12 mL/g in the EFS-511, Lawrence, KS, silty clay loam sediment to 25 mL/g in the EFS-488, Louisville, NE, silt loam soil. The Freundlich adsorption isotherms, $K_{f(ads)}$, were normalised for the organic matter and organic carbon content for each soil to calculate the soil sorption coefficients, $K_{fom(ads)}$ and $K_{foc(ads)}$. The $K_{fom(ads)}$ values ranged from 778 mL/g in the EFS-488, Louisville, NE, silt loam soil to 2107 mL/g in the EFS-511, Lawrence, KS, silty clay loam sediment while the $K_{foc(ads)}$ values ranged from 1,384 mL/g in the EFS-488, Louisville, NE, silt loam soil to 3,594 mL/g in the EFS-511, Lawrence, KS, silty clay loam sediment. UK RMS considers the results from EFS-488 soil not to be reliable due to poor mass balance and EFS-511 due to the selection of a sediment as opposed to a soil.

Table B.1.2.1.2-7: Adsorption constants of isoflucypram in soils proposed by the applicant and not accepted by the RMS

Soil	$K_{f(ads)}$ [mL/g]	$K_{fom(ads)}$ [mL/g]	$K_{oc(ads)}$ [mL/g]	1/n	r^2
EFS-487, Sanger, CA, sandy loam (soil)^	13	837	1394	1.0150	0.9881
EFS-488, Louisville, NE, silt loam (soil)*	25	778	1384	0.9101	0.9978
EFS-511, Lawrence, KS, silty clay loam (sediment)*	12	2107	3594	0.9387	0.9992
Arith. Mean*	16.7	1240.7	2124	0.9546	0.9950
Geo. Mean*			1907		

^soil accepted but not at all concentration, revised endpoints are presented below by RMS.

* soils and associated mean values are not accepted by the UK RMS, see conclusion section below.

C. DESORPTION RESULTS

The percent of isoflucypram desorbed from the soil was calculated for each soil/sediment at each of the five test solution concentrations. A summary of the averaged percent desorption's for each soil can be found in the table below. The average values ranged from 15.9% in the EFS-488, Louisville, NE, silt loam soil to 33.4% in the EFS-487, Sanger, CA, sandy loam soil.

Table B.8.1.2.1.2- 8: Average percent desorbed of isoflucypram on each soil/sediment

Soil	Percentage desorbed (average[a)])
EFS-487, Sanger, CA, sandy loam (soil)	33.4
EFS-488, Louisville, NE, silt loam (soil)	15.9
EFS-511, Lawrence, KS, silty clay loam (sediment)	30.9

a) Calculations performed using the data from all concentrations 0.005-0.5 µg/mL

A summary of the desorption isotherms for each soil can be found in the table below. The values for the Freundlich desorption isotherms, $K_{f(des)}$, ranged from 19 mL/g in the EFS-511, Lawrence, KS, silty clay loam sediment to 33 mL/g in the EFS-488, Louisville, NE, silt loam soil. The Freundlich desorption isotherms, $K_{f(des)}$, were corrected for the organic matter and organic carbon content for each soil to calculate the soil sorption coefficients, $K_{fom(des)}$ and $K_{foc(des)}$.

Table B.1.2.1.2- 9: Desorption constants of isoflucypram in soils

Soil	$K_{f(ads)}$ [mL/g]	$K_{fom(ads)}$ [mL/g]	$K_{oc(ads)}$ [mL/g]	1/n	r^2
EFS-487, Sanger, CA, sandy loam (soil)	23	1555	2591	1.0442	0.9942
EFS-488, Louisville, NE, silt loam (soil)	33	1018	1810	0.9116	0.9978
EFS-511, Lawrence, KS, silty clay loam (sediment)	19	3212	5479	0.9538	0.9993

III. CONCLUSIONS

The adsorption constants $K_{f(ads)}$ of isoflucypram for two test soils and one sediment determined by the applicant are calculated based on the Freundlich isotherms ranged from 12 to 25 mL/g (mean: 16.7 mL/g). The respective $K_{oc(ads)}$ values were in the range of 1384 to 3594 mL/g (mean: 2124 mL/g). UK RMS considers the results of the EFS-488 soil not to be reliable due to poor mass balance of 79.3% to 89.8% in 3 concentrations, with the remaining 2 concentrations insufficient to derive a Freundlich isotherm. EFS-511 sediment is not considered to be acceptable for E.U. assessment due to the OECD guideline stating soils only are suitable for use. The applicant has not identified a use where sorption to sediment would be critical.

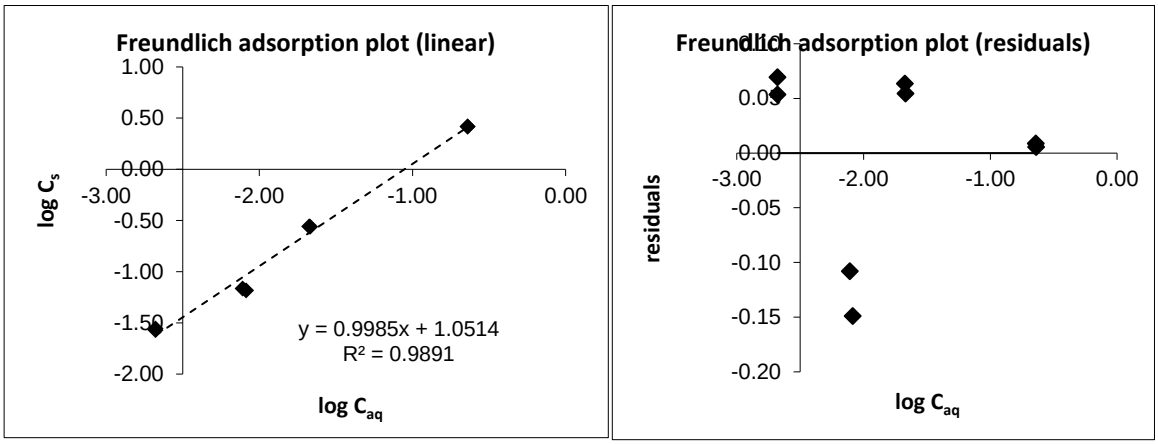
UK RMS has performed an evaluation using the EFSA OECD106 evaluators checklist for the 2 soils. LOD for the LSC counter is determined as 38 dpm and twice background for the HPLC analysis or 0.002µg/ml. HSE considers that the instruments were able to achieve accurate measurement at all concentrations. Mass balance has been determined for the adsorption and desorption stages, this also included the PES value. The percentage loss at the adsorption stage is not reported, therefore a Kfe/Kf calculation could not be determined as a chemical mass balance is not available. However mass balance outside the range accepted in the guidance was noted in all soils, with 3 concentrations in the EFS-488 soil (0.015, 0.05 and 0.150 µg/ml) being determined as unreliable (89.8, 89.0 and 79.3% respectively) and 1 concentration in the EFS-487 (0.015 µg/ml) showing a 111.2% recovery. No explanation is provided in the report for the highly variable mass balance.

UK RMS has recalculated the Kfoc for the 4 acceptable concentrations for the EFS-487 soil using the calculation tool supplied with the evaluators checklist. Results are supplied in table B.8.1.2.1.2-10, with acceptable r^2 the Kfoc value is considered to be acceptable. Kfoc values are in the same range at the soil with 5 concentrations, but the result is considered to be more conservative. RMS therefore proposes to use the Kfoc and 1/n from the 4 concentration analysis of the data.

Table B.8.1.2.1.2-10: Endpoints for isoflucypram adsorption in the EFS-487, Sanger, CA, sandy loam (soil) using the 4 concentrations where an acceptable mass balance was achieved.

Slope =	0.9985	**logK_F =**	1.0514
Intercept =	1.0514	**K_F =**	11.257
Correlation coefficient (r) =	0.9945	**K_{Foc} =**	1250.8
Coefficent of determination (r^2) =	0.9891	**1/n =**	0.9985

Figure B.8.1.2.1.2-1: Log graph and residual plots for the Sanger soil for the 4 acceptable concentrations.



B.8.1.2.2.1: Adsorption of isoflucypram metabolite, M12

Previous evaluation:	None, new active substance.

Author: D'Ambrosio, A.; 2014;
Title: [pyrazolyl-4-14C] BCS-CY26497: Adsorption/desorption in five different soils
Report No.: AS357
Document No.: M-499692-01-1
Guideline(s): OECD Guideline for Testing of Chemicals, No 106 "Adsorption/Desorption" -Using a Batch Equilibrium Method", Jan. 21, 2000
US EPA, Fate, Transport and Transformation Test Guidelines OPPTS 835.1230 Sediment and Soil Adsorption/Desorption Isotherm, January 1998
Guideline deviation(s): None
GLP/GEP: yes

Study Summary

The adsorption behaviour of M12 was studied in five soils in batch equilibrium experiments in the dark at 20.2°C: 3 E.U. soils and 2 U.S. soils, U.S. soils are considered to be appropriate for E.U. assessment by the UK RMS.

Table B.8.1.2.2.1- 1: Selected soils

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Wurmwiese	Monheim, Germany	sandy loam	5.3	1.9
Hoefchen am Hohenseh 4a	Burscheid, Germany	silt loam	6.3	2.0
Dollendorf II	Blankenheim, Germany	loam	7.3	4.5
Guadelupe	CA, USA	sandy loam	6.7	0.7
Springfield	NE, USA	silt loam	6.6	1.7

The adsorption phase of the study (definitive test) was carried out using pre-equilibrated air-dried soil with pyrazolyl-labelled M12 at concentrations of nominal 1.00, 0.30, 0.10, 0.03, and 0.01 mg/L in the dark at 20°C for 24 hours.
The equilibration solution used was 0.01 M aqueous $CaCl_2$ solution for all soils.

After the preliminary test I following soil to solution ratios were defined to the soils: Wurmwiese, Höfchen am Hohenseh, Dollendorf II and Springfield, NE 1:2 (10g soil 20 mL $CaCl_2$) and Guadalupe, CA 1:1 (20 g soil and 20 mL CaCl2).
Desorption phase of the study was carried out by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle for 24 hours.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and the M12 residues in the supernatant were analysed by liquid scintillation counting (LSC). The adsorption/desorption parameters were calculated using the Freundlich adsorption isotherm.

Test systems without soil were used as control in preliminary test and did not show adsorption to the vessels or degradation.

For all soils the parental mass balance after 72 h showed that > 90% of applied pyrazolyl-labelled M12 could be recovered. This demonstrates that the test item was sufficient stable for the test in these soils.

The mass balance in the definitive test of the soils was determined by LSC of the supernatants after adsorption/desorption and by combustion of the remaining soils. The overall material balance for all concentrations for individual specimens was in the range of 91.3 - 101.8%, 95.9 - 103.3%, 96.5 - 103.2%, 99.1 - 111.2% and 93.0 - 100.9% of the applied radioactivity in soils Wurmwiese, Höfchen am Hohenseh 4a, Dollendorf II, Guadalupe, CA and Springfield, NE, respectively.

In the definitive adsorption test 48.9 - 58.4%, 26.0 - 38.3%, 37.3 - 47.0%, 19.3 - 6.8%, and 35.5 - 46.5% of the applied test material was adsorbed in soils Wurmwiese, Höfchen am Hohenseh 4a, Dollendorf II, Guadalupe, CA, Springfield, NE, respectively.
The calculated adsorption constants $K_{f(ads)}$ of the Freundlich isotherms for the five test soils ranged from 0.3 mL/g to 2.0 mL/g. The Freundlich exponents 1/n were in the range of 0.8952 to 0.9311, indicating that the concentration of the test item did affect the adsorption behaviour.

At the end of one adsorption and one desorption phase, 24.7 - 34.2%, 34.2 - 47.2%, 27.7 - 36.5%, 43.0 - 53.7% and 29.9 - 43.7% of the initially adsorbed amount were desorbed in soils Wurmwiese, Höfchen am Hohenseh 4a, Dollendorf II, Guadalupe, CA, Springfield, NE, respectively.

The mean desorption $K_{f(des)}$ ranged from 0.2 - 2.2 mL/g and and the normalised $K_{oc(des)}$ ranged from 34.5 – 116.3 mL/g.

The following table summarises the key soil properties and results from the study proposed by the applicant:

Table B.8.1.2.2.1- 2: Summary of the adsorption data of M12

Soil	Texture (USDA)	pH ($CaCl_2$)	OC [%]	$K_{f(ads)}$ [mL/g]	1/n	$K_{oc(ads)}$ [mL/g]
Wurmwiese	sandy loam	5.3	1.9	2.0	0.9297	105.8
Hoefchen am Hohenseh 4a	silt loam	6.3	2.0	0.8	0.8952	37.9
Dollendorf II	loam	7.3	4.5	1.3	0.9243	28.1
Guadelupe	sandy loam	6.7	0.7	0.3	0.9311	38.4
Springfield	silt loam	6.6	1.7	1.2	0.9185	70.7
Arith. mean				1.1	0.9198	56.2

According to Briggs[1] depending on the soil type the mobility of M12 can be classified as mobile to intermediate mobile in the tested soils.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazolyl-labelled BCS-CN88460-carboxylic acid *(M12)*
Sample-ID: KML 9692
Specific activity: 3.92 MBq/mg (105.97 µCi/mg)
Radiochemical purity: > 99% by TLC

Reference item

Non-labelled BCS-CN88460-carboxylic acid *(M12)*
Batch code: BCS-CY26497-01-01
Chemical purity: 97.0%

[1] Briggs, G. G. (1973) A Simple Relationship Between Soil Adsorption of Organic Chemicals and their Octanol/Water Partition Coefficients *Proc. 7th British Insecticide and Fungicide Conference, Nottingham/UK.*

2. Test Soils

Five test soils (three of European origin, two of USA origin) were used within this study, chosen to cover a representative range in soil physico-chemical properties. The physico-chemical properties of the test soils are given in the following table:

Table B.8.1.2.2.1- 3: Physico-chemical properties of test soils

Parameter	Results		
Soil designation	Wurmwiese	Hoefchen am Hohenseh 4a	Dollendorf II
Geographic location City State Country	 Monheim North Rhine-Westphalia Germany	 Burscheid North Rhine-Westphalia Germany	 Blankenheim North Rhine-Westphalia Germany
Latitude and longitude	N 51° 04.857' E 06° 55.251'	N 51° 04.014' E 07° 06.324'	N 50° 22.899' E 06° 43.001'
Soil taxonomic classification (USDA)	no information available		
Soil series	no information available		
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	sandy loam 55 29 16	silt loam 21 65 14	loam 39 35 26
pH - in $CaCl_2$ - in water	5.3 5.6	6.3 6.6	7.3 7.5
Organic carbon (combustion) [% OC] Organic matter [% OM]	1.9 3.27	2.0 3.44	4.5 7.74
Cation exchange capacity [meq/100 g]	9.9	11.0	19.8
Soil designation	Guadalupe CA	Springfield NE	
Geographic location City State Country	 Guadalupe California USA	 Springfield Nebraska USA	
Latitude and longitude	N 35° 01' 05.6'' W 120° 36' 10.1''	N 96.15085 W 41.03725	
Soil taxonomic classification (USDA)	no information available		
Soil series	no information available		
Textural class (USDA) Sand [%] 56 Silt [%] 32.6 Clay [%] 11.4	sandy loam 12.7 60.8 26.5	silt loam 12.7 60.8 26.5	
pH - in $CaCl_2$ - in water	6.7 6.8	6.6 7.2	
Organic carbon (combustion) [% OC] Organic matter [% OM]	0.7 1.1	1.7 2.9	
Cation exchange capacity [meq/100 g]	16.1	16.1	

B. STUDY DESIGN

1. Experimental Conditions

Important parameters for the test e.g. stability of the test item, soil-to-solution ratio and equilibration time for adsorption were determined prior to the definitive test in preliminary tests.

In the definitive test soil/solution ratios of 1:2 for the soils Wurmwiese, Höfchen am Hohenseh 4a, Dollendorf II and Springfield, NE and 1:1 for the soil Guadalupe, CA and equilibration time (24 hours) established for each soil in the preliminary tests were used.

Borosilicate glass centrifuge tubes (42 or 83 mL) with Teflon®, lined screw top lids were used as test vessels. They were shaken by an overhead shaker in the dark at controlled temperature (20°C).

Adsorption/desorption tests were conducted at nominal concentrations of 1.00, 0.30 0.10, 0.03 and 0.01 µg/mL. For the preparation of the application solution it was taken into account that the final concentration of the test item was decreased by a factor of 10 after application into the equilibrated test system.
In the definitive test 10 g for the soils Wurmwiese, Höfchen am Hohenseh 4a, Dollendorf II and Springfield, NE and 20 g for the soil Guadalupe, CA were weighed into centrifuge tubes, and 18 mL of aqueous 0.01 M $CaCl_2$ stock solution were added. After pre-equilibration for $\geq$ 16 hours, 2 mL of the respective application solution were spiked in.

Following the indirect method, the batches were equilibrated. Following the determined shaking period of 24 hours, the test vessels were centrifuged and the supernatant was completely decanted. The volumes were measured gravimetrically (density of the solution was set equivalent to 1 g/mL) and recorded, and aliquots of 2 x 1 mL from all soils were taken for LSC of the supernatant. The pH was measured in all supernatants.
One single point desorption was performed on all concentrations. The volume of solution removed was replaced by an equal volume of Stock Solution. The test vessels were then shaken for the predetermined period of 24 hours for single point desorption and handled as described in the previous section. The pH was measured in the test vessels containing 1mg/L.
After the desorption cycle the soils were mixed with approximately 0.4 g cellulose / g soil, air-dried, homogenised and aliquots were combusted. Mass balance was established on all specimens from the definitive tests.

2. Analytical Methodology
Radioactivity determinations: The liquid specimens were measured with a liquid scintillation counter.
Soil specimens were mixed with approximately 0.4 g cellulose/g soil, air dried, homogenised, and combusted in a Sample Oxidiser. Radioactivity was measured with a liquid scintillation counter.
The distribution of the radioactive regions of interest was measured with a Radio-HPLC-detector and quantified via manual evaluation of the area integrals and an evaluation program.

3. Calculations
Adsorption and desorption isotherms were calculated by linear regression analysis of the adsorption or desorption data according to the Freundlich equation.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE
The recovery of radioactivity for all soils is presented in Table B.8.1.2.2.1- 4.
The radioactive material balance in the test soils was calculated as sum of the radioactivity detected within the decanted supernatant solutions after the adsorption / desorption step and the radioactivity found in the air-dried and combusted soil residues. The total radioactivity recovery with respect to the individual vessel ranged from 91.3% to 111.2% of the applied radioactivity.
The complete material balance observed for all test systems therefore demonstrated that no significant amount of radioactivity dissipated from the test vessels or was lost upon processing.

Table B.8.1.2.2.1- 4: Recovery of radioactivity after adsorption, desorption and extraction (as percentage of applied radioactivity, mean of two replicates)

Concentration [mg/L]	Soil				
	Wurmwiese	Höfchen am Hohenseh 4a	Dollendorf II	Guadalupe	Springfield
0.97	99.25	103.5	103.15	103.65	100.85
0.30	93.05	97.2	97.65	99.2	94,15
0.10	94.7	96.85	97.2	99.3	94.25
0.03	91.95	96.0	97.2	100.25	95.65
0.01	99.5	97.75	99.5	107.05	100.3
Mean[a)]	95.96	98.17	98.94	101.89	97.04

B. ADSORPTION RESULTS

In the definitive adsorption test 48.9 – 58.4% AR were adsorbed in soil Wurmwiese, 26.0 – 38.3% AR in soil Hoefchen am Hohenseh 4a, 37.3 – 47.0% AR in soil Dollendorf II, 19.3 – 26.8% in soil Guadalupe and 35.5 – 46.6% AR in soil Springfield. The respective concentrations in solution and in soil and the percentage of adsorbed test item are summarised in Table B.8.1.2.2.1- 5.

Table B.8.1.2.2.1- 5: Concentration of M12 in the solid and liquid phases at the end of adsorption period (mean of duplicates)

Concentration [µg/mL]	Soil [µg/g]	Solution [µg/mL]	Percentage adsorbed mean SD
Wurmwiese			
0.010	0.011	0.004	57.7 ± 0.5
0.029	0.034	0.012	58.1 ± 0.4
0.098	0.113	0.042	57.0 ± 0.1
0.293	0.326	0.132	55.1 ± 0.1
0.962	0.953	0.491	49.0 ± 0.1
Hoefchen am Hohenseh 4a			
0.010	0.007	0.006	37.5 ± 1.1
0.029	0.021	0.018	36.5 ± 0.5
0.098	0.069	0.064	34.8 ± 0.0
0.292	0.192	0.197	32.5 ± 0.5
0.961	0.506	0.711	26.0 ± 0.0
Dollendorf II			
0.010	0.009	0.005	46.7 ± 0.3
0.029	0.027	0.015	46.2 ± 0.9
0.097	0.089	0.054	44.8 ± 0.3
0.290	0.255	0.165	43.1 ± 0.3
0.953	0.728	0.596	37.4 ± 0.1
Guadalupe			
0.010	0.003	0.007	25.6 ± 0.1
0.029	0.008	0.021	26.3 ± 0.7
0.098	0.025	0.073	25.3 ± 0.5
0.292	0.072	0.220	24.5 ± 0.4
0.960	0.190	0.773	19.5 ± 0.3
Springfield			
0.010	0.009	0.005	46.0 ± 0.8
0.029	0.027	0.016	45.7 ± 0.2
0.098	0.088	0.054	43.9 ± 0.3
0.290	0.251	0.167	42.4 ± 0.1
0.954	0.700	0.611	36.0 ± 0.7

The adsorption behaviour of M12 in the concentration range of two orders of magnitude (i.e. from 0.01 to 1.0 µg/mL) was accurately described for all soils with the Freundlich equation. The adsorption behaviour was accurately described by the Freundlich equation for all test soils, reflected in correlation coefficients of fit of calculated adsorption isotherms to the respective measured data close to one (0.9973 - 0.9988).

The calculated adsorption constants $K_{f(ads)}$ of the Freundlich isotherms for the five test soils ranged from 0.3 to 2.0 mL/g (mean: 1.1 mL/g). The Freundlich exponents 1/n were in the range of 0.8952 to 0.9311 (mean: 0.9198).

In general the organic matter in soil, represented as organic carbon content, is the most important binding site for xenobiotics. Therefore, the Freundlich adsorption coefficients (K_{fads}) were normalised for the percentage of organic carbon content of the test soils to obtain Freundlich K_{ocads} values as a general comparability basis of the test item adsorption behaviour. For the test item the calculated K_{ocads} values in the five soils varied between 28.1 and 105.8 mL/g (mean: 56.2 mL/g).

An overview of the results according to the Freundlich equation is presented in Table B.8.1.2.1- 6.

Table B.8.1.2.2.1- 6: Adsorption constants of M12 in soils

Soil	$K_{f(ads)}$ [mL/g]	1/n	$K_{oc(ads)}$ [mL/g]	r^2
Wurmwiese	2.0	0.9297	105.8	0.9983
Hoefchen am Hohenseh 4a	0.8	0.8952	37.9	0.9978
Dollendorf II	1.3	0.9243	28.1	0.9988
Guadelupe	0.3	0.9311	38.4	0.9973
Springfield	1.2	0.9185	70.7	0.9983
Arith. mean	**1.1**	**0.9198**	**56.2**	**0.9981**
Geo. mean			**49.79**	

C. DESORPTION RESULTS

Evaluations of the desorption experiments performed for all soils at five test concentrations are given in the following table:

Table B.8.1.2.2.1- 7: Concentration of M12 in the solid and liquid phases at the end of desorption period (mean of duplicates)

Concentration [mg/L]	Soil [mg/kg]	Solution [mg/L]	Percentage adsorbed mean SD
Wurmwiese			
0.010	0.008	0.003	26.6 ± 1.0
0.029	0.025	0.008	25.5 ± 1.1
0.098	0.083	0.027	26.9 ± 0.3
0.293	0.235	0.080	27.9 ± 0.1
0.962	0.632	0.288	33.7 ± 0.7
Hoefchen am Hohenseh 4a			
0.010	0.005	0.003	34.6 ± 0.6
0.029	0.014	0.009	35.4 ± 0.8
0.098	0.044	0.030	36.0 ± 0.2
0.292	0.120	0.092	37.4 ± 1.0
0.961	0.268	0.320	47.1 ± 0.2
Dollendorf II			
0.010	0.007	0.003	28.0 ± 0.4
0.029	0.019	0.009	28.2 ± 0.8
0.097	0.062	0.032	30.1 ± 0.2
0.290	0.176	0.095	30.8 ± 0.2
0.953	0.463	0.335	36.4 ± 0.0
Guadalupe			
0.010	0.001	0.004	43.6 ± 0.7
0.029	0.004	0.011	43.8 ± 0.3
0.098	0.014	0.036	44.5 ± 0.3
0.292	0.039	0.107	45.5 ± 1.0
0.960	0.088	0.396	53.4 ± 0.5
Springfield			
0.010	0.006	0.003	34.4 ± 1.9
0.029	0.018	0.008	31.8 ± 0.9
0.098	0.058	0.029	32.1 ± 0.0
0.290	0.163	0.087	34.9 ± 1.1
0.954	0.399	0.305	43.1 ± 0.8

Desorption isotherms for desorption were calculated in analogy to the adsorption experiment. Correlation coefficients for desorption were in the range of 0.9920 –0.9980.
The Freundlich desorption coefficients K_{fdes} ranged from 0.2 mL/g (soil Guadalupe, CA) to 2.2 mL/g (soil Wurmwiese) with Freundlich exponents (1/n) ranging from 0.8632 to 0.9225. Normalisation to the soil organic carbon contents led to the following K_{focdes} values of 116.3 mL/g for soil Wurmwiese, 41.4 mL/g for soil Höfchen am Hohenseh 4a, 30.6 mL/g for soil Dollendorf II, 34.5 mL/g for soil Guadalupe, CA and 78.3 mL/g for soil Springfield, NE, respectively.
An overview of the results is presented in Table B.8.1.2.2.1- 8.

Table B.8.1.2.2.1- 5: Desorption constants of M12 in soils

Soil	**$K_{f(des)}$ [mL/g]**	**1/n**	**$K_{oc(des)}$ [mL/g]**	**r^2**
Wurmwiese	2.2093	0.9225	116.3	0.9975
Hoefchen am Hohenseh 4a	0.8286	0.8632	41.4	0.9948
Dollendorf II	1.3750	0.9101	30.6	0.9980
Guadelupe	0.2417	0.8894	34.5	0.9920
Springfield	1.3306	0.9055	78.3	0.9957
Arith. mean	**1.1970**	**0.8981**	**60.2**	**0.9956**

III. CONCLUSIONS

UK RMS notes for the definitive test the concentration in soil is not reported at the adsorption step. The weight of soil pellet plus remaining supernatant is not reported however this will not have a significant impact on the Koc (ads). Mass balance for the definitive step is reported as the mass in the adsorption supernatant plus the mass in the desorption supernatant plus the mass in soil following combustion. This is not considered to greatly affect the result as the PES during the prelim test was negligible for all soils (0.80 to 4.12% AR), with parental mass balance >90% AR in all soils.

The adsorption coefficients K_{fads} of M12 in five test soils were determined by the applicant to range from 0.3 mL/g to 2.0 mL/g based on Freundlich equation. The corresponding organic carbon normalised adsorption coefficients K_{focads} ranged from 28.1 mL/g to 105.8 mL/g (mean 56.2 mL/g). The Freundlich exponents 1/n were in the range of 0.8952 to 0.9311 indicating that the concentration of the test item affected the adsorption behaviour slightly, only.

The desorption coefficients K_{focdes} of M12 were found to be in the same range as the respective adsorption coefficients (30.6 mL/g – 116.3 mL/g).

UK RMS has validated the study using the OECD 106 evaluators checklist, with the supplied spreadsheet. The concentration of the adsorption supernatant in µg is reported for all soils, UK RMS has converted this value to µg/mL details are included in table B.8.1.2.2.1-10, using the volume of CaCl2 added + entrained water in the air dried soils detailed in table B.8.1.2.2.1-9. The LOD and LOQ of the instruments used were sufficient with all peaks over 50 cpm integrated and for LSC measurement against the quench curve was performed. Kd x Soil solution ratio ranged from 0.36 to 1.49 so is acceptable for all soils at all concentrations. Analysis of Kfe/Kf is not possible as soil extractions were not performed for the highest concentration, but soils were combusted directly therefore contained both extractable and non-extractable compounds. Results of the RMS checks are similar to those of the applicant and are supplied in table B.8.1.2.2.1-10, therefore the applicant proposed values are accepted for the generation of endpoints. UK RMS has provided fit graphs in figures B.8.1.2.2.1-1 to B.8.1.2.2.1-5.

B.8.1.2.2.1-9: Volumes of water used.

Soil	Volume of added water + water remaining in the soil (mL)
Wurmwiese	20.21
Hoefchen am Hohenseh 4a	20.24
Dollendorf II	20.41
Guadelupe	20.26
Springfield	20.38

Table B.8.1.2.2.1-10: Adsorption supernatant concentration results for metabolite M12 converted into µg/mL by RMS

Soil	Initial concentration of test solution (µg/mL)	Concentration of adsorption supernatant (µg/mL)
Wurmwiese	0.970	0.4917
	0.970	0.4905
	0.300	0.1318
	0.300	0.1313

	0.100	0.0424
	0.100	0.0423
	0.030	0.0122
	0.030	0.012
	0.010	0.0041
	0.010	0.0041
Hoefchen am Hohenseh 4a	0.970	0.7125
	0.970	0.7121
	0.300	0.1965
	0.300	0.1984
	0.100	0.0641
	0.100	0.0641
	0.030	0.0185
	0.030	0.0183
	0.010	0.006
	0.010	0.0061
Dollendorf II	0.970	0.5975
	0.970	0.5959
	0.300	0.1644
	0.300	0.1655
	0.100	0.054
	0.100	0.0536
	0.030	0.0156
	0.030	0.0152
	0.010	0.0051
	0.010	0.0051
Guadelupe	0.970	0.7707
	0.970	0.7749
	0.300	0.2214
	0.300	0.2195
	0.100	0.0735
	0.100	0.0729
	0.030	0.0215
	0.030	0.0212
	0.010	0.0072
	0.010	0.0072
Springfield	0.970	0.6151
	0.970	0.6052
	0.300	0.167
	0.300	0.1668
	0.100	0.0544
	0.100	0.0549
	0.030	0.0156
	0.030	0.0155

	0.010	0.0052
	0.010	0.0051

Based on the soil sorption parameters measured in this study and classification of soil mobility potential according to Briggs, depending on the soil type the mobility of M12 can be classified as mobile to intermediate mobile in the tested soils.

Table B.8.1.2.2.1-11: RMS soil adsorption results for metabolite M12 following analysis using the evaluators checklist, values are not relied upon for the risk assessment.

Soil	Organic carbon (%)	pH	KF, ads	KFoc, ads	1/n	r2
Wurmwiese	1.9	5.6	2.038	107.242	0.921	0.998
Hoefchen am Hohenseh 4a	2	6.6	0.782	39.102	0.884	0.997
Dollendorf II	4.5	7.5	1.323	29.400	0.915	0.998
Guadelupe	0.7	6.8	0.285	40.659	0.914	0.996
Springfield	1.7	7.2	1.259	74.063	0.910	0.998
Mean		-	1.137	58.093	0.909	-
Geomean		-	0.945	51.753	0.909	-

Figure B.8.1.2.2.1-1: Log plot graph and residuals graph for Wurmwiese soil

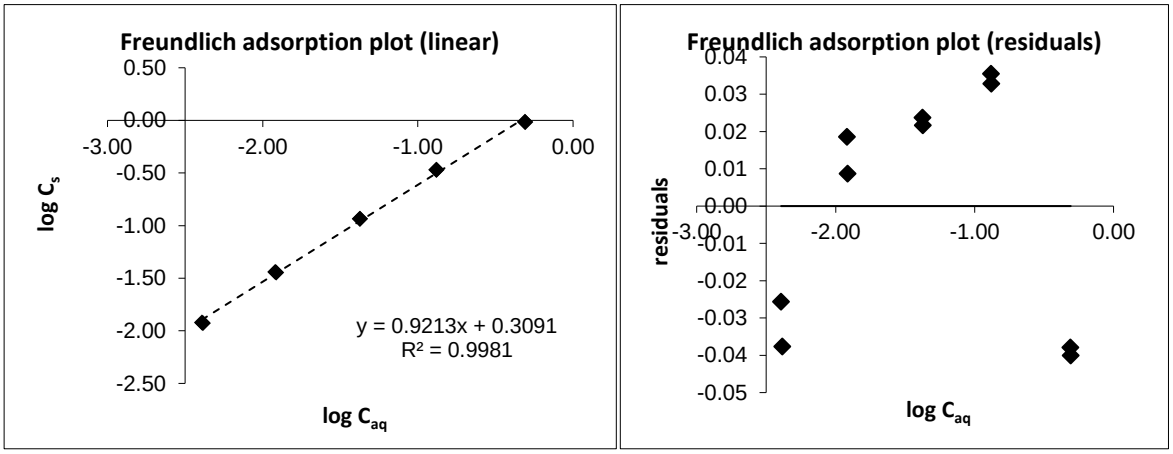


Figure B.8.1.2.2.1-2: Log plot graph and residuals graph for Hoefchen am Hohenseh 4a soil

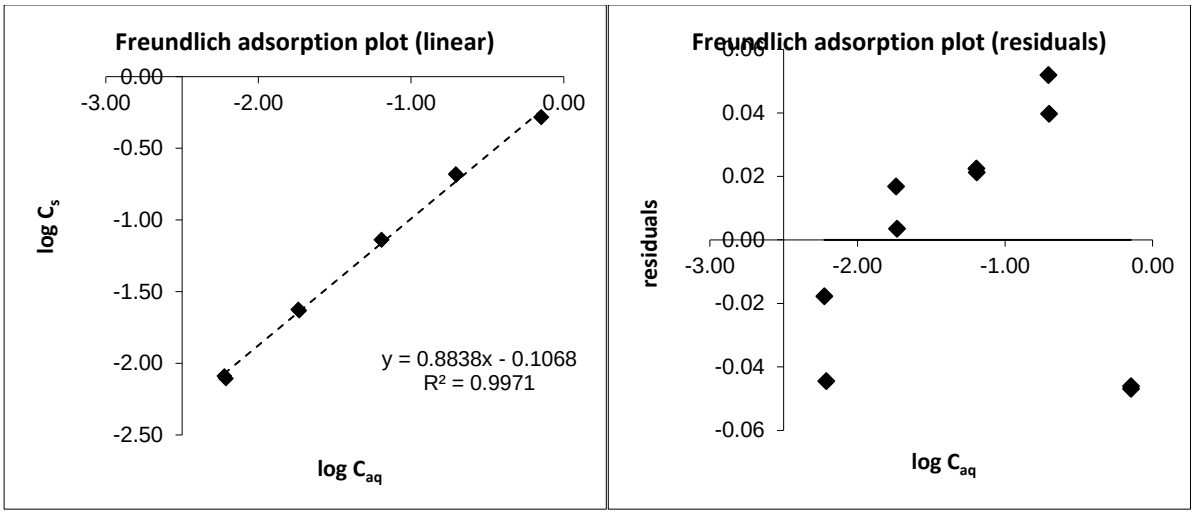


Figure B.8.1.2.2.1-3: Log plot graph and residuals graph for Dollendorf II soil

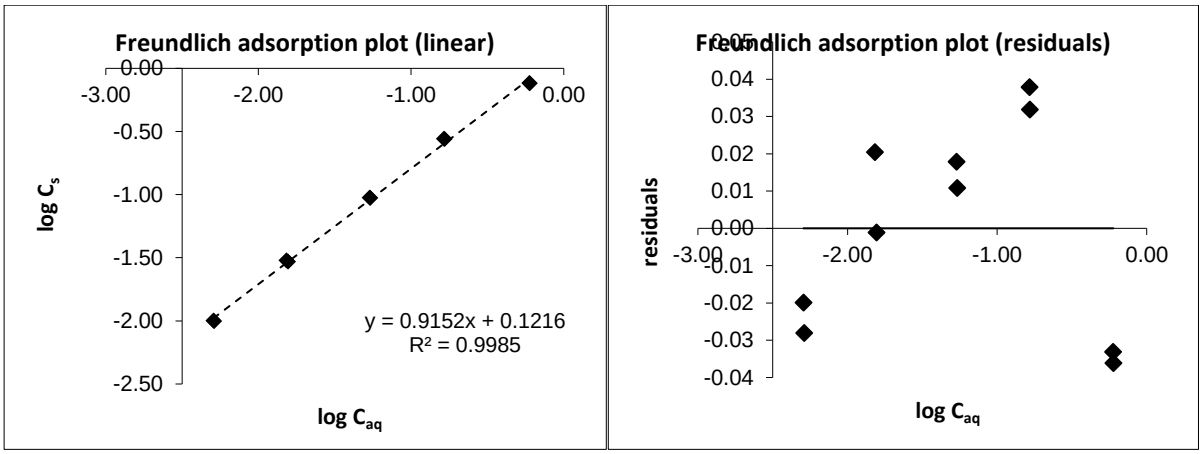


Figure B.8.1.2.2.1-4: Log plot graph and residuals graph for Guadelupe soil

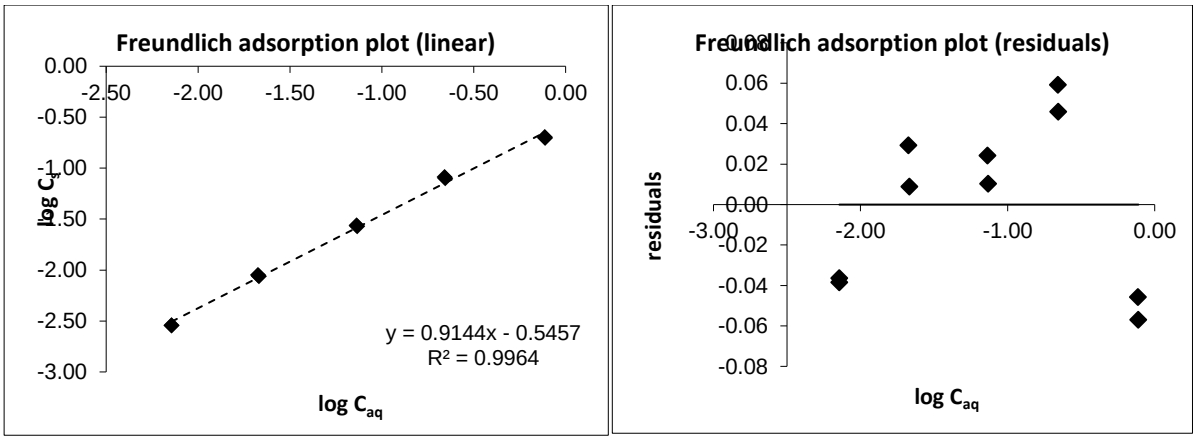
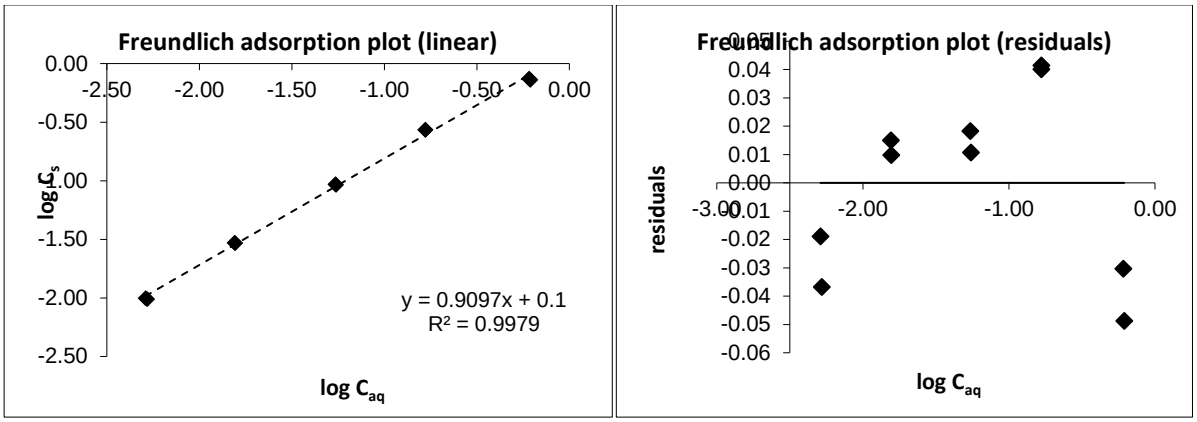


Figure B.8.1.2.2.1-5: Log plot graph and residuals graph for Springfield soil



M12 may show some pH dependency with regard to adsorption. A full assessment using all adsorption data for M12 is provided in the summary section B.8.1.3.2.5 below.

B.8.1.2.2.2: Adsorption of isoflucypram metabolite, M12

Previous evaluation:	None, new active substance.

Author: Shrestha, S.; 2017;
Title: [Pyrazolyl-4-14C]BCS-CY26497: Adsorption/desorption in four US soils
Report No.: MELNN219
Document No.: M-589856-01-1
Guideline(s): OECD Guideline for the Testing of Chemicals, No. 106, Adsorption/Desorption, 2000
Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Guideline deviation(s): None
GLP/GEP: yes

Study Summary

The adsorption behaviour of M12 was studied in four US agricultural soils in batch equilibrium experiments in the dark at 20°C: UK RMS considers the selected soils are acceptable but notes the %OC in the SCA soil is just below the 0.3% organic carbon stated in table 1 of the OECD guidance 106.

Table B.8.1.2.2.2- 1: Selected soils

Designation	Location	Texture (USDA)	pH ($CaCl_2$)	OC [%]
END	Northwood, North Dakota, USA	loamy sand	4.9	0.94
MMN	Morris, Minnesota, USA	clay loam	7.7	2.4
SCA	Sanger, California, USA	sandy loam	5.6	0.29
SKS	Stilwell, Kansas, USA	silty clay loam	5.8	1.8

The adsorption phase of the study was carried out using sieved (≤ 2 mm) and air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution. Preliminary tests were conducted for solubility of the test substance, adsorption to the test vessels, appropriate soil-to-solution ratio, equilibrium time, and stability of the test substance. Control test systems containing only 0.01 M $CaCl_2$ were used in the test for solubility and adsorption of the test substance to the test vessels. In the pre-test for parental mass balance, Northwood, North Dakota (END), Morris, Minnesota (MMN), Sanger, California (SCA), and Stilwell, Kansas (SKS) test systems had 94.4, 95.0, 94.9 and 93.9% of applied radioactivity (AR) as the test substance, respectively, at the end of a 48-h period of shaking.

For the definitive test, a soil-to-solution ratio of 1:4 for END soil (5g soil plus 20 mL $CaCl_2$), 1:2 for MMN soil (10 g soil plus 20 mL $CaCl_2$), 1:1 for SCA soil (10 g soil plus 10 mL $CaCl_2$), and 1:2 for SKS (10 g soil plus 20 mL $CaCl_2$) soil were used. The nominal test concentrations of M12 ranged over two orders of magnitude and were 1.0, 0.3, 0.1, 0.03, 0.01 mg/L. All applications of the test substance were made in aqueous 0.01 M CaCl2 solution. The tests were conducted in 30-mL Teflon® centrifuge tubes with screw caps, in an environmental chamber in the dark at 20 ± 2 °C on a reciprocal shaker.

The aqueous supernatant after adsorption and desorption was separated by centrifugation, and the supernatant was radioassayed. Supernatants from representative samples were analysed by HPLC to determine the composition of radioactive residues. Residues in the soils were determined by extraction followed by combustion and radioassay. The adsorption/desorption parameters were calculated using Freundlich adsorption/desorption isotherms. The test substance was sufficiently stable throughout the 48-h study period, with 100% test substance noted in HPLC analyses of adsorption supernatants and soil extracts.

Mean material balances for END, MMN, SCA, and SKS soils were 98.5% AR (range 96.2 to 101.9% AR), 97.8% AR (range 96.2 to 100.2% AR), 97.9% AR (range 95.4 to 106.5% AR), and 98.6% AR (range 95.0 to 102.6% AR), respectively. The overall mean material balance was 98.2% (SD = 2.3%).

In the definitive adsorption test, the mean % AR adsorbed to soil ranged from 40.4 to 46.7% in END soil, 36.1 to 41.3% in MMN soil, 35.8 to 48.0% in SCA soil, and 47.1 to 60.3% in SKS soil.

In the definitive desorption test, the mean % AR desorbed from the initially adsorbed amount ranged from 40.4 to 47.4% in END soil, 31.4 to 36.4% in MMN soil, 29.2 to 41.5% in SCA soil, and 22.4 to 33.5% in SKS soil.

The calculated adsorption constants $K_{f\text{-}ads}$ of the Freundlich isotherms ranged from 0.544 to 2.72 mL/g (mean 1.54 mL/g) for the four tested soils. The Freundlich exponents (1/n) were in the range of 0.8914 to 0.9604 (mean 0.9245), indicating that the concentration of the test substance minimally affected the adsorption behaviour of the test substance in the examined concentration range.

In general, the organic matter in soil, determined as organic carbon content, is the most important component responsible for binding organic chemicals. Therefore the adsorption coefficients (K_f) were correlated with the organic carbon content of the soils to compare the adsorption behavior in different soils. For M12 the $K_{foc\text{-}ads}$ values ranged from 49.1 to 289.8 mL/g (mean 155.6 mL/g). According to Briggs[1] classification scheme, the mobility of M12 can be classified as 'Low' for two soils (END and SCA), 'Intermediate' for one soil (SKS) and 'Mobile' for one soil (MMN).

[1] Briggs, G. G. (1973)
A Simple Relationship Between Soil Adsorption of Organic Chemicals and their Octanol/Water Partition Coefficients
Proc. 7th British Insecticide and Fungicide Conference, Nottingham/UK.

The calculated desorption constants $K_{f\text{-}des}$ of the Freundlich isotherms ranged from 1.322 to 4.870 mL/g (mean: 3.510 mL/g) for the tested matrices. The Freundlich exponents 1/n ranged from 0.8972 to 1.0434 (mean: 0.9450). The $K_{foc\text{-}des}$ values for desorption ranged from 201.1 to 455.8 mL/g (mean 327.4 mL/g).

The following table summarises the adsorption and desorption data of M12 proposed by the applicant:

Table B.8.1.2.2.2- 2: Summary of the adsorption/desorption data of M12

Soil texture (USDA)	Adsorption			Desorption		
	$K_{f\text{-}ads}$ [mL/g]	1/n	$K_{foc\text{-}ads}$ [mL/g]	$K_{f\text{-}des}$ [mL/g]	$1/n_{des}$	$K_{foc\text{-}des}$ [mL/g]
(END) loamy sand	2.724	0.9497	289.8	4.230	0.9403	450.0
(MMN) clay loam	1.178	0.9604	49.1	4.870	1.0434	202.9
(SCA) sandy loam	0.544	0.8966	187.5	1.322	0.8990	455.8
(SKS) silty clay loam	1.727	0.8914	95.9	3.619	0.8972	201.1
Arith. mean:	1.543	0.9245	155.6	3.510	0.9450	327.4

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazolyl-labelled BCS-CN88460-carboxylic acid *(M12)*
Standard-ID: C-1202
Specific activity: 3.73 MBq/mg (100.8 µCi/mg)
Radiochemical purity: 100% by HPLC

Reference item
Reference substances were not used

2. Test Soils

Four test soils (USA origin) were used within this study. The soils were taken from agricultural use areas representing different geographical regions and different soil properties. The physico-chemical properties of the test soils are given in the following table:

Table B.8.1.2.2.2- 3: Physico-chemical properties of test soils

Parameter	Soils			
Soil designation	Northwood, ND	Morris, MN	Sanger, CA	Stilwell, KS
System ID/ Soil ID	END 050216-S	MMN 100215-S	SCA 032615-S	SKS 122613-S
Geographic location City State Country	 Northwood North Dakota USA	 Morris Minesota USA	 Sanger California USA	 Stilwell KS USA
Soil coordinates	N 47.70093 W 97.51697	N 45.58333 W 95.86667	N 36.70227 W 119.46355	N 38.81528 W 94.66111
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	loamy sand 83.8 4.3 11.9	clay loam 31.6 39.8 28.6	sandy loam 67.1 27.2 5.7	silty clay loam 4.7 60.4 34.9
pH - in $CaCl_2$ - in water - in saturated paste	4.9 5.3 5.2	7.7 8.1 7.9	5.6 6.2 6.2	5.8 6.2 6.0
Organic carbon (combustion) [% OC] Organic matter [% OM][b)]	0.94 1.6	2.4 4.1	0.29 0.51	1.8 3.1
Cation exchange capacity [meq/100 g][a)]	10.4	19.2	4.9	18.5
Bulk density [g/cm^3]	1.18	0.97	1.23	0.97
Max. water holding capacity [gm/100 g]	39.3	N/A	24.7	35.2
Moisture at 1/10 bar (pF 2.0) [%]	18.6	30.9	14.8	30.6
Moisture at 1/3 bar (pF 2.5) [%]	9.3	23.9	11.3	24.0

N/A = not available

a) Gravimetric moisture content (g water per 100 g dry soil)

b) % Organic matter = % Organic carbon x 1.724

B. STUDY DESIGN

1. Experimental Conditions

Preliminary tests were performed to determine solubility, adsorption to test vessel, stability, and equilibration time prior to the definitive test in order to optimise the test conditions.

The definitive test was performed in duplicate with five test substance concentrations (nominal 0.01 to 1.0 mg/L). A soil-to-solution ratio of 1:4 was used for END soil, 1:2 was used for MMN soil, 1:1 was used for SCA soil, and 1:2 was used for SKS soil. The equilibration time was 24 hours for adsorption and was followed by a 24 hour desorption phase.

For the adsorption phase the test systems were set up with the appropriate amounts of soil and 0.01 M $CaCl_2$, and were pre-equilibrated by shaking overnight before treatment. A 1-mL aliquot of each application solution was added to the SCA test systems and a 2-mL aliquot was added to the END, MMN and SKS test systems. After application, the test systems were shaken for approximately 24 hours. Test systems were removed, soil and supernatants were separated by centrifugation, and the supernatants were decanted. The volumes of the supernatants were determined by weight and aliquots were taken for radioassay. The supernatants were replaced by approximately the same weight of fresh 0.01 M $CaCl_2$ solution, and the test systems were placed on the reciprocal shaker. The pH of adsorption supernatants

were measured. One replicate of adsorption supernatant at the highest test concentration per soil was analysed by HPLC.

After shaking for 24 hours for desorption equilibrium, test systems were removed from shaker, centrifuged, and supernatants were decanted. The volume of the supernatants was determined by weight and aliquots were analysed on LSC.
Soils were extracted once with 15-mL acetonitrile for 20 min at ambient temperature on a benchtop shaker, centrifuged at 3,000 g for 5 min, and supernatant was decanted and radioassayed. The volume of the extract was recorded. Soils were air-dried, weighed, and aliquots were combusted.

2. Analytical Methodology

Radioactivity in samples was determined in triplicate by LSC.
The liquid specimens were measured with a liquid scintillation counter.
Solid samples (after extraction) were oxidized. The generated $^{14}CO_2$ was radioassayed with 15 mL of Harvey Carbon-14 oxidizer cocktail. The samples were radioassayed for ^{14}C-content by LSC, and the results were corrected for oxidizer efficiency.

3. Calculations

The amount of test substance adsorbed to soil was calculated by subtracting the equilibrium concentration in the solution from the initial concentration (applied concentration). By establishing the material balances and the stability of the test substance with HPLC/radiodetection it was verified that, besides the adsorption to soils, no other significant processes had contributed to the decline of test substance measured in the supernatant.

Calculation of the Freundlich constant and related K_{foc} was performed according to US EPA OCSPP Fate, Transport and Transformation Test Guideline No. 835.1230.
The radioactive contents determined in the supernatants of the adsorption and desorption steps at each equilibrium were used to calculate adsorption ($K_{f\text{-}ads}$) and desorption isotherms ($K_{f\text{-}des}$), respectively, as well as the organic carbon related distribution coefficients (K_{foc}).

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

In the definitive test the overall mean material balances for END, MMN, SCA and SKS soils were 98.5% (individual replicate range 96.2 to 101.9%), 97.8% (96.2 to 100.2%), 97.9% (95.4 to 106.5%), and 98.6% (95.0 to 102.6%) AR, respectively. The overall mean material balance was 98.2 ± 2.3% AR, which demonstrates the effectiveness of the extraction method. Parental mass balances are reported as 94.4%, 95.0%, 94.9 and 93.9%. The recovery of radioactivity for all soils is presented in the following table.

Table B.8.1.2.2.2- 4: Recovery of radioactivity (as percentage of applied radioactivity, mean of two replicates) of M12

Concentration [µg/mL]	Soil			
	END	MMN	SCA	SKS
1.07	96.35	96.4	96.3	96.2
0.29	97.9	98.3	95.85	97.75
0.09	98.2	97.7	96.35	98.05
0.03	98.6	97.0	97.0	98.8
0.01	101.25	99.85	104.25	101.9
Mean	98.5	97.8	97.9	98.6

B. ADSORPTION RESULTS

In the definitive adsorption test, the mean % AR sorbed to soil ranged from 40.4 to 46.7% in END soil, 36.1 to 41.3% in MMN soil, 35.8 to 48.0% in SCA soil, and 47.1 to 60.3% in SKS soil. The respective concentrations in solution, in soil, and the percentage of adsorbed test substance are shown in the following table.

Table B.8.1.2.2.2- 5: Concentration of M12 in the solid and liquid phases at the end of adsorption period

Concentration [µg/mL]	Soil [µg/g]	Solution [µg/mL]	Percentage adsorbed mean SD
END			
0.009	0.017	0.005	46.7 ± 0.2
0.028	0.051	0.016	45.2 ± 2.0
0.09	0.172	0.052	45.3 ± 1.3
0.29	0.493	0.163	43.1 ± 0.9
1.07	1.723	0.636	40.4 ± 0.5
MMN			
0.009	0.008	0.006	40.8 ± 0.0
0.028	0.023	0.017	41.3 ± 0.4
0.09	0.077	0.056	40.8 ± 0.1
0.29	0.228	0.172	39.8 ± 0.1
1.07	0.770	0.682	36.1 ± 0.3
SCA			
0.009	0.009	0.004	0.005 48.0
0.028	0.028	0.013	0.015 45.8
0.09	0.09	0.041	0.054 43.1
0.29	0.29	0.113	0.173 39.4
1.07	1.07	0.382	0.685 35.8
SKS			
0.009	0.011	0.004	60.3 ± 0.2
0.028	0.034	0.012	59.1 ± 0.6
0.09	0.106	0.042	55.7 ± 0.1
0.29	0.298	0.137	52.1 ± 0.1
1.07	1.004	0.565	47.1 ± 0.3

The adsorption behaviour of M12 in the concentration range of two orders of magnitude (i.e. from 0.01 to 1.0 mg/L) was accurately described for all soils with the Freundlich equation. The coefficients of determination (r^2 value) for the individual adsorption isotherms ranged from 0.9989 to 0.9997 (mean: 0.9993).

The calculated adsorption constants $K_{f(ads)}$ of the Freundlich isotherms for the four test soils ranged from 0.544 to 2.724 mL/g (mean: 1.543 mL/g). The Freundlich exponents 1/n were in the range of 0.8914 to 0.9604 (mean: 0.9245), indicating that the concentration of the test substance minimally affected the adsorption behaviour in the examined concentration range.
In general, the organic matter in soil, determined as organic carbon content, is responsible for binding most organic chemicals. Therefore, the adsorption coefficients K_{fads} were correlated with the organic carbon content of the matrix to get a comparability of the adsorption behaviour in different soils. For M12, the calculated K_{ocads} values ranged from 49.1 to 289.8 mL/g (mean: 155.6 mL/g).
An overview of the results according to the Freundlich equation is presented in the following table:

Table B.8.1.2.2.2- 6: Adsorption constants of M12 in soils

Soil	**$K_{f(ads)}$ [mL/g]**	**1/n**	**$K_{oc(ads)}$ [mL/g]**	**r^2**
END	2.724	0.9497	289.8	0.9989
MMN	1.178	0.9604	49.1	0.9992
SCA	0.544	0.8966	187.5	0.9997
SKS	1.727	0.8914	95.9	0.9995
Arith. mean	**1.543**	**0.9245**	**155.6**	**0.9993**
Geo. mean			**126.5**	

C. DESORPTION RESULTS

In the definitive desorption test, the mean % AR desorbed from soil ranged from 40.4 to 47.4% in END soil, 31.4 to 36.4% in MMN soil, 29.2 to 41.5% in SCA soil, and 22.4 to 33.5% in SKS soil. The respective concentrations in solution, in soil and the percentage of desorbed test substance are shown in the following table.

Table B.8.1.2.2.2- 7: Concentration of M12 in the solid and liquid phases at the end of desorption period (mean of duplicates)

Concentration [µg/mL]	Soil [µg/kg]	Solution [µg/mL]	Percentage desorbed mean SD
END			
0.009	0.010	0.002	40.4
0.028	0.031	0.005	40.7
0.09	0.101	0.018	41.1
0.29	0.277	0.054	43.7
1.07	0.906	0.204	47.4
MMN			
0.009	0.005	0.001	36.4
0.028	0.016	0.004	32.8
0.09	0.051	0.013	33.8
0.29	0.156	0.036	31.6
1.07	0.528	0.121	31.4
SCA			
0.009	0.003	0.001	30.7
0.028	0.009	0.004	29.2
0.09	0.029	0.012	29.9
0.29	0.075	0.038	33.5
1.07	0.223	0.158	41.5
SKS			
0.009	0.009	0.001	23.2
0.028	0.026	0.004	22.4
0.09	0.080	0.013	24.4
0.29	0.216	0.041	27.6
1.07	0.668	0.168	33.5

The r^2 value of the individual desorption isotherms ranged from 0.9951 to 0.9988 (mean: 0.9972). 1.322 mL/g to 4.870 mL/g (mean: 3.510 mL/g). The Freundlich exponents 1/n ranged from 0.8972 to 1.0434 (mean: 0.9450). The calculated K_{ocdes} values ranged from 201.1 to 455.8 mL/g (mean: 327.4 mL/g). An overview of the results is presented in the following table.

Table B.8.1.2.2.2- 8: Desorption constants of M12 in soils

Soil	$K_{f(des)}$ [mL/g]	1/n	$K_{oc(des)}$ [mL/g]	r^2
END	4.230	0.9403	450.0	0.9984
MMN	4.870	1.0434	202.9	0.9988
SCA	1.322	0.8990	455.8	0.9951
SKS	3.619	0.8972	201.1	0.9964
Arith. mean	**3.510**	**0.945**	**327.4**	**0.997**

III. CONCLUSIONS

The adsorption coefficients K_{fads} of M12 in four test soils were determined to range from 0.5 to 2.7 mL/g (mean 1.5 mL/g. The corresponding organic carbon normalised adsorption coefficients K_{focads} proposed by the applicant ranged from 49.1 to 289.8 mL/g (mean 155.6 mL/g).

The Freundlich exponents 1/n were in the range of 0.8914 to 0.9604 indicating that the concentration of the test item affected the adsorption behaviour slightly, only.

The desorption coefficients K_{focdes} of M12 were 1.6 to 4.1 times higher compared as the respective adsorption coefficients (201.1 mL/g – 455.8 mL/g) indicating a strong binding of the test substance once adsorbed to the soil.

UK RMS had validated the study using the OECD 106 evaluators checklist. LOD and LOQ values for HPLC are reported as 0.5% with LOQ of 1.01% AR. LSC LOD and LOQ values are not reported, however DPM values at the lowest concentration are sufficient for accurate analysis. Parental mass balances are reported as 94.4%, 95.0%, 94.9 and 93.9% during the adsorption equilibrium time point test for END, MMN, SCA and SKS respectively. This is above the 90% stated in the guidance and HPLC chromatograms show no degradation during the test. Kfe/Kf values are not able to be calculated as parental mass balance checks were not performed during the isotherms test. Individual replicate values are reported in table B.8.1.2.2.2-9 by the UKRMS for evaluation purposes. Results from the RMS evaluation are similar to the values proposed by the applicant, therefore the applicants' values are accepted. RMS residual fit graphs are provided in figures B.8.1.2.2.2-1 to B.8.1.2.2.2-4.

Table B.8.1.2.2.2-9: Individual replicate concentration values for the adsorption supernatant for M12.

Soil	Initial concentration of test solution (µg/mL)	Concentration of supernatant (µg/mL)
Northwood, ND	1.07	0.630
	1.07	0.641
	0.29	0.165
	0.29	0.160
	0.09	0.053
	0.09	0.051
	0.03	0.015
	0.03	0.016
	0.01	0.005
	0.01	0.005
Morris, MN	1.07	0.685
	1.07	0.678
	0.29	0.172
	0.29	0.172
	0.09	0.056
	0.09	0.056
	0.03	0.017
	0.03	0.017
	0.01	0.006

	0.01	0.006
Sanger, CA	1.07	0.694
	1.07	0.676
	0.29	0.173
	0.29	0.174
	0.09	0.054
	0.09	0.054
	0.03	0.016
	0.03	0.015
	0.01	0.005
	0.01	0.005
Stilwell, KS	1.07	0.568
	1.07	0.561
	0.29	0.137
	0.29	0.137
	0.09	0.042
	0.09	0.042
	0.03	0.012
	0.03	0.011
	0.01	0.004
	0.01	0.004

Table B.8.1.2.2.2-10: UK RMS evaluators checklist results for M12, values are not relied upon in the risk assessment.

Soil	Organic carbon (%)	pH	KF, ads	KFoc, ads	1/n	r2
Northwood, ND	0.94	5.3	2.602	276.78	0.922	0.998
Morris, MN	2.4	8.1	1.179	49.12	0.963	0.998
Sanger, CA	0.3	6.2	0.526	175.45	0.879	0.998
Stilwell, KS	1.8	6.2	1.710	95.00	0.886	0.997
Mean			1.504	149.09	0.912	
Geomean			1.289	122.70	0.912	

Figure B.8.1.2.2.2-1: Log fit and residuals graph for Northwood soil

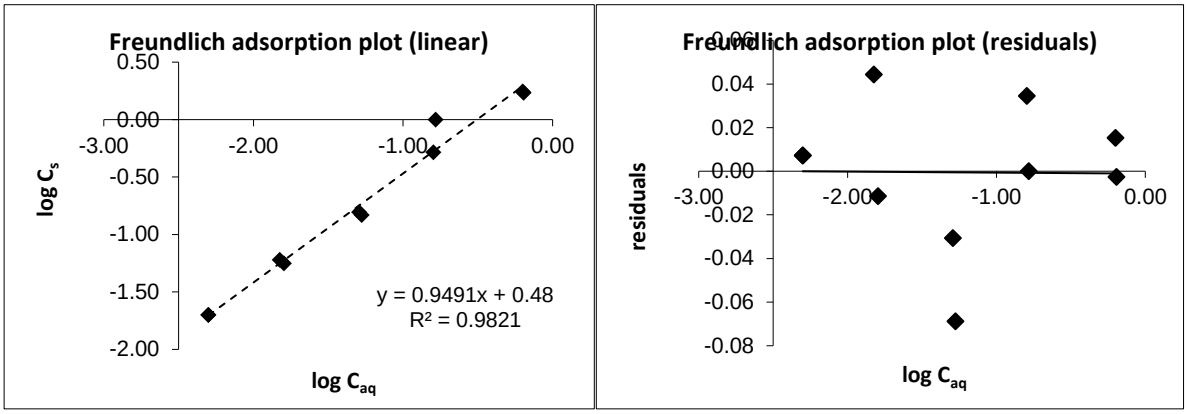


Figure B.8.1.2.2.2-2. Log fit and residuals graph for Morris soil

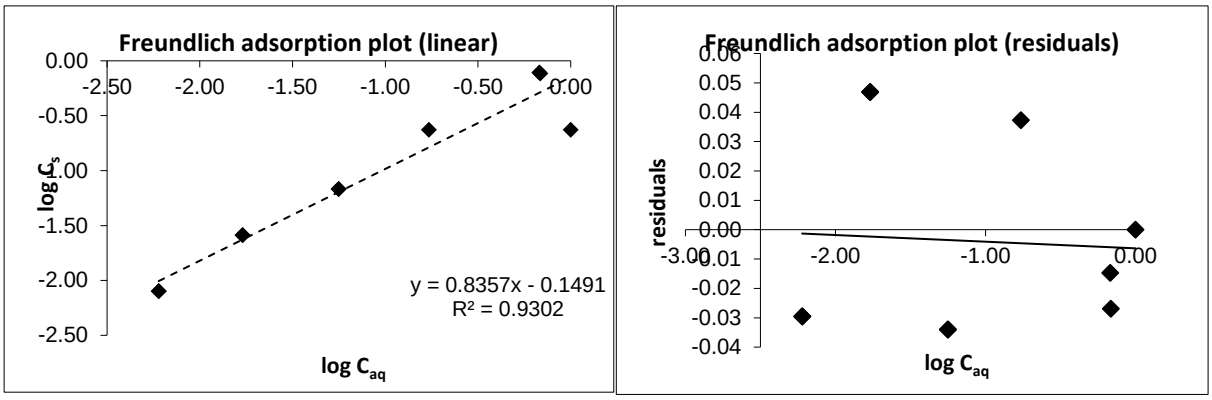


Figure B.8.1.2.2.2-3. Log fit and residuals graph for Sanger soil

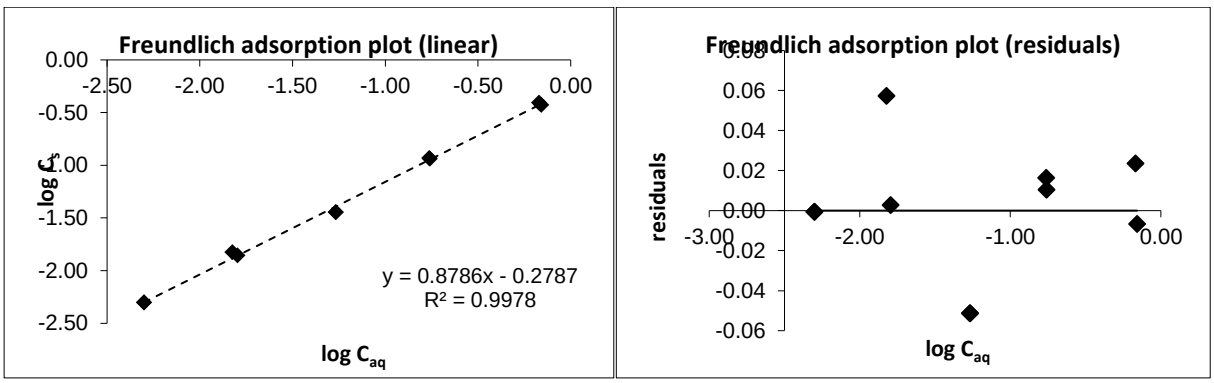
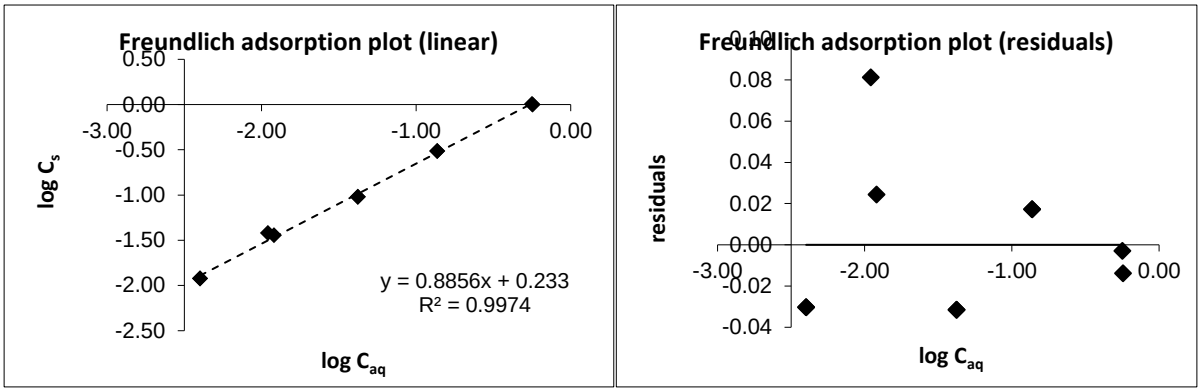


Figure B.8.1.2.2.2-4. Log fit and residuals graph for Stilwell soil



M12 may show some pH dependency. A full assessment using all adsorption data for M12 is provided in section B.8.1.3.2.5 below.

Using the Briggs classifications for the estimation of the mobility of chemicals in soil based on the mean K_f and/or K_{foc} values, M12 can be classified as mobile to low mobility for adsorption, and once sorbed will remain strongly sorbed.

B.8.1.3. Mobility in soil and plant uptake studies

The plant uptake factor and the transpiration stream concentration factor of isoflucypram are presented by the applicant using the default value of 0 and a Briggs estimate.
In addition, laboratory studies on the plant uptake factor and the transpiration stream concentration factor of isoflucypram and M12 were performed (see B.8.1.3.1.2 and B.8.1.3.1.3 respectively) using wheat plants in a hydroponic system. Whilst no agreed E.U. or other international guidelines exist for the conduct and assessment of such studies, the UK RMS has evaluated them in order to determine whether they appear to be sufficiently robust to derive endpoints for use in environmental exposure modelling..

B.8.1.3.1.1: Plant uptake of Isoflucypram and its metabolite, desk study.

Previous evaluation:	None, new active substance.

Author: Reinken, G.; Kallweit, W.; 2017;
Title: Isoflucypram (ISY): Core PECgw EUR - Modelling core info document for groundwater risk assessment in Europe
Report No.: EnSa-17-0655
Document No.: M-608724-02-1
Guideline(s): None
Guideline deviation(s): None
GLP/GEP: No

Study Summary

Plant uptake describes the amount of chemical taken up by the plant from soil during its growth and prior to harvesting. The EFSA (2013)[1] PPR panel has recognised in an opinion that plant uptake via roots is significant when calculating leaching exposure concentrations and has recommended the use of the plant uptake in exposure models if evidence for the actual occurrence of the process is demonstrated.

According to EFSA (2013), the use of a worst case default transpiration stream concentration factor (TSCF) of zero in the leaching assessment is recommended as a first step. As a second step EFSA (2013) proposes the use a TSCF derived from the equation given by Briggs et al. (1982)[2] which is based on the relationship between plant uptake and octanol water partition coefficient (Table B.8.1.3.1.1- 1). This is also in line with the approach recommended by FOCUS (2014). It is also possible to consider experimentally determined TSCF.

Table B.8.1.3.1.1- 1: Plant uptake factors for isoflucypram and its metabolite M12 derived from Briggs equation (Briggs *et al.* 1982)

Compound	log Pow[a)]	TSCF by Briggs	Reference log Pow
Isoflucypram	4.0 (pH 4-9)	0.10	Ziemer, F.; Peschke, C.; 2014; M-484656-01-1 (summarised in MCA section 2.7)
M12	at 23°C: 2.11 at pH 5 0.22 at pH 7 -1.1 at pH 9	0.75 0.29 0.03	Ziemer, F.; Peschke, C.; 2015; M-519996-01-1; (summarised in MCA section 2.7)

a) Used for estimation of TSCF

UK RMS has validated the TSCF values proposed by the applicant and agrees with the values proposed.

The Briggs estimation leads to a TSCF of 0.10 for isoflucypram (Table B.8.1.3.1.1- 1). Consequently, the Briggs estimated value of 0.10 was used as refined input for the leaching assessment.

The Briggs estimated TSCF of M12 is pH dependent. This is supported by the change in log Pow. Thus, the default PUF of zero was considered as input for the leaching assessment.

B.8.1.3.1.2: Plant uptake of Isoflucypram

Previous evaluation:	None, new active substance.

Author: Daumann, M.; 2017a;
Title: Determination of the plant uptake of [pyrazole-4-14C] BCS-CN88460 in wheat plants - Report amendment 1
Report No.: S16-05508
Document No.: M-587420-02-1

[1] EFSA, 2013: Scientific Opinion on the report of the FOCUS groundwater working group (FOCUS, 2009): assessment of higher tiers, EFSA Journal 2013; 1(6):3291

[2] Briggs G.G., Bromilow R.H., and Evans A.A., (1982): Relationships between lipophilicity and root uptake and translocation of non-ionized chemicals by barley. *Pestic. Sci. 13, 495-504*

Guideline(s): None available
Guideline deviation(s): None
GLP/GEP: yes

Study Summary (for Plant uptake factor (PUF/TSCF))

The uptake of pyrazole-labelled isoflucypram was investigated in wheat plants (variety: *Thasos*) over a study duration of ten days under controlled temperature, humidity and light conditions (mean: 20.2°C, approx. 50% humidity and a day/night cycle of 16 h/8 h). The plant uptake factor (PUF) and the transpiration stream concentration factor (TSCF) were determined. The test was performed in quadruplicates (four test systems) with additional triplicates of plant controls and triplicates of stability controls.
The initial test item concentration in the test solution was 91.79 µg/L, corresponding to 30.94 µg test item per test vessel. UK RMS notes that no experiment was performed to assess if the concentration of test material in the solution has an influence on plant uptake.

Pre-grown wheat plants (BBCH code approx. 13) were either exposed to the test solution (half strengthened Hoagland's No. 2 basal salt L mixture nutrition solution including the test item) or to nutrient solution only (controls) for the whole study duration of 10 days. Sample aliquots were analysed 0 (t_{start}), 2 (t_0), 4 (t_1) and 10 (t_{end}) days after treatment (DAT). UK RMS notes that no biocides were added to the nutrient/ test solution. This solution will be an ideal media for bacteria from the air or transferred to the roots in which to grow. No experimental assessment has been provided to determine whether the test substance is stable in the nutrient solution in the presence of the plants. Consequently there is no evidence that uptake is of parent only and not of any metabolites that may have been formed by biotic degradation of the parent substance in the nutrient solution. UK RMS considers this to be a critical omission.

Wheat plants appeared healthy over the total study duration both in treated and untreated test systems. During the course of the study the wheat plants grew from BBCH 13 at t_{start} (application and start of equilibration) to BBCH 14-24 at t_{end} (end of study at DAT-10). Oxygen saturation for all experiments was always above 86% and pH-values remained in an adequate range for treated (6.49 to 6.96) and control test systems (6.51 to 6.91).
Mean material balances were 96.2% AR for t_1 (range from 95.2 to 100.7% AR) and 92.2% AR for t_{end} (range from 88.7 to 95.4% AR).
The total net transpiration rates at the end of the incubation phase (t_{end}) ranged from 51.0 to 69.5 mL for treated plants and 45.1 to 50.6 mL for control plants. Approximately 2.4 mL of the test solutions was lost due to evaporation, which was determined as the mean of 3 control replicates.

The mean initial concentrations (t_{start}, DAT-0) of pyrazole-labelled isoflucypram in the test solutions amounted to 91.79 µg/L, increasing to 95.71 µg/L at t_1 (interim sampling at DAT-4) and decreasing to 89.74 µg/L at t_{end} (end of study at DAT-10). Root washing desorbed 0.209 µg of the test item from the roots at t_1 and 0.212 µg at t_{end}. The separate analysis of roots and shoots showed, that at t_1 40.7% and at t_{end} 45.0% of radioactivity taken up was translocated from roots into shoots.

The PUF was calculated from the respective amount of test item in the test solution and the volume of the test solution each at the end of equilibration phase (t_0) and the end of the respective incubation phases (t_1, t_{end}). The PUFs in wheat for pyrazole-labelled isoflucypram amounted to 0.40 ± 0.10 (t_0-t_1) and 0.49 ± 0.06 (t_0-t_{end}). The mean PUF was determined as 0.44 ± 0.10. The TSCF results from a calculation using the respective parameters present at the start of the study (t_{start}) and the end of incubation phases (t_1, t_{end}), additionally taking into account the radioactivity present in the plant shoot tissues, indicative for the uptake and translocation of the test item in correlation with the net amount of transpiration. The respective TSCFs were determined as 0.14 ± 0.01 (t_{start}-t_1) and 0.17 ± 0.02 (t_{start}-t_{end}) (see table B.8.1.3.1.2-1).

Table B.8.1.3.1.2-1: PUF and TSCF values of isoflucypram in wheat

Replicate no.	PUF_{wheat}		$TSCF_{wheat}$	
	$t_0 - t_1$	$t_0 - t_{end}$	$t_{start} - t_1$	$t_{start} - t_{end}$
1	0.27	0.45	0.12	0.13
2	0.47	0.46	0.14	0.16
3	0.32	0.60	0.15	0.16
4	0.55	0.46	0.15	0.19
5	0.41	-*	0.14	-*
mean	**0.40**	**0.49**	**0.14**	**0.17**
SD	0.10	0.06	0.01	0.02
CV [%]	25.00	13.03	9.65	12.84
overall mean	**0.44**		-	
SD	0.10			
CV [%]	21.94			

a) Replicate was not included into calculations due to unusual observation with respect to growth

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Pyrazole-labelled isoflucypram
Sample-ID: KML 10300
Specific activity: 4.22 MBq/mg
Radiochemical purity: > 99%
Chemical purity: > 99%

2. Test System

Germination and early growth of wheat plants (variety: *Thasos*) was conducted on Perlite until plants reaching a BBCH stage of approx. 11-12. The plants were cultivated under temperature, humidity and light controlled conditions in an incubation room. During germination and early growth, plants were grown on a moist Perlite substrate, before exposed to constant illumination using LED lights. Once germination had occurred water was replaced a with nutrient solution (NS) until sufficient growth had occurred. The characteristics of the Perlite substrate, illumination and the composition of untreated nutrient solution are given in the table below.

Table B.8.1.3.1.2-2: Test conditions

Perlite characteristics		Illumination characteristics		Nutrient solution
Granule size d [mm]	Mass p_s [kg/m³]	day / night [h/h]	Quantity [klux]	
0-6	approx. 90	16 / 8	approx. 12.0	0.8 g Hoagland's No. 2 basal salt L mixture, 1.03 g MES buffer (2-(N-morpholino)-ethanesulfonic acid) and 0.75 mL of 15% Ferric EDTA (ethylenediaminetetraacetic acid) solution were dissolved in an appropriate amount of demineralized water. pH was adjusted to 6.5 using sodium hydroxide (KOH) and finally was filled up to 1 L with demineralized water.

B. STUDY DESIGN

1. Use Pattern

The initial test item concentration in the test solution was 91.79 µg/L, corresponding to 30.94 µg test item per test vessel.

2. Experimental Conditions

The hydroponic test system for the PUF/TSCF experiment consisted of 10 brown glass vessels filled with ~340 mL test solution and 2 plants each, 3 brown glass vessels filled with ~340 mL test solution only and 3 more brown glass vessels filled with ~340 mL nutrition solution (NS) and 2 plants each. Plants were gently fixed in the glass vessels with elastomer foam and staked with a wire spiral. All vessels were bubbled with air to maintain aerobic conditions.

All used plants were pre-grown on Perlite and transferred at BBCH stage of approx. 11-12 to hydroponic conditions. Therefore, 2 plants for each brown glass vessel were selected based on health, morphology and size as suitable replicates. After acclimatisation to the new hydroponic growth conditions (10 days), plants were transferred into 10 brown glass vessels containing ~340 mL NS with pyrazole-labelled isoflucypram at a final concentration of 91.79 µg/L, as well as into 3 brown glass test vessels containing ~340 mL NS with 50.5 µL of MeOH. The latter corresponded to the organic solvent used for application of the test item. Throughout the whole experiment performed under hydroponic conditions, all used test vessels were bubbled with air to maintain aerobic conditions. Following a 10-day acclimatisation phase, plants were exposed to the test item for 2 days during the equilibration phase, before starting the incubation phase lasting for 2 (t_1) or 8 (t_{end}) more days. All plants were monitored regularly and no unusual observation with respect to growth could be detected.

3. Sampling

The study was performed with two wheat plants per brown glass vessel. Analyses of pH and redox were conducted at 0 (t_{start}), 2 (t_0), 4 (t_1) and 10 days (t_{end}) after treatment. Harvest of the plants were performed at t_1 and t_{end}. At all dates, radioactivity in the test solution, pH, oxygen saturation and volume of the solutions were determined. At t_{start}, t_1 and t_{end} biomass of the plants was determined for all (t_{start}) or harvested brown glass vessels (t_1, t_{end}).

3. Analytical Procedures

At each date of analysis (t_{start}, t_0, t_1, t_{end}), 3 aliquots of 0.5 mL were taken from each radioactive test system and the overall contained radioactivity was determined by liquid scintillation counting (LSC). Additionally, at the plant harvest after 4 (t_1) and 10 days (t_{end}) after treatment, the root surfaces were washed with ACN/H_2O (4/1; *v/v*), washings were quantified by LSC. The amount of radioactivity in the test solution as well as in the root wash solution was determined by LSC. Radioactivity taken up into root tissues as well as radioactivity located in the shoots was determined by combustion in an oxygen atmosphere using an oxidiser. The released carbon dioxide was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

The purity of the stock solution, as well as the stability of respective aliquots used for application (application solution) before and after application were checked by High Performance Liquid Chromatography (HPLC) coupled with radiodetection. It was noted by the UKRMS that chemical stability vessels had not been exposed to the potential biota contained on the plant roots, therefore the chemical purity results may not be the same as those exposed to plant roots.

II. RESULTS AND DISCUSSION

A. PROPERTIES OF TEST SYSTEM

Oxygen saturation for all experiments was always above 86% and the pH of the test solutions during the course of study ranged from 6.49 to 6.96 for the treated and 6.51 to 6.91 for the untreated test systems.

B. PLANT CONTROLS

The health and growth of the wheat plants was visually assessed regularly. In parallel to the treated test systems, untreated test systems (plant controls) were incubated to enable the detection of possible effects on plant growth and health induced by the test item. Wheat plants appeared healthy over the total study duration both in treated and untreated test systems.

Besides an increase of biomass detectable for all treated (1.07 g) and untreated plants (1.30 g) at the end of the incubation phase, the study author stated that plant vigour was evident from the detectable plant development. This was reflected by BBCH stages of approx. 13 at the start of equilibration phase and BBCH stages of approx. 14 to 24 at the end of incubation phase.

C. ANALYTICAL RESULTS

Mean material balances were 96.2% AR for t_1 (range from 95.2 to 100.7% AR) and 92.2% AR for t_{end} (range from 88.7 to 95.4% AR) (see Table B.8.1.3.1.2- 3). The complete material balances found at all sampling intervals for all test systems demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

The total transpiration rates at the end of the incubation phase (t_{end}) ranged from 51.0 to 69.5 mL for treated plants and 45.1 to 50.6 mL for control plants. This corresponds with a net transpiration of 17.2% of the initial volume (337.61 mL) for the test vessels, meeting the requirements of at least 15% net transpiration (see Table B.8.1.3.1.2- 4).

The concentration of isoflucypram in the test solution stayed nearly constant over the entire incubation phase of ten days. The mean initial concentration of pyrazole-labelled isoflucypram in the test solutions at t_{start} amounted to 91.79 µg/L, increasing to 95.71 µg/L at t_1 and decreasing to 89.74 µg/L at t_{end}. The mass of the test item decreased from 30.94 µg at t_{start} to 29.76 µg at t_1 and further decreased to 24.48 µg at t_{end}. (Table B.8.1.3.1.2- 5).

On average the root washing released 0.209 µg of the test item from the roots at t_1 and 0.212 µg at t_{end} (see Table B.8.1.3.1.2- 6).

The separate analysis of roots and shoots showed, that at t_1 40.7% and at t_{end} 45.0% of radioactivity taken up was translocated from roots into shoots (see Table B.8.1.3.1.2- 3).

Table B.8.1.3.1.2-3: Mass balance (in percentage of applied radioactivity)

	Recovery single samples					mean
Day 4 (t_1)						
Test solution t_{start}	100.0	100.0	100.0	100.0	100.0	100.0
Test solution t_1	92.0	96.6	91.4	88.6	90.7	91.9
Root wash	0.7	0.5	0.5	0.9	0.6	0.6
Test solution + root wash	92.7	97.1	91.9	89.5	91.3	92.5
Aliquots	1.3	1.4	1.3	1.3	1.3	1.3
Combustion shoots	0.8	0.9	1.1	1.1	1.0	1.0
Combustion roots	1.1	1.3	1.3	1.6	1.7	1.4
Translocation from roots to shoots	39.6	39.3	44.9	41.6	37.9	40.7
Plant total	1.9	2.2	2.4	2.7	2.7	2.4
Total recovery radioactivity	96.0	100.7	95.6	93.5	95.2	96.2
Day 10 (t_{end})						
Test solution t_{start}	100.0	100.0	100.0	100.0		100.0
Test solution t_{end}	85.3	88.5	78.7	84.3		84.2
Root wash	0.8	0.6	1.0	0.6		0.7
Test solution + root wash	86.1	89.1	79.6	84.9		84.9
Aliquots	1.3	1.4	1.3	1.4		1.4
Combustion shoots	2.2	2.5	3.9	3.1		3.0
Combustion roots	3.3	2.4	5.1	3.8		3.7
Translocation from roots to shoots	39.9	51.1	43.5	45.5		45.0
Plant total	5.5	5.0	9.1	6.9		6.6
Total recovery radioactivity	93.0	95.4	88.7	91.8		92.2

Table B.8.1.3.1.2-4: Water uptake

Sample	**sample ID**	**V t_{start} initial [mL]**	**V t_{end} initial [mL]**	**ΔV t_{start} → t_{end} [mL]**	**net ΔV t_{start} → t_{end} [mL]**	**ΔV t_{start} → t_{end} [%]**
Control plants	160818P1	338.93	285.36	53.57	50.63	14.94
	160818P2	338.62	290.55	48.07	45.13	13.33
	160818P3	338.54	287.63	50.91	47.97	14.17
	mean (P1-3)			50.85	47.91	14.15
Day 4 (t_1)	160818PT7	335.14	315.90	19.24	18.05	5.36
	160818PT8	335.76	317.15	18.61	17.42	5.17
	160818PT9	337.25	316.06	21.19	20.00	5.91
	160818PT10	336.57	311.19	25.38	24.19	7.16
	160818PT11	338.41	313.68	24.73	23.54	6.93
	mean (PT7-11)			21.83	20.64	6.10
Day 10 (t_{end})	160818PT12	334.16	276.92	57.24	54.30	16.25
	160818PT13	338.36	284.41	53.95	51.01	15.08
	160818PT15	342.70	270.29	72.41	69.47	20.27
	160818PT16	335.23	274.77	60.46	57.52	17.16
	mean (PT12-16)			61.02	58.08	17.19

P1-P3 = untreated plants, P7-P16 = treated plants
V t_{start} initial = volume of test solution at the start of the equilibrium after sampling, DAT-0
V t_{end} initial = volume of test solution at the end of the experiment, DAT-10
Δ volume lost
t_{start} = start of equilibration, DAT-0; t_{end} = final sampling, DAT-10

Table B.8.1.3.1.2-5: Concentrations of isoflucypram in test solutions

Date	Single values									Mean
	PT7	PT8	PT9	PT10	PT11	PT12	PT13	PT15	PT16	
Day 0 (t_{start})										
V t_{start} [mL]	335.14	335.76	337.25	336.57	338.41	334.16	338.3	342.7	335.2	337.06
c t_{start} [µg/L]	93.46	89.18	100.36	104.06	94.76	90.00	90.48	85.43	78.34	91.79
m t_{start} [µg]	31.32	29.94	33.84	35.02	32.07	30.07	30.62	29.28	26.26	30.94
Day 2 (t_0)										
V t_0 [mL]	325.05	327.02	326.37	323.32	325.42	323.38	327.95	329.11	320.78	325.38
c t_0 [µg/L]	91.22	91.36	97.71	100.32	92.46	86.71	89.56	80.95	75.35	89.52
m t_0 [µg]	29.65	29.88	31.89	32.43	30.09	28.04	29.37	26.64	24.17	29.13
Day 4 (t_1)										
V t_1 final [mL]	310.89	313.89	310.68	308.85	310.47					310.96
c t_1 [µg/L]	92.67	92.11	99.60	100.51	93.68					95.71
m t_1 [µg]	28.81	28.91	30.94	31.04	29.09					29.76
Day 10 (t_{end})										
V t_{end} final [mL]						273.10	281.20	264.59	271.23	272.53
c t_{end} [µg/L]						93.95	96.34	87.04	81.64	89.74
m t_{end} [µg]						25.66	27.09	23.03	22.14	24.48

PT7 – PT16 = treated plants
V t_{start} = volume of test solution at the start of the equilibrium after sampling, DAT-0
c t_{start} = concentration of the test item in the test solution at the start of the equilibrium phase after sampling, DAT-0
m t_{start} = mass of test item in test solution at the start of equilibrium phase after sampling, DAT-0
V t_0 = volume of test solution at the end of the equilibrium after sampling, DAT-2
c t_0 = concentration of the test item in the test solution at the end of the equilibrium phase after sampling, DAT-2
m t_0 = mass of test item in test solution at the end of equilibrium phase after sampling, DAT-2
V t_1 final = volume of test solution at the interim sampling after sampling, DAT-4
c t_1 = concentration of the test item in the test solution at the interim sampling after sampling, DAT-4
m t_1 = mass of test item in test solution at the interim sampling after sampling, DAT-4
V t_{end} final = volume of test solution at the end of the experiment after sampling, DAT-10
c t_{end} = concentration of the test item in the test solution at the end of the experiment, DAT-10
m t_{end} = mass of test item in test solution at the end of the experiment after sampling, DAT-10

Table B.8.1.3.1.2- 6: Concentrations of isoflucypram in root wash

Date	Single values									Mean
	PT7	PT8	PT9	PT10	PT11	PT12	PT13	PT15	PT16	
Day 4 (t_1)										
c t_1 root wash [µg/L]	5.23	2.84	3.03	4.33	3.91					3.87
m t_1 root wash [µg]	0.229	0.148	0.168	0.317	0.181					0.209
Day 10 (t_{end})										
c t_{end} root wash [µg]						2.71	2.16	1.12	3.26	2.50
m t_{end} root wash [µg]						0.229	0.178	0.092	0.286	0.212

PT7 – PT16 = treated plants
c t_1 root wash = concentration of test item in root wash solution at the interim sampling, DAT-4
m t_1 root wash = mass of test item in root wash solution at the interim sampling, DAT-4
c t_{end} root wash = concentration of test item in root wash solution at the end of the experiment, DAT-10
m t_{end} root wash = mass of test item in root wash solution at the end of the experiment, DAT-10

D. CALCULATION OF PUFs AND TSCFs

The mean PUF in wheat for pyrazole-labelled isoflucypram amounted to 0.40 ± 0.10 (t_0-t_1) and 0.49 ± 0.06 (t_0-t_{end}). The mean PUF in wheat for pyrazole-labelled isoflucypram accounted for 0.44 ± 0.10, indicative for a slightly inhibited plant uptake of the test item in comparison to water uptake (see Table B.8.1.3.1.2-7).

(equation 1 [4])

$$PUF = \frac{\ln\left(\frac{m_{sol}}{m_0}\right)}{\ln\left(\frac{V_{sol}}{V_0}\right)}$$

With

m_0: mass of test item at the start of the experiment (t_0, at the end of the equilibration phase) [µg]

V_0: volume of test solution at the start of the experiment (t_0, at the end of the equilibration phase) [L]

m_{sol}: mass of test item at a given time t after the start of the experiment [µg]

V_{sol}: volume of test solution at given time t after the start of the experiment [L]

(equation 2)

$$m_{sol} = c_{sol} \times V_{sol} + m_{aliquots} + m_{rootwash}$$

With

m_{sol}: mass of test item in test solution [µg]

c_{sol}: concentration of test item in test solution at a given time t after the start of the experiment [µg/L]

V_{sol}: volume of test solution at a given time t after the start of the experiment [L]

$m_{aliquots}$: sum of test item mass in removed aliquots [µg]

$m_{root\ wash}$: mass of test item in root wash [µg]

The volume of test solution was derived by using equation 3.

(equation 3)

$$V_{sol} = \frac{\left(m_{flask} - m_{flask,empty} - m_{plant}\right)}{\rho_{sol}} + V_{aliquots} + V_{evaporation}$$

With

V_{sol}: volume of test solution [L]

m_{flask}: weight of test vessel filled with test solution [g]

$m_{flask,\ empty}$: weight of empty test vessel [g]

m_{plant}: weight of plant at a given time t after the start of the experiment [g]

ρ_{sol}: density of test solution assumed to be 1 [kg/L]

$V_{aliquots}$: sum of aliquot volumes removed to determine test item concentration [L]

$V_{evaporation}$: volume of solution lost due to evaporation [L]

(equation 4)

$$TSCF = \frac{\ln\left(1 - \frac{m_{shoots}}{m_{shoots} + m_{sol}}\right)}{\ln\left(\frac{V_{sol}}{V_0}\right)}$$

With

V_{sol}: volume of test solution [L]

V_{start}: volume of test solution at the start of the experiment (at the start of the equilibration phase) [L]

m_{sol}: mass of test item in test solution [µg]

m_{shoots}: mass of test item in shoots [µg]

Table B.8.1.3.1.2-7: Plant uptake factor (PUF) for isoflucypram in wheat plants

	PUF_{wheat}		
	t_1	**t_{end}**	**overall**
single samples	0.27	0.45	
	0.47	0.46	
	0.32	0.60	
	0.55	0.46	
	0.41	*	
mean	**0.40**	**0.49**	**0.44**
SD	0.10	0.06	0.10
CV [%]	25.00	13.03	21.94

SD = standard deviation
CV = coefficient of variation
t_1 = interim sampling, DAT-4
t_{end} = final sampling, DAT-10
* Replicate not included due to unusual growth

The mean TSCF in wheat for pyrazole-labelled isoflucypram amounted to 0.14 ± 0.01 (t_{start}-t_1) and 0.17 ± 0.02 (t_{start}-t_{end}), indicative for the translocation of the test item (or equivalents) from root to shoot tissues.For all plants analysed, the relative amount of the test item that was taken up by the roots and allocated to the shoots ranged from 37.9 to 51.1% (see Table B.8.1.3.1.2-8).

Table B.8.1.3.1.2-8: Transpiration stream concentration factor (TSCF) for isoflucypram in wheat plants

	$TSCF_{wheat}$		
	t_1	**t_{end}**	**overall**
single samples	0.12	0.13	
	0.14	0.16	
	0.15	0.19	
	0.15	0.18	
	0.14		
mean	**0.14**	**0.17**	**0.15**
SD	0.01	0.02	0.02
CV [%]	9.65	12.84	14.25

SD = standard deviation
CV = coefficient of variation
t_1 = interim sampling, DAT-4
t_{end} = final sampling, DAT-10

III. CONCLUSIONS

The RMS acknowledges that good plant health indicated by biomass increase and water consumption throughout the testing period were demonstrated.
The mean PUF for pyrazole-labelled isoflucypram in wheat plants proposed by the applicant was determined as 0.44. The calculated TSCF values amounted to 0.14 ± 0.01 (t_{start}-t_1) and 0.17 ± 0.02 (t_{start}-t_{end}), indicative for the translocation of the test item (or equivalents) from root to shoot tissues in this test system. For all plants analysed, more than 37.9% of the test item taken up by the roots was allocated to the shoots.

UK RMS notes that no experiment was performed to assess if the concentration of test material in the solution has an influence on plant uptake. No indication is provided that the concentration of the test solution used is similar to that of the soil pore water predicted by FOCUS PEARL and PELMO.

UK RMS notes that no biocides were added to the nutrient/ test solution. This solution is potentially an ideal medium for bacteria from the air or transferred to the roots to grow. No experimental assessment has been provided, to demonstrate that breakdown of the test substance by biotic degradation (introduced via the addition of plants) in the nutrient solution has not occurred. Thus it cannot be demonstrated that any uptake is of parent only and not of metabolites formed via degradation. UK RMS considers this to be a critical omission.

UK RMS also notes that plants selected are winter wheat, which have been grown indoors from seed. These will not have been subject to typical temperatures and growth conditions in the field, such that the waxes typically formed to protect the plant and retain moisture will not be present. With plant growth in ideal laboratory conditions (20°C and 18 hrs of daylight, not typical conditions for winter wheat at BBCH 13-21) the volumes of water up taken and consumed by the plants may not be considered typical of field grown plants. The rate of growth may not be typical of field grown plants and may influence the uptake of water and nutrients to maintain that growth. Rates of water consumption may not match those used in the FOCUS ground water models.

It is a known phenomenon that plants grown under hydroponic conditions can produce a larger root mass than those grown in soil. Consequently the PUF and/or TSCF value from a hydroponic study may not reflect the values obtained from a study conducted on the same crop grown in a soil bound system.

In light of the concerns cited above, the RMS considers that accurate PUF and TSCF values cannot be determined from this study. Consequently the RMS considers that the study is not reliable with respect to derivation of robust endpoints for use in environmental exposure modelling. However the study description but is retained in the evaluation document for the purposes of peer review . As noted above the applicant proposes to use a TSCF value of 0.1 for isoflucypram (as determined using the Briggs equation) as a first tier refinement in environmental exposure modelling.

B.8.1.3.1.3: Plant uptake of Metabolite of Isoflucypram

Previous evaluation:	None, new active substance.

Author: Daumann, M.; 2017b;
Title: Determination of the plant uptake of [pyrazole-4-14C] BCS-CN88460 carboxylic acid in wheat plants - Report amendment 1

Report No.: S16-05510
Document No.: M-588284-02-1
Guideline(s): None available
Guideline deviation(s): None
GLP/GEP: yes

Study Summary (for Plant uptake factor (PUF/TSCF))

The uptake of pyrazolyl-labelled M12 (BCS-CN88460-carboxylic acid) was investigated in wheat plants (variety: *Thasos*) over a study duration of ten days under controlled temperature, humidity and light conditions (mean: 22.5°C, approx. 50% humidity and a day/night cycle of 16 h/8 h). The plant uptake factor (PUF) and the transpiration stream concentration factor (TSCF) were determined. The test was performed in quadruplicates (four test systems) with additional triplicates of plant controls and triplicates of stability controls.

The initial test item concentration in the test solution was 89.08 µg/L, corresponding to 29.85 µg test item per test vessel. Pre-grown wheat plants (BBCH code approx. 13) were either exposed to the test solution (half strengthened Hoagland's No. 2 basal salt L mixture nutrition solution including the test item) or to nutrient solution only (controls) for the whole study duration of 10 days. Sample aliquots were analysed 0 (t_{start}), 2 (t_0), 4 (t_1) and 10 (t_{end}) days after treatment (DAT). UK RMS notes that no biocides were added to the nutrient/ test solution. This solution will be an ideal media for bacteria from the air or transferred to the roots in which to grow. No experimental assessment has been provided to determine whether the test substance is stable in the nutrient solution in the presence of the plants. Consequently there is no evidence that uptake is of parent only and not of any metabolites that may have been formed by biotic degradation of the parent substance in the nutrient solution. UK RMS considers this to be a critical omission.

Wheat plants appeared healthy over the total study duration both in treated and untreated test systems. During the course of the study the wheat plants grew from BBCH 13 at t_{start} (application and start of equilibration) to BBCH 14-15 at t_{end} (end of study at DAT-10). Oxygen saturation for all experiments except for one sample (161023PT11: 68%) was always above 79% and pH-values remained in an adequate range for treated (6.53 to 6.99) and control test systems (6.48 to 7.06).
Mean material balances were 98.8% of the applied radioactivity (AR) for t_1 (range from 97.5 to 99.4% AR) and 97.0% AR for t_{end} (range from 95.9 to 98.1% AR).
The total net transpiration rates at the end of the incubation phase (t_{end}) ranged from 46.6 to 59.7 mL for treated plants and 49.6 to 58.4 mL for control plants. About 2.5 mL of the test solutions was lost due to evaporation, which was determined as the mean of 3 control replicates.
The mean initial concentrations (t_{start}, DAT-0) of pyrazolyl-labelled M12 in the test solutions amounted to 89.08 µg/L, increasing to 91.82 µg/L at t_1 (interim sampling at DAT-4) and decreasing to 101.05 µg/L at t_{end} (end of study at DAT-10). Root washing desorbed 0.115 µg of the test item from the roots at t_1 and 0.079 µg at t_{end}. The separate analysis of roots and shoots showed, that at t_1 37.2% and at t_{end} 38.4% of radioactivity taken up was translocated from roots into shoots. UK RMS notes that no biocides were added to the nutrient/ metabolite dosed solutions.

The PUF was calculated from the respective amount of test item in the test solution and the volume of the test solution each at the end of equilibration phase (t_0) and the end of the respective incubation phases (t_1, t_{end}). The PUFs in wheat for pyrazolyl-labelled M12 amounted to 0.24 (t_0-t_1) and 0.26 (t_0-t_{end}). The mean PUF was determined as 0.25.

The TSCF results from a calculation using the respective parameters present at the start of the study (t_{start}) and the end of incubation phases (t_1, t_{end}), additionally taking into account the radioactivity present in the plant shoot tissues, indicative for the uptake and translocation of the test item in correlation with the net amount of transpiration. The respective TSCF was determined as 0.07 (t_{start}-t_1) and 0.06 (t_{start}-t_{end}) (see table B.8.1.3.1.3-1 below).

Table B.8.1.3.1.3- 1: PUF and TSCF values of M12 in wheat plants

Replicate No.	PUF_{wheat}		$TSCF_{wheat}$	
	t_0-t_1	t_0-t_{end}	t_{start}-t_1	t_{start}-t_{end}
1	0.20	0.28	0.11	0.06
2	0.21	0.27	0.06	0.07
3	0.22	0.19	0.04	0.07
4	0.31	0.29	0.07	0.06
5	0.29	0.29	0.05	0.03
mean	**0.24**	**0.26**	**0.07**	**0.06**
SD	0.05	0.04	0.02	0.02
CV [%]	19.17	13.81	34.25	27.48
overall mean	**0.25**		---	
SD	0.04			
CV [%]	16.96			

SD = standard deviation, CV = coefficient of variation

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item

Pyrazolyl-labelled BCS-CN88460-carboxylic acid *(M12)*
Sample-ID: KML 10176
Specific activity: 3.73 MBq/mg
Radiochemical purity: > 98%
Chemical purity: 97.2%

2. Test System

Germination and early growth of wheat plants (variety: *Thasos*) was conducted on Perlite until plants reaching a BBCH stage of approx 11-12. The plants were cultivated under temperature, humidity and light controlled conditions in an incubation room. During germination was performed with water on Perlite substrate before exposed to constant illumination using LED lights once germination had occurred this was replaced a with nutrient solution (NS) until sufficient growth had occurred. The characteristics of the Perlite substrate, illumination and the composition of untreated nutrient solution are given in the table below.

Table B.8.1.36.1.3- 2: Test conditions

Perlite characteristics		Illumination characteristics		Nutrient solution
Granule size d [mm]	Mass p_s [kg/m^3]	day / night [h/h]	Quantity [klux]	
0-6	approx. 90	16 / 8	approx. 12.0	exemplary: 0.8 g Hoagland's No. 2 basal salt L mixture, 1.03 g MES buffer (2-(N-morpholino)-ethanesulfonic acid) and 0.75 mL of 15% Ferric EDTA (ethylenediaminetetraacetic acid) solution were dissolved in an appropriate amount of demineralised water. pH was adjusted to 6.5 using sodium hydroxide (KOH) and finally was filled up to 1 L with demineralised water.

B. STUDY DESIGN

1. Use Pattern

The initial test item concentration in the test solution was 89.08 µg/L, corresponding to 29.85 µg test item per test vessel.

2. Experimental Conditions

The hydroponic test system for the PUF/TSCF experiment consisted of 10 brown glass vessels filled with ~340 mL test solution and 2 plants each, 3 brown glass vessels filled with ~340 mL test solution only and 3 more brown glass vessels filled with ~340 mL nutrition solution (NS) and 2 plants each. Plants were gently fixed with elastomer foam and staked with a wire spiral. All vessels were bubbled with air to maintain aerobic conditions.

All used plants were pre-grown on Perlite and transferred at BBCH stage of approx. 11-12 to hydroponic conditions. Therefore, 2 plants for each brown glass vessel were selected based on health, morphology and size as suitable replicates. After acclimatisation to the new hydroponic growth conditions (10 days), plants were transferred into 10 brown glass vessels containing ~340 mL NS with pyrazolyl-labelled M12 at a final concentration of 89.08 µg/L, as well as into 3 brown glass test vessels containing ~340 mL NS with 47.6 µL of MeOH. The latter corresponded to the organic solvent used for application of the test item. Three more vessels contained NS with radiolabelled test item at the described concentration, but without plants. Throughout the whole experiment performed under hydroponic conditions, all used test vessels were bubbled with air to maintain aerobic conditions. Following a 10-day acclimatisation phase, plants were exposed to the test item for 2 days during the equilibration phase, before starting the incubation phase lasting for 2 (t_1) or 8 (t_{end}) more days. All plants were monitored regularly and no unusual observation with respect to growth could be detected.

3. Sampling

The study was performed with two wheat plants per brown glass vessel. Analyses of pH and redox were conducted at 0 (t_{start}), 2 (t_0), 4 (t_1) and 10 days (t_{end}) after treatment. Harvest of plants were performed at t_1 and t_{end}. At all dates, radioactivity in the test solution, pH, oxygen saturation and volume of the solutions were determined. At t_{start}, t_1 and t_{end} biomass of the plants was determined for all (t_{start}) or harvested brown glass vessels (t_1, t_{end}).

3. Analytical Procedures

At each date of analysis (t_{start}, t_0, t_1, t_{end}), 3 aliquots of 0.5 mL were taken from each radioactive test system and overall contained radioactivity was determined by liquid scintillation counting (LSC). Additionally, at the plant harvest after 4 (t_1) and 10 days (t_{end}) after treatment, the root surfaces were washed with ACN/H_2O (4/1; *v/v*), washings were quantified by LSC. The amount of radioactivity in the test solution as well as in the root wash solution was determined by LSC. Radioactivity taken up into root tissues as well as radioactivity allocated to the shoots was determined by combustion in an oxygen atmosphere using an oxidiser. The released carbon dioxide was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

The purity of the stock solution, as well as the stability of respective aliquots used for application (application solution) before and after application were checked by High Performance Liquid Chromatography (HPLC) coupled with radiodetection. It was noted by the UKRMS that chemical stability vessels had not been exposed to the potential biota contained on the plant roots, therefore the chemical purity results may not be the same as those exposed to plant roots.

II. RESULTS AND DISCUSSION

A. PROPERTIES OF TEST SYSTEM

Oxygen saturation for all experiments except for one sample (161023PT11: 68%) was always above 79% and pH of the test solutions during the course of study ranged from 6.53 to 6.99 for the treated and 6.48 to 7.06 for the untreated test system.

B. PLANT CONTROLS

The health and growth of the wheat plants was visually assessed regularly. In parallel to the treated test systems untreated test systems (plant controls) were incubated to enable the detection of possible effects on plant growth and health induced by the test item. Wheat plants appeared healthy over the total study duration both in treated and untreated test systems.
Besides an increase of biomass detectable for all treated (0.57 g) and untreated plants (2.26 g) at the end of the incubation phase, plant vigour was evident from the detectable plant development. This was reflected by BBCH stages of approx. 11 at the start of acclimatisation phase and BBCH stages of approx. 14 to 15 at the end of incubation phase.

C. ANALYTICAL RESULTS

Mean material balances were 98.8% AR for t_1 (range from 97.5 to 99.4% AR) and 97.0% AR for t_{end} (range from 95.9 to 98.1% AR) (see Table B.8.1.3.1.3-3). The complete material balances found at all sampling intervals for all test systems demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

The total transpiration rates at the end of the incubation phase (t_{end}) ranged from 46.6 to 59.7 mL for treated plants and 49.6 to 58.4 mL for control plants. This corresponded with a net transpiration of 15.6% of the initial volume (335.12 mL) for the test vessels at the end of incubation phase, meeting the requirements of at least 15% net transpiration (see Table B.8.1.3.1.3-4).
The concentration of the test item in the test solution stayed nearly constant over the entire incubation phase of ten days. The mean initial concentration of pyrazolyl-labelled M12 in the test solutions at t_{start} amounted to 89.08 µg/L, increasing to 91.82 µg/L at t_1 and 101.05 µg/L at t_{end}. The mass of the test item decreased from 29.85 µg at t_{start} to 28.75 µg at t_1 and further decreased to 27.88 µg at t_{end} (Table B.8.1.3.1.3-5).
On average the root washing released 0.115 µg of the test item from the roots at t_1 and 0.079 µg at t_{end} (see Table B.8.1.3.1.3-6).
The separate analysis of roots and shoots showed, that at t_1 37.2% and at t_{end} 38.4% of radioactivity taken up was translocated from roots into shoots (see Table B.8.1.3.1.3-3).

Table B.8.1.3.1.3-3: Mass balance (in percentage of applied radioactivity)

	Recovery single samples					mean
Day 4 (t_1)						
Test solution t_{start}	100.0	100.0	100.0	100.0	100.0	**100.0**
Test solution t_1	96.6	95.8	96.7	96.5	94.9	**96.1**
Root wash	0.3	0.4	0.4	0.4	0.4	**0.4**
Test solution + root wash	97.0	96.2	97.1	96.8	95.3	**96.5**
Aliquots	1.4	1.4	1.4	1.4	1.4	**1.4**
Combustion shoots	0.5	0.4	0.3	0.4	0.3	**0.4**
Combustion roots	0.6	0.7	0.6	0.7	0.6	**0.6**
Translocation from roots to shoots	45.8	36.2	36.9	33.9	33.2	**37.2**
Plant total	1.1	1.0	0.9	1.1	0.9	**1.0**
Total recovery radioactivity	**99.4**	**98.6**	**99.3**	**99.3**	**97.5**	**98.8**
Day 10 (t_{end})						
Test solution t_{start}	100.0	100.0	100.0	100.0	100.0	**100.0**
Test solution t_{end}	93.1	94.3	93.9	92.9	93.8	**93.6**
Root wash	0.3	0.2	0.3	0.3	0.2	**0.3**
Test solution + root wash	93.4	94.6	94.2	93.3	94.0	**93.9**
Aliquots	1.4	1.4	1.4	1.4	1.4	**1.4**
Combustion shoots	0.9	1.1	1.4	1.2	0.5	**1.0**
Combustion roots	1.4	1.1	1.9	2.0	1.4	**1.6**
Translocation from roots to shoots	39.5	50.1	41.7	36.7	24.0	**38.4**
Plant total	2.4	2.2	3.3	3.2	1.9	**2.6**
Total recovery radioactivity	**97.1**	**98.1**	**97.4**	**96.4**	**95.9**	**97.0**

Table B.8.1.3.1.3-4: Water uptake

Sample	**sample ID**	**V t_{start} initial [mL]a)**	**V t_{end} initial [mL]**	**ΔV t_{start} → t_{end} [mL]**	**net ΔV t_{start} → t_{end} [mL]**	**ΔV t_{start} → t_{end} [%]**
Control plants	161018P1	336.71	281.40	55.31	52.16	15.49
	161018P2	338.33	276.78	61.55	58.40	17.26
	161018P3	340.92	288.13	52.79	49.64	14.56
	mean (P1-P3)			**56.55**	**53.40**	**15.77**
Day 4 (t_1)	161018PT7	335.19	319.90	15.29	13.90	4.13
	161018PT8	332.93	312.65	20.28	18.89	5.65
	161018PT9	333.83	311.12	22.71	21.32	6.36
	161018PT10	337.43	318.52	18.91	17.52	5.17
	161018PT11	335.23	315.63	19.60	18.21	5.41
	mean (PT7-PT11)			**19.36**	**17.97**	**5.34**
Day 10 (t_{end})	161018PT12	335.74	283.77	51.97	48.82	14.54
	161018PT13	339.06	289.29	49.77	46.62	13.75
	161018PT14	334.19	273.16	61.03	57.88	17.32
	161018PT15	333.54	270.73	62.81	59.66	17.89
	161018PT16	334.10	283.31	50.79	47.64	14.26
	mean (PT12-PT16)			**55.27**	**52.13**	**15.55**

P1-P3 = untreated plants, PT7-PT16 = treated plants

Vt_{start} initial = volume of test solution at the start of the equilibration after sampling, DAT-0

V t_{end} initial = volume of test solution at the end of the experiment, DAT-10

t_{start} = start of equilibration, DAT-0; t_{end} = final sampling, DAT-10

a) for PT7-PT11 $t_{end} = t_1$

Δ volume lost

Table B.8.1.3.1.3-5: Concentrations of M12 in test solutions

Date	Single values										Mean
	PT7	PT8	PT9	PT10	PT11	PT12	PT13	PT14	PT15	PT16	
Day 0 (t_{start})											
V t_{start} [mL]	335.19	332.93	333.83	337.43	335.23	335.74	339.06	334.19	333.54	334.10	335.12
C t_{start} [µg/L]	89.12	89.54	89.28	89.17	89.54	89.71	88.96	88.61	88.43	88.45	89.08
m t_{start} [µg]	29.87	29.8	29.80	30.09	30.02	30.12	30.16	29.61	29.49	29.55	29.85
Day 2 (t_0)											
V t_0 initial [mL]	325.36	322.61	321.94	327.28	325.17	323.46	327.91	323.47	319.78	323.33	324.03
C t_0 [µg/L]	90.23	90.38	91.57	90.71	89.78	91.12	90.81	89.96	91.13	90.31	90.60
m t_0 [µg]	29.36	29.16	29.48	29.69	29.19	29.47	29.78	29.10	29.14	29.20	29.36
Day 4 (t_1)											
V t_1 final [mL]	318.12	311.23	307.50	316.97	312.05						313.17
C t_1 [µg/L]	90.75	91.78	93.72	91.56	91.27						91.82
m t_1 [µg]	28.87	28.56	28.82	29.02	28.48						28.75
Day 10 (t_{end})											
V t_{end} final [mL]						280.14	285.92	269.33	266.89	277.98	276.05
C t_{end} [µg/L]						100.10	99.53	103.22	102.70	99.71	101.05
m t_{end} [µg]						28.04	28.46	27.80	27.41	27.72	27.88

PT7-PT16 = treated plants
V t_{start} = volume of test solution at the start of the equilibration after sampling, DAT-0
V t_0 initial = volume of test solution at the end of the equilibration before sampling, DAT-2
V t_1 final = volume of test solution after sampling, DAT-4
V t_{end} final = volume of test solution at the end of the experiment after sampling, DAT-10
c t_{start} = concentration of test item in test solution at the start of equilibration phase after sampling, DAT-0
c t_0 = concentration of test item in test solution at the end of the equilibration before sampling, DAT-2
c t_1 = concentration of test item in test solution at the interim sampling after sampling, DAT-4
c t_{end} = concentration of test item in test solution at the end of the experiment, DAT-10
m t_{start} = mass of test item in test solution at the start of equilibration phase after sampling, DAT-0
m t_0 = mass of test item in test solution at the end of the equilibration before sampling, DAT-2
m t_1 = mass of test item in test solution at the interim sampling after sampling, DAT-4
m t_{end} = mass of test item in test solution at the end of the experiment after sampling, DAT-10

Table B.8.1.3.1.3-6: Concentrations of M12) in root wash.

Date	Single values										Mean
	PT7	PT8	PT9	PT10	PT11	PT12	PT13	PT14	PT15	PT16	
Day 4 (t_1)											
c t_1 [µg/L]	1.23	1.42	1.33	1.45	1.57						1.44
m $_{root\ wash}$ t_1 [µg]	0.099	0.119	0.108	0.116	0.116						0.115
Day 10 (t_{end})											
c t_{end} [µg]						1.07	0.87	1.06	1.15	0.87	0.99
m $_{root\ wash}$ t_{end} [µg]						0.087	0.068	0.084	0.095	0.070	0.079

PT7-PT11 = treated plants
c t_1 root wash = concentration of test item in root wash solution at the interim sampling, DAT-4
c t_{end} root wash = concentration of test item in root wash solution at the end of the experiment, DAT-10
m t_1 root wash = mass of test item in root wash solution at the interim sampling, DAT-4
m t_{end} root wash = mass of test item in root wash solution at the end of the experiment, DAT-10

D. CALCULATION OF PUFs AND TSCFs

The PUF in wheat for pyrazolyl-labelled M12 amounted to 0.24 ± 0.05 (t_0-t_1) and 0.26 ± 0.04 (t_0-t_{end}). The mean PUF amounted for to 0.25 ± 0.04, indicative for a slightly inhibited plant uptake of the test item in comparison to water uptake (see Table B.8.1.3.1.3-7).

(equation 1 [4])

$$PUF = \frac{\ln\left(\frac{m_{sol}}{m_0}\right)}{\ln\left(\frac{V_{sol}}{V_0}\right)}$$

With

m_0: mass of test item at the start of the experiment (t_0, at the end of the equilibration phase) [µg]

V_0: volume of test solution at the start of the experiment (t_0, at the end of the equilibration phase) [L]

m_{sol}: mass of test item at a given time t after the start of the experiment [µg]

V_{sol}: volume of test solution at given time t after the start of the experiment [L]

(equation 2)

$$m_{sol} = c_{sol} \times V_{sol} + m_{aliquots} + m_{rootwash}$$

With

m_{sol}: mass of test item in test solution [µg]

c_{sol}: concentration of test item in test solution at a given time t after the start of the experiment [µg/L]

V_{sol}: volume of test solution at a given time t after the start of the experiment [L]

$m_{aliquots}$: sum of test item mass in removed aliquots [µg]

$m_{root\ wash}$: mass of test item in root wash [µg]

The volume of test solution was derived by using equation 3.

(equation 3)

$$V_{sol} = \frac{\left(m_{flask} - m_{flask,empty} - m_{plant}\right)}{\rho_{sol}} + V_{aliquots} + V_{evaporation}$$

With

V_{sol}: volume of test solution [L]

m_{flask}: weight of test vessel filled with test solution [g]

$m_{flask,\ empty}$: weight of empty test vessel [g]

m_{plant}: weight of plant at a given time t after the start of the experiment [g]

ρ_{sol}: density of test solution assumed to be 1 [kg/L]

$V_{aliquots}$: sum of aliquot volumes removed to determine test item concentration [L]

$V_{evaporation}$: volume of solution lost due to evaporation [L]

(equation 4)

$$TSCF = \frac{\ln\left(1 - \frac{m_{shoots}}{m_{shoots} + m_{sol}}\right)}{\ln\left(\frac{V_{sol}}{V_0}\right)}$$

With

V_{sol}: volume of test solution [L]

V_{start}: volume of test solution at the start of the experiment (at the start of the equilibration phase) [L]

m_{sol}: mass of test item in test solution [µg]

m_{shoots}: mass of test item in shoots [µg]

Table B.8.1.3.1.3-7: Plant uptake factor (PUF) for M12 in wheat plants

	PUF_{wheat}		
	t_0-t_1	**t_0-t_{end}**	**overall**
single samples	0.20	0.28	
	0.21	0.27	
	0.22	0.19	
	0.31	0.29	
	0.29	0.29	
mean	**0.24**	**0.26**	**0.25**
SD	0.05	0.04	0.04
CV [%]	19.17	13.81	16.96

SD = standard deviation; CV = coefficient of variation
t_0 = end of equilibrium phase, DAT-2
t_1 = interim sampling, DAT-4
t_{end} = end of study at DAT-10

The mean TSCFs in wheat for pyrazolyl-labelled M12 amounted to 0.07 ± 0.02 (t_{start}-t_1) and 0.06 ± 0.02 (t_{start}-t_{end}), indicative for the translocation of the test item (or equivalents) from root to shoot tissues (see Table B.8.1.3.1.3-8).
For all plants analysed, the relative amount of the test item that was taken up by the roots and allocated to the shoots ranged from 24.0 to 50.1%.

Table B.8.1.3.1.3-8: Transpiration stream concentration factor (TSCF) for M12 in wheat plants

	$TSCF_{wheat}$		
	t_{start}-t_1	**t_{start}-t_{end}**	**overall**
single samples	0.11	0.06	
	0.06	0.07	
	0.04	0.07	
	0.07	0.06	
	0.05	0.03	
mean	**0.07**	**0.06**	**0.06**
SD	0.02	0.02	0.02
CV [%]	34.23	27.44	32.12

SD = standard deviation; CV = coefficient of variation
t_{start} = start of equilibration, DAT-0;
t_1 = interim sampling, DAT-4
t_{end} = end of study at DAT-10

III. CONCLUSIONS

The RMS acknowledges that good plant health indicated by biomass increase and water consumption throughout the testing period were demonstrated.
The deviations between the values of individual test replicates were low indicated by an overall coefficient of variation of 16.96% (PUF) and 32.12% (TSCF), respectively. Furthermore, the reliability of this plant uptake experiment was confirmed as the reduced test item amount in the test solution at the end of the incubation phase could be recovered in the plants with a recovery of 97.0%.

The PUF proposed by the applicant for pyrazolyl-labelled M12 in wheat plants (variety: *Thasos*) was determined as 0.25 ± 0.04. The calculated TSCF values amounted to 0.07 ± 0.02 (t_{start}-t_1) and 0.06 ± 0.02 (t_{start}-t_{end}), indicative for the translocation of the test item (or equivalents) from root to shoot tissues. For all plants analysed, more than 24.0% of the test item taken up by the roots was allocated to the shoots.

However the RMS has some concerns over some aspects of the study design.

UK RMS notes that no experiment was performed to assess if the concentration of test material in the solution has an influence on plant uptake. No indication is provided that the concentration of M12 used is similar to that of the soil pore water predicted by FOCUS PEARL and PELMO.

UK RMS notes that no biocides were added to the nutrient/ test solution. This solution is potentially an ideal medium for bacteria from the air or transferred to the roots to grow. No experimental assessment has been provided, to demonstrate that breakdown of the test substance by biotic degradation (introduced via the addition of plants) in the nutrient solution has not occurred. Thus it cannot be demonstrated that any uptake is of parent "M12" only and not of metabolites formed via degradation. UK RMS considers this to be a critical omission.

UK RMS also notes that plants selected are winter wheat, which have been grown indoors from seed. These will not have been subject to typical temperatures and growth conditions; such that the waxes typically formed to protect the plant and retain moisture will not be present. With plant growth in ideal conditions the volumes of water up taken (20°C and 18 hrs of daylight, not typical conditions for winter wheat at BBCH 13-21) the volumes of water consumed by the plants may not be considered typical or field gown plants. The rate of growth may not be typical of field grown plants and may influence the uptake of water and nutrients to maintain that growth. Rates of water consumption may not match those used in the FOCUS ground water models.

It is a known phenomena that plants grown under hydroponic conditions produce a larger root mass than those grown in soil, the TSCF value may be unreliable if the weight of root is greater than that of field grown wheat plants

Due to the pH dependency of M12 the applicant has chosen to use the default value of 0 in FOCUS modelling. The study is retained for reference and not used further in the assessment of Isoflucypram and its metabolites.

B.8.1.3.2 Column leaching studies

B.8.1.3.2.1 Column leaching of the active substance

No column leaching studies were performed for isoflucypram. The potential mobility can be determined from the adsorption/desorption studies described under B.8.1.2.1

B.8.1.3.2.2 Column leaching of metabolites, breakdown and reaction products

No column leaching studies were performed for the major soil degradation product of isoflucypram. The potential mobility can be determined from the adsorption/desorption studies described under B.8.1.2.2

B.8.1.3.2.3 Lysimeter studies

The leaching behaviour of isoflucypram and its major soil metabolite M12 are addressed by standard Focus groundwater modelling. Therefore, lysimeter studies were not conducted.

B.8.1.3.2.4 Field leaching studies

Field leaching studies have not been conducted for the active substance as sufficient information can be derived from the existing studies.

B.8.1.3.2.5 Summary of the adsorption and desorption of Isoflucypram and its metabolites.

The soil adsorption properties of isoflucypram have been assessed in 2 studies, Stupp, H. P.; Junge, T.; (2014), see section B.8.1.2.1.1 and Herczog, K. J. S.; (2015),see section B.8.1.2.1.2. 7 soils in total were tested and both studies were considered to be acceptable, however one soil in Herczog, (2015) was not accepted by the RMS as it was considered to be a sediment and not applicable. Furthermore, one further soil in Herczog (2015) had such poor mass balance (79.3-102.9-%, with 3 concentrations below 90%) that the results from this soil could not be accepted. The Freundlich isotherm parameters for an additional soil in Herczog (2015) was re-calculated by the RMS for the 4 acceptable concentrations as the 0.0015µg/ml concentration was not considered reliable due to mass balance issues. A summary table of the values accepted by the RMS is included below see table B.8.1.3.2.5-1.

The adsorption properties of metabolite M-12 have been assessed in 2 studies D'Ambrosio, A.; (2014) see section B.8.1.2.2.1 and Shrestha, S.; (2017) see section B.8.1.2.2.2. Both studies were considered to be acceptable by the RMS and the applicants proposed values accepted. A summary of the values determined are listed in table B.8.1.3.2.5-2.

No lysimeter or column leaching studies were submitted by the applicant as the OECD 106 sorption studies have addressed the data requirement.

The transpiration stream concentration factor of isoflucypram and the metabolite M12 has been assessed by a calculation method in (Reinken, G.; Kallweit, W.; (2017)) and in two laboratory assessments (Daumann, M.; (2017_{a+b})). In the Reinken & Kallweit; (2017) study the log Pow was used in the Briggs equation (Briggs et al (1982)) to calculate the TSCF value of 0.10 for isoflucypram. The log Pow for M12 was determined to be pH dependant, therefore the default value of 0 was selected. In line with FOCUS (2014) guidance an experimentally determined PUF/ TSCF value was attempted. No E.U. guidance was available a the time of study conduct and evaluation and the studies were assessed on merit. UK RMS notes some potential deficiencies with the studies and proposes that neither study is acceptable for regulatory purposes. However the RMS notes that the experimentally derived TSCF value for isoflucypram of 0.14 and the value determined using the Briggs equation of 0.1 were similar. For Predicted Environmental Concentration calculations a TSCF value of 0.1 is agreed by the RMS for isoflucypram and 0 for the metabolite M12.

Table B.8.1.3.2.5-1: Summary of the adsorption values for isoflucypram.

Soil	$K_{f(ads)}$ [mL/g]	1/n	$K_{oc(ads)}$ [mL/g]	r^2
Laacher Hof AXXa	29.184	0.8904	1389.7	0.9963
Hoefchen am Hohenseh 4a	29.812	0.8788	1569.1	0.9979
Hanscheider Hof	32.430	0.8972	1410.0	0.9976
Dollendorf II	58.711	0.8690	1151.2	0.9976
Sanger	11.257	0.9985	1250.8	0.9891
Arith. mean	**32.28**	**0.907**	**1354.2**	**0.996**
Geo. mean			**1346.6**	

Table B.8.1.3.2.5-2: Summary of the adsorption values for M-12.

Soil	pH (Cacl2)	OC %	$K_{f(ads)}$ [mL/g]	1/n	$K_{oc(ads)}$ [mL/g]	r^2
Wurmwiese	5.3	1.9	2.0	0.9297	105.8	0.9983
Hoefchen am Hohenseh 4a	6.3	2	0.8	0.8952	37.9	0.9978
Dollendorf II	7.3	4.5	1.3	0.9243	28.1	0.9988
Guadelupe	6.7	0.7	0.3	0.9311	38.4	0.9973
Springfield	6.6	1.7	1.2	0.9185	70.7	0.9983
END	4.9	0.94	2.724	0.9497	289.8	0.9989
MMN	7.7	2.4	1.178	0.9604	49.1	0.9992
SCA	5.6	0.3	0.544	0.8966	187.5	0.9997
SKS	5.8	1.8	1.727	0.8914	95.9	0.9995
Arith. mean			**1.31**	**0.922**	**100.36**	**0.999**
Geo. mean					**75.35***	

* Potential pH dependence is show, please see discussion below

pH dependence of M12

The adsorption of M12 to soil was investigated in two batch equilibrium studies, one with five soils by D'Ambrosio, A.; (2014) and one with four soils by Shrestha, S.; (2017); . The K_{foc} values ranged from 28.1 to 289.8 mL/g, with Freundlich exponents between 0.8914 and 0.9604 (Table B.8.1.2.2.5-2).

The adsorption of M12 to soil is proposed by the applicant to be pH dependent following an S shaped curve which is typical for many ionic substances with a single ionisable functional group (Figure B.8.1.3.2.5- 1). A sigmoid correlation analysis of pH and K_{foc} values predicted with the German spreadsheet 'Input Decision Tool version 3.3' resulted in a K_{foc} for M12 of 37.7 mL/g considering an "apparent" pKa of 5.6 in soil. Please note that the "apparent" pKa in soil needs to be estimated as the pKa in soil is increased compared to the pKa determined in water as sorption processes are reducing the water available substance amount. UK RMS has validated this result using the same model and reaches the same conclusions, noting the lack of values for soil pH values above 8. Whilst the German tool is not agreed for use in EU-level approval assessments, it was considered to give useful assessment of the adsorption data. However, parameters calculated by the tool have not been proposed for use in risk assessment. pH in Cacl2 is converted into pH in H_2O by the model.

The geometric mean K_{foc} value for soils at pH 7.5 is 37.1 mL/g, with an arithmetic mean Freundlich exponent of 0.9424 (Table B.8.1.3.2.5- 4). The geometric mean K_{foc} value for weak acidic soils with a pH of about 5.4 is 153.2 mL/g, with an arithmetic mean Freundlich exponent of 0.9169 (Table B.8.1.3.2.5- 3).

EFSA (2013) and FOCUS groundwater (2014) recommend that Tier 1 leaching simulations for consideration of EU approval should select adsorption values, chosen to represent a realistic worst case considering the pH of the soils in the EU that are used for the production of the pertinent crop, which in this case is cereals. For a compound with a single ionisable functional group that follows a typical S shaped relationship for adsorption with pH, such as a weak acid, two contrasting pH values for which realistic best case and realistic worst case adsorption estimates may be selected to consider the impact of variable soil pH values relevant for the crop growing situation (*e.g.* pH of 7.5 and pH of 5.4 as relevant range for cereal growing conditions; with an optimum pH of about 6.5 for cereals).

Thus, the adsorption of M12 can be described

- by the geometric mean K_{foc} value of 37.1 mL/g and the arithmetic mean Freundlich exponent 1/n of 0.9424 to represent a realistic worst case (neutral soil conditions around pH 7.5);
- by the geometric mean K_{foc} value of 153.2 mL/g and the arithmetic mean Freundlich exponent 1/n of 0.9169 to represent a realistic best case (weak acidic soil conditions around pH 5.4).

The selection of Koc values for M12 for specific environmental exposure assessments is detailed in the CP document.

Figure B.8.1.3.2.5- 1: K_{oc}-pH_{water} correlation for M12 evaluated with Input Decision Tool version 3.3

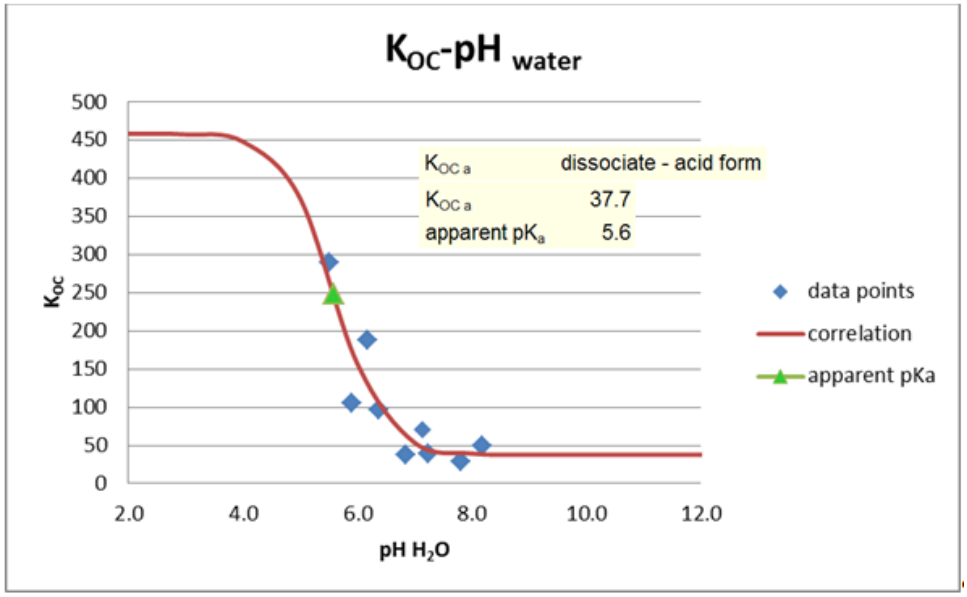


Table B.8.13.2.5-3: Soil adsorption/desorption for M12 at weak acidic soil conditions (around pH 5.4)

Soil name	Soil type	OC [%]	pH ($CaCl_2$)	K_f [mL/g]	K_{foc} [mL/g]	1/n
Wurmwiese, GER	sandy loam	1.9	5.3	2.0094	105.8	0.9297
Northwood, North Dakota, USA	loamy sand	0.94	4.9	2.724	289.8	0.9497
Sanger, California, USA	sandy loam	0.29	5.6	0.544	187.5	0.8966
Stilwell, Kansas, USA	silty clay loam	1.80	5.8	1.727	95.9	0.8914
Geometric mean (n=4)					**153.2**	
Arithmetic mean (n=4)						**0.9169**
pH-dependency					**yes – pH 5.4**	

Table B.8.1.2.2.5-4: Soil adsorption/desorption for M12 at neutral soil conditions (around pH 7.5)

Soil name	Soil type	OC [%]	pH ($CaCl_2$)	K_f [mL/g]	K_{foc} [mL/g]	1/n
Dollendorf II, GER	loam	4.5	7.3	1.2635	28.1	0.9243
Morris, Minnesota, USA	clay loam	2.40	7.7	1.178	49.1	0.9604
Geometric mean (n=2)					**37.1**	
Arithmetic mean (n=2)						**0.9424**
pH-dependency					**yes – pH 7.5**	

CONCLUSIONS

For the leaching calculations, the adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1346.6 mL/g (K_{fom} = 781.1 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.907. For the environmental exposure assessment of M12, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.

B.8.2. FATE AND BEHAVIOUR IN WATER AND SEDIMENT

The degradation of isoflucypram in aquatic systems has been addressed by the submission of one Hydrolysis study (Heinemann, O.; Kasel, D.; (2015a)), one Quantum yield study (Heinemann, O.; (2013)) and one photolytic degradation study (Heinemann, O.; Kasel, D.; (2015b)). A statement has been supplied by the applicant to address the effects on the water treatment process. No ready biodegradation study was submitted. An aerobic mineralisation study Gabbert, D.; Smith, E.; (2017) and 1 water sediment study Hein, E. M.; Kasel, D.; (2017) have been submitted. The notifer has stated that analysis in the pyrazole label is sufficient as, similar to soil degradation studies, no cleavage of the molecule is noted in any study and only 1 metabolite M12 was observed.

B.8.2.1. Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

Statement on water treatment processes

The applicant submitted a statement in respect to Article 4, 3(b) of Regulation (EC) 1107/2009. This is reproduced below in full.

"The request, complex by nature, is to be found in EC Regulation 1107/2009. However, it was not subject to specification at EU and Member State level, for example, in times of inclusion into Commission Regulation 283/2013 defining actual data requirements or, in Commission Regulation 2013/C95/01 defining and specifying the tests to serve as the data basis for evaluation. Beyond the general data requirements there was no specific guidance or interpretation given in the EU or national context. Even if standardized and reliable data were available it would be another step to define the complete steps in risk assessment including results of potential tests, their interpretations and to draw conclusions based on realistic scenarios that remain to be developed.
In the absence of tests, guidelines and guidance it thus remains with some general principles as for any chemical reaction. The progress of transformation of 'organic residues' is dependent on a full range of parameters of influence, for example: concentration of chemical (= residue) to be oxidised available, extent of other organic matter available for oxidation in water, potential catalytic influence of ions of

heavy metals, the concentration of the oxidizing chemicals (ozone/chlorine), the contact time and temperature.

Dependent on parameters of influence the transformation of residues can be expected to range from no to even complete mineralisation.
Again and with no tests specifying influence or test parameters realistically in more detail, an estimated outcome would remain fully speculative and thus not scientific. No evaluation can be made currently on the effect of water treatment processes on the nature of the residue.
In the absence of agreed and harmonised testing procedures including guidance on evaluation and interpretation of results at EU level this request is clearly out of scope for a qualified reply in the current registration process of the active substance isoflucypram.
In summary and in light of the absence of harmonised testing procedures as well as guidelines on evaluation and interpretation of these results and their use for risk assessments a scientifically qualified response to this complex requirement cannot be delivered to date".

RMS notes the lack of any guidance on conducting such an assessment.

B.8.2.1.1.: Hydrolytic degradation

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; Kasel, D.; 2015a;
Title: [Pyrazole-4-14C]BCS-CN88460: Hydrolytic degradation
Report No.: EnSa-14-1032
Document No.: M-510623-01-1
Guideline(s): OECD Test Guideline No. 111; Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009; US EPA OCSPP Test Guidelines No. 835.2120 and No. 835.2130; Japanese MAFF Test Guidelines 12 Nousan 8147, No. 2-6-1
Guideline deviation(s): None
GLP/GEP: yes

Study Summary

The hydrolytic route and rate of degradation of pyrazole-labelled isoflucypram were studied in sterile aqueous buffer solutions at three pH values (pH 4, 7 and 9) in the laboratory in the dark at 50.0°C for 7 days. The test concentrations were between 0.41 and 0.44 mg/L.
The test was performed in static systems consisting of 10 mL glass crimp-top vials each containing 5 mL of test solution closed by crimp caps with Teflon®-faced septa.
Duplicate samples were processed and analysed 0, 0.17 (4 hours), 1, 3 and 7 days after treatment (DAT). At each sampling interval, the amount of test item in test solutions was determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The test item was identified by HPLC-MS(/MS) including accurate mass determination.
Mean material balances were 100.8% AR for pH 4 (range from 97.1 to 106.9% AR), 102.8% AR for pH 7 (range from 100.0 to 107.1% AR) and 103.2% AR for pH 9 (range from 100.0 to 106.3% AR). Carbon dioxide and volatile organic compounds were not collected.
The amount of isoflucypram in the test solutions ranged from DAT 0 to DAT 7 between 97.1 and 106.9% AR for pH 4, between 100.0 and 107.1% AR for pH 7 and between 100.0 and 106.3% AR for pH 9. No degradation products of isoflucypram were observed.
Isoflucypram was stable at all pH values, therefore no DT_{50} and DT_{90} values were calculated.

It is concluded that hydrolytic degradation is unlikely to contribute to the degradation of isoflucypram under typical conditions of the environment.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item:

Pyrazole-labelled isoflucypram
Sample-ID: KML 9803
Specific activity: 4.22 MBq/mg (113.92 µCi/mg)
Radiochemical purity: > 98% (HPLC with radioactivity detector)
> 98% (TLC, scan)
Chemical purity: > 98% (HPLC with UV-detector, 210 nm)

Reference item:
No reference items were used.

2. Buffer Solutions

The study was carried out using three different buffer solutions of pH 4.0, pH 7.0 and pH 9.0. The buffer solutions were prepared with concentrations of ≤ 0.01 mol/L to minimise buffer reactions.

Table B.8.2.1.1- 1: Buffer solutions

pH	Type and final molarities
4.0	acetate, 0.01 M
7.0	TRIS, 0.01 M
9.0	borate, 0.01 M

B. STUDY DESIGN

1. Experimental Conditions

Glass crimp-top vials (*e.g.* 10 mL) closed by crimp caps with Teflon®-faced septa were used as test vessels. Carbon dioxide and volatile organic compounds were not collected.
All glassware and aqueous buffer solutions were sterilised prior to use by autoclaving twice for 20 minutes at 121°C and stored under a clean bench to prevent biodegradation of the test item during the study. The oxygen contained in the buffer solutions was depleted before sterilization by purging the buffer solutions with nitrogen.
For each pH value, 5 mL aliquots of the respective test solutions were distributed into the test vessels which were then closed with Teflon®-faced septa and placed in a temperature-controlled water bath. For DAT-0 samples, aliquots of the test solutions were processed and analysed immediately after application.
The amounts of applied test item were determined at DAT 0 as 9193 Bq (equal to 0.44 mg/L) for pH 4, 8673 Bq (equal to 0.41 mg/L) for pH 7 and 8796 Bq (equal to 0.42 mg/L) for pH 9 (see Table B.8.2.1.1-2). These values were set to 100% of applied radioactivity (AR) for the samples of the respective pH value.
The test systems were incubated in a temperature-controlled water bath in the dark at a temperature of 50°C.

Table B.8.2.1.1- 2: Application rate

0.01 M buffer pH	Radioactivity applied [Bq]	Test concentration [mg/L]
4	9193	0.44
7	8673	0.41
9	8796	0.42

2. Sampling

Five sampling intervals were distributed over the entire incubation period of 7 days. Duplicate samples were processed and analysed 0, 0.17 (4 hours), 1, 3 and 7 days after treatment (DAT).

3. Analytical Methodology

Sample preparation and processing

The pH values of the test solutions were determined at each sampling interval in the unprocessed test systems.
The sterility was checked at each sampling interval for all samples.
At each sampling interval, the radioactivity content of the samples was determined by LSC.
No isolation of degradation products was performed since no degradation product was observed.
All LSC and HPLC/radiodetection measurements were carried out without concentration steps.

Sample analysis

Radioactivity contents in samples were determined generally in duplicate by LSC.
Radioactivity contents in liquid samples (test solutions and recoveries) were determined using aliquots of up to 0.5 mL with 2 mL Quicksafe® A containing 5% water (sample counting time in general = 10 minutes, background = 12 – 14 cpm).
At each sampling interval aliquots of the test solutions were characterised by the primary chromatographic method (RP HPLC/radiodetection system).
HPLC hyphenated to electrospray ionization mass spectrometry in single or multistage mode (ESI-MS(/MS)) and radiodetection was used for confirmation of the test item identity.

II. RESULTS AND DISCUSSION

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean recovery of the test item at DAT 0 was 100% AR for all pH values demonstrating that the sample processing method was well suited to recover the applied test item from the test solution and that the test item was stable under these conditions.

2. Verification of Chromatographic Procedures

The primary chromatographic method (HPLC/radiodetection) was well suited for the quantitative analysis of the samples of this study as demonstrated by a mean HPLC recovery of 106.2% and a good linear fit for injected amounts of pyrazole-labelled isoflucypram from 8.8 to 1846 Bq absolute on column ($R^2 \geq 0.9996$).

The LOD of the primary chromatographic method was determined as 8.8 Bq absolute on column or 1.0% AR.

B. MATERIAL BALANCE

Mean material balances were 100.8% AR for pH 4 (range from 97.1 to 106.9% AR), 102.8% AR for pH 7 (range from 100.0 to 107.1% AR) and 103.2% AR for pH 9 (range from 100.0 to 106.3% AR) (Table B.8.2.1.1- 3).

The complete material balances found at all sampling intervals for all pH values demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.2.1.1- 3: Material balance of radioactivity in buffer solutions at pH 4, 7 and 9 (expressed as percentage of applied radioactivity, means of two replicates)

Material balance	Buffer solution		
	pH 4	pH 7	pH 9
Min	97.1	100.0	100.0
Max	106.9	107.1	106.3
Mean	100.8	102.8	103.2
RSD	3.4	2.4	2.1

RSD = relative standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.2.1.1- 4.

The hydrolytic route of degradation of isoflucypram at three pH values is summarised in Table B.8.2.1.1- 5 (mean values).

Table B.8.2.1.1- 4: Material balances of radioactivity under hydrolytic condition at 50°C

Buffer solution	Replicate no.	Days after treatment				
		0	0.17	1	3	7
pH 4	1	98.3	103.1	102.4	98.9	94.6
	2	101.7	110.8	100.5	98.3	99.5
	mean	**100.0**	**106.9**	**101.5**	**98.6**	**97.1**
pH 7	1	100.5	104.9	97.9	104.3	102.5
	2	99.5	109.2	105.4	99.8	104.2
	mean	**100.0**	**107.1**	**101.6**	**102.0**	**103.3**
pH 9	1	100.0	103.6	105.6	102.5	104.3
	2	100.0	104.7	107.0	101.3	103.5
	mean	**100.0**	**104.2**	**106.3**	**101.9**	**103.9**

Table B.2.1.1- 5: Hydrolytic degradation of isoflucypram at 50°C (expressed as percentage of apllied radioactivity, mean of two replicates)

Buffer solution	Compound	Days after treatment				
		0	0.17	1	3	7
pH 4	Isoflucypram	100.0	106.9	101.5	98.6	97.1
	sum of unid,/diff. residues[a)]	n.d.	n.d.	n.d.	n.d.	n.d.
	total recovery[b)]	100.0	106.9	101.5	98.6	97.1
pH 7	Isoflucypram	100.0	107.1	101.6	102.0	103.3
	sum of unid,/diff. residues[a)]	n.d.	n.d.	n.d.	n.d.	n.d.
	total recovery[b)]	100.0	107.1	101.6	102.0	103.3
pH 9	Isoflucypram	100.0	104.2	106.3	101.9	103.9
	sum of unid,/diff. residues[a)]	n.d.	n.d.	n.d.	n.d.	n.d.
	total recovery[b)]	100.0	104.2	106.3	101.9	103.9

n.d. = not detected

a) Minor degradates are summed up to sum of unidentified / diffuse residues

b) Difference to material balance values due to rounding errors as well as clean up and chromatographic losses

Carbon dioxide and volatile organic compounds

Carbon dioxide and volatile organic compounds were not collected.

Test item and degradation products in test solutions

The amount of isoflucypram in the test solutions ranged from DAT 0 to DAT 7 between 97.1 and 106.9% AR for pH 4, between 100.0 and 107.1% AR for pH 7 and between 100.0 and 106.3% AR for pH 9 (see Table B.8.2.1.1- 5 above). No degradation products of isoflucypram were observed.

D. DEGRADATION

Isoflucypram was stable at all pH values, therefore no DT_{50} and DT_{90} values were calculated.

E. DEGRADATION PATHWAY

Isoflucypram was stable at all pH values, therefore no degradation pathway is proposed.

III. CONCLUSIONS

Isoflucypram was hydrolytically stable in sterile aqueous buffer solutions at three pH values (pH 4, 7 and 9) in the laboratory in the dark at 50°C. No degradation products of isoflucypram were observed.

Hydrolytic degradation is unlikely to contribute to the degradation of isoflucypram under typical conditions of the environment.

B.8.2.1.2.: Quantum yield of isoflucypram

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; 2013
Title: [Pyrazole-4-14C]BCS-CN88460: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water
Report No.: EnSa-13-0236
Document No.: M-461939-01-1
Guideline(s): Draft SANCO 11802/2010/rev 7 in accordance with Regulation (EC) No 1107/2009, 2012; OECD Test Guideline 101, 1981; OECD Test Guideline 316, 2008
Guideline deviation(s): RMS notes the use of buffers containing acetonitrile above the organic solvent content of 1% as stated in section 34.
GLP/GEP: yes

Study Summary

The quantum yield of the direct phototransformation of isoflucypram was determined in buffered aqueous solutions using polychromatic light according to the ECETOC method. Degradation of isoflucypram in neutral aqueous solution in a range of 8 to 21% was measured by HPLC-radiodetection after a maximum irradiation period of 500 minutes. This indicated moderate degradability of isoflucypram via direct phototransformation in neutral buffered solutions. A low mean quantum yield of Φ = 0.00077 was calculated on the basis of UV absorption data and the degradation kinetics determined from both experiments.

The UV-VIS absorption spectrum of isoflucypram in phosphate buffer pH 7/acetonitrile (4/1, v/v) showed two maxima at 195 nm (abs 1.38) and 218 nm (abs 0.598). The UV-VIS absorption spectra of isoflucypram in acetate buffer pH 4/acetonitrile (4/1, v/v), in borate buffer pH 9/acetonitrile (4/1, v/v) and in water containing isoflucypram showed similar absorption properties. The molar extinction coefficient ε of isoflucypram in buffer pH 7 / ACN (4/1, *v/v*) at 290 nm was determined to 86 L x mol^{-1} cm^{-1} and at 295 nm to 75 L x mol^{-1} cm^{-1}.

The estimates based on the two modelling concepts (Zepp & Cline, Frank & Kloepffer) were comparable. Both estimates considered the quantum yield Φ and the absorption in the UV-VIS spectrum being in the range of wavelengths relevant for the environment (see tables below). Environmental half-lives of sunlight exposed top surface water layers were estimated to 8 to 22 days for a direct phototransformation of isoflucypram during periods of main use in spring to fall.
Thus, direct phototransformation in neutral aqueous solution may contribute to the dissipation of isoflucypram from the environment. This assessment does not consider other potential mechanisms which may enhance the degradation in natural water, e.g. by indirect photolytic processes. However, in neutral and alkaline aqueous solutions hydrolytic degradation is regarded as the predominant route of dissipation.

Table B.8.2.1.2- 1: Zepp and Cline Modelling (GC Solar)

Season	**Environmental DT_{50} of the direct phototransformation of isoflucypram in buffer pH 7 [days]**			
	30th degree lat.	**40th degree lat.**	**50th degree lat.**	**60th degree lat.**
Spring	8.2	8.8	**9.8**	12
Summer	7.4	7.5	**7.7**	8.2
Fall	12	15	**21**	39
Winter	15	23	45	138

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day.
The column of the 50th degree of latitude is more or less relevant to the conditions of Central Europe.

Table B.8.2.1.2- 2: Frank and Kloepffer Modelling

Month	Photolysis constant [1/sec]	Environmental DT_{50} of the direct phototransformation of isoflucypram in buffer pH 7 [days]		
		minimum	mean	maximum
January	0.692×10^{-7}	55	120	530
February	0.144×10^{-6}	27	56	240
March	0.283×10^{-6}	15	28	120
April	0.475×10^{-6}	**9.4**	17	68
May	0.605×10^{-6}	**8.3**	13	53
June	0.678×10^{-6}	**7.9**	12	47
July	0.604×10^{-6}	**8.8**	13	44
August	0.590×10^{-6}	**9.1**	14	45
September	0.342×10^{-6}	**14**	23	87
October	0.188×10^{-6}	**22**	43	190
November	0.831×10^{-7}	42	97	482
December	0.436×10^{-7}	84	180	920

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50^{th} degree lat.), no contribution of another mono- or bimolecular elimination process.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item:

Pyrazole-labelled isoflucypram

Sample-ID:	KML 9480
Specific activity:	3.90MBq/mg (105.34 µCi/mg)
Radiochemical purity:	> 99% (HPLC with radioactivity detection)
	> 99% (TLC, scan)
Chemical purity:	> 99% (HPLC with UV-detector, 210 nm)

Pyrazole-labelled isoflucypram was used for the determination of the quantum yield instead of a non-labelled test item due to a solubility issue and thus detection issue: It was not possible to detect low concentrations of the test compound using UV detection - using the ^{14}C-labeled test item radiodetection was possible. UK RMS accepts this.

Reference item:

Non-labelled isoflucypram

Certificate-ID:	AZ 18080
Batch code:	BCS-CN88460-01-02
Certified assay:	98.4% w/w (^1H-NMR spectroscopy)

2. Test Systems

The study was carried out using three different phosphate/acetonitrile (4/1, v/v) buffer solutions of pH 4.0, pH 7.0 and pH 9.0.

Table B.8.2.1.2- 3: Buffer solutions

pH	Buffer
4	acetate buffer
7	phosphate buffer
9	borate buffer

The actinometry solution consisted of 0.01 mol/L of uranyl ions and 0.05 mol/L of oxalic acid.

B. STUDY DESIGN

1. Experimental Conditions

The test systems consisted of quartz cells with teflon plugs which were placed into an merry-go-round irradiation apparatus fitted with a mercury immersion lamp. When using this artificial light source, the higher-energy UV-rays (i.e. $\lambda < 295$ nm) are almost quantitatively absorbed by incorporation of a Duran-50-filter tube. The intensity of the light acting on the test solution was measured by means of uranyl oxalate as chemical actinometer. The merry-go-round irradiation apparatus was warmed up for at least 15 minutes prior to the exposure of the samples in order to guarantee a constant radiation of the light source as well as the projected sample temperature of 25C ± 1°C already at the beginning of the experiment. Subsequently, only the merry-go-round but not the lamp or the cycle cooling was switched off for adding or removing of samples. After the equilibration phase of the system, two measuring cells with 3.0 mL of actinometry solution were first exposed in the system for 10 minutes. After that the measuring cells containing 3.0 mL of test solutions each were swiftly placed onto the 10 positions of the merry-go-round apparatus. Degradation samples were incubated for 500 minutes in maximum.
LC-MS(/MS) verification spectra for test item and reference item were performed in the positive-ion electrochemical ionisation mode (ESI).

2. Sampling

One sample of each degradation experiment was taken and analysed after 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 minutes.

3. Analytical Procedures

At each sampling interval the isoflucypram concentration in the samples was determined by reversed phase HPLC and evaluation of the respective UV signal by means of external reference standard.

Each sample for actinometry was transferred into an Erlenmeyer flask and filled up with water to about 50 mL. Then 5 mL of solution was added to each sample. The mixture was slowly titrated with the titration solution under agitation by means of a magnetic stirrer, until the colour changed from colourless to pink. The consumption in mL was determined exactly to 0.05 mL and the mean of four replicates was taken.
The consumption of the titration solution in case of the unexposed actinometry solution was determined in the same way (blank value).

II. RESULTS AND DISCUSSION

A. UV-VIS ABSORPTION PROPERTIES

The UV-VIS absorption spectrum of a solution of 11.12 mg/L isoflucypram in phosphate buffer pH 7/acetonitrile (4/1, *v/v*) showed two maxima at 195 nm (abs 1.38) and 218 nm (abs 0.598. The UV-VIS absorption spectra of isoflucypram in acetate buffer pH 4/acetonitrile (4/1, *v/v*), in borate buffer pH 9/acetonitrile (4/1, *v/v*) and in water containing 11.12 mg/L isoflucypram showed similar absorption properties.

The molar extinction coefficient ε of isoflucypram in buffer pH 7/acetonitrile (4/1, *v/v*) at 290 nm was determined to 86 L x mol^{-1} cm^{-1} and at 295 nm to 75 L x mol^{-1} cm^{-1}.

In general, the absorption properties indicate a potential for direct photolytic interactions of isoflucypram with sunlight in aqueous solutions.

B. PHOTODEGRADATION OF PARENT COMPOUND

Degradation of isoflucypram was measured by HPLC-radiodetection during the maximum irradiation period of 500 minutes. For respective data see Table B.8.2.1.2- 4, for evaluation of data see Table B.8.2.1.2- 5.

Table B.8.2.1.2- 4: Photodegradation of isoflucypram in buffer at pH 7 (expressed as mg/L)

Experiment	Duration of irradiation [minutes]										
	0	**50**	**100**	**150**	**200**	**250**	**300**	**350**	**400**	**450**	**500**
#1	0.23[a)]	0.25	0.21	0.22	0.22	0.23	0.21	0.20	0.23	0.21	0.21[b)]
#2	0.24[a)]	0.22	0.22	0.22	0.21	0.21	0.22	0.21	0.22	0.20	0.19[c)]

a) 100%
b) 92%
c) 79%

Table B.8.2.1.2- 5: Statistics of photodegradation tests in buffer pH 7

	Experiment	
	#1	**#2**
No. of data pairs	11	11
Rate constant (k)	0.0002 1/min	0.0003 1/min
Half-life (DT_{50})	3905 min	2511 min
$t_{10\%}$ (DT_{10})	594 min	382 min
Correlation coefficient	-0.5073	-0.7993

C. QUANTUM YIELD OF THE DIRECT PHOTODEGRADATION

Based on both degradation experiments performed in buffer pH 7 quantum yields Φ of 5.96 x 10^{-4} (experiment #1) and 9.44 x 10^{-4} (experiment #2) were calculated. This results in a quite low mean quantum yield Φ of 0.00077 for the direct phototransformation in neutral aqueous solution, when the test item is regarded most stable with respect to hydrolysis.

D. ASSESSMENT OF SO-CALLED ENVIRONMENTAL HALF-LIFE OF DIRECT PHOTO-DEGRADATION IN WATER

Besides compound specific factors like the quantum yield Φ, the extent of absorption of a compound in the relevant range of the tropospheric sunlight spectrum, the degradation by sunlight is influenced by geographic, climatic, seasonal and matrix-specific conditions.

The environmental half-life for different conditions can be assessed by means of arithmetic models. A prerequisite for the assessments made in the following is the presence of the substance in aqueous

solution, i.e. surface water, rain, fog or aerosols water so that exposure to sunlight is given. Moreover it is taken for granted that water constituents do not reduce the intensity of sunlight.

With the arithmetic model developed by Zepp & Cline[1] it is possible to transfer laboratory data concerning direct photodegradation in water to field conditions. The model estimates on the basis of a clear summer sky with no influence of clouds. The half-lives calculated therefore may be regarded as minimum half-lives depending on frequency and extent of cover of sky by clouds.
Based on a mean quantum yield of $\Phi = 0.00077$ and the molar extinction coefficients determined for wavelengths of 297.5 to 490 nm, environmental half-lives were calculated. The results are summarised in the following table.

Table B.8.2.1.2- 6: Environmental half-lives calculated according to Zepp & Cline

Season	Environmental direct photolysis half-life of isoflucypram [days] buffer pH 7			
	30th degree lat.	40th degree lat.	50th degree lat.	60th degree lat.
Spring	8.2	8.8	9.8	12
Summer	7.4	7.5	7.7	8.2
Fall	12	15	21	39
Winter	15	23	45	138

In contrast to the model approach by Zepp and Cline, the arithmetic model developed by Frank & Klöpffer[2] considers the influence of clouded sky for the region Central Europe, i.e. Germany.

Using the mean quantum yield of $\Phi = 0.00077$ and the molar extinction coefficients from 292.5 to 490 nm, environmental half-lives were calculated as summarised in the following table.

Table B.8.2.1.2- 7: Frank & Klöpfer modelling

Month	Photolysis constant [1/sec] pH 7	Environmental DT_{50} of the direct phototransformation of isoflucypram [days]		
		Minimum pH 7	Mean pH 7	Maximum pH 7
January	0.692×10^{-7}	55	120	530
February	0.144×10^{-6}	27	56	240
March	0.283×10^{-6}	15	28	120
April	0.475×10^{-6}	9.4	17	68
May	0.605×10^{-6}	8.3	13	53
June	0.678×10^{-6}	7.9	12	47
July	0.604×10^{-6}	8.8	13	44
August	0.590×10^{-6}	9.1	14	45
September	0.342×10^{-6}	14	23	87
October	0.188×10^{-6}	22	43	190
November	0.831×10^{-7}	42	97	482
December	0.436×10^{-7}	84	180	920

[1] Zepp, R.G. & Cline, D.M.: Environ. Sci. Technol. 11, 359 (1977).
[2] Frank, R. & Klöpffer, W.: UBA Research Report No. 10602046 (1985).

III. CONCLUSIONS

Degradation of isoflucypram in neutral aqueous solution in a range of 8 to 21% was measured by HPLC-radiodetection after a maximum irradiation period of 500 minutes. This indicated moderate degradability of isoflucypram via direct phototransformation in neutral buffered solutions. UKRMS notes the use of an organic solvent in the buffer solution at an elevated concentration not in accordance with the OECD guideline 316. A low mean quantum yield of Φ = 0.00077 was calculated on the basis of UV absorption data and the degradation kinetics determined from both experiments. UK RMS notes that no dark samples were assessed to determine whether degradation was via photoloysis only. No sterility testing was performed, however the high volumes of organic solvent make growth of micro-organisms unlikely. No mass balance calculations were presented by the applicant.

A comparison of the estimates derived from models of Zepp & Cline and Frank & Kloepffer shows that both approaches were comparable. The two approaches considered the quantum yield and the absorption in a range of wavelengths relevant for the environment. Environmental half-lives of sunlight exposed top surface water layers were estimated to 8 to 22 days for a direct phototransformation of isoflucypram during periods of main use in spring to fall.
Thus, direct phototransformation in neutral aqueous solution may contribute to the dissipation of isoflucypram from the environment.

The elevated concentration of organic solvent may have influenced the outcome of the study however the RMS is unsure as to the extent of this influence. However an aqueous photolysis study has also been submitted. Experience of the E.U. evaluation procedure for pesticides suggests that greater emphasis will be placed on the results of the aqueous photolysis study rather than the quantum yield study.

B.8.2.1.3.: Photolytic degradation.

Previous evaluation:	None, new active substance.

Author: Heinemann, O.; Kasel, D.; 2015b
Title: [Pyrazole-4-14C]BCS-CN88460: Phototransformation in water
Report No.: EnSa-14-1033
Document No.: M-510627-01-1
Guideline(s): OECD Test Guideline No. 316; Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009; US EPA OCSPP Test Guideline No. 835.2240; Japanese MAFF Test Guidelines 12 Nousan 8147, No. 2-6-2
Guideline deviation(s): none
GLP/GEP: yes

Study Summary

The photolytic route and rate of degradation of pyrazole-labelled isoflucypram were studied in sterile aqueous buffer solution at pH 7 under exposure to simulated sunlight and aerobic conditions in the laboratory at 24.5°C for 10 days. In comparison, samples were incubated at 24.7°C in the dark for 13 days. The test concentration was 0.46 mg/L.

The test was performed in static systems consisting of quartz glass vessels each containing 10 mL of test solution and equipped with traps (permeable for oxygen) for the collection of carbon dioxide and

volatile organic compounds. The test systems were continuously exposed to artificial sunlight (Xenon lamp with a < 290 nm cut-off filter). 10 days of continuous irradiation were equivalent to 32.3 and 50.1 solar summer days in Phoenix (Arizona, USA) and Athens (Greece), respectively. For comparison, additional samples were incubated in the dark.

Duplicate samples were processed and analysed 0, 1, 2, 3, 4, 7 and 10 days after treatment (DAT) for irradiated samples and 0, 1, 2, 3, 4, 7 and 13 days after treatment for dark samples. At each sampling interval, the amounts of test item and degradation products in test solutions were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles were determined by LSC. The identity of the test item was confirmed by HPLC-MS(/MS) including accurate mass determination.
Mean material balances were 96.3% AR for irradiated samples (range from 93.4 to 100.6% AR) and 95.6% AR for dark samples (range from 91.9 to 100.6% AR).

The maximum amount of carbon dioxide was 0.1% AR in irradiated and dark samples. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for irradiated and dark samples.
The amount of isoflucypram in the test solutions decreased from DAT 0 to DAT 10 from 100.6 to 93.1% AR in irradiated samples and ranged from DAT 0 to DAT 13 between 91.8 and 100.6% AR in dark samples. The total unidentified residues amounted to a maximum of 2.7% AR in irradiated samples.
The DT_{50} and DT_{90} values of isoflucypram in irradiated samples were calculated using single first order (SFO) kinetics. The experimental half-life for isoflucypram was 150 days in irradiated samples. Due to the stability of isoflucypram in the dark, a kinetic evaluation could not be performed for dark samples. Therefore, the corresponding net photodegradation rate constant (difference between irradiated and dark samples) could not be calculated. Based on the experimental DT_{50} value of 150 days for irradiated samples, the DT_{50} value of isoflucypram under environmental conditions was calculated to be e.g. 484 solar summer days at Phoenix (Arizona, USA) or 750 solar summer days at Athens (Greece).

It is concluded that photodegradation is unlikely to contribute to the degradation of isoflucypram under typical light conditions of the environment.

Table B.8.2.1.3-1: Degradation kinetics of isoflucypram in irradiated and dark samples

Test system	**SFO**					
	DT_{50} (exp.) [days]	**DT_{90} (exp.) [days]**	**Chi^2 error [%]**	**Rate constant [$days^{-1}$]**	**DT_{50} under natural conditions [days]**	**Net photodegradation rate constant[a] / DT_{50} [day^{-1} / days]**
Irradiated	150	497	1.6	0.005	484 (Phoenix, USA) 750 (Athens, Greece)	n.c.[b]
Dark[b]	n.c.	n.c.	n.c.	n.c.	n.c.	

n.c. = not calculated

a) net rate constant = rate constant of irradiated samples - rate constant of dark samples

b) Due to the stability of isoflucypram in the dark, a kinetic evaluation could not be performed for dark samples

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item:

Pyrazole-labelled isoflucypram	
Sample-ID:	KML 9823
Specific activity:	4.22MBq/mg (113.92.34 µCi/mg)
Radiochemical purity:	> 98% (HPLC with radioactivity detection)
	> 99% (TLC, scan)
Chemical purity:	> 99% (HPLC with UV-detector, 210 nm)

Reference item:
No reference items were used.

2. Test System

The study was carried out at pH 7.0 using a 0.01 M phosphate buffer solution. First, a buffer stock solution (0.04 M) was prepared. Therefore, 1.36 g of KH_2PO_4 were dissolved in 100 mL water, diluted with 74 mL of 0.04 M aqueous sodium hydroxide solution and made up to a final volume of 250 mL with water. After homogenisation, the pH of the solution was adjusted to a value of 7.0. Finally, this buffer stock solution was diluted (1/3, *v/v*) to result in the desired 0.01 M buffer solution.

B. STUDY DESIGN

1. Experimental Conditions

The test system for photolytic degradation in water consisted of Quartz glass vessels (50 mm x 26 mm x 16 mm) fitted with a trap attachment (permeable for oxygen), containing soda lime for absorption of carbon dioxide and a polyurethane foam plug for adsorption of volatile organic compounds. All glassware and the buffer solution were sterilised in an autoclave in order to prevent biodegradation of the test solutions during the study.
The test solution was prepared by pipetting 50 µL of stock solution (prepared in acetonitrile) to 500 mL sterile phosphate buffer solution at pH 7. Test units and solutions were sterilised by autoclaving prior to application. For preparation of the test systems the quartz glass vessels were filled with 10 mL test solution. The test concentration was 0.46 mg/L and was set to 100% of applied radioactivity.

The irradiated and dark test systems were incubated under aerobic conditions for 10 days in a Suntest® unit under continuous exposure to simulated sunlight and for 13 days in a climatic cabinet in the dark, respectively. The incubation temperatures were 24.5°C and 24.7°C for irradiated and dark samples, respectively.

The light intensity of the Suntest® unit used for artificial irradiation was constant throughout the incubation period. The spectral distribution of the artificial sunlight was similar to the distribution of natural sunlight. A UV filter was fitted to remove all wave lengths < 290 nm. The average irradiance for irradiated samples was 1160 W/m^2. 10 days of continuous irradiation at this light intensity was equivalent to 32.3 and 50.1 solar summer days in Phoenix (Arizona, USA) and Athens (Greece), respectively.

2. Sampling

Seven sampling intervals were distributed over the entire incubation period of 10 days for irradiated samples and 13 days for dark samples. Duplicate samples were processed and analysed 0, 1, 2, 3, 4, 7 and 10 days after treatment (DAT) for irradiated samples and 0, 1, 2, 3, 4, 7 and 13 days after treatment for dark samples.

3. Analytical Procedures

The samples were monitored for aerobic conditions at DAT-0 and DAT-10 and the pH values were determined at each sampling interval.
The sterility was checked at all sampling intervals for each replicate. Therefore, 100 µL of the test solutions were applied onto agar plates and incubated in the dark for at least two weeks at ambient temperature. Then the plates were visually inspected and no microbial growth was reported

Carbon dioxide absorbed by soda lime was liberated with 18% aqueous hydrochloric acid and trapped. The liberated carbon dioxide was purged into the trapping vessels by a stream of nitrogen. The radioactivity contents of these vessels were determined by liquid scintillation counting (LSC) and summed up to determine the total radioactivity liberated from soda lime.

The PU foam plug was extracted with 20 mL ethyl acetate to desorb volatile organic compounds. The radioactivity content was determined by LSC.

Samples were monitored for aerobic conditions at DAT-0 and DAT-10 and the pH values were determined at each sampling interval.

At each sampling interval, the amounts of test item and degradation products in test solutions were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles were determined by LSC. The identity of the test item was confirmed by HPLC-MS(/MS) including accurate mass determination.
No isolation of degradation products was performed since no degradation product > 10% AR was observed.

II. RESULTS AND DISCUSSION

The buffer and test solutions at pH 7.0 were prepared under sterile conditions with concentrations of ≤ 0.01 mol/L to minimize buffer reactions.
The irradiated and dark test systems were incubated under aerobic conditions for 10 days in a Suntest® unit under continuous exposure to simulated sunlight and for 13 days in a climatic cabinet in the dark, respectively. The incubation temperatures were 24.5°C and 24.7°C for irradiated and dark samples, respectively.
The light intensity of the Suntest® unit used for artificial irradiation was constant throughout the incubation period. The spectral distribution of the artificial sunlight was similar to the distribution of natural sunlight. The average irradiance for irradiated samples was 1160 W/m^2. 10 days of continuous irradiation at this light intensity was equivalent to 32.3 and 50.1 solar summer days in Phoenix (Arizona, USA) and Athens (Greece), respectively.

The pH values of the test solutions for irradiated and dark samples were determined at each sampling interval as 7.0.
The oxygen contents were determined at DAT-0 and DAT-10 in irradiated samples and ranged between 7.7 and 8.7 mg/L.
The sterility tests at each sampling interval demonstrated that sterile conditions were maintained throughout the incubation period. No contamination was observed in the test solutions.

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean DAT-0 recovery for the test item was 100.6% AR demonstrating that the sample processing method was well suited to recover the applied test item from the test solution and that the test item was stable under these conditions.

2. Verification of Chromatographic Procedures

The primary chromatographic method (HPLC/radiodetection) was well suited for the quantitative analysis of the samples of this study as demonstrated by a mean HPLC recovery of 106.2% and a good linear fit for injected amounts of pyrazole-labelled isoflucypram on column ($R^2 > 0.9996$). The LOD of the primary chromatographic method was determined as 8.8 Bq absolute on column or 0.9% AR.

B. MATERIAL BALANCE

Mean material balances were 96.3% AR (range of 93.4 to 100.6% AR) for irradiated samples and 95.6% AR (range of 93.4 to 100.6% AR) for dark samples (Table B.8.2.1.3-2).

The complete material balances found at all sampling intervals for both irradiated and dark samples demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.2.1.3-2: Material balance of radioactivity in irradiated and dark samples (expressed as percentage of applied radioactivity, mean of two replicates)

Samples	Material balance			
	min.	max.	mean	RSD [%]
irradiated	93.4	100.6	96.3	2.3
dark	91.9	100.6	95.6	3.0

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.2.1.3-3.
The route of degradation of isoflucypram in aqueous buffer solution at pH 7 under aerobic irradiated and dark conditions is summarised in Table B.8.2.1.3-4.

Table B.8.2.1.3-3: Material balance of radioactivity in irradiated and dark samples (expressed as percentage of applied radioactivity, mean of two replicates)

	DAT						
	0[a)]	**1**	**2**	**3**	**4**	**7**	**10**
Irradiated samples							
Volatiles							
carbon dioxide	n.a.	0.1	0.1	< 0.1	0.1	0.1	0.1
volatile organic compounds	n.a.	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
total volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	0.1
Solution	100.6	96.4	93.6	93.3	96.3	97.5	95.8
Material balance	100.6	96.5	93.7	93.4	96.4	97.6	95.9
Dark samples							
Volatiles							
carbon dioxide	n.a.	0.1	0.1	0.1	0.1	0.1	< 0.1
volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	< 0.1
Solution	100.6	96.9	92.3	91.8	96.4	93.6	97.3
Material balance	100.6	96.9	92.4	91.9	96.5	93.7	97.4

n.a.: nota analysed; DAT: days after treatment
a) The same duplicates were used ass irradiated and dark DAT-0 samples

Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide was 0.1% AR in irradiated and dark samples. Formation of volatile organic compounds was insignificant as demonstrated by values of ≤ 0.1% AR at all sampling intervals for both irradiated and dark samples (Table B.8.2.1.2-4).

Test item and degradation products in test solution

The amount of isoflucypram in the test solutions decreased from DAT-0 to DAT-10 from 100.6 to 93.1% AR in irradiated samples and ranged from DAT-0 to DAT-13 between 91.8 and 100.6% AR in dark samples. The total unidentified residues amounted to a maximum of 2.7% AR in irradiated samples (Table B.8.2.1.2-4). No degradation products of isoflucypram > 10% AR were observed.

Table B.8.2.1.3-4: Degradation of isoflucypram in irradiated and dark samples (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	Samples	DAT						
		0[a)]	1	2	3	4	7	10 / 13[b)]
Isoflucypram	irradiated	100.6	96.4	93.6	93.3	95.6	95.3	93.1
	dark		96.9	92.3	91.8	96.4	93.6	97.3
Sum of unid./diff. residues[c)]	irradiated	n.d.	n.d.	n.d.	n.d.	< LOD	2.2	2.7
	dark		n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
Total residues in solution[d)]	irradiated	100.6	96.4	93.6	93.3	96.3	97.5	95.8
	dark		96.9	92.3	91.8	96.4	93.6	97.3
Carbon dioxide[e)]	irradiated	n.a.	0.1	0.1	< 0.1	0.1	0.1	0.1
	dark		0.1	0.1	0.1	0.1	0.1	< 0.1
Volatile organic compounds[e)]	irradiated	n.a.	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
	dark		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total recovery[d)]	irradiated	100.6	96.5	93.7	93.4	96.4	97.6	95.9
	dark		96.9	92.4	91.9	96.5	93.7	97.3

n.d.: not detected, n.a.: not analysed, DAT: days after treatment

a) The same duplicates were used as irradiated and dark DAT-0 samples
b) DAT-10 for irradiated samples, DAT-13 for dark samples
c) Minor degradates are summed up to unidentified residues
d) Difference to material balance values due to rounding errors as well as clean up and chromatographic losses
e) Values taken from material balance

D. KINETIC ANALYSIS OF DATA

The experimental DT_{50} and DT_{90} values of isoflucypram in irradiated samples were calculated using single first order (SFO) kinetics. The table below summarises the best fit results of the DT_{50} and DT_{90} calculations:

Table B.8.2.1.3-5: Degradation kinetics of isoflucypram in irradiated and dark samples

Test system	SFO					
	DT_{50} (exp.) [days]	DT_{90} (exp.) [days]	Chi^2 error [%]	Rate constant [$days^{-1}$]	DT_{50} under natural conditions [days]	Net photodegradation rate constant [a)] / DT_{50} [day^{-1} / days]
Irradiated	150	497	1.6	0.005	484 (Phoenix, USA) 750 (Athens, Greece)	n.c.[b)]
Dark [b)]	n.c.	n.c.	n.c.	n.c.	n.c.	

n.c. = not calculated

a) net rate constant = rate constant of irradiated samples - rate constant of dark samples
b) Due to the stability of isoflucypram in the dark, a kinetic evaluation could not be performed for dark samples

The experimental half-life for isoflucypram was 150 days in irradiated samples. Due to the stability of isoflucypram in the dark, a kinetic evaluation could not be performed for dark samples. Therefore, the corresponding net photodegradation rate constant (difference between irradiated and dark samples) could not be calculated. Based on the experimental DT_{50} value of 150 days for irradiated samples, the DT_{50} value of isoflucypram under environmental conditions was calculated to be e.g. 484 solar summer days at Phoenix (Arizona, USA) or 750 solar summer days at Athens (Greece).

E. DEGRADATION PATHWAY

No degradation products of isoflucypram > 10% AR were observed and identified. Therefore, no degradation pathway is proposed.

III. CONCLUSIONS

Isoflucypram was slowly degraded in aqueous buffer solution at pH 7 under exposure to simulated sunlight and aerobic conditions in the laboratory. No degradation products > 10% AR were observed. The experimental half-life for isoflucypram was 150 days in irradiated samples. Due to the stability of isoflucypram in the dark, a kinetic evaluation could not be performed for dark samples. Therefore, the corresponding net photodegradation rate constant (difference between irradiated and dark samples) could not be calculated. Based on the experimental DT_{50} value of 150 days for irradiated samples, the DT_{50} value of isoflucypram under environmental conditions was calculated to be e.g. 484 solar summer days at Phoenix (Arizona, USA) or 750 solar summer days at Athens (Greece).

UK RMS has validated the kinetic assessment using the individual replicate values listed in table B.8.2.1.3-6: using the model CAKE v3.1 and agrees with the results proposed by the applicant. No metabolites could be kinetically assessed. Fit and residual graphs for the light and dark samples are provided in Figures B.8.2.1.2-1 and B.8.1.2.1-2 for the irradiated and dark samples respectively.

Table B.8.2.1.3-6: Individual recovery percentages for the active substance.

Compound	**Samples**	**DAT**						
		0[a)]	**1**	**2**	**3**	**4**	**7**	**10 / 13[b)]**
Isoflucypram	irradiated	100.3	98.1	93.6	92.9	95.9	94.4	93.7
		100.9	94.8	93.6	93.7	95.4	96.2	92.4
Isoflucypram	dark	100.3	96.5	92.2	88.2	97.9	92.4	98.2
		100.9	97.2	92.3	95.4	95.0	94.9	96.5

a) The same duplicates were used as irradiated and dark DAT-0 samples
b) DAT-10 for irradiated samples, DAT-13 for dark samples

Photodegradation is unlikely to contribute to the degradation of isoflucypram under typical light conditions of the environment.

Figure B.8.2.1.3-1: SFO fit graph and residuals plot for the irradiated samples

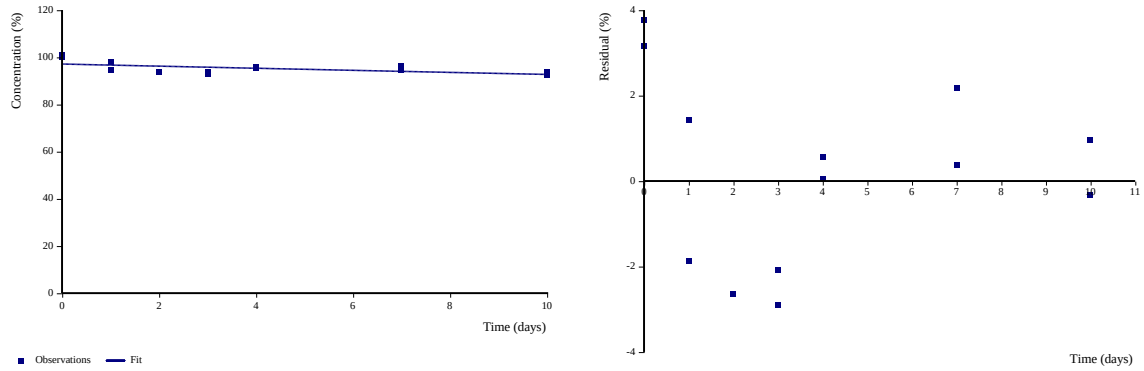
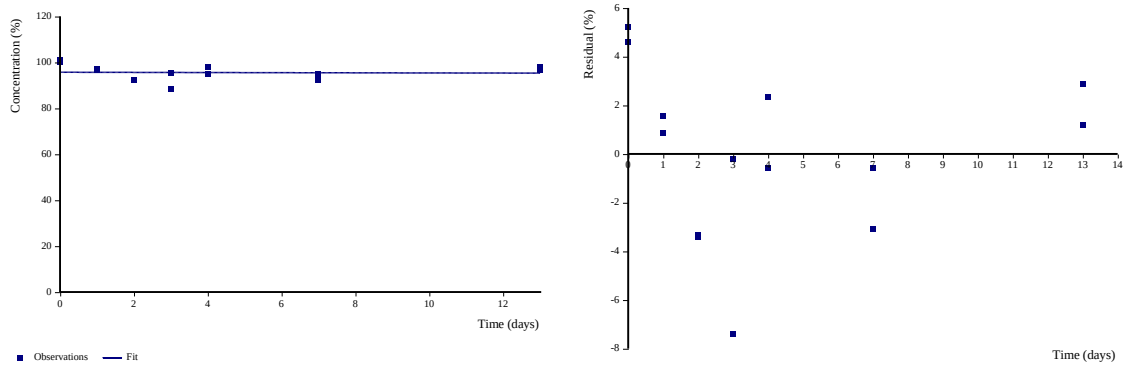


Figure B.8.2.1.3-2: SFO fit graph and residuals plot for the dark samples.



B.8.2.1.4 Indirect photochemical degradation

No study for the determination of the photolytic route and rate of degradation of isoflucypram in natural water has been performed and is not required under Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009.

B.8.2.2. Route and rate of biological degradation in aquatic systems

B.8.2.2.1 "Ready biodegradability"

A study on the "Ready Biodegradability" of isoflucypram was not performed. However, water-sediment studies under aerobic conditions was performed which are described in section B.8.2.2.3. By default isoflucypram is proposed to be classified as ‘not readily biodegradable’.

B.8.2.2.2 Aerobic mineralisation in surface water

The route and rate of degradation of isoflucypram were studied in surface water under aerobic conditions using the pyrazole-label. A summary of the route and rate of degradation of isoflucypram in the aquatic environment is given in section B.8 and Figure B.8- 2.

Previous evaluation:	None, new active substance.

Author: Gabbert, D.; Smith, E.; 2017
Title: [Pyrazole-4-14C] BCS-CN88460: Aerobic mineralization in surface water
Report No.: MELNN017
Document No.: M-582106-01-1
Guideline(s): OECD Test Guideline No. 309, Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC), No 1107/2009
Guideline deviation(s): none
GLP/GEP: yes

Study Summary

The route and rate of degradation of pyrazole-labelled isoflucypram were studied in surface water under aerobic conditions (pelagic test). Two test concentrations were incubated in the laboratory in the dark at 20 ± 2°C for 61 days.

Two study application rates were used and included 10.0 µg/L and 103.6 µg/L surface water for low and high concentration test systems, respectively. The test system consisted of a 250 mL flasks each containing 100 mL of surface water which was equipped with a static volatile trap (permeable to oxygen) for the collection of carbon dioxide and volatile organic compounds. The surface water in the test systems was kept in motion during the entire study period to maintain aerobicity.

Duplicate test systems of each test concentration were processed and analysed at 0, 8, 14, 22, 29, 43, 48, and 61 days after treatment (DAT). Sterile test systems for both concentrations were processed and analysed at DAT-0 and 61. The amounts of test substance and degradation products in surface water were determined by liquid scintillation counting (LSC) and by HPLC/ radiodetection analysis. The amount of volatiles was determined by LSC. Identification was performed by HPLC-MS including accurate mass determination and/or by co-chromatography with reference substances. The redox potential, pH and oxygen content of the surface water were measured throughout the study on 0, 8, 14, 22, 29, 43, 48, and 61 days.

Mean material balances were acceptable for low concentration test systems (range from 98.5 to 103.9% AR) and for high concentration test systems (range from 94.7 to 101.9% AR).

For all test systems in this study (low, high and sterile control test systems), the amount of CO_2 and organic volatiles formed during this study was negligible, with the exception of DAT-14 (low concentration) which had a mean of 4.4% AR as CO_2.

Isoflucypram was stable in all test systems, with a mean of 100.6% AR and 98.3% AR in low and high concentration test systems, respectively. No degradation products were formed in any test systems in this study. In sterile test systems, the mean amount of isoflucypram in surface water at DAT-61 was 93.4% and 94.9% AR in the low and high concentration test systems, respectively.

The experimental data could be described by a single first order (SFO) kinetic model. The DT_{50} values of isoflucypram in the tested surface water under aerobic conditions were > 1000 days for both low and high concentrations.

Table B.8.2.2.2-1: Degradation kinetics of isoflucypram in surface water under aerobic conditions

Concentration	Best fit kinetic model	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]
Low	SFO	> 1000	> 1000	1.264
High	SFO	> 1000	> 1000	1.62

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item and Control Substance

Test item:

Pyrazole-labelled isoflucypram
Sample ID: C-1173
Specific activity: 45.550 mCi/mMole (113.92 µCi/mg)
Radiochemical purity: 100% (determined within this study)

Control substance:
To confirm the microbial activity of the surface water in this study phenyl-UL-^{14}C and carboxyl-^{14}C-labelled benzoic acid was used as the control substance, and its degradability in surface water was measured and compared to an expected DT_{90} of typically < 14 days. Radiolabelled benzoic acid was used in the aqueous treatment solution in order to obtain the appropriate concentration, sampling was taken at 0, 14 and 39 days.

Phenyl-UL-^{14}C- and carboxyl-^{14}C-labelled benzoic acid
Sample ID: C-1189
Specific activity: 125.00 mCi/mMole
Radiochemical purity: 100%

The degradation of Benzoic acid at 39 days was sufficient to conclude that the surface water was suitability biologically active 102.4 % day 0 to 7.6% day 39.

2. Test Water

Natural surface water from a location known not to be exposed to discharges or effluents or near human activity was used.

Table B.8.2.2.2- 2: Physico-chemical properties of test water

Parameter	Results
Water designation	Beaver Dam lake
Origin	Wake Forest, North Carolina, USA
GPS coordinates	N 36°02'00.2" W 78°41'01.6"
Site description	North Carolina State Park system, part of Falls Lake. Separated by a dam, no gasoline boats allowed.
pH[a)] - measured at sampling site - measured at Bayer CropScience	 7.95 8.284
Oxygen saturation [mg/L] [1]	8.72
Redox potential at 20°C, pH 6.9 E_{obs} [mV]	169.0
Total organic carbon (TOC) [mg C/L]	8.1
Disolved organic carbon (DOC) [mg C/L]	6.4
Biological oxygen demand (BOD) [mg C/L]	1.2
Total nitrogen [mg/L}	0.4
Total phosphorous [mg/L]	0.6

[a)] determined on-site at day of sampling

The surface water was collected fresh from the natural water system just below water surface. At the sampling site, temperature and pH were determined. The water was filtered through a 0.100 mm mesh at the test facility. Redox, pH, and dissolved oxygen were determined at the test facility two days after sampling.

B. STUDY DESIGN

1. Experimental Conditions

The test system consisted of a 250 mL flasks which was equipped with a static volatile traps (permeable to oxygen) containing soda lime for the collection of carbon dioxide and polyurethane foam for trapping volatile organic compounds.
For preparation of the test systems, 100 mL aliquots of the surface water (filtered through a 0.100 mm mesh) were added to each test vessel. The test vessels were then fitted with the trap attachments.
The untreated test systems were equilibrated to study conditions for two days prior to application.
Study application rates of 10.0 µg/L and 103.6 µg/L per test system were applied for the low and the high concentration, respectively.
The test item was applied onto the water surface of the respective equilibrated test systems using a gastight syringe. After application, the test vessels (except DAT-0 samples) were fitted with trap attachments and placed into a walk-in incubator. The test systems were incubated in the dark for 62 days at 20°C.

2. Sampling

Eight sampling intervals were distributed over the entire incubation period of 61 days. Duplicate samples were processed and analysed 0, 8, 14, 22, 29, 43, 48 and 61 days after treatment (DAT) for both low and high concentration. Sterile controls were processed and analysed at DAT-61 for both concentrations, microbial activity samples at DAT-0, DAT-14 and DAT-39.

3. Analytical Procedures

The water was removed from the test systems with an additional rinse of 20 mL ACN. The amounts of

isoflucypram and its degradation products in water were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amount of volatiles was determined by LSC. Identification was performed by HPLC-MS including accurate mass determination and/or by co-chromatography with reference substances.
At each sampling interval, pH, oxygen content and redox potential of the surface water were determined.

4. Calculations

Amounts of test substance and degradation products were calculated as percentage of applied radioactivity ([% AR]. Values are presented as single values and as means if replicates were made. The data for the test substance were evaluated according to FOCUS kinetics (2006)[1] using the software KinGUI 2.

II. RESULTS AND DISCUSSION

The pH in water ranged from 8.1 to 9.1 (mean: 8.6) in test systems with low concentration and from 8.0 to 8.8 (mean: 8.5) in test systems with high concentration.

The redox potential (E_H-values) in surface water were 376.0 (mean, range from 327.2 to 397.2) for low concentration test systems and 372.9 (mean, range from 343.7 to 394.2) for high concentration test systems. The redox potentials of the sterile samples were 362.1 in low concentration systems and 358.8 in the high concentration, averaged over two replicates.

The oxygen contents in surface water samples were 8.8 mg/L (mean, range from 8.4 to 9.1 mg/L) in low concentration test systems and 8.9 mg/L (mean, range from 8.5 to 9.3 mg/L) in high concentration test systems. The oxygen contents of the sterile samples were in a similar range.

The values for the redox potentials and oxygen contents indicate aerobic conditions throughout the entire incubation period for both concentrations.

The sterility tests for the sterile samples at DAT-61 demonstrated the absence of viable microorganisms in these samples during the entire incubation period.

A. ANALYTICAL METHODOLOGY

1. Verification of Sample Processing Method

The mean recovery of the test substance at DAT-0 was 98.5% and 94.7% AR for low concentration and high concentration samples, respectively.
These results demonstrate that the sample processing method was well suited to recover the applied test substance from the surface water and that the test substance was stable under these conditions.

2. Verification of Chromatographic Procedures

The HPLC method was used as the primary method for data evaluation. A good selectivity and reproducibility demonstrated the suitability for separation and quantification.
The off-column HPLC recovery was measured on random samples, which averaged 96.3% in the low concentration (n = 8) test systems and 101.0% in the high concentration (n = 8) test systems, respectively. LOD/LOQ were assessed as peaks/ values above 3x background (LOQ 2.0% A.R.), this is accepted by UK RMS.

[1] 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 2.0, 434 pp

B. MATERIAL BALANCE

Mean material balances were 101.7% AR for low concentration test systems (range from 98.5 to 103.9% AR) and 98.4% AR for high concentration test systems (range from 94.7 to 101.9% AR).
The complete material balances found at all sampling intervals for both concentrations demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table B.8.2.2.2-3: Material balance from mean values - not including sterile samples (expressed as % AR)

	Material balance	
	low concentration	high concentration
Min	98.5	94.7
Max	103.9	101.9
Mean	101.7	98.4
RSD	1.7	2.1

RSD = relative standard deviation

C. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.2.2.2-4 and Table B.8.2.2.2-5. The formation of carbon dioxide was minimal in the study. The formation of volatile organic compounds was insignificant as demonstrated by values of ≤0.1% AR at all sampling intervals for both concentrations. Therefore, no identification of the volatile organic compounds was performed.

The route of degradation of isoflucypram in surface water under aerobic conditions is summarised in Table B.8.2.2.2- 6 and Table B.8.2.2.2-7. No degradation products were observed in the surface water.

Table B.8.2.2.2- 1: Material balance of radioactivity in surface water under aerobic conditions at low concentration - including sterile samples (expressed as % AR)

Component	Replicate	Days after treatment								
	no.	0	8	14	22	29	43	48	61	61 sterile
Volatiles										
carbon dioxide	A	n.a.	0.6	6.9	0.9	0.7	1.0	1.0	0.9	0.8
	B	n.a.	0.5	1.9	0.7	0.3	0.7	0.8	0.9	0.9
	mean	**n.a.**	**0.6**	**4.4**	**0.8**	**0.5**	**0.8**	**0.9**	**0.9**	**0.9**
volatile organic compounds	A	n.a.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	B	n.a.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	mean	**n.a.**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
total volatiles	A	n.a.	0.7	7.0	1.1	0.7	1.1	1.1	1.1	0.9
	B	n.a.	0.6	2.0	0.8	0.4	0.8	0.9	1.0	1.0
	mean	**n.a.**	**0.7**	**4.5**	**0.9**	**0.6**	**0.9**	**1.0**	**1.0**	**1.0**
Water[a)]	A	97.1	103.7	98.4	100.4	100.1	100.7	100.7	97.6	90.8
	B	99.9	102.2	100.3	101.8	99.8	102.8	101.4	100.7	96.0
	mean	**98.5**	**102.9**	**99.4**	**101.1**	**100.0**	**101.7**	**101.1**	**99.2**	**93.4**
Material balance	A	97.1	104.4	105.5	101.5	100.8	101.7	101.8	98.6	91.8
	B	99.9	102.8	102.3	102.6	100.2	103.6	102.2	101.7	97.0
	mean	**98.5**	**103.6**	**103.9**	**102.0**	**100.5**	**102.7**	**102.0**	**100.2**	**94.4**

n.a. = not analysed
a) includes acetonitrile rinse, min 1.8% to max. 8.8% AR

Table B.2.2.2-5: Material balance of radioactivity in surface water under aerobic conditions at high concentration - including sterile samples (expressed as % AR)

Component	Replicate no.	Days after treatment 0	8	14	22	29	43	48	61	61 sterile
Volatiles										
carbon dioxide	A	n.a.	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	B	n.a.	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	mean	**n.a.**	**0.1**	**0.2**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
volatile organic compounds	A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	mean	**n.a.**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
total volatiles	A	n.a.	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	B	n.a.	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1
	mean	**n.a.**	**0.1**	**0.2**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
Water[a)]	A	97.1	93.8	99.6	101.4	99.1	98.4	98.2	98.3	95.5
	B	95.5	99.9	101.9	99.1	99.9	98.4	96.2	97.0	97.4
	mean	**94.7**	**99.7**	**101.7**	**99.1**	**99.2**	**98.3**	**97.3**	**96.3**	**94.9**
Material balance	A	97.1	93.8	99.7	101.6	99.2	98.5	98.4	98.4	95.7
	B	95.5	100.0	102.2	99.2	100.0	98.5	96.3	97.1	97.5
	mean	**94.7**	**99.8**	**101.9**	**99.2**	**99.2**	**98.4**	**97.4**	**96.4**	**95.0**

n.a. = not analysed

a) includes acetonitrile rinse, min 1.8% to max. 8.8% AR

Table B.2.2.2-6: Degradation of isoflucypram in surface water under aerobic conditions (low concentration, mean values and SD expressed as % AR)

Compound	Mean SD	Days after treatment 0	8	14	22	29	43	48	61	61 sterile
Isoflucypram	Mean	98.5	103.7	99.4	101.1	100.0	101.7	101.1	99.2	93.4
	SD	± 1.4	± 0.0	± 1.0	± 0.7	± 0.1	± 1.1	± 0.3	± 1.6	± 2.6
Total water residues	Mean	98.5	103.7	99.4	101.1	100.0	101.7	101.1	99.2	93.4
	SD	± 1.4	± 0.0	± 1.0	± 0.7	± 0.1	± 1.1	± 0.3	± 1.6	± 2.6
Carbon dioxide	Mean	n.a.	0.5	4.4	0.8	0.5	0.8	0.9	0.9	0.9
	SD		± 0.1	± 2.5	± 0.1	± 0.2	± 0.1	± 0.1	± 0.0	± 0.0
Volatile organic compounds	Mean	n.a.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	SD		± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Material balance	Mean	98.5	104.4	103.9	102.0	100.5	102.7	102.0	100.2	94.4
	SD	± 1.4	± 0.0	± 1.6	± 0.5	± 0.3	± 0.9	± 0.2	± 1.5	± 2.6

n.a. = not analysed, SD = standard deviation

Table B.2.2.2-7: Degradation of isoflucypram in surface water under aerobic conditions (high concentration, mean values and SD expressed as % AR)

Compound	Mean SD	Days after treatment 0	8	14	22	29	43	48	61	61 sterile
Isoflucypram	Mean	94.7	99.7	101.7	99.1	99.2	98.3	97.3	96.3	94.9
	SD	± 0.8	± 0.2	± 0.3	± 0.0	± 0.8	± 0.1	± 1.1	± 0.7	± 2.5
Total water residues	Mean	94.7	99.7	101.7	99.1	99.2	98.3	97.3	96.3	94.9
	SD	± 0.8	± 0.2	± 0.3	± 0.0	± 0.8	± 0.1	± 1.1	± 0.7	± 2.5
Carbon dioxide	Mean	n.a.	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	SD		± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Volatile organic compounds	Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	SD		± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Material balance	Mean	94.7	99.8	101.9	99.2	99.2	98.4	97.4	96.4	95.0
	SD	± 0.8	± 0.2	± 0.3	± 0.0	± 0.8	± 0.1	± 1.1	± 0.7	± 2.5

n.a. = not analysed, SD = standard deviation

D. DEGRADATION PATHWAY

Based on the results of the study, isoflucypram does not degrade in surface water under aerobic conditions.

E. KINETIC ANALYSIS

The degradation of isoflucypram followed single first order (SFO) kinetics. The table below summarises the results of the DT_{50} and DT_{90} calculations. The DT_{50} values of isoflucypram in the tested surface water under aerobic conditions were > 1000 days for both low and high concentrations.

Table B.8.2.2.2-8: Degradation of isoflucypram in surface water under aerobic conditions

Concentration	Best fit kinetic model	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]
Low	SFO	> 1000	> 1000	1.264
High	SFO	> 1000	> 1000	1.62

SFO = single first order

III. CONCLUSIONS

Isoflucypram was degraded minimally at both the low test concentration and high concentration to CO_2. The DT_{50} values of isoflucypram in the tested surface water under aerobic conditions were > 1000 days for both low and high concentrations.

Formation of volatiles such as carbon dioxide was minimal and the interval mean reached a maximum of 4.4% AR (DAT-14, low concentration) which appeared to be elevated compared to other sampling occasions, with a mean value of 1.2% for the entire study.

The RMS concludes that there was virtually no degradation of isoflucypram in the aerobic mineralisation in surface water study

B.8.2.2.3: Water sediment study

The route and rate of degradation of isoflucypram in water/sediment systems under aerobic conditions were investigated using the pyrazole-label in a single study. A separate kinetic study provided by the applicant has also been supplied and validated by the RMS. An assessment in only a single label has been provided, justification for the acceptance of this has been provided by the RMS in section B.8.1.

Previous evaluation:	None, new active substance.

Author: Hein, E. M.; Kasel, D.; 2017
Title: [pyrazole-4-14C]BCS-CN88460: Aerobic aquatic metabolism
Report No.: EnSa-15-0965
Document No.: M-580411-01-1
Guideline(s): OECD Test Guideline No. 308, Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Guideline deviation(s): none
GLP/GEP: yes

Study Summary

The route and rate of degradation of pyrazole-labelled isoflucypram were studied in two water-sediment systems under aerobic conditions in the laboratory in the dark at 20°C for 100 days: The water-sediment systems used are detailed in table B.8.2.2.3-1. The study followed the OECD guidance for the testing of chemicals No.308.

Table B.8.2.2.3-1: Water-sediment systems used

Water-sediment system	System ID	Source	Texture[a)] (USDA)	pH		TOC	
				water[b)]	sediment[c)]	water[d)] [mg/L]	sediment[e)] [g/kg]
Anglersee	A	Leverkusen, Germany	sand	7.1	6.6	< 2	8.8
Wiehltalsperre	W	Nespen, Germany	loam	7.3	5.1	< 2	58.5

TOC: total organic carbon
a) sediment textural class
b) water pH value determined on-site immediately after sampling
c) sediment value derived from aqueous 0.01 M $CaCl_2$ suspensions
d) water TOC determined at start of study (application of test item)
e) sediment TOC determined at start of study (application of test item

A nominal study application rate of 19.5 µg/test system (corresponding to 37.5 µg/L) was applied based on a 5 fold maximum single field application rate of isoflucypram of 75 g/ha due to analytical reasons and this is accepted by the RMS.

The test was performed in systems consisting of cylindrical glass containers containing a water-to-sediment volume ratio of approx. 3/1 (*v/v*) and equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds. During incubation, the water was in smooth motion.
Duplicate samples were processed and analysed 0, 3, 7, 14, 29, 51, 72 and 100 days after treatment (DAT). At each sampling interval, the water was separated from the sediment by centrifugation and decantation. The sediment was extracted three times at ambient temperature, once using acetonitrile and twice using acetonitrile/water 4/1 (*v/v*). Furthermore, two microwave-assisted extraction steps were performed using acetonitrile/water 4/1 (v/v) at 70 °C and methanol/water 1/1 (*v/v*) at 50°C. The amounts of test item and degradation products in water and sediment extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. Test item and degradation products were identified by HPLC-MS/MS) including accurate mass determination and/or by NMR.

Mean material balances were 95.4% AR for system Anglersee (range from 93.0 to 97.9% AR) and 96.0% AR for system Wiehltalsperre (range from 92.9 to 98.3% AR).

The maximum amounts of carbon dioxide were 0.3 and 0.1% AR at any sampling interval in system Anglersee and Wiehltalsperre, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of < 0.1% AR at all sampling intervals for both water/sediment systems. Residues in water decreased from DAT-0 to DAT-100 from 82.3 to 20.6% AR in system Anglersee and from 78.4 to 6.5% AR in system Wiehltalsperre. Extractable residues in sediment of system Anglersee increased from DAT-0 to DAT-29 from 15.2 to 76.2% AR and then decreased to 70.6% AR at DAT 100. Extractable residues in sediment of system Wiehltalsperre increased from DAT-0 to DAT-51 from 17.6 to 84.6% AR and then decreased to 82.7% AR at DAT 100. Extractable residues in the total system (water and sediment extracts) decreased from DAT-0 to DAT-100 from 97.5 to 91.2% AR in system Anglersee and from 96.0 to 89.2% AR in system Wiehltalsperre.

Non-extractable residues (NER) increased from DAT-0 to DAT-100 from 0.4 to 6.4% AR in system Anglersee and from 0.7 to 6.2% AR in system Wiehltalsperre.
Isoflucypram dissipated from the water due to degradation and translocation into the sediment. The amount of isoflucypram in the water decreased from DAT-0 to DAT-100 from 82.3 to 8.2% AR in system Anglersee and from 78.4 to 4.4% AR in system Wiehltalsperre.
The amount of isoflucypram in the sediment extracts increased in system Anglersee from DAT-0 to DAT-29 from 15.2 to 76.2% AR and decreased then to 63.2% AR at DAT-100. In system Wiehltalsperre, the amount of isoflucypram in the sediment extracts increased from DAT-0 to DAT-51 from 17.6 to 83.0% AR and decreased then to 80.1% AR at DAT-100.

The amount of isoflucypram in the total system decreased from DAT-0 to DAT-100 from 97.5 to 71.4% AR in system Anglersee and from 96.0 to 84.5% AR in system Wiehltalsperre.
Degradation of isoflucypram in the total system was accompanied by the formation of one degradation product identified as M12 with a maximum occurrence of 6.6% AR at DAT-100 in system Anglersee (see Table B.8.2.2.3-2). The total unidentified residues amounted to a maximum of 12.4% AR and no single component exceeded 4.6% AR at any sampling interval in both water/sediment systems.

The experimental data could be best described by a first order multi compartment (FOMC) kinetic model for dissipation from the water and a single first order (SFO) kinetic model for degradation in the total system. The DT_{50} values for the dissipation of isoflucypram from the water were 2.0 and 1.8 days in system Anglersee and Wiehltalsperre, respectively. The DT_{50} values for the degradation of isoflucypram in the total water/sediment system were 218 and 681 days in system Anglersee and Wiehltalsperre, respectively (Table B.8.2.2.3-3).

Table B.8.2.2.3-2: Identified degradation product (maximum occurrence in total system)

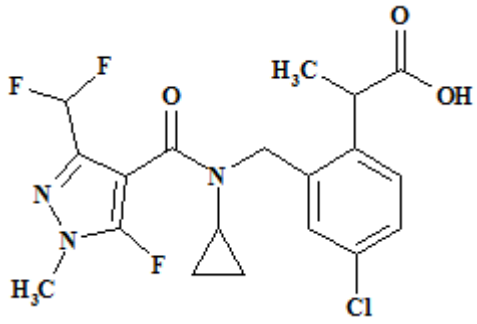
Compound	Chemical structure	Maximum occurrence in total system [%]
M12 (BCS-CN88460-carboxylic acid)		6.6

Table B.8.2.2.3-3: Degradation kinetics of isoflucypram in water-sediment systems under aerobic conditions

Water-sediment system		Best fit kinetic model [a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual assessment [b)]
Anglersee	water layer	FOMC	2.0	89.5	4.5	+
	total system	SFO	218	725	2.1	+
Wiehltalsperre	water layer	FOMC	1.8	41.4	2.6	+
	total system	SFO	681	> 1000	1.3	+

a) SFO = single first order, FOMC = first order multi compartment
b) Visual assessment: + = good

I. MATERIALS AND METHODS

A. MATERIALS

1. Test and Reference Items

Test item

Pyrazole-labelled isoflucypram
Sample ID: KML 9710
Specific activity: 4.22 MBq/mg (113.92 µCi/mg)
Radiochemical purity: > 98% (HPLC with radioactivity detector)
> 99% (TLC, scan)
Chemical purity: > 99% (HPLC with UV-detector, 210 nm)

Reference item
Reference substances were not used

2. Test Systems

The study was carried out using two different natural water-sediment systems (Anglersee and Wiehltalsperre). Water and sediment were sampled fresh. The natural systems were characterised at the site of collection with respect to temperature, pH and redox potential of the water and sediment as well as oxygen content of the water.
Water and sediment were taken in approximately 0.5 m water depth and filled separately in plastic containers. Sediment was obtained from the upper sediment layer.
In the laboratory, the sediments were sieved to ≤ 2 mm, filled into plastic trays and stored at ambient temperature overnight for sedimentation. The water was filtered through a 0.063 mm mesh before preparation of the test systems.

Aliquots of the water/sediment systems were characterized with respect to the total organic carbon of the water and the sediment. In addition, aliquots of both sediments were characterised with respect to textural class, pH, and cation exchange. The results of the characterisation are presented in Table B.8.2.2.3-4. The sediment microbial activity was determined at start of equilibration as well as start and end of the study (Table B.8.2.2.3-5).

Table B.8.2.2.3-4: Physico-chemical characteristics of the water-sediment systems

Parameter		Results	
		Anglersee	Wiehltalsperre
Properties of water			
Temperature [°C]		9.9	6.3
pH		7.1	7.3
Redox potential [mV]		241	257
Oxygen saturation [%]		105	102
Total organic carbon (TOC) [mg/L]	start of the study a)	< 2	< 2
	study end	3.4	2.5
Properties of sediment			
Textural class (USDA)		sand	loam
sand [%]		97	49
silt [%]		2	42
clay [%]		1	9
pH (sediment / 0.01 M $CaCl_2$ 1/2)		6.6	5.1
pH (sediment/water 1/1		7.0	5.3
TOC [g/kg dw]	start of the study a)	8.8	58.5
	study end	7.9	51.3
OC [%]		0.8	5.8
Cation exchange capacity [meq/100g]		4.4	7.7
Redox potential [mV]		237	42
Moisture [g H_2O *ad* 100 g dry weight]		41	139

a) = application of the test item

Table B.8.2.2.3-5: Results of microbial activity determinations (expressed as mg microbial carbon dioxide per hour per kg of sediment dry weight)

System	Sampling date		
	Start of equilibrium	DAT-1 Bio1-	DAT-100 Bio2- / Bio2+
Anglersee	15.0	9.7	5.5 / 3.9
Wiehltalsperre	82.5	64.8	49.2 / 73.5

BIO- = samples were left untreated
BIO+ = samples were applied with solvent of application solution (229 µL methanol)

B. STUDY DESIGN

1. Experimental Conditions

Special cylindrical glass containers (volume approx. 1000 mL, inner diameter approx. 10.5 cm, surface area approx. 86.6 cm^2) were used as test vessels and each container was fitted with trap attachments (permeable for oxygen) containing soda lime for absorption of carbon dioxide and a polyurethane foam plug for adsorption of volatile organic compounds.
For preparation of the test systems a water-to-sediment volume ratio of approx. 3/1 was used corresponding to a water layer of approximately 6 cm and a sediment layer of approximately 2 cm. Therefore, wet sediment with a weight equivalent to a volume of 175 mL was weighed into each vessel and 520 mL of the corresponding water were added. The flasks were then fitted with trap attachments, valves and stirrers.

The test item was applied dropwise onto the water surface of the respective equilibrated test systems. After application, the test vessels were fitted with trap attachments (except of DAT-0 samples).
The test systems were incubated in the dark for 100 days at 20°C in walk-in climatic chamber.

2. Sampling

Eight sampling intervals were distributed over the entire incubation period of 100 days. Duplicate samples were processed and analysed 0, 1, 3, 7, 14, 29, 51, 72 and 100 days after treatment (DAT).
Microbial activity was determined at start of equilibration as well as start (DAT-1) and end of the study (DAT-100).

3. Analytical Procedures

At each sampling interval, the water was separated from the sediment by centrifugation and decantation. The sediment was extracted three times at ambient temperature, once using acetonitrile and twice using acetonitrile/water 4/1 (*v/v*). Furthermore, two microwave-assisted extraction steps were performed using acetonitrile/water 4/1 (*v/v*) at 70°C and methanol/water 1/1 (*v/v*) at 50°C. The amounts of test item and degradation products in water and sediment extracts were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. The amounts of volatiles and non-extractable residues were determined by LSC and combustion/LSC, respectively. Test item and degradation products were identified by HPLC-MS(/MS) including accurate mass determination and/or by NMR. Samples were concentrated as required. For LSC the LOD was set to twice and the LOQ three times the background of the instrument (0.3 to 0.5 BQ), resulting in values ranging from <0.1% A.R to 0.9% A.R. depending on the extraction type. For HPLC LOD and LOQ were calculated for each sample are resulted in values between 1.0% A.R. and 3.0% A.R.

4. Determination of Degradation Kinetics

Whilst calculations were provided, the applicant submitted a separate report on kinetic calculations. The UK RMS has made an assessment following the description of this water/ sediment study.

II. RESULTS AND DISCUSSION

The test systems were incubated under aerobic conditions in the dark in a walk-in climatic chamber at a mean temperature of 20.4°C for 100 days. Determinations of microbial activity were performed at start of equilibration as well as start (DAT-1) and end of the study (DAT-100) and demonstrated that the used sediments were microbially viable.
The pH values in the water ranged from 7.8 to 8.6 in Anglersee test systems and from 6.4 to 8.3 in Wiehltalsperre test systems. The corresponding pH values in the sediment ranged from 6.3 to 7.4 in Anglersee test systems and from 6.0 to 7.0 in Wiehltalsperre test systems.

The oxygen contents in the water ranged from 8.0 to 8.6 mg/L in Anglersee test systems and from 7.8 to 8.8 mg/L in Wiehltalsperre test systems. The redox potentials determined in water and sediment were at highly positive E_H-values during the incubation period. However, variations between different test systems were observed. In Anglersee test systems, the E_H-values in water ranged from +365 to +423 mV. The corresponding E_H-values in sediment were between +159 and +536 mV. In Wiehltalsperre test systems, the E_H-values in water ranged from +352 to +446 mV. The corresponding E_H-values in sediment were between +242 and +410 mV.
The positive values for the redox potentials and the oxygen contents suggest aerobic conditions during the incubation period.

A. MATERIAL BALANCE

Mean material balances were 95.4% AR for system Anglersee (range from 93.0 to 97.9% AR) and 96.0% AR for system Wiehltalsperre (range from 92.9 to 98.3% AR) (Table B.8.2.2.3-8 and Table B.8.2.2.3-9).

The complete material balances found at all sampling intervals for both water/sediment systems demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

B. DISTRIBUTION AND COMPOSITION OF RESIDUES

The detailed figures of the radioactivity distribution are presented in Table B.8.2.2.3-6 and Table B.8.2.2.3-7.

The route of degradation of isoflucypram in Anglersee and Wiehltalsperre water-sediment systems under aerobic conditions is summarised in Table B.8.2.2.3-6 and Table B.8.2.2.3-7. The proposed degradation pathway is presented in Figure B.8.2.2.3-1.

Carbon dioxide and volatile organic compounds

The maximum amounts of carbon dioxide were 0.3 and 0.1% AR at any sampling interval in system Anglersee and Wiehltalsperre, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of < 0.1% AR at all sampling intervals for both water/sediment systems (Table B.8.2.2.3-6 and Table B.8.2.2.3-7).

Test item and degradation products in the water

Residues in water decreased from DAT-0 to DAT-100 from 82.3 to 20.6% AR in system Anglersee and from 78.4 to 6.5% AR in system Wiehltalsperre.

Isoflucypram dissipated from the water due to degradation and translocation into the sediment. The amount of isoflucypram in the water decreased from DAT-0 to DAT-100 from 82.3 to 8.2% AR in system Anglersee and from 78.4 to 4.4% AR in system Wiehltalsperre.

Degradation of isoflucypram in the water was accompanied by the formation of one degradation product identified as M12, with a maximum occurrence of 5.4% AR at DAT-100 in the water of system Anglersee. The total unidentified residues in the water amounted to a maximum of 7.1% AR at any sampling interval for both water/sediment systems and no single compound exceeded 2.5% AR (Table B.8.2.2.3-6 and Table B.8.2.2.3-7).

Test item and degradation products in the sediment

Extractable residues in sediment of system Anglersee increased from DAT-0 to DAT-29 from 15.2 to 76.2% AR and then decreased to 70.6% AR at DAT-100. Extractable residues in sediment of system Wiehltalsperre increased from DAT-0 to DAT-51 from 17.6 to 84.6% AR and then decreased to 82.7% AR at DAT-100.

The amount of isoflucypram in the sediment extracts increased in system Anglersee from DAT-0 to DAT-29 from 15.2 to 76.2% AR and decreased then to 63.2% AR at DAT-100. In system Wiehltalsperre, the amount of isoflucypram in the sediment extracts increased from DAT-0 to DAT- 51 from 17.6 to 83.0% AR and decreased then to 80.1% AR at DAT-100.

Degradation of isoflucypram in the sediment was accompanied by the formation of one degradation product identified as M12, with a maximum occurrence of 1.3% AR at DAT-100 in the sediment extracts of system Anglersee. The total unidentified residues in the sediment extracts amounted to a maximum of 5.3% AR at any sampling interval for both water/sediment systems and no single compound exceeded 2.7% AR (Table B.8.2.2.3-6 and Table B.8.2.2.3-7).

Test item and degradation products in the total water-sediment system

Extractable residues in the total system (water and sediment extracts) decreased from DAT-0 to DAT- 100 from 97.5 to 91.2% AR in system Anglersee and from 96.0 to 89.2% AR in system Wiehltalsperre.

The amount of isoflucypram in the total system decreased from DAT-0 to DAT-100 from 97.5 to 71.4% AR in system Anglersee and from 96.0 to 84.5% AR in system Wiehltalsperre.

Degradation of isoflucypram in the total system was accompanied by the formation of one degradation product identified as M12 with a maximum occurrence of 6.6% AR at DAT-100 in system Anglersee. The total unidentified residues amounted to a maximum of 12.4% AR and no single component exceeded 4.6% AR at any sampling interval in both water/sediment systems (Table B.8.2.2.3-6 and Table B.8.2.2.3-7).

Non-extractable residues

Non-extractable residues (NER) increased from DAT-0 to DAT-100 from 0.4 to 6.4% AR in system Anglersee and from 0.7 to 6.2% AR in system Wiehltalsperre (Table B.8.2.2.3-6 and Table B.8.2.2.3-7).

Table B.8.2.2.3- 6: Degradation of isoflucypram in system Anglersee under aerobic conditions (expressed as percentage of applied radioactivity)

Compound	Source		DAT 0	3	7	14	29	51	72	100
Isoflucypram	water	A	83.2	39.8	26.5	22.0	12.7	11.7	10.8	8.4
		B	81.3	40.9	38.2	27.8	16.0	13.4	9.7	7.9
		Mean	82.3	40.4	32.4	24.9	14.3	12.5	10.3	8.2
	sediment	A	12.9	51.8	66.2	71.5	79.4	65.9	65.1	63.1
		B	17.5	52.0	55.4	65.9	73.0	64.6	66.4	63.4
		Mean	15.2	51.9	60.8	68.7	76.2	65.3	65.7	63.2
	entire system [d]	A	96.1	91.6	92.7	93.5	92.1	77.6	75.9	71.4
		B	98.9	93.0	93.6	93.7	89.0	78.0	76.1	71.3
		Mean	97.5	92.3	93.1	93.6	90.6	77.8	76.0	71.4
M12	water	A	n.d.	n.d.	n.d.	n.d.	2.2	2.7	3.3	5.5
		B	n.d.	n.d.	n.d.	n.d.	1.7	2.2	3.2	5.3
		Mean	n.d.	n.d.	n.d.	n.d.	1.9	2.4	3.2	5.4
	sediment	A	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.2	1.0
		B	n.d.	n.d.	n.d.	n.d.	n.d.	1.2	1.2	1.5
		Mean	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	1.2	1.3
	entire system [d]	A	n.d.	n.d.	n.d.	n.d.	2.2	2.7	4.5	6.5
		B	n.d.	n.d.	n.d.	n.d.	1.7	3.3	4.4	6.8
		Mean	n.d.	n.d.	n.d.	n.d.	1.9	3.0	4.4	6.6
Sum of unid./diff. residues [a]	water	A	n.d.	n.d.	< LOD	n.d.	0	6.1	5.3	7.5
		B	n.d.	n.d.	n.d.	n.d.	1.1	5.3	6.1	6.7
		Mean	n.d.	n.d.	< LOD	n.d.	< LOD	5.7	5.6	7.1
	sediment	A	n.d.	n.d.	< LOD	n.d.	n.d.	2.7	2.3	5.5
		B	n.d.	n.d.	n.d.	n.d.	n.d.	1.2	2.3	5.0
		Mean	n.d.	n.d.	< LOD	n.d.	n.d.	2.0	2.3	5.3
	entire system [d]	A	n.d.	n.d.	< LOD	n.d.	n.d.	8.8	7.5	13.1
		B	n.d.	n.d.	n.d.	n.d.	1.1	6.6	8.4	11.7
		Mean	n.d.	n.d.	< LOD	n.d.	< LOD	7.7	7.9	12.4
Total extractable residues [b]	water	A	83.2	39.8	26.5	22.0	14.9	20.5	19.3	21.3
		B	81.3	40.9	38.2	27.8	18.7	20.9	18.9	20.0
		Mean	82.3	40.4	32.4	24.9	16.8	20.7	19.1	20.6
	sediment	A	12.9	51.8	66.2	71.5	79.4	68.5	68.6	69.6
		B	17.5	52.0	55.4	65.9	73.0	67.0	69.8	70.0
		Mean	15.2	51.9	60.8	68.7	76.2	67.8	69.2	69.8
	entire system [d]	A	96.1	91.6	92.7	93.5	94.3	89.0	87.9	90.9
		B	98.9	93.0	93.6	93.7	91.7	87.9	88.7	89.9
		Mean	97.5	92.3	93.1	93.6	93.0	88.5	88.3	90.4
Carbon dioxide [c]		A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1
		B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	0.4	0.1	0.1
		Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	0.3	0.1	0.1
Volatile organic compounds [c]		A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
		B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
		Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
Non-extractable residues [c]		A	0.4	0.9	1.7	1.9	2.9	3.6	4.9	6.3
		B	0.3	0.9	1.3	2.0	2.8	3.6	4.3	6.4
		Mean	0.4	0.9	1.5	1.9	2.8	3.6	4.6	6.4
Total recovery [b]		A	96.6	92.5	94.4	95.4	97.3	92.7	92.9	97.3
		B	99.2	93.9	94.9	95.7	94.5	91.9	93.1	96.5
		Mean	97.9	93.2	94.6	95.5	95.9	92.3	93.0	96.9

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, LOD: limit of detection (1.0% AR)

a) Minor degradates are summed up to sum of unidentified / diffuse residues (single max. < 5 %AR in the entire system)

b) Difference to material balance values due to rounding errors as well as clean up and chromatographic losses

c) Values taken from material balance

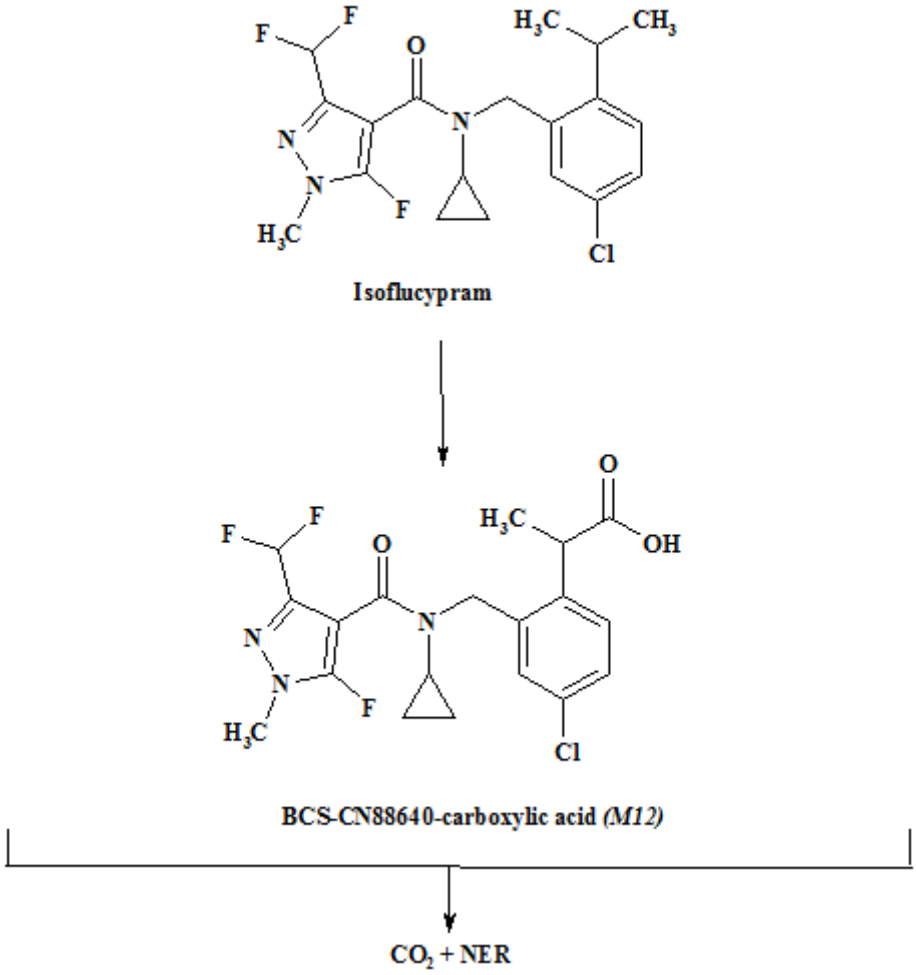
d) Mean values of the entire system could be unequal compared to the sum of the mean values of water and sediment, as values of the entire system are calculated individually for each replicate by summation of values from water and sediment before averaging the entire system values

Table B.8.2.2.3- 7: Degradation of isoflucypram in system Wiehltalsperre under aerobic conditions (expressed as percentage of applied radioactivity)

Compound	Source		DAT							
			0	3	7	14	29	51	72	100
Isoflucypram	water	A	81.6	39.5	27.4	18.3	12.4	8.7	7.7	6.7
		B	75.3	35.8	26.1	16.5	12.0	8.2	7.3	2.0
		Mean	78.4	37.7	26.7	17.4	12.2	8.5	7.5	4.4
	sediment	A	18.2	51.3	68.0	74.2	80.5	81.4	80.1	79.4
		B	16.9	55.7	66.4	76.2	79.9	84.5	80.9	80.8
		Mean	17.6	53.5	67.2	75.2	80.2	83.0	80.5	80.1
	entire system [d]	A	99.8	90.8	95.4	92.6	93.0	90.1	87.8	86.1
		B	92.2	91.5	92.5	92.7	91.9	92.7	88.1	82.8
		Mean	96.0	91.2	93.9	92.6	92.4	91.4	88.0	84.5
M12	water	A	n.d.	n.d.	n.d.	n.d.	1.2	< LOD	< LOD	1.1
		B	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.2	< LOD
		Mean	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD	< LOD
	sediment	A	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD
		B	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	1.2	< LOD
		Mean	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD
	entire system [d]	A	n.d.	n.d.	n.d.	n.d.	1.2	< LOD	< LOD	1.1
		B	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	2.4	< LOD
		Mean	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.2	< LOD
Sum of unid./diff. residues [a]	water	A	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.1
		B	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.1 [4]
		Mean	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.7
	sediment	A	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD
		B	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.1
		Mean	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD
	entire system [d]	A	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	2.2
		B	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	2.2
		Mean	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	2.2 [4]
Total extractable residues [b]	water	A	81.6	39.5	27.4	18.3	13.6	8.7	7.7	8.9
		B	75.3	35.8	26.1	16.5	12.0	8.2	8.5	3.2
		Mean	78.4	37.7	26.7	17.4	12.8	8.5	8.1	6.0
	sediment	A	18.2	51.3	68.0	74.2	80.5	81.4	80.1	80.4
		B	16.9	55.7	66.4	76.2	79.9	84.5	82.1	80.8
		Mean	17.6	53.5	67.2	75.2	80.2	83.0	81.1	80.6
	entire system [d]	A	99.8	90.8	95.4	92.6	94.1	90.1	87.8	89.3
		B	92.2	91.5	92.5	92.7	91.9	92.7	90.6	83.9
		Mean	96.0	91.2	93.9	92.6	93.0	91.4	89.2	86.6
Carbon dioxide [c]		A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1
		B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1
		Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1
Volatile organic compounds [c]		A	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
		B	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
		Mean	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
Non-extractable residues [c]		A	0.6	1.6	2.4	2.5	4.0	3.7	4.4	5.0
		B	0.8	1.7	2.1	2.3	4.3	4.0	4.6	7.3
		Mean	0.7	1.7	2.2	2.4	4.1	3.8	4.5	6.2
Total recovery [b]		A	100.4	92.5	97.7	95.1	98.1	93.8	92.2	94.5
		B	93.1	93.2	94.5	95.0	96.2	96.7	95.1	91.4
		Mean	96.7	92.9	96.1	95.1	97.2	95.3	93.7	92.9

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, LOD: limit of detection (1.0% AR)
a) Minor degradates are summed up to sum of unidentified / diffuse residues (single max. < 2 %AR in the entire system)
b) Difference to material balance values due to rounding errors as well as clean up and chromatographic losses
c) Values taken from material balance
d) Mean values of the entire system could be unequal compared to the sum of the mean values of water and sediment, as values of the entire system are calculated individually for each replicate by summation of values from water and sediment before averaging the entire system values
4) Multiple metabolites <LOD, some rounding errors noted.

Figure B.8.2.2.3- 1: Proposed degradation pathway of pyrazole-labelled isoflucypram in water-sediment systems under aerobic conditions



C. DEGRADATION OF PARENT COMPOUND

Dissipation kinetics of isoflucypram from the water

The dissipation of isoflucypram from the water followed first order multi compartment (FOMC) kinetics in both water/sediment systems according to the lowest chi^2 error values and visual assessments. The table below summarises the best fit results of the DT_{50} and DT_{90} calculations for the dissipation of isoflucypram from the water.
The DT_{50} values for isoflucypram were 2.0 and 1.8 days in the water of the tested water/sediment systems under aerobic conditions.

Table B.8.2.2.3- 8: Dissipation of isoflucypram from the water phase

Water-sediment system (sediment texture (USDA))	Best fit kinetic model [a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual Assessment [b)]
Anglersee (sand)	FOMC	2.0	89.5	4.5	+
Wiehltalsperre (loam)	FOMC	1.8	41.4	2.6	+

a) FOMC: first order multi compartment
b) visual assessment: + = good

Degradation kinetics of isoflucypram in the entire water-sediment system

The degradation of isoflucypram in the total system followed single first order (SFO kinetics in system Anglersee and Wiehltalsperre, respectively, according to the lowest chi^2 error values and visual assessments. The table below summarises the best fit results of the DT_{50} and DT_{90} calculations for the degradation of isoflucypram in the total system.
The DT_{50} values for isoflucypram were 218 and 681 days in the total system of the tested water-sediment systems under aerobic conditions.

Table B.8.2.2.3- 9: Degradation of isoflucypram in the entire water-sediment system

Water-sediment system (sediment texture (USDA))	Best fit kinetic model [a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual Assessment [b)]
Anglersee (sand)	SFO	218	725	2.1	+
Wiehltalsperre (loam)	SFO	681	> 1000	1.3	+

a) SFO: single first order
b) visual assessment: + = good

III. CONCLUSIONS

UK RMS has assessed the supplied study and finds the study to be acceptable. Isoflucypram dissipated from the water in water/sediment systems under aerobic conditions in the laboratory in the dark. The concentration of isoflucypram in sediment increased to 68% after 7 days, reaching a maximum of 80.9 % after 72 days. From this peak the decline in sediment was slow. The calculated best fit DT_{50} values for the dissipation of isoflucypram proposed in the final report are from water 2.0 and 1.8 days in the tested water/sediment systems. In the total water-sediment system, isoflucypram was degraded slowly. The calculated best fit DT_{50} values for the total system were 218 and 681 days in the tested water-sediment systems. UK RMS has provided a kinetic assessment evaluation in section B.8.2.2.4 below. Degradation values should be taken from this assessment.

Formation of carbon dioxide accounted to ≤ 0.3% AR in both water/sediment systems.
Non-extractable residues accounted for a maximum of 6.4% AR in both water/sediment systems.
One degradation product of isoflucypram was identified: M12 with a maximum occurrence of 6.6% AR in the total system.

B.8.2.2.4: Kinetic assessment of the water sediment study

Previous evaluation:	None, new active substance.

Author: Reinken, G.; Kallweit, W.; 2017;
Title: Isoflucypram (ISY) and metabolite - Kinetic evaluation of aerobic aquatic metabolism in water/sediment systems
Report No.: EnSa-17-0356
Document No.: M-608356-02-1
Guideline(s): Not applicable
Guideline deviation(s): none
GLP/GEP: Not required modelling study

Study Summary

The degradation behaviour of isoflucypram in water-sediment systems was investigated in two aerobic laboratory water/sediment test systems in one experimental studies at 20°C in the dark (Hein, E. M.; Kasel, D.; 2017; see section B.8.2.2.3).
The objective of this study was to obtain degradation or dissipation half-lives of isoflucypram and its aquatic metabolite M12 in the water phase, sediment phase as well as in the total system of water and sediment in the dark.
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), according to FOCUS kinetics (FOCUS 2006[1], 2014[2]). The kinetic modelling analysis was conducted using the software tool KinGUI 2.1, implementing the IRLS error model (Iteratively reweighted least square) and validated by the UK RMS using CAKE v3.1. using ILRS with the extra solver if required.
The identification of the appropriate kinetic model followed the recommendations given by the FOCUS Degradation Kinetics Workgroup (FOCUS 2006, 2014) based on a detailed statistical analysis including visual assessment, chi²err statistics, significance t-test and correlation analysis.
The FOCUS kinetics report distinguishes between two levels of kinetics: At Level 1 a single compartment is used to derive (i) degradation endpoints from the total system or (ii) dissipation or decline endpoints from each compartment separately, in water, sediment or total system from maximum onwards. Level 2 considers two-compartmental approaches to estimate the real degradation in water and sediment, in parallel, considering exchange rates between water and sediment.
The resulting degradation and dissipation half-lives in total system, water or sediment phase (trigger and modelling purpose) and formation fractions of isoflucypram and its metabolite are given in Table B.2.2.4- 1 to Table 7.2.2.3- 4. The values presented are accepted by the UK RMS as extremely similar values were achieved in the RMS validation process.
For metabolite M12, no fully reliable and statistically significant dissipation kinetics in water or in total system could be derived (based on chi²err error, t-test) for modelling purpose. Only a formation fraction

[1] 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, v.2.0, June 2006
[2] FOCUS, 2014: Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. v1.1., 18. Dec. 2014. EU Document

for the total system could be derived for the system Anglersee.

Table B.8.2.2.4- 1: Degradation and dissipation in water / sediment systems: <u>trigger</u> endpoints of isoflucypram, Level P-I

Isoflucypram	**Distribution: max. in sediment 84.5%[a)] after 51 days (Wiehltalsperre)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**Temp. [ºC]**	**DT_{50} / DT_{90} whole sys. [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} water [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} sed [days]**	**St. (χ^2err) [%]**	**Method of calculation whole sys. / water / sed**
Anglersee, sand[b)]	7.1	6.6	20	211 / 702 (SFO)	2.09	2.79 / 74.2 (FOMC)	2.78	282 / 938 (SFO)	3.25	SFO / FOMC recalc / SFO
Wiehltalsperre, loam[b)]	7.3	5.1	20	593 / >1000 (SFO)	0.88	2.06 / 39.6 (FOMC)	2.10	n.r.	-	SFO / FOMC recalc / -
Geometric mean at 20ºC				**354**		**2.40**		**282**		

n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
a) maximum value of a single replicate
b) Hein, E. M.; Kasel, D.; 2017;

Table B.8.2.2.4- 2: Degradation and dissipation in water / sediment systems: <u>modelling</u> endpoints of isoflucypram, Level P-I

Isoflucypram	**Distribution: max. in sediment 84.5%[a)] after 51 days (Wiehltalsperre)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**Temp. [ºC]**	**DT_{50} / DT_{90} whole sys. [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} water [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} sed [days]**	**St. (χ^2err) [%]**	**Method of calculation whole sys. / water / sed**
Anglersee, sand[b)]	7.1	6.6	20	211 / 702 (SFO)	2.09	22.3 / 74.2 (FOMC recalc)	2.78	282 / 938 (SFO)	3.25	SFO / FOMC recalc / SFO
Wiehltalsperre, loam[b)]	7.3	5.1	20	593 / >1000 (SFO)	0.88	11.9 / 39.6 (FOMC recalc)	2.10	n.r.	-	SFO / FOMC recalc / -
Geometric mean at 20ºC				**354**		**16.3**		**282**		

n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
a) maximum value of a single replicate
b) Hein, E. M.; Kasel, D.; 2017;

Table B.8.2.2.4- 3: Degradation in water and sediment: <u>modelling</u> endpoints of isoflucypram, Level P-II

Isoflucypram	**Distribution: max. in sediment 84.5%[a] after 51 days (Wiehltalsperre)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**Temp. [°C]**	**DT_{50} / DT_{90} whole sys. [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} water [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} sed [days]**	**St. (χ^2err) [%]**	**Method of calculation water / sed**
Anglersee, sand[b]	7.1	6.6	20	n.r.	-	n.r.	-	n.r.	-	- / -
Wiehltalsperre, loam[b]	7.3	5.1	20	n.r.	-	n.r.	-	n.r.	-	- / -
Geometric mean at 20°C										

n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
a) maximum value of a single replicate
b) Hein, E. M.; Kasel, D.; 2017

Table B.2.2.4- 4: Degradation and dissipation in water / sediment systems: <u>trigger</u> endpoints of M12, Level M-I

Metabolite M12	**Distribution: max in total system 6.6 % after 100 d (Anglersee) max in water 5.4 % after 100 d (Anglersee) max in sediment 1.3 % after 100 d (Anglersee) Kinetic formation fraction (k_f/k_{dp}) from parent in total system: ff = 0.228 (n=1)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**Temp. [°C]**	**DT_{50} / DT_{90} whole sys. [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} water [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} sed [days]**	**St. (χ^2err) [%]**	**Method of calculation**
Anglersee, sand[a]	7.1	6.6	20	n.r.	-	n.e.	-	n.e.	-	- / - / -
Wiehltalsperre, loam[a]	7.3	5.1	20	n.r.	-	n.e.	-	n.e.	-	- / - / -
Geometric mean at 20°C				**n.r.**		**n.e.**		**n.e.**		

n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
n.e. = Not evaluable, not sufficient data points
a) Hein, E. M.; Kasel, D.; 2017

Table B.2.2.4-5: Degradation and dissipation in water / sediment systems: modelling endpoints of M12, Level M-I (pathway fit)

Metabolite M12	**Distribution: max in total system 6.6 % after 100 d (Anglersee) max in water 5.4 % after 100 d (Anglersee) max in sediment 1.3 % after 100 d (Anglersee) Kinetic formation fraction (k_f/k_{dp}) from parent in total system: ff = 0.228 (n=1)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**Temp. [°C]**	**DT_{50} / DT_{90} whole sys. [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} water [days]**	**St. (χ^2err) [%]**	**DT_{50} / DT_{90} sed [days]**	**St. (χ^2err) [%]**	**Method of calculation whole sys. / water / sed**
Anglersee, sand[a]	7.1	6.6	20	n.r.	-	n.e.	-	n.e.	-	- / - / -
Wiehltalsperre, loam[a]	7.3	5.1	20	n.r.	-	n.e.	-	n.e.	-	- / - / -
Geometric mean at 20°C				**n.r.**		**n.e.**		**n.e.**		

n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
n.e. = Not evaluable, not sufficient data points
a) Hein, E. M.; Kasel, D.; 2017;

I. METHODS

The objective of this study was to obtain degradation or dissipation half-lives of isoflucypram and its aquatic metabolite M12 in the water phase, sediment phase as well as in the total system of water and sediment in the dark. 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), according to FOCUS kinetics (FOCUS 2006, 2014).
The FOCUS kinetics report distinguishes between two levels of kinetics: At Level 1 a single compartment is used to derive (i) degradation endpoints from the total system or (ii) dissipation or decline endpoints from each compartment separately, in water, sediment or total system from maximum onwards. Level 2 considers two-compartmental approaches to estimate the real degradation in water and sediment, in parallel, considering exchange rates between water and sediment.

Isoflucypram and its aquatic metabolite M12 were addressed for the total system, water and sediment phases.
The degradation behaviour of isoflucypram in water-sediment systems was investigated in two aerobic laboratory water/sediment test systems in one experimental study at 20°C in the dark (Hein, E. M.; Kasel, D.; 2017;). Duplicate samples were taken at 0, 3, 7, 14, 29, 51, 72, and 100 days after treatment (DAT). In all trials, the parent substance isoflucypram was applied.
Further information on study conditions, observed metabolites and physico-chemical properties is summarised in Table B.2.2.4-6. Data entry used by the applicant and accepted by the RMS is presented in tables B.8.2.2.4-7 to B.8.2.2.4-10.

Table B.2.2.3-6: General information on aerobic aquatic laboratory studies with isoflucypram

Water-sediment system	Texture of sediment	Radioactive label	Duration [days]	Metabolites observed
Anglersee, GER	sand	pyrazole-label	100	M12
Wiehltalsperre, GER	loam			

Table B.8.2.2.4-7 Residue data inputs (%AR) for isoflucypram in aerobic water/ sediment system Anglersee.

DAT	Non time shifted data			DAT (time shifted)	Time shifted data for sediment analysis #		
	water	sediment	total system		water	sediment	total system
0	96.6	0	96.6	-	-	-	-
0	99.2	0	99.2	-	-	-	-
3	39.8	51.8	91.6	-	-	-	-
3	40.9	52	93	-	-	-	-
7	26.5	66.2	92.7	-	-	-	-
7	38.2	55.4	93.6	-	-	-	-
14	22	71.5	93.5	-	-	-	-
14	27.8	65.9	93.7	-	-	-	-
29	12.7	79.4	92.1	0	12.7	79.4	92.1
29	16	73	89	0	16	73	89
51	11.7	65.9	77.6	22	11.7	65.9	77.6

51	13.4	64.6	78	22	13.4	64.6	78
72	10.8	65.1	75.9	43	10.8	65.1	75.9
72	9.7	66.4	76.1	43	9.7	66.4	76.1
100	8.4	63.1	71.4	71	8.4	63.1	71.4
100	7.9	63.4	71.3	71	7.9	63.4	71.3

Samples from the peak occurrence in sediment onwards to enable "top down" fitting.

Table B.8.2.2.4-8 Residue data inputs (%AR) for M12 in aerobic water/ sediment system Anglersee.

	Residue data reported			Residue data used		
DAT	water	sediment	total system	water	sediment	total system
0	n.d.	n.d.	n.d.	0	0	0
0	n.d.	n.d.	n.d.	0	0	0
3	n.d.	n.d.	n.d.	-	-	-
3	n.d.	n.d.	n.d.	-	-	-
7	n.d.	n.d.	n.d.	-	-	-
7	n.d.	n.d.	n.d.	-	-	-
14	n.d.	n.d.	n.d.	0.5 a)	-	0.5 a)
14	n.d.	n.d.	n.d.	0.5 a)	-	0.5 a)
29	2.2	n.d.	2.2	2.2	-	2.2
29	1.7	n.d.	1.7	1.7	0.5 a)	1.7
51	2.7	n.d.	2.7	2.7	0.5 a)	2.7
51	2.2	1.2	3.3	2.2	1.2	3.3
72	3.3	1.2	4.5	3.3	1.2	4.5
72	3.2	1.2	4.4	3.2	1.2	4.4
100	5.5	1.0	6.5	5.5	1.0	6.5
100	5.3	1.5	6.8	5.3	1.5	6.8

a) 0.5X LOD

Table B.8.2.2.4-9 Residue data inputs (%AR) for isoflucypram in aerobic water/ sediment system Wiehltalsperre.

	Non time shifted data			DAT (time shifted)	Time shifted data for sediment analysis #		
DAT	water	sediment	total system		water	Sediment*	total system
0	100.4	0	100.4	-	-	-	-
0	93.1	0	93.1	-	-	-	-
3	39.5	51.3	90.8	-	-	-	-
3	35.8	55.7	91.5	-	-	-	-
7	27.4	68.0	95.4	-	-	-	-
7	26.1	66.4	92.5	-	-	-	-
14	18.3	74.2	92.6	-	-	-	-

14	16.5	76.2	92.7	-	-	-	-
29	12.4	80.5	93.0	-	-	-	-
29	12.0	79.9	91.9	-	-	-	-
51	8.7	81.4	90.1	0	8.7	81.4	90.1
51	8.2	84.5	92.7	0	8.2	84.5	92.7
72	7.7	80.1	87.8	21	7.7	80.1	87.8
72	7.3	80.9	88.1	21	7.3	80.9	88.1
100	6.7	79.4	86.1	49	6.7	79.4	86.1
100	2.0	80.8	82.8	49	2.0	80.8	82.8

* Insufficient numbers of time points to derive a kinetic fit.
# Samples from the peak occurrence in sediment onwards to enable "top down" fitting.

Table B.8.2.2.4-10 Residue data inputs (%AR) for M12 in aerobic water/ sediment system Anglersee.

	Residue data reported			Residue data used		
DAT	**water**	**sediment**	**total system**	**water**	**sediment**	**total system**
0	n.d.	n.d.	n.d.	0	0	0
0	n.d.	n.d.	n.d.	0	0	0
3	n.d.	n.d.	n.d.	-	-	-
3	n.d.	n.d.	n.d.	-	-	-
7	n.d.	n.d.	n.d.	-	-	-
7	n.d.	n.d.	n.d.	-	-	-
14	n.d.	n.d.	n.d.	0.5 a)	-	0.5 a)
14	n.d.	n.d.	n.d.	-	-	-
29	1.2	n.d.	1.2	1.2	-	1.2
29	<LOD	n.d.	<LOD	-	-	-
51	<LOD	<LOD	<LOD	0.5 a)	-	0.5 a)
51	<LOD	<LOD	<LOD	0.5 a)	0.5 a)	0.5 a)
72	<LOD	<LOD	<LOD	0.5 a)	-	0.5 a)
72	1.2	1.2	2.4	1.2	1.2	2.4
100	1.1	<LOD	1.1	1.1	-	1.1
100	<LOD	<LOD	<LOD	0.5 a)	0.5 a)	0.5 a)

The kinetic evaluation of the laboratory degradation behaviour was done following a tiered approach, based on various model assumptions according to FOCUS kinetics (FOCUS 2006, 2014) using the software KinGUI 2.1 with four different kinetic models: Single First-Order (SFO) and the bi-exponential models FOMC (First-Order Multi-Compartment model), DFOP (double first order parallel) and HS (Hockey-stick).
For the kinetic evaluation of water-sediment studies FOCUS (2006, 2014) distinguishes two levels of kinetics:
- Level I: One compartmental approach to estimate the dissipation from the water column, the sediment (from maximum onwards) or the degradation from the total system, as a single compartment.
- Level II: Multi-compartmental approach to estimate the degradation in the water column and sediment compartments in parallel, including partitioning processes via reaction rates or sorption isotherms.

For the aquatic exposure assessment, a Level II evaluation is not mandatory. FOCUS recommends e.g. for parent compound to use the Level I total-system degradation half-life for both compartments at Step 2 level, or in combination with the conservative worst-case default degradation half-life of 1000 days for the respective other compartment at Step 3 level. For lower tier calculations or the comparison with trigger values often a Level I evaluation of the dissipation is appropriate.

Dissipation kinetics:
Dissipation half-lives of the parent compound and metabolites, separately for the water- and sediment phase, as well as for the total system, mainly for metabolites, can be derived starting from the maximum onwards. The time axis might be shifted by the time t_{max}, where the maximum occurred. Generally, free fitting of the initial amount is used as default for all substances or phases.

Degradation kinetics:
- Level P-I degradation: Additionally, overall degradation rates of each substance from the total water-sediment system should be derived from an overall compartment modelling approach.
  The proposed metabolic route of isoflucypram in water-sediment systems was converted into compartment systems. The compartments were associated with the sum of measured amounts in the water and the sediment phase of the compounds. No values were associated with sink compartment. If obviously no kinetic evaluation of a metabolite was possible, e.g. too low measurements or no significant decay during the duration of the study, the compartment system can be simplified correspondingly. Correspondingly, if a degradation pathway is observed not to be significant or relevant, it can be deleted in a further evaluation.
- Level P-II degradation: A 2-compartmental approach was taken into account to estimate the degradation in the water column and sediment compartments, in parallel, inclusive partitioning processes via reaction rates. Simple first-order (SFO) kinetics was used to describe degradation separately in the water and the sediment phase as well as reversible transfer between these compartments.

II. RESULTS AND DISCUSSION

A summary of all results for modelling and trigger purpose is given in the executive summary. The most appropriate kinetic parameters are summarised per water-sediment system, for modelling and trigger purpose, for parent isoflucypram and its metabolite M12.

Dissipation kinetics

Dissipation from water, isoflucypram (level P-I)
Residues of isoflucypram in water have been measured at study end in both trials below 10% of the initial residues. A $DissT_{50}$ from biphasic models is estimated by DT_{90} / 3.32. This is a dissipation rate and not used in FOCUS modelling.

For both systems, initially all data points were included in the kinetic evaluation. In a modified approach, the residue data of DAT 3 were excluded from the fit as the concentrations are noticeable decreased at this day in both systems by the applicant. The statistics (chi² err) were improved for most of the fits (compare Figure B.2.2.4-1 and Figure B.2.2.4- 2 for system Anglersee; Figure B.2.2.4- 3 and Figure B.2.2.4- 4 for system Wiehltalsperre). For derivation of modelling and trigger endpoints, the modified fits were taken into account. UK RMS does not accept that the 3 DAT time point is sufficiently low to remove the data points and uses the initial fit data. All kinetic fit assessments are provided in table B.8.2.2.4-11 for reference.

Table B.8.2.2.4-11: Isoflucypram: kinetic and statistical results of dissipation from water

Kinetic model	DT_{50} trigger [days]	DT_{90} trigger [days]	DT_{50} mod* [days]	VA	χ^2err [%]	k_1/α [1/d/-]	k_2/β [1/d/-]	tb/g [d/-]	t-test of k_1/k_2	MS
Anglersee										
SFO[a)]	4.60	15.3	4.60	-	30.3	0.1507			< 0.001	
FOMC[a)]	**2.03**	**89.5**	**26.9**	+	4.54	0.4536	0.5622			**M/T**
DFOP[a)]	2.13	66.6	20.1	+	7.03	0.6079	0.01782	0.6726	< 0.001 / < 0.001	
HS[a)]	2.35	65.1	19.6	o	7.41	0.2955	0.01889	3.8833	< 0.001 / < 0.001	
SFO[b)]	5.93	19.7	5.93	-	25.4	0.1168			< 0.001	
FOMC[b)]	2.79	74.2	22.3	+	2.78	0.5452	1.078			
DFOP[b)]	3.84	73.9	22.3	+	5.19	0.2734	0.01233	0.7514	< 0.001 / 0.005	
HS[b)]	4.38	71.2	21.4	o	6.03	0.1582	0.01444	8.866	< 0.001 / 0.001	
Wiehltalsperre										
SFO[a)]	3.32	11.0	3.32	-	26.8	0.2088			< 0.001	
FOMC[a)]	**1.76**	**41.4**	**12.5**	+	2.60	0.5696	0.74043			**M/T**
DFOP[a)]	2.06	47.4	14.3	+	5.51	0.5261	0.02025	0.7388	< 0.001 / < 0.001	
HS[a)]	2.20	46.2	13.9	o	6.58	0.3146	0.02285	4.276	< 0.001 / < 0.001	
SFO[b)]	4.52	15.0	4.52	-	22.5	0.1534			< 0.001	
FOMC[b)]	2.06	39.6	11.9	+	2.10	0.6231	1.0081			
DFOP[b)]	3.16	46.9	14.1	+	1.81	0.3007	0.0148	0.8001	< 0.001 / < 0.001	
HS[b)]	3.77	47.8	14.4	-	2.42	0.1837	0.01615	9.137	< 0.001 / < 0.001	

* $DT_{50\ mod}$ Half-life for modelling before normalisation
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)
a) Initial fit including all residue data
b) Modified fit excluding the residue data of DAT 3, not accepted by the RMS.
VA) Visual assessment: + = good, o = moderate, - = poor
Dissipation rate is not used in FOCUS modelling.

For both systems, SFO resulted in a visually not acceptable fit as the later data points were clearly underestimated and the residuals not systematically distributed. FOMC fits resulted in the best chi² tests and best visual assessments. FOMC results were therefore proposed for trigger and modelling purpose.

Figure B.8.2.2.4-1: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Anglersee (initial fit)

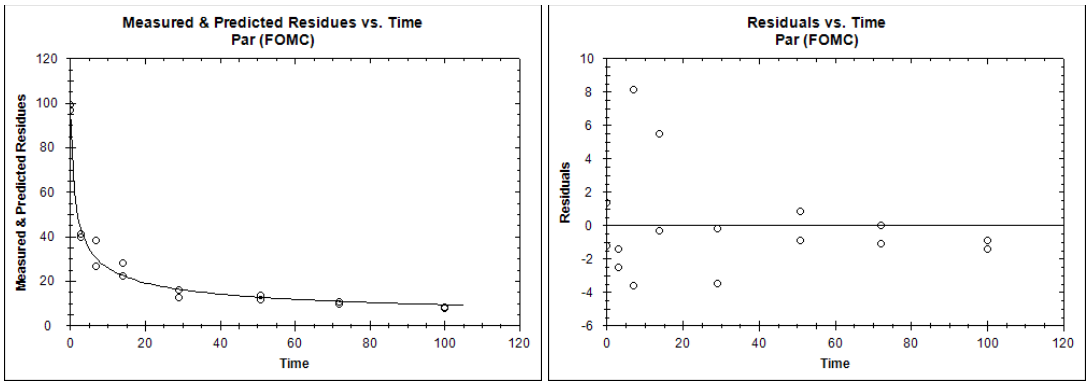


Figure B.8.2.2.4-2: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Anglersee (modified fit). Not accepted by RMS

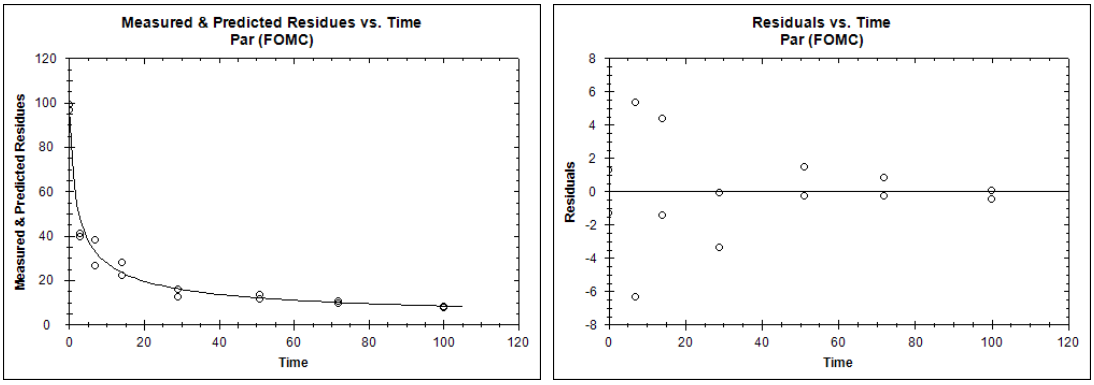


Figure B.8.2.2.4-3: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Wiehltalsperre (initial fit)

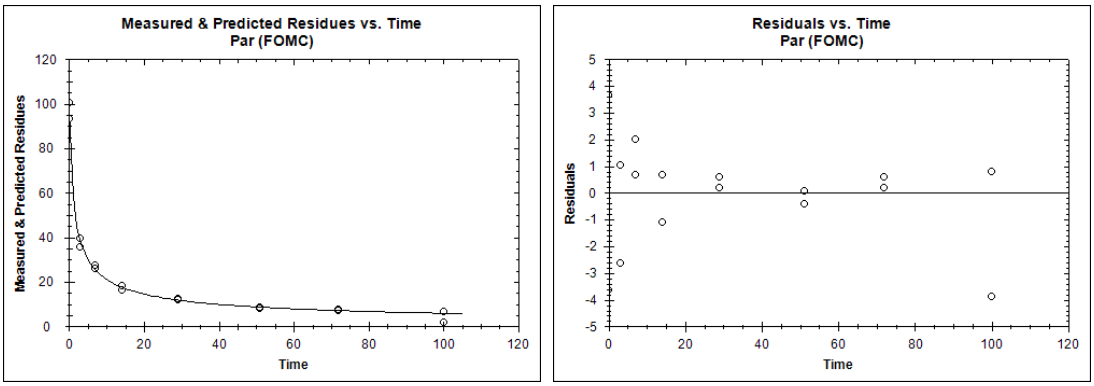
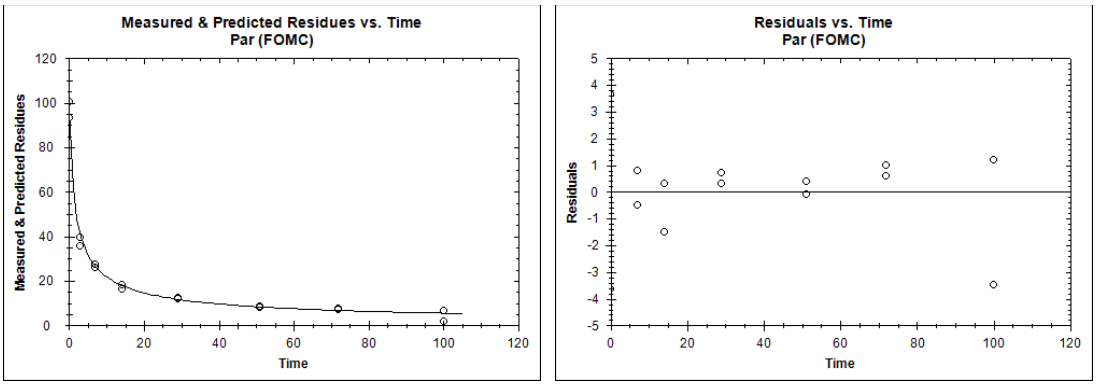


Figure B.8.2.2.4- 4: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Wiehltalsperre (modified fit). Not accepted by RMS.



Dissipation from sediment, isoflucypram (level P-I)

Table B.8.2.2.4-12: Isoflucypram: kinetic and statistical results of dissipation from sediment

Kinetic model	DT_{50} trigger [days]	DT_{90} trigger [days]	DT_{50} mod* [days]	VA	χ^2err [%]	k_1/α [1/d/-]	k_2/β [1/d/-]	tb/g [d/-]	t-test of k_1/k_2	MS
Anglersee										
SFO	**282**	**938**	**282**	o	3.25		0.002455		0.0079	**M/T**
FOMC	>1000	>1000	>1000	+	1.01	0.02415	0.04785			
Wiehltalsperre										
SFO	>1000	>1000	>1000	o	n.e.		-		-	
FOMC	>1000	> 1000	>1000	o	n.e.	-	-			

* $DT_{50\ mod}$ = Half-life for modelling: if residues at end < 10 %, $DT_{50} = DT_{90} / 3.32$; otherwise DT_{50} of slow phase

n.e. = Not evaluable, not sufficient data points

MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

VA) Visual assessment: + = good, o = moderate, - = poor

For the system Anglersee the SFO fit is statistically acceptable but visually borderline based on the low number of available data points (four, each with two replicates). While the fit was visually improved using the FOMC model, no degradation parameters could be derived. Thus the SFO model is considered appropriate for trigger and modelling purpose.

For the system Wiehltalsperre only three data points (each with two replicates) from the maximum onwards were available. Based on these low numbers and some scattering of the data no reliable degradation parameters could be derived. UK RMS accepts this approach.

Dissipation from water or sediment, M12, decline (level M-I)

A attempt was made to evaluate the dissipation of the metabolite M12 in water or sediment phase from the observed maximum onwards.

However, in the system Anglersee an evaluation was not possible due to the fact that the concentration of the metabolite was still increasing at the end of the study.

In the system Wiehltalsperre M12 was only detected in three (water) or one (sediment) samples. Based on these few data points a kinetic evaluation was not possible. This is accepted by the RMS.

Degradation kinetics

Degradation in total system, isoflucypram (level P-I)

Table B.8.2.2.4-13: Isoflucypram: kinetic and statistical results of degradation in total system

Kinetic model	DT_{50} trigger [days]	DT_{90} trigger [days]	DT_{50} mod* [days]	VA	χ^2err [%]	k_1/α [1/d/-]	k_2/β [1/d/-]	tb/g [d/-]	t-test of k_1/k_2	MS
Anglersee										
SFO[a)]	**222**	**736**	**222**	o	2.23	0.003128			<0.001	**M/T**
FOMC[a)]	222	738	222	o	2.41	390.8	124900			
SFO[b)]	211	702	211	o	2.09	0.003281			<0.001	
FOMC[b)]	211	702	211	o	2.31	12850	3916000			
Wiehltalsperre										
SFO[a)]	**681**	**>1000**	**681**	o	1.43	0.001018			<0.001	**M/T**
FOMC[a)]	681	>1000	-	o	1.54	3.491E+5	3.429E+8			
SFO[b)]	593	>1000	593	o	0.88	0.001168			<0.001	
FOMC[b)]	593	>1000	-	o	0.97	3.665E+6	3.137E+9			

* $DT_{50\ mod}$ = Half-life for modelling: if residues at end < 10 %, DT_{50} = DT_{90} / 3.32; otherwise DT_{50} of slow phase
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)
a) Initial fit including all residue data
b) Modified fit excluding the residue data of DAT 3, not accepted by the RMS.
VA) Visual assessment: + = good, o = moderate, - = poor

For the systems Anglersee and Wiehltalsperre a pathway fit of isoflucypram with M12 has been carried out. Initially, all data points were included in the kinetic evaluation. As the residue data measured at DAT 3 are low compared to those measured before and afterwards, a modified fit was conducted additionally where the residue data measured at DAT 3 were excluded by the applicant. This was not accepted by the RMS as it cannot be concluded that the DAT 3 is genuine, data should not be excluded to simply improve kinetic fits . For derivation of modelling and trigger endpoints, the modified fits were not taken into account. The SFO fit is visually and statistically acceptable. As the FOMC model does not significantly improve the fit the SFO model is chosen for derivation of modelling and trigger endpoints.

Figure B.8.2.2.4-5: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Anglersee (initial fit)

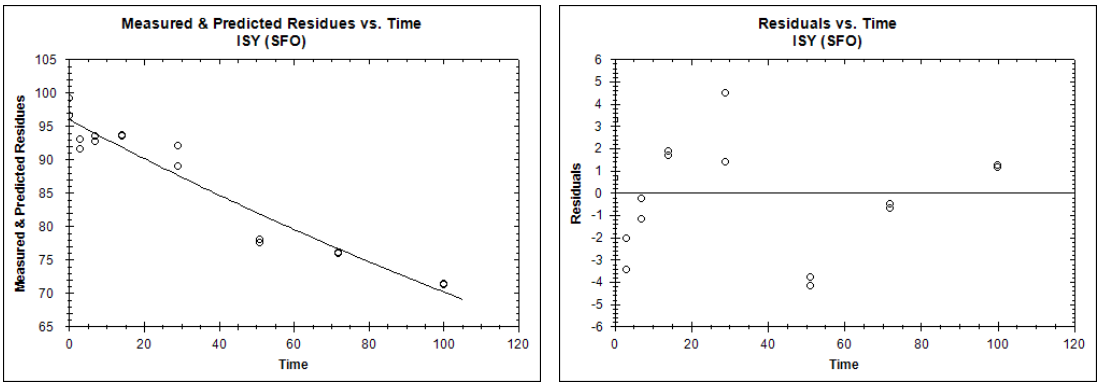


Figure B.8.2.2.4-6: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Anglersee (modified fit not accepted by RMS)

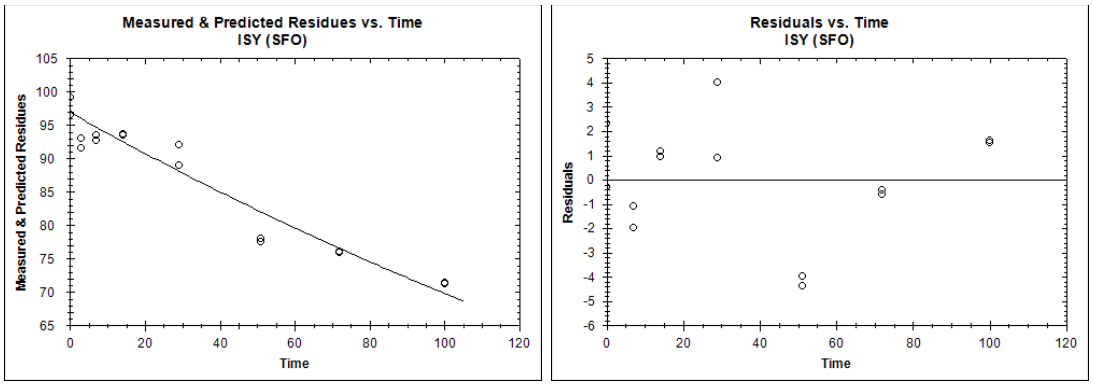


Figure B.8.2.2.4-7: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Wiehltalsperre (initial fit)

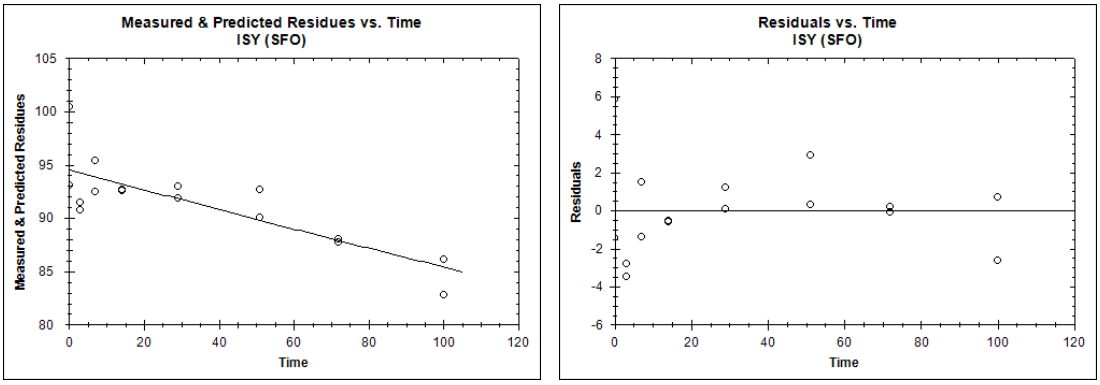
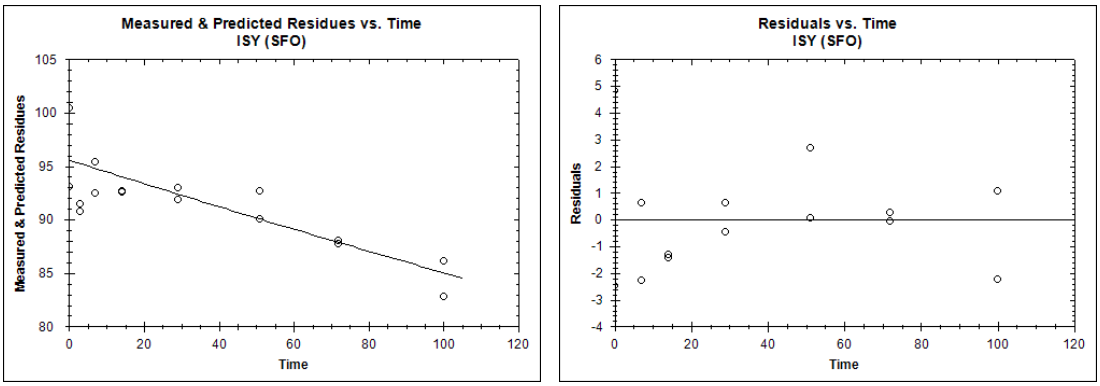


Figure B.8.2.2.4-8: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Wiehltalsperre (modified fit not accepted by RMS)



Degradation in total system, M12, pathway (level M-I)

In general, the kinetic evaluation of total system degradation of the metabolite M12 was based on the pathway fit in combination with the parent compound isoflucypram. For the metabolite always an SFO kinetic model was chosen in the pathway fit. Finally, the metabolite results reported here are based on the corresponding appropriate parent fit, for modelling (m) or trigger purpose (p).

It should be noted, that the 15% threshold value for the scaled error ε of the chi² err test should not be employed as absolute cut-off criterion, as this value is strictly appropriate only for optimal experimental conditions. It might be that the error to pass the chi² err test is higher than 15%, but the model fit still represents a reasonable description of the degradation behaviour. Especially in case of field data or for

metabolites it may be justified to accept larger values, due to generally low measurements compared to the mean of all measurements, which strongly influences the chi² err test.

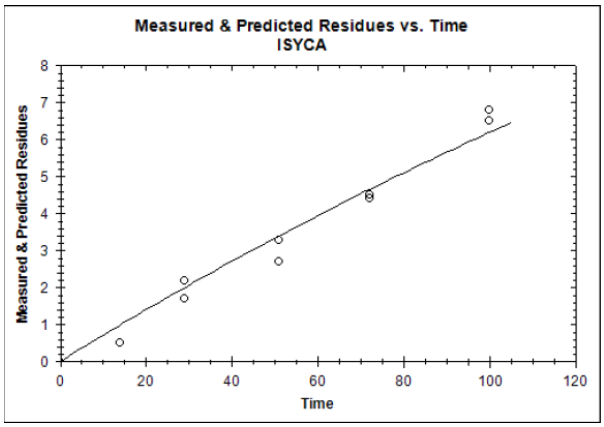
Table B.8.2.2.4-14: M12: kinetic and statistical results of degradation in total system, pathway fit: SFO degradation

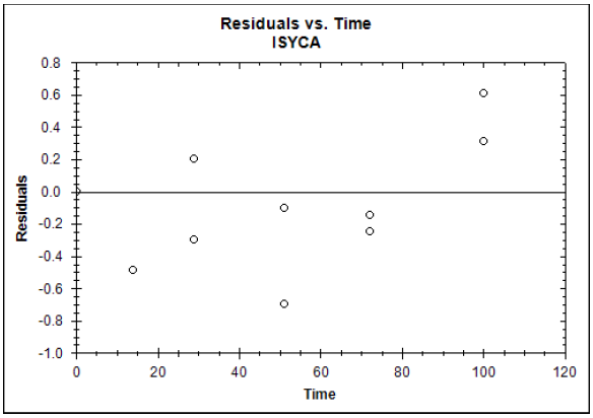
Kinetic model of parent	DT_{50} trigger [days]	DT_{90} trigger [days]	DT_{50} mod* [days]	ff	VA	χ^2err [%]	k_1/α [1/d/-]	k_2/β [1/d/-]	tb/g [d/-]	t-test of k_1/k_2	MS
Anglersee											
SFO-SFO[a)]	> 1000[n.r.]	> 1000[n.r.]	> 1000[n.r.]	0.240 ± 0.027	+	8.71	2.250E-14	-	-	0.5	-
SFO-SFO[b)]	> 1000[n.r.]	> 1000[n.r.]	> 1000[n.r.]	**0.228 ± 0.056**	+	8.84	2.295E-14	-	-	0.5	-
Wiehltalsperre											
SFO-SFO[a)]	12.0	39.7	12.0	0.632 ± 0.811	-	31.1	0.05799	-	-	0.248	-
SFO-SFO[b)]	12.2	40.6	12.2	0.538 ± 0.675	-	31.1	0.05671		--	0.247	-

n.r. = Not fully reliable, mathematically not significantly different from 0, not usable
n.e. = Not evaluated, not enough data points for kinetic evaluation available
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)
* $DT_{50\ mod}$ Half-life for modelling before normalisation
a) Initial fit including all residue data
b) Modified fit excluding the residue data of DAT 3, not accepted by the RMS.

For the system Anglersee, even though the visual fit is good the degradation rate is not significantly different from zero and statistically not reliable. Consequently, no reliable degradation half-life could be estimated. The formation fraction, however, is considered reliable based on the good fit and an acceptable standard deviation.
For the system Wiehltalsperre, residue data of M12 were included in the pathway fit even though the metabolite had only been detected in three samples. It was not possible to derive reliable degradation half-lives or formation fractions from these data. This is accepted by the RMS and the system is not considered further.

Figure B.8.2.2.4.9 SFO-SFO fit and residual graphs for M12 (BCS-CN88460- carboxylic acid) Anglersee, all data points.





Degradation in water and sediment phase (level P-II)

The applicant attempted to perform a P-II assessment. The applicant concluded that no separate degradation rates for water and sediment could be derived and it was not possible to derive degradation parameters from these fits.

III. CONCLUSIONS

The trigger and modelling endpoints for isoflucypram are presented in Table B.8.2.2.4-15 and Table B.8.2.2.4-16 to Table B.8.2.2.4-17, respectively.
For metabolite M12 no fully reliable and statistically significant dissipation kinetics in water or in total system could be derived (based on χ^2err error, t-test) for modelling purpose. Only a formation fraction for the total system (ff = 0.240) could be derived for the system Anglersee, default values of 1000 days are proposed for both modelling and persistence/ triggering endpoints.

Table B.8.2.2.4- 15: Degradation and dissipation in water / sediment systems: <u>trigger</u> endpoints of isoflucypram, Level P-I

Water / sediment system	Whole system			Water			Sediment		
	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation
Anglersee	222	736	SFO	2.79	74.2	FOMC recalc.	222	736	SFO
Wiehltalsperre	681	>1000	SFO	2.06	39.6	FOMC recalc.	n.r.	n.r.	-
Geometric mean	**388**			**2.40**			**222**		

Table B.8.2.2.4- 16: Degradation and dissipation in water / sediment systems: <u>modelling</u> endpoints of isoflucypram, Level P-I

Water / sediment system	Whole system			Water			Sediment		
	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation
Anglersee	222	736	SFO	1000	1000	Default.	222	736	SFO
Wiehltalsperre	681	> 1000	SFO	1000	1000	Default	681	> 1000	SFO
Geometric mean	**388**			**1000**			**388**		

Table B.8.2.2.4- 17: Degradation and dissipation in water / sediment systems: <u>modelling</u> endpoints and trigger of M12, Level P-I

Water / sediment system	Whole system			Water			Sediment		
	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation
Anglersee*	1000	1000	default	1000	1000	Default.	1000	1000	SFO
Wiehltalsperre	1000	1000	default	1000	1000	Default	1000	1000	SFO
Geometric mean	1000			**1000**			1000		

* Formation fraction of 0.240 was calculated.

B.8.2.2.5: Summary of the Fate and Behaviour in water and Sediment

Isoflucypram is hydrolytically stable in sterile aqueous buffer solutions at three pH values (pH 4, 7 and 9) in the laboratory in the dark. No degradation products of isoflucypram were observed.

Hydrolytic degradation is unlikely to contribute to the degradation of isoflucypram under typical conditions of the environment.

Photodegradation is unlikely to contribute to the degradation of isoflucypram under typical light conditions of the environment. Isoflucypram was slowly degraded in aqueous buffer solution at pH 7 under exposure to simulated sunlight and aerobic conditions in the laboratory. No degradation products > 10% AR were observed.

In surface water under aerobic conditions, isoflucypram does not degrade. Isoflucypram dissipated rapidly from the water in water/sediment systems under aerobic conditions. One degradation product of isoflucypram was identified: M12 with a maximum occurrence in the total system of 6.6% AR (water layer 5.4%; sediment 1.3%, respectively) at the end of the study (100 days). Formation of carbon dioxide accounted to ≤ 0.3% AR in both water/sediment systems. Non-extractable residues accounted for a maximum of 6.4% AR in both water/sediment systems. The proposed metabolic pathway of isoflucypram in the aerobic water/sediment systems is shown in Figure B.8.-2.

The dissipation rates in water have been assessed as 12.5 days and 26.9 days (DT_{50} geomean) of 18.33 days, dissipation rates are included for Member States wishing to rely on those values. The DT_{50} for the whole system degradation (SFO) of 354 days was determined.

B.8.2.3. Degradation in the saturated zone

The degradation of isoflucypram in the saturated zone was not studied since isoflucypram is not expected to reach the saturated zone after its use according to good agricultural practices. A summary of the route and rate of degradation of isoflucypram in water and sediment is given in section B.8.2.2.5.2 and Figure B.8-2.

B.8.3. FATE AND BEHAVIOUR IN AIR

B.8.3.1. Route and rate of degradation in air

Isoflucypram has a very low vapour pressure of 1.2×10^{-7} Pa (Dreisch, S.; (2014) at 20°C, summarised in Volume 3, section B.2). Therefore, it can be concluded that significant volatilisation of isoflucypram is not to be expected.
In addition, estimates of the chemical lifetime in the troposphere resulted in half-lives < 2 days for isoflucypram.

B.8.3.1 Route and rate of degradation in air

Previous evaluation:	None, new active substance.

Author: Beckmann, M.; 2015
Title: BCS-CN88460: Calculation of the chemical half-life in the troposphere
Report No.: EnSa-15-1015
Document No.: M-544687-01-1
Guideline(s): Not applicable
Guideline deviation(s): none
GLP/GEP: Not required modelling study

Study Summary

Based on the estimation according to structure-activity relationship (SAR) methods developed by Atkinson *et al.*, the half-life time in air of isoflucypram was assessed with the computer program AOPWIN™ (version 1.92).
The half-life time ($t_{1/2}$) was estimated with 0.344 days (long-term scenario) assuming the typical OH radical concentration averaged over 24 hours (0.5×10^6 radicals/cm³).
The half-life time ($t_{1/2}$) was estimated with 0.229 days (long-term scenario) assuming the typical OH radical concentration averaged over 12 hours (1.5×10^6 radicals/cm³).

I. METHODS

The objective of this report is the assessment of the potential chemical half-life ($t_{1/2}$) of isoflucypram in the troposphere. The model calculation was based on structure-activity relationship (SAR) methods developed by Atkinson *et al.* and available as the computer program AOPWIN ("Atmospheric Oxidation Program for Microsoft Windows"), version 1.92a.
The program is able to estimate reaction rate constants in the atmospheric gas-phase between light- and thus photochemically generated hydroxyl radicals and organic chemicals. It is also able to estimate rate constants for gas-phase reactions between ozone and compounds containing double (olefinic) or triple (acetylenic) bonds. The rate constants estimated by the program are used in the following for the calculation of half-lives of organic compounds in the atmosphere on the basis of average atmospheric concentrations of hydroxyl radicals and ozone. AOPWIN™ requires only the chemical structure and atmospheric concentrations of the potential reaction partners as inputs.
Considering the chemical structure of isoflucypram, it can be concluded that reactions with photochemical produced hydroxyl radicals will mainly determine its degradation rate ($K_{\text{total, indirect photoreaction}} \approx k_{OH}$) in the air.
No ozone reaction is expected and therefore not included for the determination of isoflucypram.

The AOPWIN program allows the user to select 12 or 24 hour time frames and any average hydroxyl radical concentrations. For the current report the 0.5×10^6 radicals/cm^3 per day (24-h) was taken for the long term estimations.

II. RESULTS AND DISCUSSION

The overall reaction rate of isoflucypram with hydroxyl radicals is estimated to be 46.6558 x 10^{-12} cm^3 x $molecule^{-1}$ x s^{-1}. This rate is derived mainly from incremental reactions like hydrogen abstraction (6.5543 x 10^{-12} cm^3 x $molecule^{-1}$ x s^{-1}) and an addition reaction to the aromatic ring (assumed value of 40.1015 x 10^{-12} cm^3 x $molecule^{-1}$ x s^{-1}, value estimated).

Based on the overall hydroxyl radical reaction rate constant in combination with the "long term" concentration of these radicals in the atmosphere (*i.e.*24 h day, 0.5 x 10^6 OH radicals/cm^3, 12 h day, 1.5 x 10^6 OH radicals/cm^3) the half-life ($t_{1/2}$) of isoflucypram in air is derived to:

- Half-life ($t_{1/2}$) = 0.344 days (24 h day)
- Half-life ($t_{1/2}$) = 0.229 days (12 h day)

That estimate should be regarded as worst-case assumption as the approach does not consider the contribution of any other reactive species to the overall atmospheric degradation of isoflucypram in air.

III. CONCLUSIONS

UK RMS has repeated the calculations using the same model and version and achieves the same result.

B.8.3.2. Transport via air

The transport via air of isoflucypram was not studied since its vapour pressure is below the FOCUS air trigger value of 10^{-5} Pa.

B.8.3.3. Local and global effects

On account of the short chemical lifetime of isoflucypram in the air it is to be expected that the substances cannot be transported in the gaseous phase over large distances or can accumulate in the air. Thus, no difference in the behaviour isoflucypram and other organic substances emitted into the air from natural sources (*e.g.* from plants and soil) is indicated.

B.8.4. MONITORING DATA CONCERNING FATE AND BEHAVIOUR OF THE ACTIVE SUBSTANCE, METABOLITES, DEGRADATION AND REACTION PRODUCTS

B.8.4.1 Definition of the residue

A definition of the residue is provided in table B.8.4.1-1. Isoflucypram is a new active substance as such no monitoring data is available.

Table B.8.4.1-1: Residue definitions for relevant risk assessment.

Compartment	Residue definition for risk assessment
Soil	Isoflucypram and BCS-CN88460-carboxylic acid *(M12) (M10)*?*
Groundwater	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)* ***(M10)*?***
Surface water	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Sediment	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Air	Isoflucypram

* less than 5% AR but increasing very slightly at the last timepoint.

B.8.4.2: Definition of the residue for monitoring

Not proposed, to be decided at the conclusion of the E.U. peer review procedure.

B.8.5. REFERENCES RELIED ON

B.8.5.1: Literature review

For Isoflucypram and its metabolites no publication has been identified which would indicate a side effect on the environment. The applicant has conducted a literature search using appropriate search criteria including the phrases IUPAC name, CAS name/number, common names, codes and abbreviations, molecular structure, molecular formula, molar mass and other names codes. A large database of scientific literature has been searched. Since this is a new active substance no literature data were found and no results were returned for Isoflucypram and its metabolite M12. This is the sum of the information provided by the applicant for the literature review.

B.8.5.2: References relied upon.

Table B.8.5.2-1. Table of references relied upon.

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	Hellpointner, E.; Junge, T.	2014	[14C]BCS-CN88460: Aerobic metabolism/degradation in four soils Bayer Report No.: EnSa-13-1043 Edition Number: M-486690-01-1 Date: 2014-05-05 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.1.1 / 02	Gabbert, D.; McConnell, L. L.; Arthur, E. L.	2017	[Pyrazole-4-14C]BCS-CN88460: Aerobic soil metabolism in two US soils Bayer CropScience LP, Stilwell, KS, USA Bayer Report No.: MELNN013	No	Yes	New data for a new active substance	Bayer	

			Edition Number: M-588260-01-1 Date: 2017-05-11 GLP/GEP: Yes, unpublished					
KCA 7.1.1.1 / 03	Heineman n, O.; Kasel, D.	2017	[Phenyl-UL-14C]BCS-CN88460: Aerobic degradation / metabolism in one soil Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-16-0986 Edition Number: M-599926-01-1 Date: 2017-09-05 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.1.2 / 01	Heineman n, O.; Kasel, D.	2015	[Pyrazole-4-14C]BCS-CN88460: Anaerobic degradation / metabolism in one soil Bayer Report No.: EnSa-14-0146 Edition Number: M-513456-01-1 Date: 2015-03-03 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.1.3 / 01	Heineman n, O.	2013	[Pyrazole-4-14C]BCS-CN88460: Phototransformation on soil Bayer Report No.: EnSa-13-0200 Edition Number: M-467307-01-1 Date: 2013-10-11 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.2 / 01	Reinken, G.; Kallweit, W.	2017a	Isoflucypram (ISY): Core PECsoil EUR - Modelling core info document for soil risk assessment in Europe Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0654 Edition Number: M-608723-01-1 Date: 2017-12-04 GLP/GEP: No, unpublished	No	No		Bayer	
KCA 7.1.2 / 02	Reinken, G.; Kallweit, W.	2017 b	Isoflucypram (ISY): Core PECgw EUR - Modelling core info document for groundwater risk assessment in Europe Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0655	No	No		Bayer	

			Edition Number: M-608724-02-1 Date: 2017-12-04 **... amended: 2017-12-08** GLP/GEP: No, unpublished					
KCA 7.1.2 / 03	Reinken, G.; Kallweit, W.	2017c	Isoflucypram (ISY): Core PECsw EUR - Modelling core info document for surface water risk assessment in Europe Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0656 Edition Number: M-608725-02-1 Date: 2017-12-04 **... amended: 2017-12-08** GLP/GEP: No, unpublished	No	No		Bayer	
KCA 7.1.2.1.1 / 04	Reinken, G.; Kallweit, W.	2017 d	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under aerobic laboratory conditions Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0102 Edition Number: M-608255-01-1 Date: 2017-11-30 GLP/GEP: No, unpublished	No	No		Bayer	
KCA 7.1.2.1.2 / 01	Reinken, G.; Kallweit, W.	2017e	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under aerobic laboratory conditions Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0102 Edition Number: M-608255-01-1 Date: 2017-11-30 GLP/GEP: No, unpublished **... also filed:** **KCA 7.1.2.1.1 / 04**	No	No		Bayer	
KCA 7.1.2.2.1 / 01	Heineman n, O.; Junge, T.	2017	Terrestrial field dissipation study with BCS-CN88460 + prothioconazole EC 200 in Germany, United Kingdom, France (North), France (South), Italy and Spain	No	Yes	New data for a new active substance	Bayer	

			Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: 14-2750 Report includes Trial Nos.: 14-2750-01 14-2750-02 14-2750-03 14-2750-04 14-2750-05 14-2750-06 Edition Number: M-595964-01-1 Date: 2017-07-13 GLP/GEP: Yes, unpublished					
KCA 7.1.2.2.1 / 02	Reinken, G.; Mikolasch, B.	2017	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under field conditions for trigger purpose Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0634 Edition Number: M-608368-01-1 Date: 2017-11-30 GLP/GEP: No, unpublished	No	No		Bayer	
KCA 7.1.2.2.1 / 03	Reinken, G.; Mikolasch, B.	2017	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under field conditions for modelling purpose Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0533 Edition Number: M-608370-01-1 Date: 2017-11-30 GLP/GEP: No, unpublished	No	No		Bayer	
KCA 7.1.2.2.1 / 04	Koch, V.	2016	Determination of the storage stability of BCS-CN88460 and the metabolite BCS-CN88460-carboxylic acid in soil for 24 months Bayer Report No.: P641 14 1803 Edition Number: M-574766-01-1 Date: 2016-12-12 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.3.1.1 / 01	Stupp, H. P.; Junge, T.	2014	[Pyrazole-14C]BCS-CN88460: Adsorption/desorption on four European soils	No	Yes	New data for a new active substance	Bayer	

			Bayer Report No.: EnSa-13-0521 Edition Number: M-499024-01-1 Date: 2014-09-16 GLP/GEP: Yes, unpublished					
KCA 7.1.3.1.1 / 02	Herczog, K. J. S.	2015	[Pyrazole-4-14C]BCS-CN88460: Adsorption/Desorption on Two US Soils and One Sediment Ricerca Biosciences LLC, Concord, OH, USA Bayer Report No.: 032774-1 Edition Number: M-518345-01-1 Date: 2015-04-23 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.3.1.2 / 01	D'Ambrosio, A.	2014	[pyrazolyl-4-14C] BCS-CY26497: Adsorption/desorption in five different soils RLP AgroScience GmbH, Neustadt a. d. Weinstraße, Germany Bayer Report No.: AS357 Edition Number: M-499692-01-1 Date: 2014-09-16 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.3.1.2 / 02	Shrestha, S.	2017	[Pyrazolyl-4-14C]BCS-CY26497: Adsorption/desorption in four US soils Bayer CropScience LP, RTP, NC, USA Bayer Report No.: MELNN219 Edition Number: M-589856-01-1 Date: 2017-05-30 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.1.4 / 02	Daumann, M.	2017	Determination of the plant uptake of [pyrazole-4-14C] BCS-CN88460 in wheat plants - Report amendment 1 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer Report No.: S16-05508 Edition Number: M-587420-02-1 Date: 2017-03-24 **... amended: 2017-05-23** GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	

KCA 7.1.4 / 03	Daumann, M.	2017	Determination of the plant uptake of [pyrazole-4-14C] BCS-CN88460 carboxylic acid in wheat plants - Report amendment 1 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer Report No.: S16-05510 Edition Number: M-588284-02-1 Date: 2017-05-03 **... amended: 2017-05-23** GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.2.1.1 / 01	Heineman n, O.; Kasel, D.	2015a	[Pyrazole-4-14C]BCS-CN88460: Hydrolytic degradation Bayer Report No.: EnSa-14-1032 Edition Number: M-510623-01-1 Date: 2015-02-12 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.2.1.2 / 01	Heineman n, O.	2013	[Pyrazole-4-14C]BCS-CN88460: Determination of the quantum yield and assessment of the environmental half-life of the direct photo-degradation in water Bayer Report No.: EnSa-13-0236 Edition Number: M-461939-01-1 Date: 2013-08-08 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.2.1.2 / 02	Heineman n, O.; Kasel, D.	2015 b	[Pyrazole-4-14C]BCS-CN88460: Phototransformation in water Bayer Report No.: EnSa-14-1033 Edition Number: M-510627-01-1 Date: 2015-02-12 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	
KCA 7.2.2.2 / 01	Gabbert, D.; Smith, E.	2017	[Pyrazole-4-14C]BCS-CN88460: Aerobic mineralization in surface water Bayer CropScience LP, RTP, NC, USA Bayer Report No.: MELNN017 Edition Number: M-582106-01-1 Date: 2017-02-23 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	

KCA 7.2.2.3 / 02	Hein, E.M.; Kasel, D.	2017	pyrazole-4-14C]BCS-CN88460: Aerobic aquatic metabolism Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-15-0965 Edition Number: M-580411-01-1 Date: 2017-02-01 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	