Document Title

Summary of the Fate and behaviour in the environment for Isoflucypram

(Code: BCS-CN88460)

Data Requirements

Regulation (EC) No 1107/2009 & Regulation (EU) No 283/2013

Document MCA

Section 7: Fate and behaviour in the environment

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2018-01-24	Original Document MCA - Section 7	M-612536-01-1
2018-04-17	Dossier update - CA 7 Introduction: new information added	M-612536-02-1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

INTRODUCTION

Isoflucypram (CAS-No. 1255734-28-1) is a new fungicidal active substance developed by Bayer.
This document supports the application for regulatory approval of Isoflucypram in Europe under Regulation (EC) No 1107/2009.

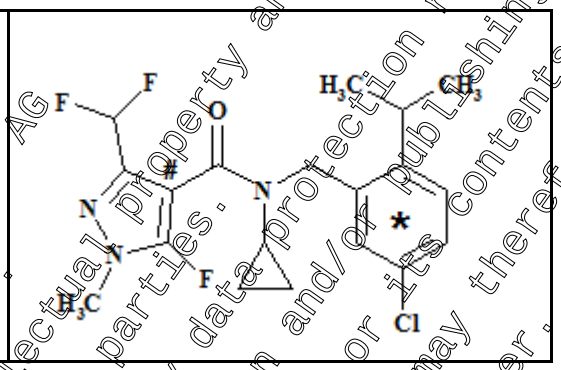
The document MCA Section 7 summarises all data on the fate of isoflucypram in all environmental compartments, assessment relating to the cut-off criteria POP, PBT or vPvB as well as definitions of residues for risk assessments and monitoring which are relevant for the approval of Isoflucypram alongside 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.

Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides with an outstanding efficacy against the major economically important diseases of cereal crops (wheat, triticale, rye, barley and oats) and excellent crop safety.
Since isoflucypram is an SDH inhibitor and thus assigned to the FRAC resistance Group 7 the application scope of isoflucypram-containing products on cereals with only one foliar spray at a maximum of 75 g a.s./ha supports an effective anti-resistance management strategy.
Tailor-made and broad spectrum Isoflucypram combinations show highly beneficial properties in terms of plant physiology beside the long-lasting and certain curative efficacy to control funghi diseases and to maximize the full yield potential of the cereal crops.

Details of the literature search undertaken are summarized in MCA Section 9. For isoflucypram and its metabolites, no publication and relevant scientifically peer-reviewed open literature reference has been identified which would indicate that a side-effect on human health, the environment and non-target species may exist, which would then need to be considered in the risk assessment of this new active substance dossier.

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.

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:

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 split of the molecule. BCS-CN88460-carboxyclic acid *(M12)* was found as the only major metabolite.

Soil:
As mentioned above all soil metabolism studies were performed using the pyrazole-label.

In soil metabolism studies under aerobic conditions the metabolites were formed possibly via carboxylation of isoflucypram to result in BCS-CN88460-carboxylic acid *(M12)* as major metabolite, hydroxylation of BCS-CN88460-carboxylic acid *(M12)* to result in BCS-CN88460-lactic acid *(M10)* and demethylation of BCS-CN88460-carboxylic acid *(M12)* to result in BCS-CN88460-desmethyl-carboxylic acid *(M11)* (see Figure 7.1.1.1- 1 and Figure 7.1.1.1- 2). No split of the molecule could be observed.
As precaution in addition an aerobic soil metabolism study with the phenyl-label was performed using one soil. In this study BCS-CN88460-carboxylic acid *(M12)* was also found as major metabolite (see Figure 7.1.1.1- 3). In this study also no split of the molecule could be observed.

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

In the soil photolysis study no products of pyrazole-labelled isoflucypram above the identification triggers were formed. The total unidentified residues amounted to a maximum of 2.8% AR.

Therefore, in soil the entire pathway and all possible main metabolites are covered 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.

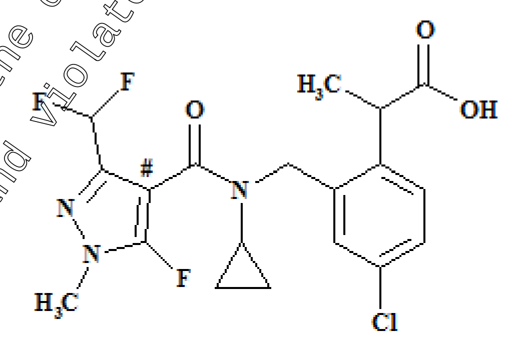
In surface water under aerobic conditions isoflucypram was stable in all test systems. No degradation products were formed in any test systems in this study.

Degradation of isoflucypram in the total system was accompanied by the formation of one degradation product identified as BCS-CN88460-carboxylic acid *(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 7.2- 1).

Therefore, in water and water/sediment the entire pathway and all possible main metabolites are covered using the mentioned pyrazole-label position only.

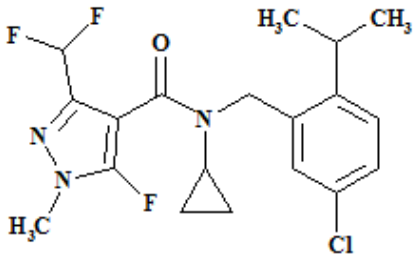
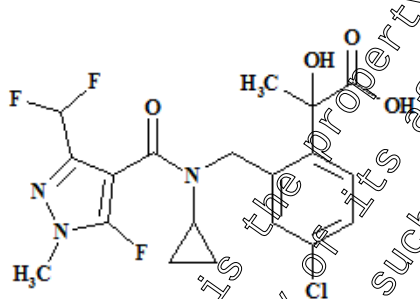
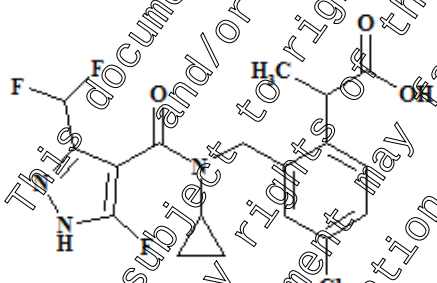
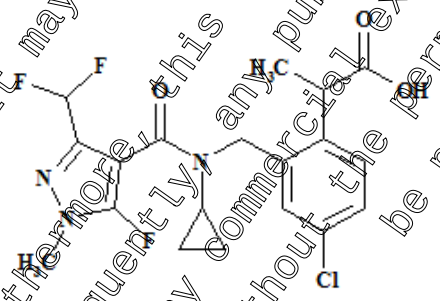
The results of the studies are summarised in the following sections 7.1 to 7.5. The proposed degradation pathways in soil, water and sediment are given in Figure 7.1.1-1 and Figure 7.2- 1, respectively.

In addition, studies have been performed with the radiolabelled metabolite BCS-CN88460-carboxylic acid *(M12)*:

Structural formula of BCS-CN88460-carboxylic acid *(M12)*: #: [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). A full list containing structural formula, various names, short forms, codes and occurrences of degradation products is provided as Document N3.

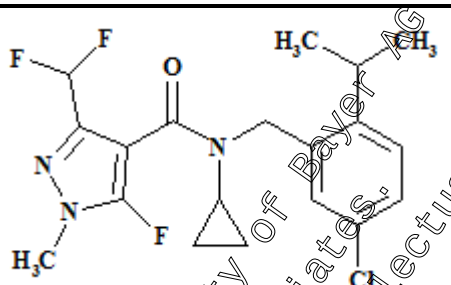
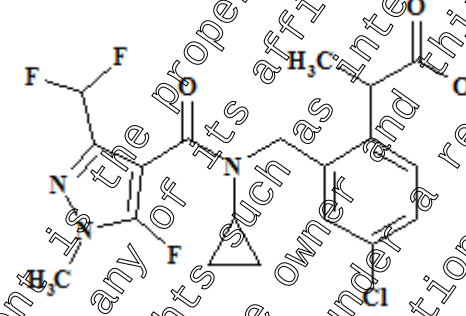
Isoflucypram: Substances and environmental fate metabolites; structures, codes, synonyms

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-methylethyl)phenyl]methyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl- *[CA]*	soil: aerobic & anaerobic, field dissipation, photolysis water: hydrolysis, photolysis, water-sediment
M10	 $C_{19}H_{19}ClF_3N_3O_3$ *[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.

Active substance and metabolite addressed in this document with environmental fate studies

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

% AR = % of applied radioactivity

CA 7.1 Fate and behaviour in soil

Isoflucypram is slowly but steadily degraded in soil under aerobic conditions to the major degradation product BCS-CN88460-carboxylic acid *(M12)* and to final degradation product carbon dioxide and non-extractable residues. Under anaerobic conditions no degradation products < 5% were identified. The degradation of isoflucypram is driven by microbial degradation under typical conditions in the environment but photodegradation will play no role in the overall fate of isoflucypram. More details for route and rate of isoflucypram and its major degradation product in soil are given in section CA 7.1.1 and section CA 7.1.2, respectively.
The degradation pathway of isoflucypram in soil is given in Figure 7.1.1-1.

CA 7.1.1 Route of degradation in soil

Summary: 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.

From the studies on the route of degradation in soil it can be concluded that isoflucypram was slowly but steadily 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

(BCS-CN88460-lactic acid *(M10)* and BCS-CN88460-desmethyl-carboxylic acid *(M11)*) were found only in amounts < 5% of the applied radioactivity (AR). The highest concentrations were found for the major metabolite BCS-CN88460-carboxylic acid *(M12)*, with a maximum of 9.6% AR.
Under anaerobic conditions no degradation products > 5% were found.
Photodegradation will play no role in the overall fate of isoflucypram.

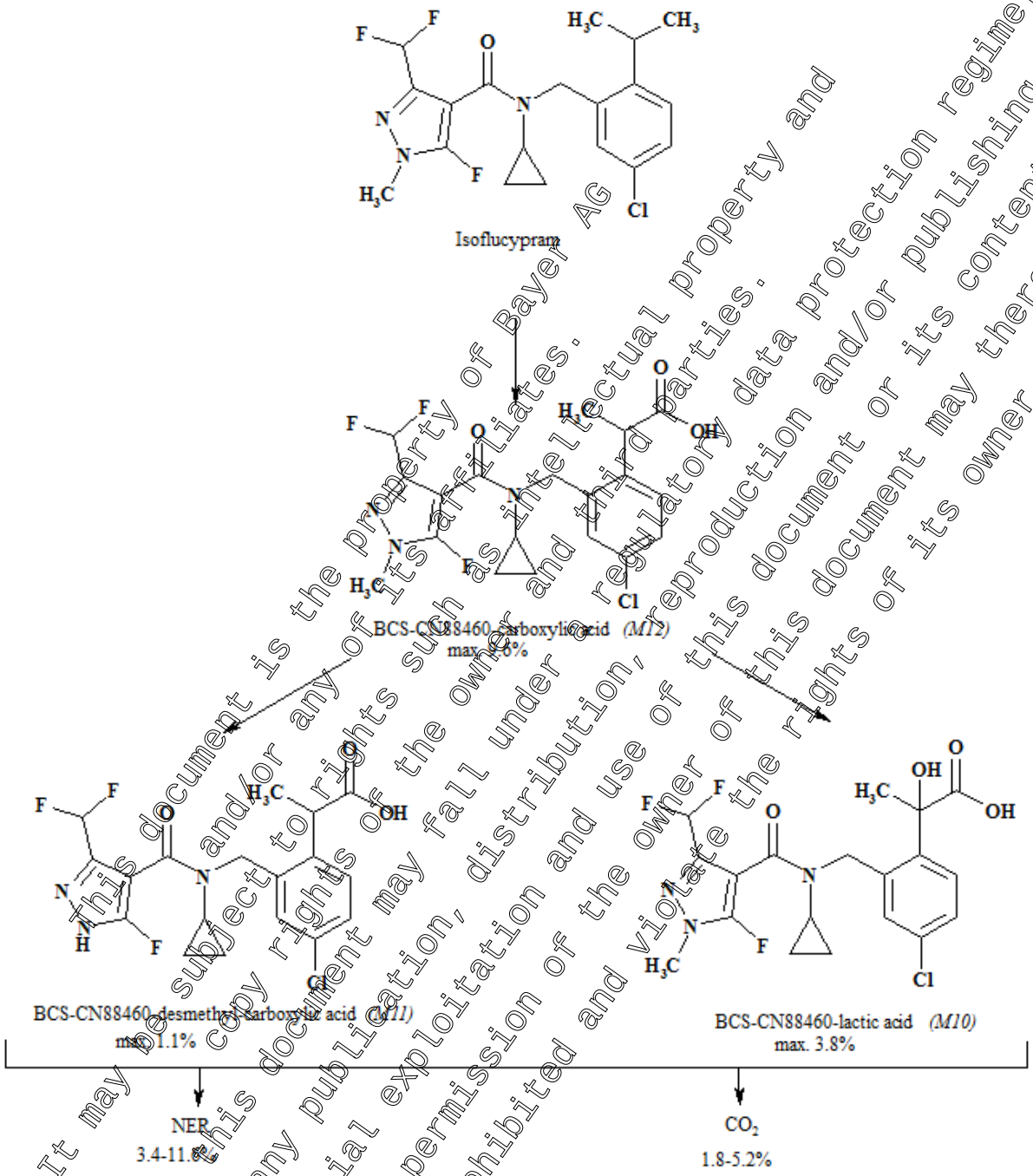
A summary of maximum occurrences of the major metabolite BCS-CN88460-carboxylic acid *(M12)*, CO_2 and non-extractable residues in soil is given in Table 7.1.1- 1.

Table 7.1.1- 1: Summary of maximum occurrences of the major metabolite BCS-CN88460-carboxylic acid *(M12)*, carbon dioxide and non-extractable residues in soil (in percent of applied radioactivity)

Compound	Soil metabolism, aerobic [%]	Soil metabolism, anaerobic [%]	Soil photolysis [%]
BCS-CN88460-carboxylic acid *(M12)*	9.6	-	-
Carbon dioxide	5.2	0.2	0.2
Non-extractable residues	11.6	0.2	1.2

The proposed degradation pathway of isoflucypram in soil is shown in Figure 7.1.1-1.

Figure 7.1.1-1: Proposed degradation pathway of isoflucypram in soil



CA 7.1.1.1 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 CA 7.1.1 and Figure 7.1.1-1.

Report:	KCA 7.1.1.1/01; [redacted]; [redacted]; 2014; M-486690-01-1
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**

Executive 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:

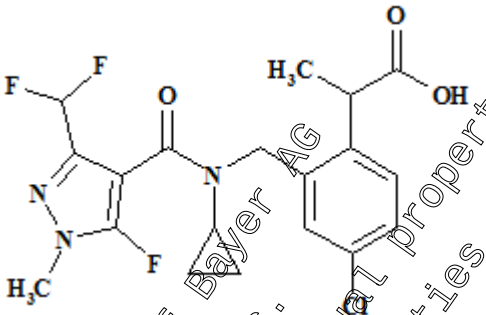
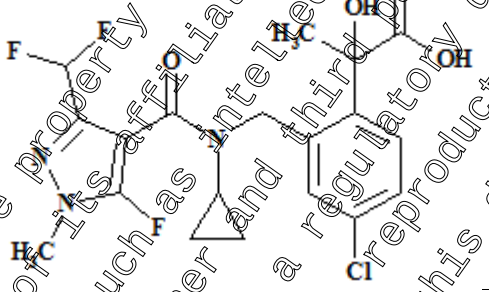
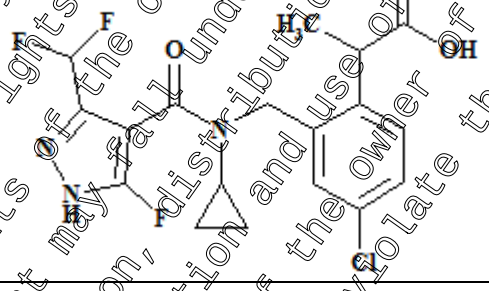
Table 7.1.1.1- 1: Selected soils

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.
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: BCS-CN88460-carboxylic acid *(M12)* with 5.8% AR at DAT-104 in soil Dollendorf II, BCS-CN88460-lactic acid *(M10)* with 3.8% AR at DAT-104 in soil Dollendorf II and BCS-CN88460-desmethyl-carboxylic acid *(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 7.1.1.1- 2: Identified degradation products (maximum occurrence) in soils (in percent of applied radioactivity)

Compound	Chemical structure	Maximum occurrence in soil [%]
BCS-CN88460-carboxylic acid *(M12)*		5.8
BCS-CN88460-lactic acid *(M10)*		3.8
BCS-CN88460-desmethyl-carboxylic acid *(M11)*		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 7.1.1.1- 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. 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 7.1.1.1- 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 taxonomic classification (USDA)	Loamy skeletal, mixed, semiactive, mesic Dystric Eutrudept	Sandy, mixed, mesic Typic Cambudoll
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/cm³]	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

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

cont.

Table 7.1.1.1- 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 taxonomic classification (USDA)	Loamy, mixed, mesic Typic Argudalf	fine-loamy, mixed, active, frigid Typic Eutrudept
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/cm³]	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.

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 7.1.1.1- 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

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.

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. 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 well suited 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 7.1.1.1- 5 and Table 7.1.1.1- 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 7.1.1.1- 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 7.1.1.1- 6.

The route of degradation of isoflucypram in soil under aerobic conditions is summarised in Table 7.1.1.1- 7 to Table 7.1.1.1- 10. The proposed degradation of isoflucypram in soil is presented in Figure 7.1.1.1- 1.

Table 7.1.1.1- 6: Material balance of radioactivity in soils under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

	DAT									
	0	2	6	15	28	50	62	84	104	120
Hanscheider Hof										
Volatiles										
carbon dioxide	n.a.	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.6	1.8
volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	n.a.	< 0.1	< 0.1	0.1	0.3	0.6	0.8	1.2	1.6	1.8
Extractable residues										
combined extract	97.6	96.1	96.1	95.3	95.9	94.7	94.0	91.3	90.4	90.0
mircowave extract	1.3	1.5	1.6	2.3	1.4	1.6	1.6	1.9	1.9	1.9
total extractable residues	99.0	97.6	97.7	97.6	97.3	96.3	95.6	93.2	92.3	91.9
Non-extractable residues	1.5	2.4	2.0	2.7	2.9	4.2	4.9	5.4	6.3	5.8
Material balance	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	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.2	1.7	2.2	2.5
volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.2	1.8	2.2	2.5
Extractable residues										
combined extract	99.3	96.8	95.0	94.6	94.6	92.5	89.5	87.4	86.0	86.4
mircowave extract	1.1	1.1	1.2	1.8	1.0	1.4	1.5	1.8	1.7	1.9
total extractable residues	100.4	97.8	96.2	96.4	95.6	93.8	91.0	89.3	87.7	88.3
Non-extractable residues	0.8	1.2	1.3	1.8	2.3	3.4	4.1	5.2	5.4	5.8
Material balance	101.2	99.0	97.5	98.3	98.3	98.1	96.3	96.2	95.2	96.7
Hoefchen am Hohensela										
Volatiles										
carbon dioxide	n.a.	< 0.1	< 0.1	0.1	0.3	0.9	1.0	1.7	2.3	2.8
volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.0	1.7	2.3	2.8
Extractable residues										
combined extract	97.7	93.9	96.3	93.8	94.7	94.2	91.0	88.8	86.3	86.8
mircowave extract	1.0	1.4	1.2	2.0	1.1	1.1	1.5	1.7	1.6	1.5
total extractable residues	98.7	95.3	97.5	95.9	95.8	95.3	92.5	90.6	87.9	88.3
Non-extractable residues	1.3	1.8	1.8	2.2	2.5	4.0	4.5	5.9	8.0	7.7
Material balance	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	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.8	2.5	3.0
volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.8	2.5	3.0
Extractable residues										
combined extract	94.1	93.3	94.5	93.8	90.7	91.0	89.3	85.8	84.2	83.9
mircowave extract	1.8	1.8	1.6	1.9	1.2	1.2	1.3	1.7	1.9	1.7
total extractable residues	95.8	95.1	96.1	95.6	92.0	92.1	90.7	87.5	86.0	85.6
Non-extractable residues	3.2	5.0	2.8	3.7	3.6	4.9	5.8	7.4	11.6	10.7
Material balance	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 7.1.1.1- 7 to Table 7.1.1.1- 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: BCS-CN88460-carboxylic acid *(M12)* with 5.8% AR at DAT-104 in soil Dollendorf II, BCS-CN88460-lactic acid *(M10)* with 3.8% AR at DAT-104 in soil Dollendorf II and BCS-CN88460-desmethyl-carboxylic acid *(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 7.1.1.1- 7 to Table 7.1.1.1- 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 7.1.1.1- 7 to Table 7.1.1.1- 10).

Table 7.1.1.1- 7: Degradation of isoflucypram in soil Hanscheider Hof under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	DAT									
	0	2	6	15	28	50	62	84	104	120
Isoflucypram	98.2	97.3	97.3	95.8	93.7	92.3	90.0	86.3	82.2	82.6
BCS-CN88460-carboxylic acid *(M12)*	n.d.	n.d.	n.d.	0.8	1.4	1.6	1.7	2.2	2.6	2.4
ROI 2	n.d.	n.d.	n.d.	0.7	0.9	1.3	1.6	1.4	1.1	1.8
BCS-CN88460-lactic acid *(M10)*	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.7	0.7	0.8	0.8
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	< LOD	0.6	n.d.
BCS-CN88460-desmethyl-carboxylic acid *(M11)*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.8	0.6
ROI 8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6	n.d.
ROI 9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD
Sum of unid./diff. residues	0.9	< LOD	< LOD	0.9	2.2	2.2	3.2	4.0	6.4	5.8
Total extractable residues[a)]	99.0	97.6	97.6	97.5	97.3	96.3	95.6	93.2	92.0	91.8
Carbon dioxide[b)]	n.a.	< 0.1	< 0.1	0.1	0.2	0.6	0.8	1.2	1.6	1.8
Volatile organic compounds[b)]	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues[b)]	1.5	2.4	2.0	2.7	2.9	4.2	4.9	5.4	6.3	5.8
Total recovery[a)]	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, SD: standard deviation, 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 7.1.1.1- 8: Degradation of isoflucypram in soil Laacher Hof AXXa under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	DAT									
	0	2	6	15	28	50	62	84	104	120
Isoflucypram	99.8	96.9	95.6	93.9	90.4	84.8	81.0	76.7	72.5	70.1
BCS-CN88460-carboxylic acid *(M12)*	n.d.	n.d.	n.d.	1.3	1.9	3.2	3.3	4.0	4.9	5.4
ROI 2	n.d.	n.d.	n.d.	0.9	1.5	2.3	2.6	2.5	2.5	3.4
BCS-CN88460-lactic acid *(M10)*	n.d.	n.d.	n.d.	n.d.	0.7	1.5	1.9	2.1	2.5	2.8
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	< LOD	< LOD	< LOD	0.6
BCS-CN88460-desmethyl-carboxylic acid *(M11)*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.1	1.0
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.5	0.6	0.6
ROI 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.1	1.1	1.3
ROI 8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD
ROI 9	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	0.6	0.9	0.4	0.9	2.4	4.3	4.3	6.1	6.7	8.9
Total extractable residues[a)]	100.4	97.8	96.1	96.1	95.4	93.8	90.9	89.3	87.7	88.2
Carbon dioxide[b)]	n.a.	< 0.1	< 0.1	0.1	0.4	0.9	1.2	1.7	2.2	2.5
Volatile organic compounds[b)]	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues[b)]	0.8	1.2	1.3	1.8	2.3	3.4	4.1	5.2	5.4	5.8
Total recovery[a)]	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, SD: standard deviation, 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 7.1.1.1- 9: Degradation of isoflucypram in soil Hoefchen Am Hohenseh under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	DAT									
	0	2	6	15	28	50	62	84	104	120
Isoflucypram	98.2	94.8	96.4	93.9	91.1	89.5	85.1	82.4	78.6	77.2
BCS-CN88460-carboxylic acid *(M12)*	n.d.	n.d.	0.6	0.9	1.3	1.3	1.5	1.8	1.4	1.6
ROI 2	n.d.	n.d.	n.d.	0.7	1.3	2.0	1.9	0.8	2.5	2.6
BCS-CN88460-lactic acid *(M10)*	n.d.	n.d.	n.d.	n.d.	0.6	0.9	1.4	1.5	1.7	1.6
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BCS-CN88460-desmethyl-carboxylic acid *(M11)*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.6	0.7	0.6
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.5	n.d.	< LOD
ROI 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.1	0.7	1.0
ROI 8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 9	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	< LOD	0.5	< LOD	0.7	2.9	3.5	3.5	4.2	5.5	5.3
Total extractable residues[a)]	98.6	95.3	97.3	95.6	95.8	95.3	92.0	90.6	87.9	88.3
Carbon dioxide[b)]	n.a.	< 0.1	< 0.1	0.1	0.3	0.9	1.0	1.7	2.3	2.8
Volatile organic compounds[b)]	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues[b)]	1.4	1.8	1.8	2.2	2.3	4.0	4.5	5.9	8.0	7.7
Total recovery[a)]	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, SD: standard deviation, 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 7.1.1.1- 10: Degradation of isoflucypram in soil Dollendorf II under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	DAT									
	0	2	6	15	28	50	62	84	104	120
Isoflucypram	95.3	95.0	94.6	93.1	88.1	85.1	82.4	77.9	69.3	72.2
BCS-CN88460-carboxylic acid *(M12)*	n.d.	n.d.	1.1	1.3	1.9	2.5	2.4	2.9	5.8	2.5
ROI 2	n.d.	n.d.	n.d.	0.9	1.4	2.1	2.0	2.1	2.6	2.6
BCS-CN88460-lactic acid *(M10)*	n.d.	n.d.	n.d.	n.d.	0.6	1.3	1.5	1.8	3.8	2.9
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	0.4	n.d.
BCS-CN88460-desmethyl-carboxylic acid *(M11)*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	< LOD	0.8	0.7
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.1	1.9	1.1
ROI 8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 9	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	< LOD	< LOD	0.4	0.9	2.2	3.2	3.7	4.1	6.4	7.4
Total extractable residues[a)]	95.7	95.0	96.1	95.3	91.8	92.1	90.7	86.9	86.0	85.6
Carbon dioxide[b)]	n.a.	< 0.1	< 0.1	0.1	0.3	0.7	1.1	1.8	2.5	3.0
Volatile organic compounds[b)]	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues[b)]	3.2	5.0	2.8	3.7	3.6	4.9	5.8	7.4	11.6	10.7
Total recovery[a)]	98.9	99.9	98.9	99.1	95.7	97.7	97.6	96.1	100.1	99.3

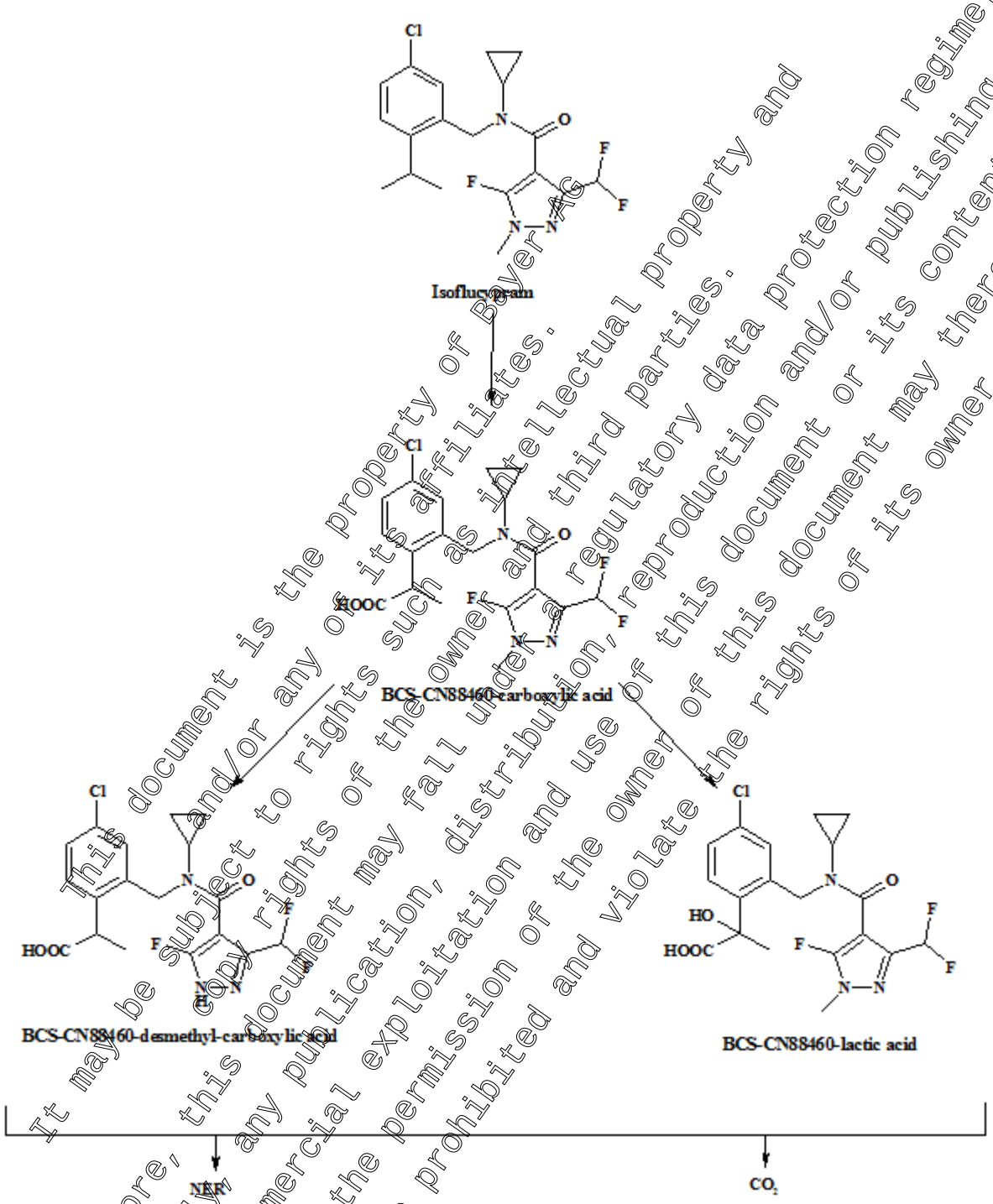
n.d.: not detected, n.a.: not analysed, DAT: days after treatment, SD: standard deviation, ROI: regions of interest
a) Difference to Material Balance values due to rounding errors as well as clean up and chromatographic losses
b) Values taken from Material Balance

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 7.1.1.1- 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 7.1.1.1- 1: Proposed degradation pathway of pyrazole-labelled isoflucypram in soils under aerobic conditions



III. CONCLUSIONS

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: BCS-CN88460-carboxylic acid *(M12)* with 5.8% AR, BCS-CN88460-lactic acid *(M10)* with 3.8% AR and BCS-CN88460-desmethyl-carboxylic acid *(M11)* with 1.1% AR.
Formation of non-extractable residues (NER) was up to 10.7% AR at study end (DAT-120), which is an indication for biotic degradation of isoflucypram.

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Report:	KCA 7.1.1.1/02; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
Title:	[Pyrazole-4-14C]BCS-CN88460: Aerobic soil metabolism in two US soils
Report No.:	MELNN013
Document No.:	M-588260-01-1
Guideline(s):	US EPA OCSPP 835.4100, Aerobic Soil Metabolism, 2008, OECD Guideline 307, Aerobic and Anaerobic Transformation in Soil, 2002 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 PMRA: Daco No. 8.2.3.4.2 Biotransformation in Soil (TGAI), Aerobic Soil 20-30degrees C
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive 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 a moisture content of between pF 2.0 and 2.5.

Table 7.1.1.1- 11: 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

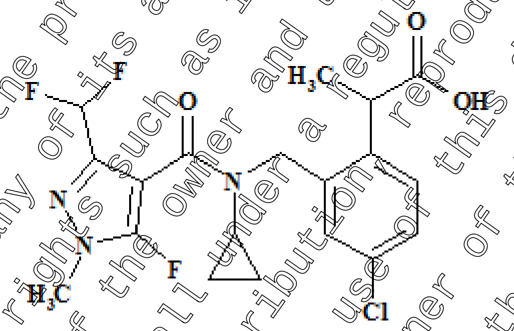
The study application rate was based on the anticipated maximum single field-use rate for isoflucypram of 75 g a.s. per hectare which corresponded to a concentration in soil of 0.2 µg of isoflucypram per g of soil as dry weight. In order to bridge to a higher rate, additional test systems were treated at 0.43 µg/g (equivalent to approximately 150 g a.s. per hectare). These test systems were also used for metabolite identification purposes.
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 – BCS-CN88460-carboxylic acid *(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 7.1.1.1- 12: Identified degradation products (maximum occurrence) in soils (in percent of applied radioactivity)

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

Based on results of this laboratory study, isoflucypram degrades slowly under aerobic conditions to form BCS-CN88460-carboxylic acid *(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 7.1.1.1- 13). 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.

Table 7.1.1.1- 13: Physico-chemical properties of test soils

Parameter	Results	
Soil designation	CA soil	Ne soil
Geographic location City State Country	 Sanger California USA	 Louisville Nebraska USA
Soil taxonomic classification (USDA)	Coarse-Loamy, mixed, superactive, nonacid, thermic Typic Xerorthents	Fine-silty, mixed, superactive, mesic Typic Hapludolls
Soil series	Hanford series	no information available
Textural class (USDA) Sand [%] (50 µm – 2 mm) Silt [%] (2 µm – 50 µm) Clay [%] (< 2 µm)	sandy loam 68.6 27.3 4.1	silty clay loam 15.0 50.1 34.9
pH - in 0.01 M $CaCl_2$ 1/2 - in water 1/1 - in saturated paste	6.3 6.7 6.6	6.3 6.7 6.6
Organic carbon (combustion) [% OC] Organic matter [a] [% OM]	0.77 1.3	2.0 3.5
Cation exchange capacity [meq/100 g]	6.7	17.2
Water holding capacity maximum (MWHC) [g H_2O *ad* 100 g DW] at 1/10 bar (pF 2.0) [%] at 1/3 bar (pF 2.5) [%] at 15 bar [%]	 29.1 21.8 9.8 4.2	 52.4 43.0 32.7 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 - Fungi ….-.Bacteria DAT-123 biomass UT[b] / biomass SC[c] - Actinomycetes - Fungi ….-.Bacteria	 1,140,000 11,900 2,150,000 42,700 / 79,500 9,590 / 10,400 698,000 / 1,210,000	 943,000 9,620 1,510,000 378,000 / 407,000 14,100 / 8,140 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 silanized 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 color 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 Fisher Optima water. 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.
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 analyzed 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 analyed 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 7.1.1.1- 14: 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 7.1.1.1- 13). 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 481 dpm (rounded up to 500 dpm), which is 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 7.1.1.1- 15 and Table 7.1.1.1- 16).
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 7.1.1.1- 15: 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 7.1.1.1- 16.

Table 7.1.1.1- 16: Distribution of radioactivity in soils under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

	Sampling times [days]							
	0	6	14	21	28	60 a)	88	123
CA soil								
Volatiles								
carbon dioxide	-	0.1	0.1	0.2	0.5	0.5	0.9	1.9
volatile organic compounds	-	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
total volatiles	-	0.1	0.2	0.2	0.6	0.5	0.9	2.0
Extractable radioactivity								
ambient extract	93.1	94.7	94.9	94.0	92.7	90.8	88.0	87.9
aggresive extract	1.6	2.4	2.4	3.0	3.1	3.3	3.7	3.6
ethyl acetate/hexane	-	-	-	-	-	-	-	0.8
subtotal extractable	94.7	97.1	97.4	97.0	95.8	94.1	91.7	92.3
Non-extractable residues	0.2	0.5	0.7	0.9	1.0	1.9	3.0	3.4
Material balance	94.9	97.7	98.2	98.1	97.4	96.5	95.6	97.6
NE soil								
Volatiles								
carbon dioxide	-	0.1	0.3	0.4	0.2	1.2	2.0	3.2
volatile organic compounds	-	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
total volatiles	-	0.1	0.3	0.4	0.3	1.3	2.0	3.3
Extractable radioactivity								
ambient extract	91.8	89.4	88.8	88.9	87.1	83.5	78.7	73.8
microwave extract	3.0	6.3	5.7	6.0	5.8	7.0	6.5	7.5
ethyl acetate/hexane	-	-	-	-	-	-	-	1.8
subtotal extractable	94.8	95.7	94.6	94.9	92.9	90.5	85.2	83.0
Non-extractable residues	0.3	1.9	2.0	2.5	3.2	6.0	8.4	10.7
Material balance	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 7.1.1.1- 17 and Table 7.1.1.1- 18. The proposed degradation of isoflucypram in soil is presented in Figure 7.1.1.1- 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 7.1.1.1- 17 and Table 7.1.1.1- 18).

- 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 BCS-CN88460-carboxylic acid *(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 BCS-CN88460-carboxylic acid *(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 7.1.1.1- 17 and Table 7.1.1.1- 18.

Table 7.1.1.1- 17: Degradation of isoflucypram in CA soil under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	Sampling times [days]							
	0	6	14	21	28	60	88	123
Isoflucypram	94.7	97.1	97.4	97.0	95.8	90.4	89.8	86.2
BCS-CN88460-carboxylic acid *(M12)*	< LOD	< LOD	< LOD	< LOD	< LOD	1.1	< LOD	1.3
Unknown 2	< LOD	< LOD	< LOD	< LOD	< LOD	2.6	1.9	4.0
Unknown 3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Unidentified radioactivity	< LOD	< LOD	< LOD	< LOD	< LOD	2.6	1.9	4.0
Total extractable radioactivity	94.7	97.1	97.4	97.0	95.8	94.1	91.7	92.3
Carbon dioxide	-	0.1	0.1	0.2	0.5	0.5	0.9	1.9
Volatile organics	-	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
Total volatile	-	0.1	0.2	0.2	0.6	0.5	0.9	2.0
Bound residues	0.2	0.5	0.7	0.9	1.0	1.9	3.0	3.4
Total recovery	94.9	97.7	98.2	98.1	97.4	96.5	95.6	97.6

Table 7.1.1.1- 18: Degradation of isoflucypram in NE soil under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	Sampling times [days]							
	0	6	14	21	28	60 a)	88	123
Isoflucypram	94.8	94.8	94.6	93.5	92.9	86.4	75.0	64.4
BCS-CN88460-carboxylic acid *(M12)*	< LOD	< LOD	< LOD	1.4	< LOD	4.1	6.7	9.6
Unknown 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.6	4.0
Unknown 3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.2
Unidentified radioactivity	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	3.6	7.2
Total extractable radioactivity	94.8	94.8	94.6	94.9	92.9	90.5	85.2	83.0
Carbon dioxide	-	0.1	0.3	0.4	0.2	1.2	2.0	3.2
Volatile organics	-	< LOD	0.1	< LOD	0.1	< LOD	< LOD	0.1
Total volatile	-	0.1	0.3	0.4	0.3	1.3	2.0	3.3
Bound residues	0.3	0.3	2.0	2.5	3.2	6.0	8.4	10.7
Total recovery	95.1	95.1	96.9	97.8	96.4	97.8	95.6	97.0

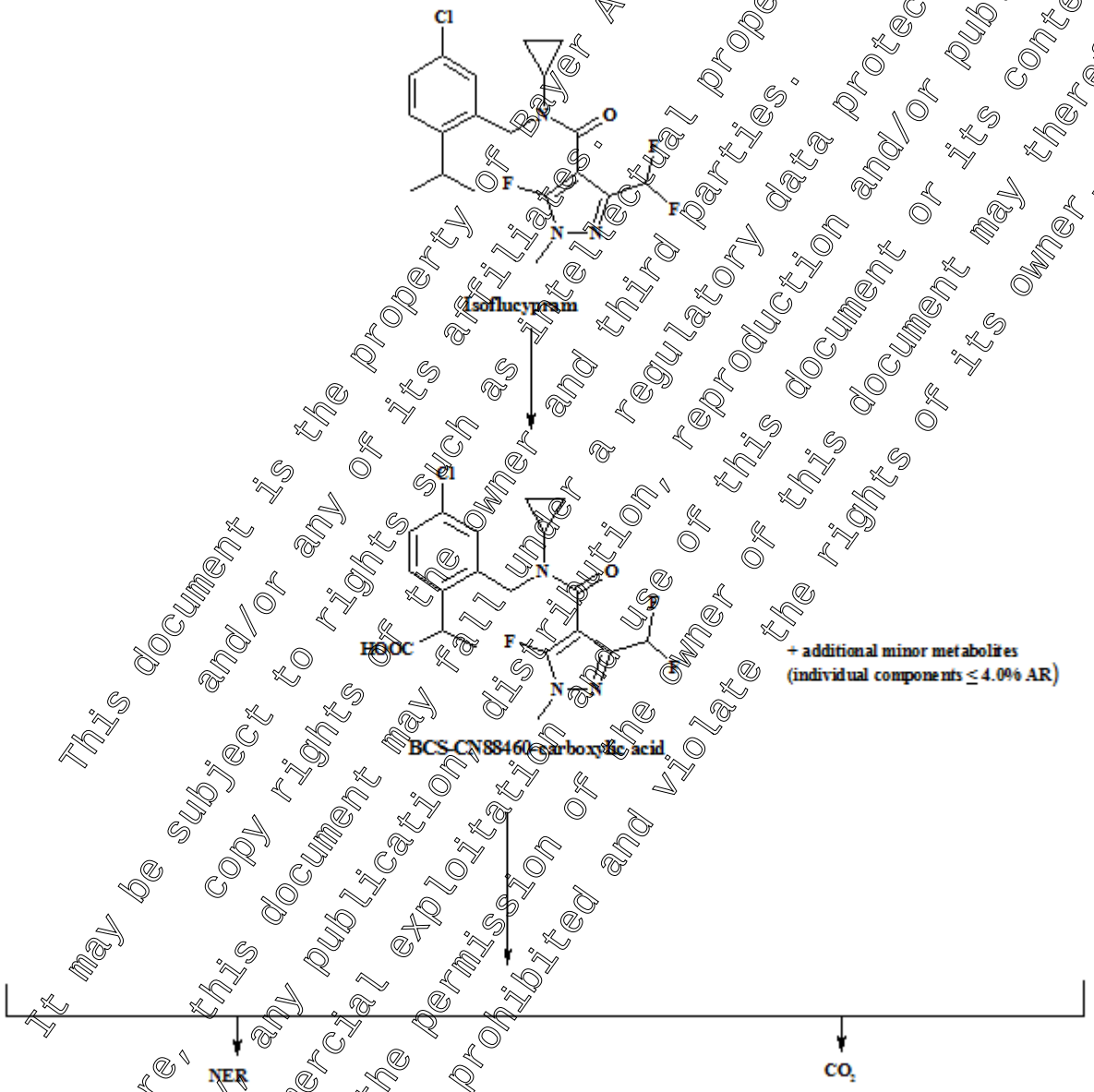
a) Only one replicate was analysed for the day 60 interval

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 7.1.1.1- 2.

Under aerobic soil conditions, BCS-CN88460-carboxylic acid *(M12)* was formed. Furthermore, CO_2 (max 3.3%) and non-extractable residue (max 10.7%) were also formed during this study.

Figure 7.1.1.1- 2: Proposed degradation pathway of pyrazole-labelled isoflucypram in soils under aerobic conditions



III. CONCLUSIONS

Isoflucypram degrades slowly under aerobic conditions in the laboratory.
One soil metabolite, BCS-CN88460-carboxylic acid *(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.

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Report:	KCA 7.1.1.1/03; [redacted]; [redacted]; 2017; M-599926-01-1
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 / 835.4200; Japanese MAFF Test Guidelines 12 Nousan 8147, No. 2-5-2
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive 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 for 125 days.

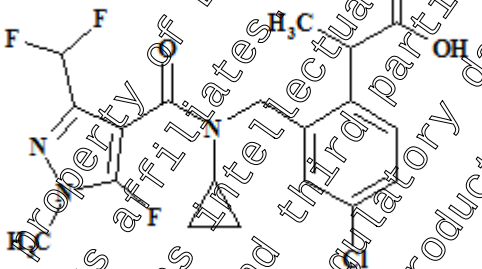
Table 7.1.1.1- 19: Selected soil

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

An actual study application rate of 18.8 µg/kg soil dry weight was applied 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.
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, BCS-CN88460-carboxylic acid *(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 7.1.1.1- 20: Identified degradation products (maximum occurrence) in soil (in percent of applied radioactivity)

Compound	Chemical structure	Maximum occurrence in soil [%]
BCS-CN88460-carboxylic acid *(M12)*		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 7.1.1.1- 21). The soils are well characterised and the plant protection product use history of the soils for at least 5 years is known.

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].

Table 7.1.1.1- 21: 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 taxonomic classification (USDA)	sandy, mixed, active, nonacid, mesic Inceptic Hapludalf	fine-loamy, mixed, active, frigid Typic Eutrudept
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/cm³]	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. The test vessels were then fitted with trap attachments.

[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

The untreated test systems were equilibrated to study conditions for 5 days prior to application.

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 lager 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 7.1.1.1- 22: 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 combined first three ambient extracts was combined and filled 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.

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 19.4°C for 125 days. The test was performed at a soil moisture of 53.0% of the maximum water holding capacity. 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 well suited 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 7.1.1.1- 23).
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 7.1.1.1- 23: 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 7.1.1.1- 24.
The route of degradation of isoflucypram in soil under aerobic conditions is summarised in Table 7.1.1.1- 25.
The proposed degradation of isoflucypram in soil is presented in Figure 7.1.1.1- 3.

Carbon dioxide and volatile organic compounds

The maximum amount of carbon dioxide was 5.2% AR at study end (DAT-125) (Table 7.1.1.1- 24).
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 7.1.1.1- 24).
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 BCS-CN88460-carboxylic acid *(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 7.1.1.1- 24: Material balance of radioactivity in soils under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

	Days after treatment									
	0	**2**	**6**	**14**	**28**	**50**	**65**	**85**	**100**	**125**
Volatiles										
carbon dioxide	n.a.	< 0.1	0.2	0.3	1.1	2.3	2.9	3.5	4.3	5.2
volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1
total volatiles	n.a.	< 0.1	0.2	0.3	1.1	2.4	2.9	3.5	4.4	5.2
Extractable residues										
ambient extract	95.6	97.3	95.7	93.7	93.0	89.9	91.5	85.2	87.7	83.5
microwave extract 1	3.6	4.3	3.9	4.5	4.3	4.4	4.6	4.8	5.0	6.5
microwave extract 2	1.1	1.2	1.4	1.3	1.4	1.3	1.6	1.8	1.7	2.0
total extractable residues	100.3	102.8	101.0	99.5	98.6	95.6	97.7	91.9	94.4	92.0
Non-extractable residues	0.7	0.9	1.2	1.5	2.2	3.2	3.8	5.6	5.3	6.4
Material balance	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 7.1.1.1- 25: Degradation of isoflucypram in soil Laacher Hof AXXa under aerobic conditions (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	Days after treatment									
	0	**2**	**6**	**14**	**28**	**50**	**65**	**85**	**100**	**125**
Isoflucypram	100.3	102.8	101.0	97.6	94.2	88.2	88.0	73.0	81.5	75.5
BCS-CN88460-carboxylic acid *(M12)*	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	n.d.	n.d.	n.d.	<LOD	1.9	4.7	6.5	13.1	8.4	10.3
Total extractable residues[a]	100.3	102.8	101.0	99.2	98.3	95.6	97.5	91.9	94.1	92.0
Carbon dioxide[b]	n.a.	< 0.1	0.2	0.3	1.1	2.3	2.9	3.5	4.3	5.2
Volatile organic compounds[b]	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1
Non-extractable residues[b]	0.7	0.9	1.2	1.5	2.2	3.2	3.8	5.6	5.3	6.4
Total recovery[a]	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

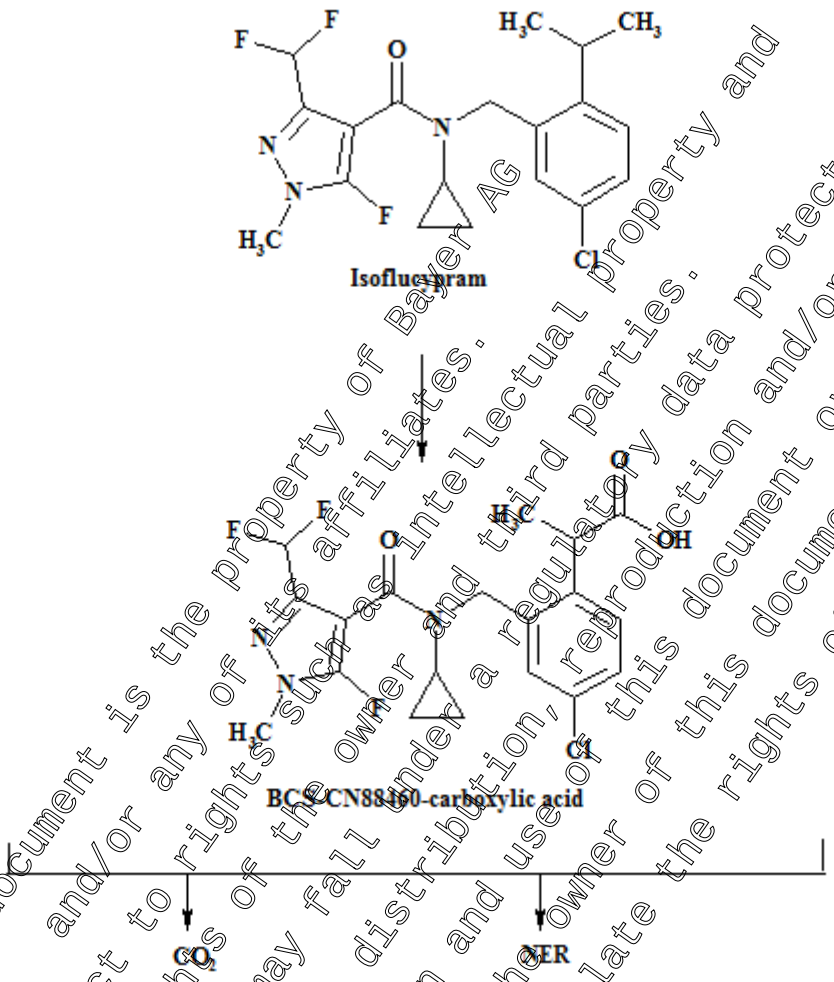
b) Values taken from material balance

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 7.1.1.1- 3), with the following possible processes involved:

- oxidation of isoflucypram to result in BCS-CN88460-carboxylic acid *(M12)*;
- mineralisation (carbon dioxide formation);
- formation of non-extractable residues (NER).

Figure 7.1.1.1- 3: Proposed degradation pathway of phenyl-labelled isoflucypram in soil under aerobic conditions



III. CONCLUSIONS

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 indicating the potential for a complete mineralisation of isoflucypram and its degradation products.
Besides the formation of carbon dioxide, BCS-CN88460-carboxylic acid *(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 is an indication for biotic degradation of isoflucypram.
The formation of non-extractable residues and carbon dioxide indicates a participation in the natural carbon cycle of soil and the potential for a complete mineralisation of isoflucypram.

CA 7.1.1.2 Anaerobic degradation

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 section CA 7.1.1.

Report:	KCA 7.1.1.2/01; [redacted]; [redacted]; 2015; M-513456-01-1
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 / 835.4200; Japanese MAFF Test Guidelines 12 Nousan 8147, No. 2-5-3
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive 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 7.1.1.2- 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:	KMI 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 7.1.1.2- 2). The plant protection product use history of the soils for at least 5 years is known. 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 7.1.1.2- 3 and Table 7.1.1.2- 4, respectively).

Table 7.1.1.2- 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	6.5 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/cm³]	1.25

a) % organic matter = % organic carbon x 1.724

Table 7.1.1.2- 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 perfomed after 2 and 32 days using DAT-0 and DAT-30 samples, respectively.

Table 7.1.1.2- 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 perfomed after 157 days using DASF-120 (DAT-150) sampes.

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.

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 7.1.1.2- 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 7.1.1.2- 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 7.1.1.2- 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 7.1.1.2- 7.
The route of degradation of isoflucypram in soil under anaerobic conditions is summarised in Table 7.1.1.2- 8.

Table 7.1.1.2- 7: Material balance of radioactivity under anaerobic conditions after an aerobic incubation phase (expressed as percentage of applied radioactivity, mean of 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)]	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
volatile organic compounds[a)]	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles aerobic phase	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	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
volatile organic compounds	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
total volatiles anaerobic phase	N/A		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
- total carbon dioxide	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
- total volatile organic compounds	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
- total volatiles	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Extractable residues										
- water	N/A		4.4	4.5	4.4	4.2	4.1	4.1	3.9	4.2
- soil										
ambient extract	99.8	91.3	87.3	93.1	86.8	87.1	85.9	83.5	88.0	86.5
mircowave extract	3.3	4.1	4.8	3.5	4.2	4.6	5.6	5.8	6.2	6.2
mircowave extract 2	0.9	1.4	1.8	1.2	1.4	1.7	1.7	1.9	2.2	1.9
total soil extractable residues	104.1	96.9	93.9	97.9	92.5	93.3	93.1	91.2	96.4	94.6
- total extractable residues	104.1	96.9	98.3	102.3	96.8	97.6	97.2	95.2	100.3	98.8
Non-extractable residues	0.4	1.5	1.7	1.4	1.7	2.2	3.0	3.8	4.0	4.2
Material balance	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 7.1.1.2- 7 and Table 7.1.1.2- 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 7.1.1.2- 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 7.1.1.2- 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 7.1.1.2- 8).

Table 7.1.1.2- 8: Degradation of isoflucypram under anaerobic conditions after an aerobic incubation phase (expressed as percentage of applied radioactivity, mean of 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 (entire system)	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)	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)	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)	n.a.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volatile organic compounds (sum aerobic and anaerobic)	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues[c)]	0.4	1.5	1.7	1.4	1.7	2.2	3.0	3.8	4.0	4.2
Total recovery[b)]	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
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 can not be proposed based on the results of the study.

III. CONCLUSIONS

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 identified.

CA 7.1.1.3 Soil photolysis

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 section CA 7.1.1 and Figure 7.1.1-1.

Report:	KCA 7.1.1.3/01; [redacted]; 2013; M-467307-01-1
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):	not specified
GLP/GEP:	**yes**

Executive 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 7.1.1.3- 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/m² 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 7.1.1.3- 2.

Table 7.1.1.3- 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)	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 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 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:

Table 7.1.1.3- 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 7.1.1.3- 4). No significant loss of moisture was observed throughout the study.

Table 7.1.1.3- 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 7.1.1.3- 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 7.1.1.3- 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 7.1.1.3- 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 7.1.1.3- 6.

The route of degradation of isoflucypram in soil under aerobic irradiated and dark conditions is summarised in Table 7.1.1.3- 7.

Table 7.1.1.3- 6: Material balance of radioactivity in irradiated and dark samples (expressed as percentage of applied radioactivity, mean of two replicates)

	DAT						
	0	**1**	**2**	**3**	**6**	**8**	**10**
Irradiated samples							
Volatiles							
carbon dioxide	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.2
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	0.2
Extractable residues							
ambient extract	100.3	101.5	105.8	101.2	98.6	97.6	98.3
mircowave extract 1	0.2	0.6	0.7	1.1	1.2	1.4	1.2
mircowave extract 2	0.1	0.2	0.2	0.3	0.3	0.3	0.5
total extractables	100.6	102.4	106.7	102.6	100.1	99.3	99.9
Non-extractable residues	< 0.1	0.3	0.3	0.7	0.9	1.2	1.2
Material balance	100.7	102.6	107.0	103.4	101.2	100.7	101.3
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
Extractable residues							
ambient extract	100.3	102.2	106.6	105.7	99.5	98.2	101.1
mircowave extract 1	0.2	0.4	0.6	0.5	0.8	0.8	0.8
mircowave extract 2	0.1	0.1	0.1	0.2	0.2	0.1	0.2
total extractables	100.6	102.7	107.4	106.4	100.5	99.1	102.1
Non-extractable residues	< 0.1	0.1	0.1	0.2	0.2	0.2	0.2
Material balance	100.7	102.9	107.5	106.6	100.7	99.3	102.4

n.d.: not detected; n.a.: nota analysed; DAT: days afater treatment

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 7.1.1.3- 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 7.1.1.3- 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 7.1.1.3- 7).

Table 7.1.1.3- 7: Degradation of isoflucypram in irradiated and dark samples (expressed as percentage of applied radioactivity, mean of two replicates)

Compound	Samples	DAT						
		0	1	2	3	6	8	10
Isoflucypram	irradiated	100.6	102.0	106.1	101.8	98.8	96.5	97.9
	dark	100.6	102.7	107.4	106.4	99.5	97.4	100.7
Sum of unid./diff. residues[a)]	irradiated	n.d.	< LOD	< LOD	< LOD	1.3	2.8	2.0
	dark	n.d.	n.d.	n.d.	n.d.	< LOD	0.6	1.5
Total extractable residues[b)]	irradiated	100.6	102.0	106.5	102.3	100.1	99.3	99.9
	dark	100.6	102.7	107.4	106.4	99.5	98.1	102.1
Carbon dioxide[c)]	irradiated	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.2
	dark	n.a.	< 0.1	0.1	0.1	< 0.1	< 0.1	< 0.1
Volatile organic compounds[c)]	irradiated	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	dark	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues[c)]	irradiated	< 0.1	0.3	0.3	0.7	0.9	1.2	1.2
	dark	< 0.1	0.1	0.1	0.2	0.2	0.2	0.2
Total recovery[b)]	irradiated	100.6	102.3	106.8	103.1	101.2	100.7	101.3
	dark	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 malance 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 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. Thus kinetic analysis was not performed.

III. CONCLUSIONS

The amount of isoflucypram 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.

The degradation of isoflucypram is driven by microbial degradation under typical conditions in the environment but photodegradation will play no role in the overall fate of isoflucypram.

CA 7.1.2 Rate of degradation in soil

The degradation behaviour of isoflucypram in soil was investigated under aerobic and anaerobic conditions in the laboratory as well as under field conditions. The kinetic models and DT_{50} values in soil of isoflucypram and its major degradation product in soil are summarised in sections CA 7.1.2.1 and CA 7.1.2.2.

Modelling input values for the calculation of predicted environmental concentrations (PECs) 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 and in sections CA 7.1.3.1 and CA 7.2, and are submitted within this Dossier.

The DT_{50} values and maximum occurrences / formation fractions in soil and aquatic systems of isoflucypram and its major degradation product used as modelling input values for the calculation of PECs are summarised in the following modelling core info documents.

- **PEC_{soil} Modelling Core Info**

Report: KCA 7.1.2/01; [redacted], G., [redacted], W., 2017, M-608723-01-1
Title: Isoflucypram (ISY): Core PECsoilEUR Modelling core info document for soil risk assessment in Europe
Report No.: EnSa-17-0654
Document No.: M-608723-01-1
Guideline(s): not applicable
Guideline deviation(s): none
GLP/GEP: no

Executive Summary
The input parameters which are used in the **PEC_{soil}** calculations are summarised in the following table.

Table 7.1.2-1: Input parameters related to active substance isoflucypram and metabolite for PEC_{soil} calculations

Compound	Molecular mass [g/mol]	Max. occurrence [%]	DT_{50} [days]	Value in accordance to EU endpoint y/n / Reference
Isoflucypram	399.85	100	630 trigger, maximum lab, not-normalised	n.a.
BCS-CH88460-carboxylic acid *(M12)*	429.8	9.6	113 trigger, maximum lab, not-normalised	

n.a. = not applicable for a new substance submission

[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

- **Isoflucypram**

Rate of degradation

The route and rate of degradation of isoflucypram under laboratory aerobic conditions was investigated in three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1).

The laboratory degradation data of these studies were kinetically evaluated according to FOCUS (2006, 2014) by [redacted], G.; [redacted], W.; 2017; M-608255-01-1. The non-normalised DT_{50} values ranged from 224 to 630 days (Table 7.1.2- 2). The maximum non-normalised DT_{50} value of 630 days was used in the soil exposure assessment.

Table 7.1.2- 2: Summary of aerobic degradation rates (non-normalised) for isoflucypram – laboratory studies trigger endpoints

Soil name	Soil type (USDA)	pH ($CaCl_2$)	temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	St. (χ^2 err) [%]	Kinetic model	Evaluated on EU level y/n / Reference
Hanscheider Hof, D[a)]	loam	5.7	20	53.1	438	> 1000	0.89	SFO	n / [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a)]	loamy sand	6.3	20	53.1	236	782	1.00	SFO	
Hoefchen am Hohenseh, GER[a)]	silt loam	6.6	20	53.4	348	> 1000	1.16	SFO	
Dollendorf II, GEr[a),e)]	loam	7.4	20	53.1	277	921	1.24	SFO	
Sanger, CA, USA[b)f)]	sandy loam	6.3	20.4	64.9[d)]	**630**	> 1000	0.68	SFO	
Louisville, NE, USA[b),g)]	silty clay loam	6.3	20.4	70.0[d)]	224	743	2.30	SFO	
Laacher Hof AXXa, GER[c)]	loamy sand	5.8	20	55.0	263	873	2.74	SFO	
Geometric mean (n=7)					323				
pH dependency:					no				

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) Calculated based on study moisture and MWHC
e) From modified pathway fit excluding the residue data of DAT 104
f) From modified parent only fit excluding the residue data of DAT 0
g) From initial pathway fit including all residue data

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

Rate of degradation

The degradation of BCS-CN88460-carboxylic acid *(M12)* under laboratory aerobic conditions was investigated in the studies with the parent substance isoflucypram ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1).

The kinetic evaluation according to FOCUS (2006, 2014) was conducted by [redacted], G.; [redacted], W.; 2017; M-608255-01-1. The non-normalised DT_{50} values ranged from 14.8 to 113 days (Table 7.1.2- 3). The maximum non-normalised DT_{50} value of 113 days was taken into account for the soil exposure assessment, together with the maximum occurrence of BCS-CN88460-carboxylic acid in soil of 9.6% AR.

Table 7.1.2- 3: Summary of aerobic degradation rates (non-normalised) and formation fractions (f.f.) for BCS-CN88460-carboxylic acid *(M12)* – laboratory studies trigger endpoints

Soil name	Soil type (USDA)	pH ($CaCl_2$)	temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	Kinetic model	St. (χ^2err) [%]	f.f.	Evaluated on EU level y/n / Reference
Hanscheider Hof, GER[a)]	Loam	5.7	20	53.1	45.4	151	SFO	7.08	0.330	n / [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a)]	Loamy sand	6.3	20	53.1	**113**	377	SFO	5.27	0.257	
Hoefchen am Hohenseh, GER[a)]	Silt loam	6.6	20	53.1	14.8	49.1	SFO	8.70	0.440	
Dollendorf II, GER[a),e)]	Loam	7.4	20	53.1	18.4	61.2	SFO	8.55	0.500	
Sanger, CA, USA[b)]	Sandy loam	6.3	20.4	64.9[d)]	-[f)]	-[f)]	SFO	-[f)]	-[f)]	
Louisville, NE, USA[b)]	Silty clay loam	6.3	20.4	70.0[d)]	n.r.	n.r.	SFO	20.01	0.286	
Laacher Hof AXXa, GER[c)]	Loamy sand	5.8	20	55.0	n.r.	n.r.	SFO	16.05	0.255	
Geometric mean (n=4)					34.4					
Arithmetic mean (n=6)									0.345	
pH dependency:					no					

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) Calculated based on study moisture and MWHC
e) From modified pathway fit excluding the residue data of DAT 104
f) Metabolite only found in two samples – parent only fit conducted
n.r. = not reliable

CONCLUSIONS

The maximum non-normalised DT_{50} values based on laboratory studies of 630 days for isoflucypram and 113 days for the major soil metabolite BCS-CN88460-carboxylic acid *(M12)* were used in the soil exposure assessment.

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- **PEC_{gw} Modelling Core Info**

Report: KCA 7.1.2/02; [redacted], G.; [redacted], W.; 2017; M-608724-02-1
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): not applicable
Guideline deviation(s): none
GLP/GEP: **no**

Executive Summary

The input parameters which are used in the **PEC_{gw}** calculations are summarised in the following table.

Table 7.1.2- 4: Input parameters related to active substance isoflucypram and metabolite for PEC_{gw} calculations

Parameter	Compound		Value in accordance with EU endpoint yes / Reference
	Isoflucypram	**BCS-CN88460-carboxylic acid *(M12)***	
Molecular mass [g/mol]	399.85	429.8	n.a.
Water solubility [mg/L]	1.8 (20°C)	10100 (20°C)	n.a.
Saturated vapour pressure [Pa]	1.2×10^{-7} (20°C)	2.6×10^{-8} (20°C)	n.a.
DT_{50} in soil [days]	Tier 1: 3.14 geometric mean lab, normalisation to pF2, 20°C with Q_{10} of 2.58, n =7 Tier 2: 3.23 geometric mean lab and field, normalisation to pF2, 20°C with Q_{10} of 2.58, n=13	Tier 1: 34.4 geometric mean lab, normalisation to pF2, 20°C with Q_{10} of 2.58, n=4 Tier 2: 84.1 geometric mean lab and field, normalisation to pF2, 20°C with Q_{10} of 2.58, n=10	n.a.
Transformation rate k	Tier 1: 0.0022075 Tier 2: 0.0021460	Tier 1: 0.0201496 Tier 2: 0.0082419	n.a.
K_{foc} / K_{fom}[a)] [mL/g]	1580 / 916.3 geometric mean, n=7	37.7 / 21.5 geometric mean, pH 7.5, n=2	n.a.
1/n[a)]	0.9142 arithmetic mean, n=7	0.9424 arithmetic mean, pH 7.5, n=2	n.a.
Plant uptake factor	Default: 0 (Tier 1a, Tier 2) Briggs estimate: 0.10 (Tier 1b)	Default: 0	n.a.
Formation fraction	-	Tier 1: 0.345 from parent Tier 2: 0.043 from parent	n.a.

n.a. = not applicable for a new active substance submission
a) Summary is given in chapter 7.1.3.1

Rate of degradation

- **Isoflucypram**

Tier 1:
The route and rate of degradation of isoflucypram under laboratory aerobic conditions was investigated in three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1).
The laboratory degradation data of these studies were kinetically evaluated according to FOCUS (2006, 2014) by [redacted], G.; [redacted], W.; 2017; M-608255-01-1. DT_{50} values were normalised to 20°C (Q_{10} = 2.58) and field capacity (pF2) following the standard procedure described in FOCUS (2000). The normalised DT_{50} values ranged from 200 to 570 days (Table 7.1.2- 5). The **geometric mean of 314 days** was used in the **leaching assessment at tier 1**.

Table 7.1.2- 5: Summary of aerobic degradation rates for isoflucypram from laboratory studies, modelling endpoints

Soil name	Soil type (USDA)	pH ($CaCl_2$)	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	DT_{50} [days] 20°C, pF2[h]	St. (χ^2err) [%]	Kinetic model	Evaluated on EU level y/n / Reference
Hanscheider Hof, GER[a]	loam	5.7	20	53.1	438	>1000	438	0.89	SFO	n / [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a]	loamy sand	6.3	20	53.1	236	782	236	1.00	SFO	
Hoefchen am Hohenseh, GER[a]	silt loam	6.6	20	53.1	348	>1000	348	1.16	SFO	
Dollendorf II, GER[a),e)]	loam	7.4	20	53.1	277	921	277	1.24	SFO	
Sanger, CA, USA[b)f)]	sandy loam	6.3	20.4	64.9[d)]	630	>1000	570	0.68	SFO	
Louisville, NE, USA[b),g)]	silty clay loam	6.3	20.4	70.0[d)]	224	743	200	2.30	SFO	
Laacher Hof AXXa, GER[c)]	loamy sand	5.8	20	55.0	263	873	263	2.74	SFO	
Geometric mean (n=7)							**314**			
pH dependency:							no			

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) Calculated based on study moisture and MWHC
e) From modified pathway fit excluding the residue data of DAT 104
f) From modified parent only fit excluding the residue data of DAT 0
g) From initial pathway fit including all residue data
h) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7

Tier 2:
The degradation of isoflucypram in the field was investigated by [redacted]; [redacted]; 2017; M-595964-01-1. A kinetic evaluation was conducted by [redacted], G.; [redacted], B.; 2017; M-608370-01-1. The degradation kinetics were normalised to a soil moisture corresponding to field capacity (pF2) and a temperature of 20°C (Q_{10} = 2.58) following FOCUS (2000). The normalised DT_{50} values

ranged from 137 to 649 days (Table 7.1.2- 6).
Based on the EFSA endpoint selector (EFSA 2014) degradation rates of isoflucypram derived from laboratory and field degradation studies are not systematically different and can therefore be pooled to derive a $DegT_{50matrix}$ value for input to simulation models. According to EFSA (2014), it may be acceptable not to perform a difference test between laboratory and field data if the geometric mean laboratory $DegT_{50matrix}$ value is higher than 240 days.
In this particular case the geometric mean laboratory $DegT_{50matrix}$ value of 314 days and the geometric mean field $DegT_{50matrix}$ value of 335 days are very close to each other. In order to make best use of all available data DT_{50} values have been pooled. Consequently, the geometric mean $DegT_{50matrix}$ value of **323 days** derived from lab and field data (n=13) was used for the leaching assessment.

Table 7.1.2- 6: Summary of aerobic degradation rates for isoflucypram from laboratory and field studies, modelling endpoints

Isoflucypram, aerobic conditions							
Soil name	**Soil type (USDA)**	**pH ($CaCl_2$)**	**Depth [cm][i]**	**DT_{50} matrix [days] norm[h]**	**St. (χ^2err) [%]**	**Method of calculation**	**Evaluated on EU level y/n / Reference**
Laboratory studies kinetic evaluation ([redacted], G.; [redacted], W.; 2017; M-608255-01-1)							
Hanscheider Hof, GER[a]	loam	5.7	0-20	438	0.89	SFO	n / [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a]	loamy sand	6.3	0-20	236	1.00	SFO	
Hoefchen am Hohenseh, GER[a]	silt loam	6.6	0-20	348	1.16	SFO	
Dollendorf II, GER[a),e]	loam	7.4	0-20	277	1.24	SFO	
Sanger, CA, USA[b),f]	sandy loam	6.3	0-20	570	0.68	SFO	
Louisville, NE, USA[b),g]	silty clay loam	6.3	0-20	200	2.30	SFO	
Laacher Hof AXXa, GER[c]	loamy sand	5.8	0-20	263	2.74	SFO	
Field dissipation studies kinetic evaluation ([redacted], G.; [redacted], B.; 2017; M-608370-01-1)							
Burscheid, GER[d]	silt loam, bare soil	5.3	0-60	281	3.1	DFOP slow phase	n / [redacted], G.; [redacted], B.; 2017; M-608370-01-1
Great Chishill, UK[d]	clay loam, bare soil	7.0	0-60	489	6.1	DFOP slow phase	
Parcay Meslay, F[d]	loam, bare soil	5.9	0-60	449	2.7	DFOP slow phase	
St. Etienne du Gres, F[d]	clay loam, bare soil	7.5	0-60	137	3.7	HS slow phase	
Albaro, IT[d]	clay, bare soil	7.0	0-60	649	5.9	DFOP slow phase	
Vilobi, ES[d]	loam, bare soil	5.8	0-60	258	10.3	DFOP slow phase	
Geometric mean (n=13) from combined lab and field studies				**323**			
pH dependency:					no		

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) [redacted]; [redacted]; 2017; M-595964-01-1
e) From modified pathway fit excluding the residue data of DAT 104
f) From modified parent only fit excluding the residue data of DAT 0
g) From initial pathway fit including all residue data
h) Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7
i) Experimental soil sampling depth considered to derive total residues for kinetic evaluation

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

Tier 1:

The degradation of BCS-CN88460-carboxylic acid *(M12)* under laboratory aerobic conditions was investigated in the studies with the parent substance isoflucypram ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1).

The kinetic evaluation according to FOCUS (2006, 2014) and normalisation to 20°C (Q_{10} = 2.58) and field capacity (pF2) (FOCUS 2000) were conducted by [redacted], G.; [redacted], W.; 2017; M-608255-01-1.

The normalised DT_{50} values ranged from 14.8 to 113 days (Table 7.1.2- 7). In **tier 1 of the leaching assessment**, only the data from the laboratory studies were considered. Consequently, the **geometric mean DT_{50} value** of **34.4 days** and the **arithmetic mean formation fraction** of **0.345** were taken into account.

Table 7.1.2- 7: Summary of aerobic degradation rates (normalised) and formation fractions (f.f.) for BCS-CN88460-carboxylic acid *(M12)* – laboratory studies modelling endpoints

Soil name	Soil type (USDA)	pH ($CaCl_2$)	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	Kinetic model	DT_{50} [days] 20°C, pF2[g]	St. (χ^2err) [%]	f.f. k_f / k_{dp}	Evaluated on EU level y/n / Reference
Hanscheider Hof, GER[a]	loam	5.7	20	53.1	45.4	151	SFO	45.4	7.08	0.330	n / [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a]	loamy sand	6.3	20	53.1	113	377	SFO	113	5.27	0.257	
Hoefchen am Hohenseh, GER[a]	silt loam	6.6	20	53.1	14.8	49.1	SFO	14.8	8.70	0.440	
Dollendorf II, GER[a),e)]	loam	7.4	20	53.1	18.4	61.2	SFO	18.4	8.55	0.500	
Sanger, CA, USA[b]	sandy loam	6.3	20.4	64.9[d]	-[f]	-[f]	SFO	-[f]	-[f]	-[f]	
Louisville, NE, USA[b]	silty clay loam	6.3	20.4	70.0[d]	n.r.	n.r.	SFO	n.r.	20.01	0.286	
Laacher Hof AXXa, GER[c]	loamy sand	5.8	20	55.0	n.r.	n.r.	SFO	n.r.	16.05	0.255	
Geometric mean (n=4)								**34.4**			
Arithmetic mean (n=6)										**0.345**	
pH dependency:								no			

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) Calculated based on study moisture and MWHC
e) From modified pathway fit excluding the residue data of DAT 104
f) Metabolite only found in two samples – parent only fit conducted
g) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
n.r. = not reliable

Tier 2:

The degradation of BCS-CN88460-carboxylic acid *(M12)* in the field was investigated in study with

the parent substance ([redacted]; [redacted]; 2017; M-595964-01-1) on six European soils. The kinetic evaluation according to FOCUS (2006, 2014) and normalisation to 20°C (Q_{10} = 2.58) and field capacity (pF2) (FOCUS 2000) were conducted by [redacted], G.; [redacted], B.; 2017; M-608370-01-1. The normalised DT50 values ranged from 67.3 to 340 days (Table 7.1.2- 8).

Based on the EFSA endpoint selector (EFSA 2014) degradation rates of BCS-CN88460-carboxylic acid derived from laboratory and field degradation studies are not systematically different and can therefore be pooled to derive a $DegT_{50matrix}$ value for input to simulation models. Consequently, the geometric mean $DegT_{50matrix}$ value of **84.1 days** derived from lab and field data was used for the leaching assessment.

Table 7.1.2- 8: Summary of aerobic degradation rates and formation fractions for BCS-CN88460-carboxylic acid *(M12)* from laboratory and field studies: Modelling endpoints

BCS-CN88460-carboxylic acid, aerobic conditions								
Soil name	**Soil type (USDA)**	**pH ($CaCl_2$)**	**Depth [cm][h)]**	**DT_{50} matrix [days] norm[g)]**	**St. (χ^2err) [%]**	**Method of cal-culation**	**f.f. k_f / k_{dp}**	**Evaluated on EU level y/n / Reference**
Laboratory studies kinetic evaluation ([redacted], G.; [redacted], W.; 2017; M-608255-01-1)								
Hanscheider Hof, GER[a)]	loam	5.7	0-20	45.4	7.08	SFO	0.330	n / [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a)]	loamy sand	6.3	0-20	113	5.27	SFO	0.257	
Hoefchen am Hohenseh, GER[a)]	silt loam	6.6	0-20	14.8	8.70	SFO	0.440	
Dollendorf II, GER[a),e)]	loam	7.4	0-20	18.4	8.55	SFO	0.500	
Sanger, CA, USA[b)]	sandy loam	6.3	0-20	-[f)]	-[f)]	SFO	-	
Louisville, NE, USA[b)]	silty clay loam	6.5	0-20	n.r.	20.01	SFO	0.286	
Laacher Hof AXXa, GER[c)]	loamy sand	5.8	0-20	n.r.	16.05	SFO	0.255	
Field dissipation studies kinetic evaluation ([redacted], G.; [redacted], B.; 2017; M-608370-01-1)								
Burscheid, GER[d)]	silt loam, bare soil	5.3	0-60	172	4.3	SFO	0.0386	n / [redacted], G.; [redacted], B.; 2017; M-608370-01-1
Great Chishill, UK[d)]	clay loam, bare soil	7.9	0-60	67.3	12.8	SFO	0.0563	
Parcay Meslay, F[d)]	loam, bare soil	5.9	0-60	340	8.9	SFO	0.0345	
St. Etienne du Gres, F[d)]	clay loam, bare soil	7.5	0-60	82.4	11.0	SFO	0.0651	
Albaro, IT[d)]	clay, bare soil	7.0	0-60	228	23.1	SFO	0.0430	
Vilobi, ES[d)]	loam, bare soil	5.8	0-60	172	23.9	SFO	0.0183	
Geometric mean (n=10) from combined lab and field studies				**84.1**	**Arithmetic mean (n=6) from field studies**		**0.043**	
pH dependency:					no			

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) [redacted]; [redacted]; 2017; M-595964-01-1
e) From modified pathway fit excluding the residue data of DAT 104
f) Metabolite only found in two samples – parent only fit conducted
g) Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7
h) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
n.r. = not reliable

In addition a population test was also conducted for the kinetic formation fraction (f. f. k_f / k_{dp}) of BCS-CN88460-carboxylic acid. The arithmetic mean formation fraction in laboratory studies is 0.345 (n = 6) whereas the arithmetic mean formation fraction in field studies is 0.043 (n = 6). Based on the

EFSA endpoint selector kinetic formation fraction of BCS-CN88460-carboxylic acid derived from laboratory and field degradation studies are systematically different. Consequently, the arithmetic mean value of **0.043** derived from field data was used for the leaching assessment at Tier 2. Summary of combined laboratory and field kinetic endpoints for modelling at Tier 2 is given in Table 7.1.2- 9.

Table 7.1.2- 9: Summary of combined laboratory and field kinetic endpoints for modelling at Tier 2

Parent / Transformation product	Rate of degradation in soil normalised geometric mean (if not pH dependent)	Kinetic formation fraction (f_f, k_f / k_{dp}) arithmetic mean
Isoflucypram	323 d (n = 13) from combined lab and field studies	n.a.
BCS-CN88460-carboxylic acid *(M12)*	84.1 d (n = 10) from combined lab and field studies	0.043 (n = 6) from field studies formed from parent

n.a. = not applicable

Tiered approach

For PEC_{gw} assessments a tiered approach concerning the DT_{50} values of isoflucypram and its metabolite BCS-CN88460-carboxylic acid, the formation fraction of BCS-CN88460-carboxylic acid, and the PUF values should be considered. In Tier 1 only laboratory data are considered for DT_{50} values and formation fractions, which can be modified by the PUF values. Field data are included in Tier 2 where a further modification by PUF values is not possible. A detailed description is presented in Table 7.1.2- 10.

Table 7.1.2- 10: Tiered approach for isoflucypram and its metabolite used for modelling

Compound	Tier 1a			Tier 1b			Tier 2		
	DT_{50} [a)] [days]	ff [a)]	PUF [d)]	DT_{50} [a)] [days]	ff [a)]	PUF [e)]	DT_{50} [b)] [days]	ff [c)]	PUF [d)]
Isoflucypram	314	n.a.	0.0	314	n.a.	**0.1**	323	n.a.	0.0
BCS-CN88460-carboxylic acid	34.4	0.345	0.0	34.4	0.345	**0.0**	84.1	0.043	0.0

a) From laboratory data
b) rom laboratory and field data
c) rom field data
d) PUF representing worst case default
e) PUF based on Briggs equation

Adsorption

For the leaching calculations, the adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1580 mL/g (K_{fom} = 916.3 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.9142. For the leaching assessment of BCS-CN88460-carboxylic acid *(M12)*, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.
A more detailed summary is given in chapter CA 7.1.3.1.

CONCLUSIONS

For isoflucypram the geometric mean (normalised to 20°C and pF2) of laboratory data of 314 days was used in the leaching assessment at Tier 1. For Tier 2 the geometric mean $DegT_{50matrix}$ value of 323 days derived from lab and field data was used for the leaching assessment.
A default plant uptake factor of zero was used at Tier 1a and Tier 2, and for Tier 1b the Briggs estimate of 0.10.
For the leaching calculations, the adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1580 mL/g (K_{fom} = 916.3 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.9142.

In tier 1 of the leaching assessment of the major metabolite BCS-CN88460-carboxylic acid *(M12)*, only the data from the laboratory studies were considered. The geometric mean DT_{50} value (normalized to 20°C and PF2) of 34.4 days and the arithmetic mean formation fraction of 0.345 were taken into account. For Tier 2 the geometric mean $DegT_{50matrix}$ value of 84.1 days derived from lab and field data and a formation fraction of 0.043 were used
For the leaching assessment of BCS-CN88460-carboxylic acid, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.

- **PEC_{sw} Modelling Core Info**

Report: KCA 7.1.2/03; [redacted], G.; [redacted], W.; 2017; M-608725-02-1
Title: Isoflucypram (JSY): Core PECsw EUR - Modelling core info document for surface water risk assessment in Europe
Report No.: EnSa-17-0656
Document No.: M-608725-02-1
Guideline(s): not applicable
Guideline deviation(s): none
GLP/GEP: **no**

Executive Summary
The input parameters which are used in the **$PEC_{sw/sed}$** calculations are summarised in the following table.

Table 7.1.2- 11: Input parameters related to active substance isoflucypram and metabolite for $PEC_{sw/sed}$ calculations

Parameter	Compound		Value in accordance with EU endpoint / Reference
	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*	
Molecular mass [g/mol]	399.85	429.8	n.a.
Water solubility [mg/L]	1.8 (20°C)	10100 (20°C)	n.a.
Saturated vapour pressure [Pa]	1.2×10^{-7} (20°C)	2.6×10^{-13} (20°C)	n.a.
Diffusion coefficient in water [m²/d]	required for Steps 3-4: 4.3 x 10^{-5}	not required	n.a.
Diffusion coefficient in air [m²/d]	required for Steps 3-4: 0.43	not required	n.a.
K_{foc}/K_{fom} [mL/g][c)]	1580 / 916.3 (geometric mean, n = 7)	37.1 / 21.5 (geometric mean, pH 7.5, n = 2)	n.a.
1/n[c)]	0.9142 (arithmetic mean, n =7)	0.9424 (arithmetic mean, pH 7.5, n = 2)	n.a.
Plant uptake factor	0	0	n.a.
Wash off factor from crop [1/m]	required for Steps 3-4: 50	not required	n.a.
DT_{50} in soil [days]	314 (geometric mean lab, normalisation to pF2, 20°C with Q_{10} of 2.58, n =7)	34.4 (geometric mean lab, normalisation to pF2, 20°C with Q_{10} of 2.58, n=4)	n.a.
DT_{50} in water [days][d)]	354 (Step 2) 354[a)]/1000[b)] (Step 3)	1000	n.a.
DT_{50} in sediment [days][d)]	354 (Step 2) 354[a)]/1000[b)] (Step 3)	1000	n.a.
DT_{50} in total system [days][d)]	354 (Step 1)	1000	n.a.
Maximum occurrence observed (% molar basis with respect to parent)	soil: 100 water: 100 sediment: 83.0 total system: 100	soil: 9.6 water: 5.4 sediment: 1.3 total system: 6.6	n.a.

According to FOCUS (2015) for substances with a K_{oc} between 100 and 2000 mL/g two options should be tested:
a) $DegT_{50}$system used for degradation in water, default DT_{50} of 1000 days used for degradation in sediment
b) $DegT_{50}$system used for degradation in sediment, default DT_{50} of 1000 days used for degradation in water
c) A more detailed summary is given in chapter CA 7.1.3.1
d) A more detailed summary is given in chapter CA 7.2.2.3
n.a. = not applicable for a new active substance submission

Rate of degradation in soil

- **Isoflucypram**

The route and rate of degradation of isoflucypram under laboratory aerobic conditions was investigated in three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1).

The laboratory degradation data of these studies were kinetically evaluated according to FOCUS (2006, 2014) by [redacted], G.; [redacted], W.; 2017; M-608255-01-1. DT_{50} values were normalised to 20°C (Q_{10} 2.58) and field capacity (pF2) following the standard procedure described in FOCUS (2000). The normalised DT_{50} values ranged from 200 to 570 days (Table 7.1.2- 12). The **geometric mean of 314 days** was used in the risk assessment.

Table 7.1.2- 12: Summary of aerobic degradation rates for isoflucypram from laboratory studies, modelling endpoints

Soil name	Soil type (USDA)	pH ($CaCl_2$)	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	DT_{50} [days] 20°C, pF2[h)]	St. (χ^2err) [%]	Kinetic model	Evaluated on EU level y/n Reference
Hanscheider Hof, GER[a)]	loam	5.7	20	53.1	438	>1000	438	0.89	SFO	n / [redacted] G.; [redacted], W.; 2017; M-608255-01-1
Laacher Hof AXXa, GER[a)]	loamy sand	6.3	20	53.1	236	782	236	1.00	SFO	
Hoefchen am Hohenseh, GER[a)]	silt loam	6.6	20	53.1	348	>1000	348	1.16	SFO	
Dollendorf II, GER[a),e)]	loam	7.4	20	53.1	277	921	277	1.24	SFO	
Sanger, CA, USA[b)f)]	sandy loam	6.3	20.4	64.9[d)]	630	>1000	570	0.68	SFO	
Louisville, NE, USA[b),g)]	silty clay loam	6.3	20.4	70.0[d)]	224	743	200	2.30	SFO	
Laacher Hof AXXa, GER[c)]	loamy sand	5.8	20	55.0	263	873	263	2.74	SFO	
Geometric mean (n=7)							**314**			
pH dependency: y/n							n			

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) Calculated based on study moisture and MWHC
e) From modified pathway fit excluding the residue data of DAT 104
f) From modified parent only fit excluding the residue data of DAT 0
g) From initial pathway fit including all residue data
h) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7

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

The degradation of BCS-CN88460-carboxylic acid *(M12)* under laboratory aerobic conditions was investigated in the studies with the parent substance isoflucypram ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted], [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1).

The kinetic evaluation according to FOCUS (2006, 2014) and normalisation to 20°C (Q_{10} = 2.58) and field capacity (pF2) (FOCUS 2000) were conducted by [redacted], G.; [redacted], W.; 2017; M-608255-01-1. The normalised DT_{50} values ranged from 14.8 to 113 days (Table 7.1.2- 13). The geometric mean DT_{50} value of 34.4 days was taken into account. The maximum occurrence of BCS-CN88460-carboxylic acid in soil in the laboratory studies was 9.6% AR.

Table 7.1.2- 13: Summary of aerobic degradation rates (normalised) and formation fractions (f.f.) for BCS-CN88460-carboxylic acid *(M12)* – laboratory studies modelling endpoints

Soil name	Soil type (USDA)	pH ($CaCl_2$)	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	Kinetic model	DT_{50} [days] 20°C, pF2[g)]	St. (χ^2err) [%]	f.f. k_f/k_{dp}	Evaluated on LF level y/n / Reference
Hanscheider Hof, GER[a)]	loam	5.7	20	53.1	45.4	151	SFO	45.4	7.08	0.330	n / [redacted] G, [redacted]
Laacher Hof AXXa, GER[a)]	loamy sand	6.3	20	53.1	113	377	SFO	113	5.27	0.257	W., 2015; M-608285-01-1
Hoefchen am Hohenseh, GER[a)]	silt loam	6.6	20	53.1	14.8	49.1	SFO	14.8	8.70	0.440	
Dollendorf II, GER[a),e)]	loam	7.4	20	53.1	18.4	61.2	SFO	18.4	8.55	0.500	
Sanger, CA, USA[b)]	sandy loam	6.3	20.4	64.9[d)]	-[f)]	-[f)]	SFO	-[f)]	-[f)]	-[f)]	
Louisville, NE, USA[b)]	silty clay loam	6.3	20.4	76.0[d)]	n.r.	n.r.	SFO	n.r.	20.01	0.286	
Laacher Hof AXXa, GER[c)]	loamy sand	5.8	20	55.0	n.r.	n.r.	SFO	n.r.	16.05	0.255	
Geometric mean (n=4)								**34.4**			
Arithmetic mean (n=6)										**0.345**	
pH dependency: y/n								n			

a) [redacted]; [redacted]; 2014; M-486690-01-1
b) [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
c) [redacted]; [redacted]; 2017; M-599926-01-1
d) Calculated based on study moisture and MWHC
e) From modified pathway fit excluding the residue data of DAT 104
f) Metabolite only found in two samples – parent only fit conducted
g) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
n.r. = not reliable

Adsorption
For the leaching calculations, the adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1580 mL/g (K_{fom} = 916.3 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.9142.
For the leaching assessment of the major metabolite BCS-CN88460-carboxylic acid *(M12)*, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.
A more detailed summary is given in chapter CA 7.1.3.1.

Degradation in aquatic systems

The geometric mean total system degradation DT_{50} of 354 days for isoflucypram was used for all phases in the Steps 1-2 calculations. For FOCUS Step 3 degradation DT_{50} for the single phases are required. Since these are not available the geometric mean total system DT_{50} of 354 days was taken into account. According to FOCUS (2015), for substances with a K_{foc} value between 100 and 2000 mL/g such as isoflucypram two options have to be tested to derive the worst case PEC_{sw} values:

1. The geometric mean total system DT_{50} of 354 days was used for the water phase and a default DT_{50} value of 1000 days was used for the sediment phase.
2. The geometric mean total system DT_{50} of 354 days was used for the sediment phase and a default DT_{50} value of 1000 days was used for the water phase.

For the major metabolite BCS-CN88460-carboxylic acid *(M12)* no reliable degradation half-lives could derive from the aerobic water-sediment study with the parent isoflucypram, due to limited formation in aquatic systems. Therefore, the DT_{50} in water, sediment, and total system was set to a conservative value of 1000 days for the use in FOCUS Steps 1-2 calculations. The maximum occurrence of BCS-CN88460-carboxylic acid in water/sediment systems was 6.6%.

A more detailed summary is given in chapter CA 7.2.2.3.

CONCLUSIONS

For isoflucypram the geometric mean (normalised to 20°C and pF2) of laboratory soil data of 314 days was used for the risk assessments.
The adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1580 mL/g (K_{fom} = 916.3 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.9142.
The geometric mean total water/sediment system degradation DT_{50} of 354 days for isoflucypram was used for all phases in the Steps 1-2 calculations. For FOCUS Step 3 degradation DT_{50} for the single phases are required. Since these are not available the geometric mean total system DT_{50} of 354 days was taken into account. According to FOCUS (2015), for substances with a K_{foc} value between 100 and 2000 mL/g such as isoflucypram two options have to be tested to derive the worst case PEC_{sw} values:

1. The geometric mean total system DT_{50} of 354 days was used for the water phase and a default DT_{50} value of 1000 days was used for the sediment phase.
2. The geometric mean total system DT_{50} of 354 days was used for the sediment phase and a default DT_{50} value of 1000 days was used for the water phase.

For the major metabolite BCS-CN88460-carboxylic acid *(M12)*, the geometric mean DT_{50} value of 34.4 days (based on soil lab data) was taken into account. The maximum occurrence of BCS-CN88460-carboxylic acid in soil in the laboratory studies was 9.6% AR.
For the $PEC_{sw/sed}$ calculations of BCS-CN88460-carboxylic acid, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.
For BCS-CN88460-carboxylic acid *(M12)* no reliable degradation half-lives could derive from the aerobic water-sediment study with the parent isoflucypram, due to limited formation in aquatic systems. Therefore, the DT_{50} in water, sediment, and total system was set to a conservative value of 1000 days for the use in FOCUS Steps 1-2 calculations. The maximum occurrence of BCS-CN88460-carboxylic acid in water/sediment systems was 6.6%.

CA 7.1.2.1 Laboratory studies

The degradation rates of isoflucypram in soil were studied using two different radiolabel positions for the parent compound (phenyl- and pyrazole-label). The studies have been performed in a number of soils in the dark in the laboratory. For isoflucypram the not-normalised trigger endpoints are summarised in Table 7.1.2.1- 1. The modelling endpoints not-normalised and normalised to pF2 and 20°C are summarised in Table 7.1.2.1- 2.

For the major soil metabolite BCS-CN88460-carboxylic acid *(M12)* the not-normalised trigger endpoints are summarised in Table 7.1.2.1- 3. The modelling endpoints not-normalised and normalised to pF2 and 20°C are summarised in Table 7.1.2.1- 4.

Table 7.1.2.1- 1: Soil degradation not-normalised DT_{50} values of isoflucypram to trigger additional studies (trigger endpoints)

Study	Soil	Model fitted	DT_{50} not-normalised [days]
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof	SFO	438
	Laacher Hof AXXa	SFO	236
	Hoefchen am Hohenseh	SFO	348
	Dollendorf II	SFO	277
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA	SFO	630
	Louisville, NE	SFO	224
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa	SFO	263
Geometric mean:			**323**

Table 7.1.2.1- 2: Soil degradation not-normalised and normalised (to pF2 and 20°C, Q_{10}: 2.58) DT_{50} values of isoflucypram for modelling purposes (modelling endpoints)

Study	Soil	Model fitted	DT_{50} not normalised [days]	DT_{50} normalised [days]
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof	SFO	438	438
	Laacher Hof AXXa	SFO	236	236
	Hoefchen am Hohenseh	SFO	348	348
	Dollendorf II	SFO	277	277
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA	SFO	630	570
	Louisville, NE	SFO	224	200
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa	SFO	263	263
Geometric mean:			**323**	**314**

Table 7.1.2.1- 3: Soil degradation not-normalised DT_{50} values of BCS-CN88460-carboxylic acid *(M12)* to trigger additional studies (trigger endpoints)

Report	Soil origin	Soil type	pH[a)]	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	f.f.	St. (χ^2err) [%]	Method of calc.
[redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	5.7	20	53.1	45.4	151	0.330	0.89	SFO
	Laacher Hof AXXa, Germany	loamy sand	6.3	20	53.1	113	377	0.257	1.00	SFO
	Hoefchen am Hohenseh, Germany	silt loam	6.6	20	53.1	114.8	749.1	0.440	1.16	SFO
	Dollendorf II, Germany[b)]	loam	7.4	20	53.1	18.4	61.2	0.500	1.24	SFO
[redacted]; 2017; M-588260-01-1	Sanger, CA, USA	sandy loam	6.3	20.4	64.9[c)]	-[d)]	-[d)]	-	0.68	SFO
	Louisville, NE, USA	silty clay loam	6.3	20.4	70.0[c)]	n.r.	n.r.	0.286	2.30	SFO
[redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	20	55.0	n.r.	n.r.	0.255	2.74	SFO
Geometric mean:						**34.4**	**114**			
Arithmetic mean:								**0.345**		

n.r. = not reliable
a) Measured in $CaCl_2$
b) From modified pathway fit excluding the residue data of DAT 104
c) Calculated based on study moisture and MWHC
d) Metabolite only found in two samples, parent only fit conducted

Table 7.1.2.1- 4: Soil degradation not-normalised and normalised (to pF2 and 20°C, Q_{10}: 2.58) DT_{50} values of BCS-CN88460-carboxylic acid *(M12)* for modelling purpose (modelling endpoints)

Report	Soil origin	Soil type	pH[a)]	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	f.f.	DT_{50} [days] 20°C, pF2/ 10 kPa[e)]	St. (χ^2err) [%]	Method of calc.
[redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	5.7	20	53.1	45.4	151	0.330	45.4	0.89	SFO
	Laacher Hof AXXa, Germany	loamy sand	6.3	20	53.1	113	377	0.257	113	1.00	SFO
	Hoefchen am Hohenseh, Germany	silt loam	6.6	20	53.1	14.8	49.1	0.440	14.8	1.16	SFO
	Dollendorf II, Germany[b)]	loam	7.4	20	53.1	18.4	61.2	0.500	18.4	1.24	SFO
[redacted]; 2017; M-588260-01-1	Sanger, CA, USA	sandy loam	6.3	20.4	64.9[c)]	-[d)]	-[d)]	-[d)]	-	0.68	SFO
	Louisville, NE, USA	silty clay loam	6.3	20.4	70.0[c)]	n.r.	n.r.	0.286	n.r.	2.30	SFO
[redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	20	55.0	n.r.	n.r.	0.255	n.r.	2.74	SFO
Geometric mean:						**34.4**	**114**		**34.4**		
Arithmetic mean:								**0.345**			

n.r. = not reliable
a) Measured in $CaCl_2$
b) From modified pathway fit excluding the residue data of DAT 104
c) Calculated based on study moisture and MWHC
d) Metabolite only found in two samples - parent only fit conducted
e) Normalised using a Q_{10} of 2.58 and Walker equation 0.7

CA 7.1.2.1.1 Aerobic degradation of the active substance

The degradation rates of isoflucypram in soil were studied using two different radiolabel positions for the parent compound (phenyl- and pyrazole-label). Three studies have been performed in a number of soils in the dark in the laboratory. In addition the data of these studies have been evaluated for trigger and modelling endpoints according to FOCUS guidance (2006, 2014).
A summary of the degradation rates of isoflucypram and its major degradation product in soil in the laboratory is given in section CA 7.1.2.1.

- **Studies soil metabolism, aerobic**

Report:	KCA 7.1.2.1.1/01; [redacted]; [redacted]; 2014; M-486690-01-1
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**

Executive Summary

The degradation data of isoflucypram as reported in [redacted]; [redacted]; 2014; M-486690-01-1 in section 7.1.1.1 (page 13) were kinetically evaluated according to FOCUS Kinetics report (FOCUS, 2006). The experimental data could be well described by a single first order (SFO) kinetic model. The half-life of isoflucypram under aerobic conditions was 458, 239, 358 and 267 days in soil Hanscheider Hof; Laacher Hof AXXa; Hoefchen Am Hohenseh and Dollendorf II, respectively (Table 7.1.2.1.1- 1).

Table 7.1.2.1.1- 1: Degradation kinetics of isoflucypram in soils under aerobic conditions

Soil	Texture (USDA)	Best fit model[a)]	DT_{50} [days]	DT_{90} [days]	Chi² error	Visual assessment[b)]
Hanscheider Hof	loam	SFO	458	> 1000	0.7	+
Laacher Hof AXXa	loamy sand	SFO	239	795	0.7	+
Hoefchen Am Hohenseh	silt loam	SFO	358	> 1000	0.9	+
Dollendorf II	loam	SFO	267	887	1.5	+

a) SFO: single first order
b) Visual assessment: + = good

I. MATERIALS AND METHODS

Details on the study conduct and its results are summarised under KCA 7.1.1.1/01, page 13.
The data for the test item were evaluated according to FOCUS kinetics (2006)[1] using the software KinGUI 2. Model input datasets were the residual amounts found in each replicate test system at each sampling interval. The initial total recovery (material balance) at DAT-0 was included in the parameter optimization procedure, but for optimal goodness of fit, the value was allowed to be estimated by the model. DT_{50} and DT_{90} values were calculated from the resulting kinetic parameters.

[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.

II. RESULTS AND DISCUSSION

The degradation of isoflucypram followed single first order (SFO) kinetics in all soils according to the lowest chi^2 error values and visual assessments.
A summary of all kinetic data is given in Table 7.1.2.1.1- 2, the best fits are highlighted in bold letters there. The half-lives for isoflucypram were between 239 and 458 days in the tested soils under aerobic conditions.

Table 7.1.2.1.1- 2: Summary of the kinetic evaluation (for trigger values according to FOCUS) of the degradation of isoflucypram in soils under aerobic conditions
(best fits are highlighted in **bold** letters)

Soil (Texture (USDA))	Kinetic model[a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual assessment[b)]
Hanscheider Hof (loam)	**SFO**	**458**	**> 1000**	**0.7**	**+**
	FOMC[c)]	448	> 1000	0.7	+
	DFOP	459	-[d)]	0.7	+
Laacher Hof AXXa (loamy sand)	**SFO**	**239**	**795**	**0.7**	**+**
	FOMC[c)]	285	> 1000	0.6	+
	DFOP[c)]	238	808	0.4	+
Hoefchen Am Hohenseh (silt loam)	**SFO**	**358**	**> 1000**	**0.9**	**+**
	FOMC	353	> 1000	1.0	+
	DFOP	359	-[d)]	0.9	+
Dollendorf II (loam)	**SFO**	**267**	**887**	**1.5**	**+**
	FOMC	259	873	1.6	+
	DFOP	267	888	1.7	+

a) SFO: Single first order, FOMC: First order multi compartment, DFOP: Double first order in parallel
b) Visual assessment: + = good, o = moderate, - = poor
c) Statistically non-reliable kinetic evaluations
d) Could not be calculated/determined

III. CONCLUSIONS

Isoflucypram was slowly degraded in soil under aerobic conditions in the dark in the laboratory. The calculated best fit half-lives were between 239 and 458 days in the tested soils.

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Report:	KCA 7.1.2.1.1/02; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1
Title:	[Pyrazole-4-14C]BCS-CN88460: Aerobic soil metabolism in two US soils
Report No.:	MELNN013
Document No.:	M-588260-01-1
Guideline(s):	US EPA OCSPP 835.4100, Aerobic Soil Metabolism, 2008, OECD: Guideline 307: Aerobic and Anaerobic Transformation in Soil, 2002 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 PMRA Daco No. 8.2.3.4.2 Biotransformation in Soil (TGAI), Aerobic Soil 20-30degrees C
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The degradation data of isoflucypram as reported in study [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1 in section 7.1.1.1 (page 26) were kinetically evaluated according to FOCUS Kinetics report (FOCUS, 2006). The experimental data could be well described by a single first order (SFO) kinetic model. The half-lives of isoflucypram under aerobic conditions were 223 and 714 days in NE and CA soil, respectively (Table 7.1.2.1.1- 3).

Table 7.1.2.1.1- 3: Degradation kinetics of isoflucypram in soils under aerobic conditions

Soil	Texture (USDA)	Best fit model[a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual assessment[b)]
CA	sandy loam	SFO	714	1000	1.15	+
NE	silty clay loam	SFO	223	741	2.35	+

a) SFO: single first order
b) Visual assessment: + = good

I. MATERIALS AND METHODS

Details on the study conduct and its results are summarised under KCA 7.1.1.1/02, page 26.
The data for the test item were evaluated according to FOCUS Kinetics (2006)[1] using the software KinGUI 2. Model input datasets were the residual amounts found in each replicate test system at each sampling interval. The initial total recovery (material balance) at DAT-0 was included in the parameter optimization procedure, but for optimal goodness of fit, the value was allowed to be estimated by the model. DT_{50} and DT_{90} values were calculated from the resulting kinetic parameters.

II. RESULTS AND DISCUSSION

The degradation of isoflucypram followed single first order (SFO) kinetics in all soils according to the lowest chi^2 error values and visual assessments.
A summary of all kinetic data is given in Table 7.1.2.1.1- 4, the best fits are highlighted in bold letters there. The half-lives for isoflucypram were between 223 and 741 days in the tested soils under aerobic conditions.

[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.

Table 7.1.2.1.1- 4: Summary of the kinetic evaluation (for trigger values according to FOCUS) of the degradation of isoflucypram in soils under aerobic conditions
(best fits are highlighted in **bold** letters)

Soil (Texture (USDA))	Kinetic model[a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual assessment[b)]
CA soil (sandy loam)	**SFO**	**714**	**>1000**	**1.15**	+
	FOMC[c)]	714	>1000	1.23	+
	DFOP	714	>1000	1.33	+
NE soil (silty clay loam)	**SFO**	**223**	**741**	**2.35**	+
	FOMC	223	741	2.51	+
	DFOP	223	740	2.71	+

a) SFO: Single first order, FOMC: First order multi compartment, DFOP: Double first order in parallel
b) Visual assessment: + = good, o = moderate, - = poor

III. CONCLUSIONS

Isoflucypram was slowly degraded in soil under aerobic conditions in the dark in the laboratory. The calculated best fit half-lives were 223 and 714 days in the tested soils.

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Report: KCA 7.1.2.1.1/03; [redacted]; [redacted]; 2017; M-599926-01-1
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 / 835.4200; Japanese MAFF Test Guidelines 12 Nousan 8147, No. 2-5-2
Guideline deviation(s): none
GLP/GEP: **yes**

Executive Summary

The degradation data of isoflucypram as reported in study [redacted]; [redacted]; 2017; M-599926-01-1 in section 7.1.1.1 (page 35) were kinetically evaluated.
The experimental data could be best described by a single first order (SFO) kinetic model. The DT_{50} value of isoflucypram under aerobic conditions was 263 days in the tested soil (Table 7.1.2.1.1- 5).

Table 7.1.2.1.1- 5: Degradation kinetics of isoflucypram in soil under aerobic conditions

Soil	Texture (USDA)	Best fit model[a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error	Visual assessment[b)]
Laacher Hof AXXa	loamy sand	SFO	263	873	2.7	o

a) SFO: single first order
b) Visual assessment: o = moderate

I. MATERIALS AND METHODS

Details on the study conduct and its results are summarised under KCA 7.1.1.1/03, page 35.

The data for the test item were evaluated according to FOCUS kinetics (2006)[1] using the software KinGUI 2. Model input datasets were the residual amounts found in each replicate test system at each sampling interval. The initial total recovery (material balance) at DAT-0 was included in the parameter optimization procedure, but for optimal goodness of fit, the value was allowed to be estimated by the model. DT_{50} and DT_{90} values were calculated from the resulting kinetic parameters.

II. RESULTS AND DISCUSSION

The degradation of isoflucypram followed single first order (SFO) kinetics in all soils according to the lowest chi² error values and visual assessments.
A summary of all kinetic data is given in Table 7.1.2.1.1- 6, the best fit is highlighted in bold letters there. The DT_{50} value of isoflucypram under aerobic conditions was 263 days in the tested soil.

Table 7.1.2.1.1- 6: Summary of the kinetic evaluation (for trigger values according to FOCUS) of the degradation of isoflucypram in soil under aerobic conditions
(best fits are highlighted in **bold** letters)

Soil (Texture (USDA)	Kinetic model[a)]	DT_{50} [days]	DT_{90} [days]	Chi² error [%]	Visual assessment[b)]
Laacher Hof AXXa (loamy sand)	**SFO**	**263**	**873**	**2.7**	**o**
	FOMC	425	> 1000	2.8	o
	DFOP	> 1000	1000	2.9	o

a) SFO: Single first order, FOMC: First order multi compartment, DFOP: Double first order in parallel
b) Visual assessment: + = good, o = moderate, - = poor

III. CONCLUSIONS

Isoflucypram was slowly degraded in soil under aerobic conditions in the dark in the laboratory. The calculated best fit DT_{50} value was 263 days in the tested soil.

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[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.

- **Kinetic evaluation of the aerobic soil metabolism studies**

Report:	KCA 7.1.2.1.1/04; [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Title:	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under aerobic laboratory conditions
Report No.:	EnSa-17-0102
Document No.:	M-608255-01-1
Guideline(s):	not applicable
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive Summary

The route and rate of degradation of isoflucypram under laboratory aerobic conditions was investigated in three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1). In the current report, a kinetic analysis of residue data of isoflucypram was conducted in order to derive kinetic parameters suitable to trigger additional studies (trigger endpoints) and for modelling and environmental risk assessments (modelling endpoints).

The model fit as well as the statistical evaluation of the results were carried out with the in-house developed software KinGUI, version 2.1. The selection of the most appropriate kinetic model was based on a detailed statistical analysis including visual assessment, χ^2-err statistics, randomness of residuals, and t-test significance following the FOCUS guidance (2006[1], 2014[2]).

The resulting DT_{50} values (i.e. trigger and modelling endpoints) of isoflucypram are given in Table 7.1.2.1.1- 7 and Table 7.1.2.1.1- 8.

The parts concerning the major degradation product BCS-CN88460-carboxylic acid *(M12)* are reported in section CA 7.1.2.1.2 of this document.

[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.

Table 7.1.2.1.1- 7: Soil degradation not-normalised DT_{50} values of isoflucypram to trigger additional studies (trigger endpoints)

Report	Soil origin	Soil type	pH[a)]	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	St. (χ^2err) [%]	Method of calculation
[redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	5.7	20	53.1	438	> 1000	0.89	SFO
	Laacher Hof AXXa, Germany	loamy sand	6.3	20	53.1	236	782	1.00	SFO
	Hoefchen am Hohenseh, Germany	silt loam	6.6	20	53.1	348	> 1000	1.16	SFO
	Dollendorf II, Germany[b)]	loam	7.4	20	53.1	277	921	1.24	SFO
[redacted]; 2017; M-588260-01-1	Sanger, CA, USA[c)]	sandy loam	6.3	20.4	64.9[e)]	630	> 1000	0.68	SFO
	Louisville, NE, USA[d)]	silty clay loam	6.3	20.4	70.0[e)]	224	743	2.30	SFO
[redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	20	55.0	263	873	2.74	SFO
Geometric mean:						**323**	**897**		

a) Measured in $CaCl_2$
b) From modified pathway fit excluding the residue data of DAT 104
c) From modified parent only fit excluding the residue data of DAT 0
d) From initial pathway fit including all residue data
e) Calculated based on study moisture and MWHC

Table 7.1.2.1.1- 8: Soil degradation not-normalised and normalised (to pF2 and 20°C, Q_{10}: 2.58) DT_{50} values of isoflucypram for modelling purpose (modelling endpoints)

Report	Soil origin	Soil type	pH[a)]	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	DT_{50} [days] 20°C, pF2/ 10 kPa[f)]	St. (χ^2err) [%]	Method of calc.
[redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	5.7	20	53.1	438	> 1000	438	0.89	SFO
	Laacher Hof AXXa, Germany	loamy sand	6.3	20	53.1	236	782	236	1.00	SFO
	Hoefchen am Hohenseh, Germany	silt loam	6.6	20	53.1	348	> 1000	348	1.16	SFO
	Dollendorf II, Germany[b)]	loam	7.4	20	53.1	277	921	277	1.24	SFO
[redacted]; 2017; M-588260-01-1	Sanger, CA, USA[c)]	sandy loam	6.3	20.4	64.9[e)]	620	> 1000	570	0.68	SFO
	Louisville, NE, USA[d)]	silty clay loam	6.1	20.4	70.0[e)]	224	743	200	2.30	SFO
[redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	20	55.0	263	873	263	2.74	SFO
Geometric mean:						**323**	**897**	**314**		

a) Measured in $CaCl_2$
b) From modified pathway for excluding the residue data of DAT 104
c) From modified parent only fit excluding the residue data of DAT 0
d) From initial pathway fit including all residue data
e) Calculated based on study moisture and MWHC
f) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7

I. METHODS

The objective of the work reported here was a kinetic evaluation of the degradation behaviour of isoflucypram in European and US soils under laboratory conditions 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).

The kinetic parameters which lead to the appropriate fit of measured to calculated values were identified based on a mathematical optimisation algorithm and statistical analysis. The modelling analysis was based on residue data from three studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1) covering different soil types. The model fit as well as the statistical evaluation of the results were carried out with the in-house developed software KinGUI, version 2.1.

The soil metabolite BCS-CN88460-carboxylic acid *(M12)* was found at relevant amounts (> 5%) in the soils Laacher Hof AXXa and Dollendorf II in [redacted]; [redacted]; 2014; M-486690-01-1, in the soil Louisville, NE in [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1,

as well as in the soil Laacher Hof in [redacted]; [redacted]; 2017; M-599926-01-1. Because residue data of BCS-CN88460-carboxylic acid was at sufficient data points above the limit of detection (LOD) in all soils in [redacted]; [redacted]; 2014; M-486690-01-1, the soil Louisville, NE ([redacted]; [redacted]; [redacted]; 2017; M-588260-01-1), and the soil Laacher Hof AXXa in [redacted]; [redacted]; 2017; M-599926-01-1, degradation of BCS-CN88460-carboxylic acid was also included into the pathway. In the soil Sanger, CA ([redacted]; [redacted]; [redacted]; 2017; M-588260-01-1), BCS-CN88460-carboxylic acid was found in concentrations above LOD only in two measurements; consequently, for this soil a parent only fit was conducted. Further metabolites were only observed in minor amounts (< 5%) and were therefore not included in the kinetic evaluation.

The degradation behaviour of isoflucypram was investigated in several soils under laboratory aerobic conditions in the dark. The compound was applied on soil at an application rate corresponding to 75 g/ha. In Table 7.1.2.1.1- 9 the main parameters of soils and study conditions are summarised.

Table 7.1.2.1.1- 9: Important characteristics of aerobic laboratory soil degradation studies with isoflucypram

Report	Soil origin	Soil type (USDA)	pH[a)]	Organic carbon [%]	Temp. [°C]	MWHC [%]
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	5.7	2.9	20	53.1
	Laacher Hof AXXa, Germany	loamy sand	6.3	2.0	20	53.1
	Hoefchen am Hohenseh, Germany	silt loam	6.6	1.9	20	53.1
	Dollendorf II, Germany	loam	7.4	5.2	20	53.1
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA, USA	sandy loam	6.3	0.77	20.4	64.9[b)]
	Louisville, NE, USA	silty clay loam	6.3	2.00	20.4	70.0[b)]
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	1.6	20	55.0

a) in $CaCl_2$
b) Calculated based on study moisture and MWHC

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 estimation of trigger endpoints the SFO model and the FOMC model are calculated. If the SFO model leads to an overall (visually and statistically) better fit, the SFO model is selected. If not the DFOP and HS models are calculated. The bi-phasic models are compared (visually and statistically) and the one model resulting in the better fit is chosen.
The aim of trigger endpoints is to obtain the curve that best interpolates the observed behaviour, and not to generate parameters for further calculations. Therefore, no assessment of parameter significance is performed, unless endpoints are extrapolated.

For modelling purposes the preferred model is the single first-order decay. If the SFO fit to parent data is visually acceptable and the χ^2err does not significantly exceed 15%, the SFO fit and parameters are

accepted.
If χ^2err is still significantly greater than 15%, then other models may be tested and/or model parameters may be fixed based on available information (*e.g.*, initial amount) on a case by case basis. The model with the smaller χ^2err is finally chosen as the appropriate model. But the 15% threshold value for the scaled error should not be employed as absolute cut-off criteria as this value is strictly appropriate only for optimal experimental conditions. There can be cases where the error value to pass the χ^2err test is higher than 15% but the model fit still represents a reasonable description of the degradation behaviour. However, the standard biphasic models recommended by FOCUS viz., DFOP and HS are recommended, if residues remain above 10% of the initial concentration. Then, a very conservative equivalent single first-order half-life can be calculated from the lower of the two kinetic rates. By this method the equivalent SFO-curve always overpredicts the residues. FOMC model is only recommended, if residues reach less than 10% of the initial concentration. Then, an equivalent single first-order half-life is recalculated, based on DT_{90FOMC} /3.32. By this method the equivalent SFO-curve meets the FOMC-curve at the time when DT_{90} is reached and consequently overpredicts the residues before.
It should be noted, however, that these corrected DT_{50} values can only be used to simulate the leaching of a parent compound alone, and they must not be used to simulate the fate of the parent and a metabolite in a linked model run, as such an approach does not assure worst-case situation for metabolites. In such cases, if the SFO model is not appropriate for the parent, the parent should be fitted with an appropriate bi-phasic model which may be implemented in environmental models (FOCUS 2014)

The DT_{50} values were normalised to the soil moisture corresponding to field capacity and a temperature of 20°C and Q_{10} = 2.58. Temperature and moisture correction factors calculated for all the soils are given in Table 7.1.2.1.1- 10.

Table 7.1.2.1.1- 10: Normalisation correction factors for the soils used in the kinetic evaluation. "FOCUS field capacity, θ_{ref}" denotes the default values for reference soil moisture as given in FOCUS (2014)

Report	Soil origin	Soil type	Study moist. MWHC [%]	Study soil MWHC g/100 g]	Study moist, θ [g/100 g]	Study temp. [°C]	FOCUS field capacity, θ_{ref} [g/100 g]	f_θ	f_T	$f_\theta \cdot f_T$
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	53.1	63.0	33.5	20	29.3	1.00	1.00	1.00
	Laacher Hof AXXa, Germany	loamy sand	53.1	50.3	26.7	20	15.8	1.00	1.00	1.00
	Hoefchen am Hohenseh, Germany	silt loam	53.1	56.1	29.8	20	31.7	1.00	1.00	1.00
	Dollendorf II, Germany	loam	53.1	84.5	44.9	20	43.1	1.00	1.00	1.00
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA, USA	sandy loam	64.9[a)]	29.1	18.9	20.4	21.8	0.90	1.00	0.90
	Louisville, NE, USA	silty clay loam	70.0[a)]	52.4	36.7	20.4	43.0	0.90	1.00	0.90
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	55.0	49.6	27.3	20	17.7	1.00	1.00	1.00

a) Calculated based on study moisture and MWHC

II. RESULTS AND DISCUSSION

Results of the kinetic evaluation of three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1) including isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* are given below. In the current study, kinetic parameters were derived for trigger and modelling endpoints based on the pathway fit. For the evaluation of trigger endpoints, the comparison of the pathway fits using the SFO and FOMC models for the parent isoflucypram is provided for all soils among the studies considered here.
The degradation rates were evaluated for the corresponding study conditions. For the soil Sanger, CA, a parent only fit was conducted as the metabolite BCS-CN88460-carboxylic acid was only found at concentrations above LOD at two time points.

Study [redacted] and [redacted] (2014):
For derivation of trigger endpoints, the kinetic evaluation was started by comparing results of the SFO and FOMC models for isoflucypram included in the pathway fit. For the soils Hanscheider Hof, Laacher Hof AXXa and Hoefchen am Hohenseh the SFO model resulted in a similar fit compared to the FOMC model (Table 7.1.2.1.1- 11) and was therefore chosen for derivation of trigger endpoints.

For derivation of modelling endpoints, the kinetic evaluation was started by assuming a single first-order (SFO) degradation. For residue data of the soils Hanscheider Hof, Laacher Hof AXXa and Hoefchen am Hohenseh the SFO fit was deemed acceptable as the residuals were well distributed and the error value of the χ^2err test was very low (Table 7.1.2.1.1- 11).
Inspection of parent and metabolite residue data of the soil Dollendorf II indicates an irregular behaviour at DAT 104. Parent residues at DAT 104 tend to be too low whereas residues of

BCS-CN88460-carboxylic acid are significantly too high (approximately factor 2 higher compared to determined residues at DAT 84 and DAT 120). This could be caused by an error in the analytical procedure. Thus, the kinetic evaluations for modelling as well as trigger endpoints have been conducted in 2 steps, considering all data and excluding DAT 104 residues, respectively (Figure 7.1.2.1.1- 1 and Figure 7.1.2.1.1- 2).
As the fit was clearly improved by the exclusion of the DAT 104 residue data, kinetic parameters derived from the SFO pathway fit (Dollendorf II: modified pathway fit) were selected as trigger and modelling endpoints and are presented in Table 7.1.2.1.1- 11.

Table 7.1.2.1.1- 11: Isoflucypram: kinetic and statistical results of the SFO and FOMC curve fits (pathway fit) best fits highlighted in **bold letters**

Soil	Kinetic model	DT_{50} [days]	DT_{90} [days]	VA	χ^2_{err} [%]	k_1 / α [1/d / -]	k_2 / β [1/d / -]	g	t-test k_1 / k_2	MS
Hanscheider Hof	**SFO**	**438**	> 1000	+	0.89	0.001583			< 0.001	**T/M**
	FOMC	440	> 1000	+	0.93	57.94	36540			
Laacher Hof AXXa	**SFO**	**236**	782	+	1.00	0.002943			< 0.001	**T/M**
	FOMC	310	> 1000	+	0.92	0.7637	209.6			
Höfchen am Hohenseh	**SFO**	**348**	> 1000	+	1.16	0.001993			< 0.001	**T/M**
	FOMC	348	> 1000	+	1.22	2011	100900			
Dollendorf II	SFO[a)]	255	848	+	1.73	0.002715			< 0.001	
	FOMC[a)]	307	> 1000	+	1.79	1.167	378.2			
	SFO[b)]	**277**	921	+	1.24	0.002501			< 0.001	**T/M**
	FOMC[b)]	463	> 1000	+	1.14	0.4861	146.5			

a) Initial fit including all residue data
b) Modified fit excluding residue data for DAT 104
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

Figure 7.1.2.1.1- 1: KinGUI results for isoflucypram in soil Dollendorf II from the SFO pathway fit – initial fit

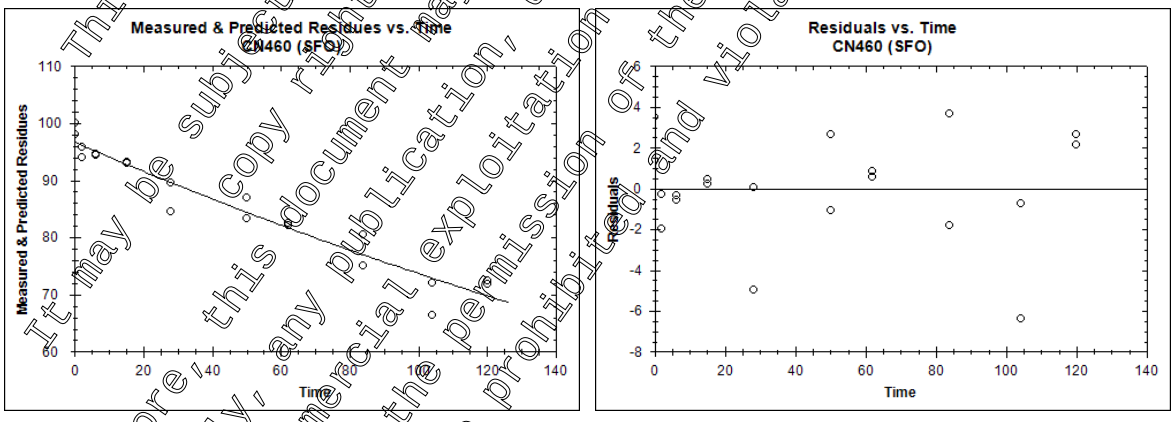
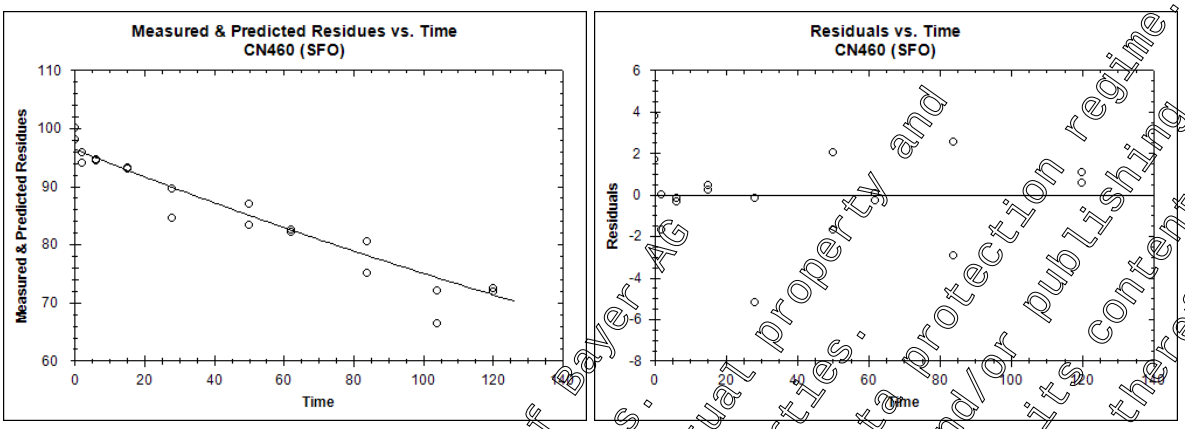


Figure 7.1.2.1.1- 2: KinGUI results for isoflucypram in soil Dollendorf II from the SFO pathway fit – modified fit



Study [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1:
For derivation of trigger and modelling endpoints, all residue data were included in a first step while in a modified approach residue data at DAT 0 were excluded from the fit (both replicates for Sanger, CA, second replicate only for Louisville, NE). As the metabolite BCS-CN88460-carboxylic acid was only found at two time points in concentrations above the LOD, the metabolite was not included in the assessment and a parent only fit was conducted for soil Sanger, CA.

For derivation of trigger endpoints, the kinetic evaluation was started by comparing results of the SFO and FOMC models for isoflucypram included in the parent only fit (Sanger, CA) or the pathway fit (Louisville, NE). For all investigated soils the SFO model resulted in a similar fit compared to the FOMC model (Table 7.1.2.1.1- 12).

For derivation of modelling endpoints, the kinetic evaluation was started by assuming a single first-order (SFO) degradation. For residue data of all soils the SFO fit was deemed acceptable as the residuals were well distributed and the error value of the χ^2err test was very low (Table 7.1.2.1.1- 12).

For the soil Sanger, CA, the fit was clearly improved by excluding the residue data for DAT 0, visually as well as statistically (compare Figure 7.1.2.1.1- 3 and Figure 7.1.2.1.1- 4, Table 7.1.2.1.1- 12). Therefore, kinetic parameters from the modified SFO parent only fit were selected as trigger and modelling endpoints.
For the soil Louisville, NE, the exclusion of the DAT 0 residue date did not result in a significant improvement of the fit (compare Figure 7.1.2.1.1- 5 and Figure 7.1.2.1.1- 6, Table 7.1.2.1.1- 12). Therefore, in this case the kinetic parameters from the initial SFO pathway fit were selected as trigger and modelling endpoints.

Table 7.1.2.1.1- 12: Isoflucypram: kinetic and statistical results of the SFO and FOMC curve fits
best fits highlighted in **bold letters**

Soil	Kinetic model	DT_{50} [days]	DT_{90} [days]	VA	χ^2err [%]	k_1 / α [1/d / -]	k_2 / β [1/d / -]	g	t-test k_1 / k_2	MS
Sanger, CA	SFO[a),b)]	709	> 1000	o	1.11	0.0009781			< 0.001	
	FOMC[a),b)]	709	> 1000	o	1.18	2392	2446000			
	SFO[a),c)]	**630**	> 1000	o	0.68	0.00110			< 0.001	**T/M**
	FOMC[a)c,)]	> 1000	> 1000	o	0.71	0.3337	244.4			
Louisville, NE	**SFO[b)]**	**224**	743	o	2.30	0.003099			< 0.001	**T/M**
	FOMC[b)]	224	744	o	2.45	1245	401600			
	SFO[c)]	215	713	o	2.02	0.00323			< 0.001	
	FOMC[c)]	215	713	o	2.16	1.1420	3536000			

a) Parent only fit
b) Initial fit including all residue data
c) Modified fit excluding residue data for DAT 0
MS: Model selected (T: for trigger evaluation; M: for modeling evaluation)

Figure 7.1.2.1.1- 3: KinGUI results for isoflucypram in soil Sanger, CA from the SFO parent only fit – initial fit

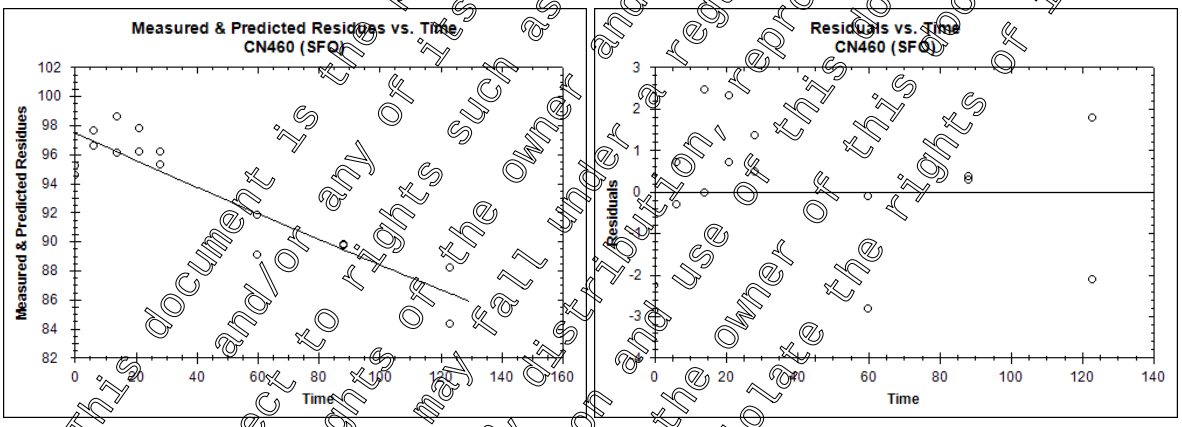


Figure 7.1.2.1.1- 4: KinGUI results for isoflucypram in soil Sanger, CA from the SFO parent only fit – modified fit

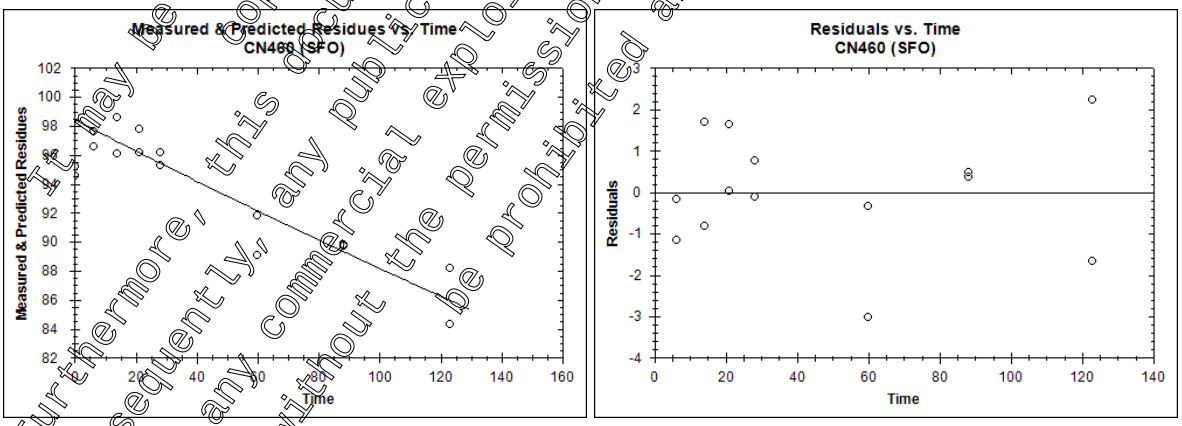


Figure 7.1.2.1.1- 5: KinGUI results for isoflucypram in soil Louisville, NE from the SFO pathway fit – initial fit

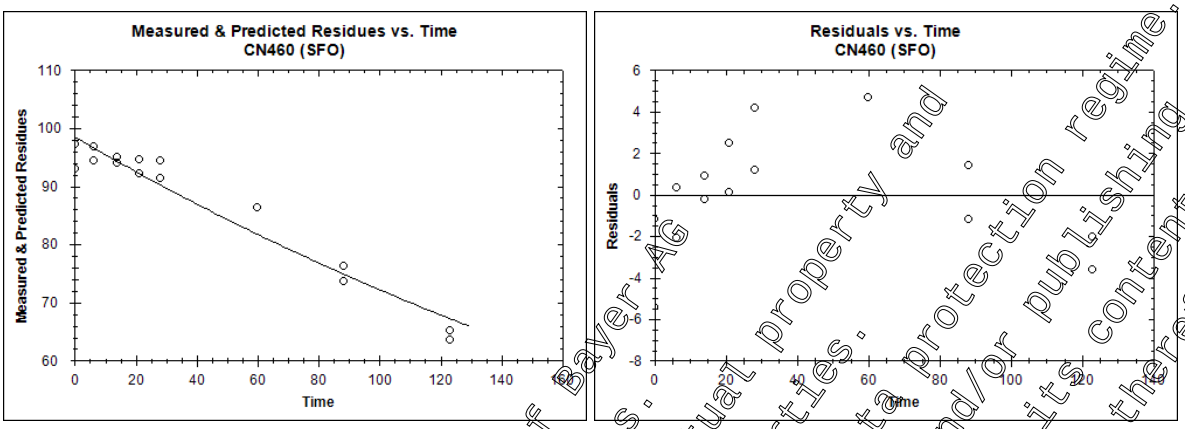
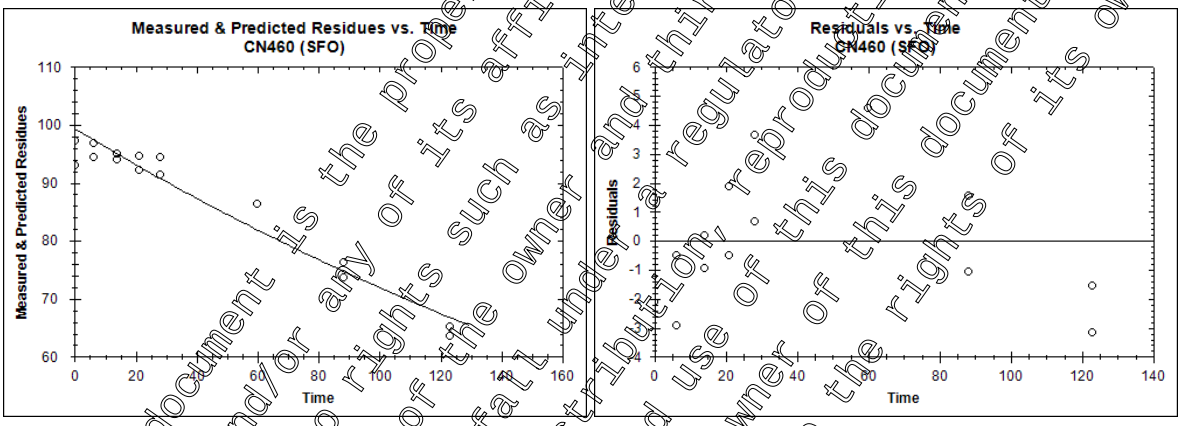


Figure 7.1.2.1.1- 6: KinGUI results for isoflucypram in soil Louisville, NE from the SFO pathway fit – modified fit



Study [redacted]; [redacted]; 2017; M-599926-01-1:

For derivation of trigger endpoints, the kinetic evaluation was started by comparing results of the SFO and FOMC models for isoflucypram included in the pathway fit. For the soil Laacher Hof AXXa the SFO model resulted in a similar fit compared to the FOMC model (Table 7.1.2.1.1- 13).

For derivation of modelling endpoints, the kinetic evaluation was started by assuming a single first-order (SFO) degradation. For residue data of the soil Laacher Hof AXXa the SFO fit was deemed acceptable as the residuals were well distributed and the error value of the χ^2err test was low (Figure 7.1.2.1.1- 7, Table 7.1.2.1.1- 13).

However, the fits might have been deteriorated by the residue data measured at DAT 85 where concentrations are exceptionally low for the parent substance, while concentrations of the metabolite are increased. If these data are removed from the fit the χ^2err decreases, but no improvement of the visual assessment or the statistical significance of the t-test is observed (Figure 7.1.2.1.1- 8, Table 7.1.2.1.1- 13).

Consequently, the degradation parameters derived from the initial SFO fit including all residue data was chosen for derivation of trigger and modelling endpoints (Table 7.1.2.1.1- 13).

Table 7.1.2.1.1- 13: Isoflucypram: kinetic and statistical results of the SFO and FOMC curve fits (pathway fit) best fits highlighted in **bold letters**

Soil	Kinetic model	DT_{50} [days]	DT_{90} [days]	VA	χ^2err [%]	k_1 / α [1/d / -]	k_2 / β [1/d / -]	g	t-test k_1 / k_2	MS
Laacher Hof AXXa	**SFO[a)]**	**263**	873	+	2.74	0.002639			< 0.001	T/M
	FOMC[a)]	397	> 1000	+	2.76	0.5611	162.7			
	SFO[b)]	295	981	+	0.98	0.002347			0.001	
	FOMC[b)]	403	> 1000	+	0.96	0.7691	274.9			

a) Initial fit including all residue data
b) Modified fit excluding residue data for DAT 85
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

Figure 7.1.2.1.1- 7: KinGUI results for isoflucypram in soil Laacher Hof AXXa from the SFO pathway fit – initial fit

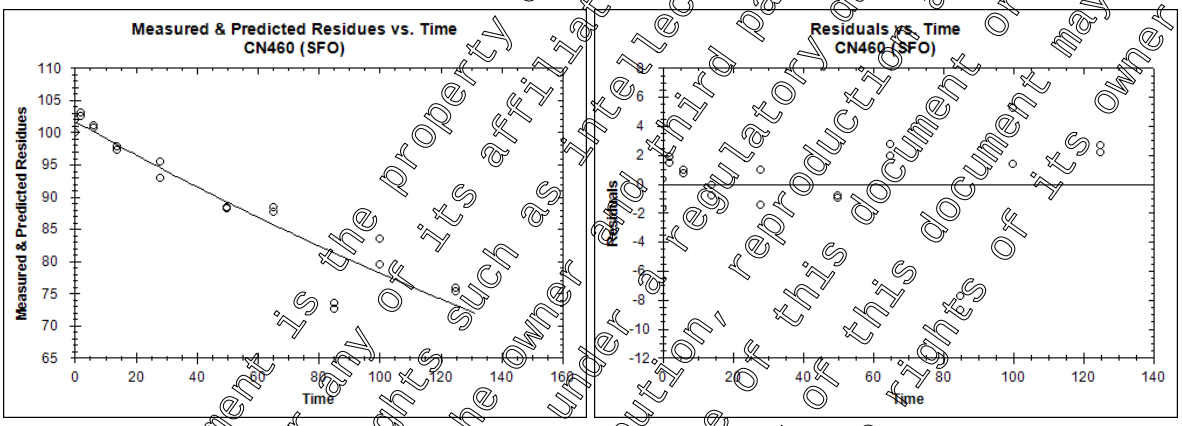
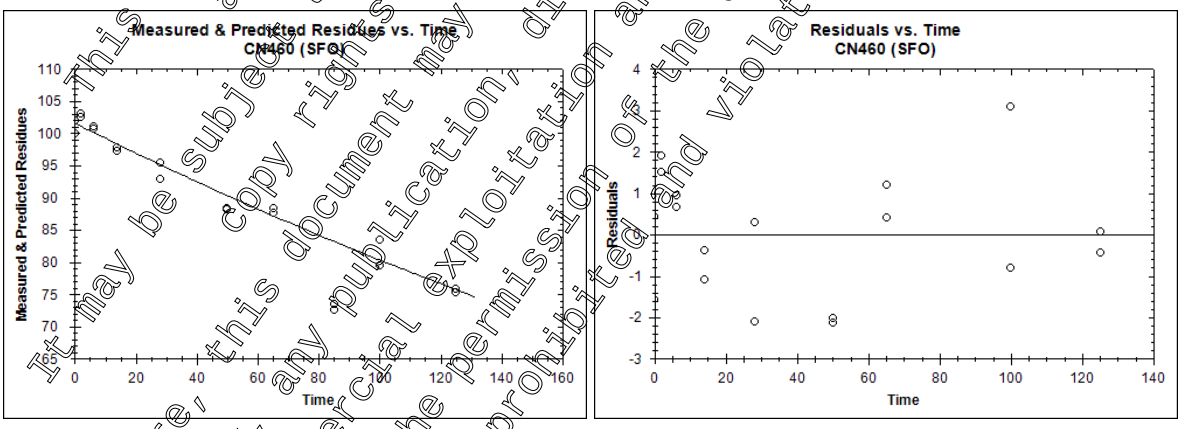


Figure 7.1.2.1.1- 8: KinGUI results for isoflucypram in soil Laacher Hof AXXa from the SFO pathway fit – modified fit



None of the bi-phasic evaluations did improve significantly the quality of the fit (neither statistically or visually).

III. CONCLUSIONS

The trigger and modelling endpoints for isoflucypram are presented in Table 7.1.2.1.1- 14 and Table 7.1.2.1.1- 15, respectively.

Table 7.1.2.1.1- 14: Soil degradation not-normalised DT_{50} values of isoflucypram to trigger additional studies (trigger endpoints)

Study	Soil	Model fitted	DT_{50} not normalised [days]
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof	SFO	438
	Laacher Hof AXXa	SFO	236
	Hoefchen am Hohensch	SFO	348
	Dollendorf II	SFO	277
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA	SFO	630
	Louisville, NE	SFO	224
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa	SFO	263
Geometric mean:			**323**

Table 7.1.2.1.1- 15: Soil degradation not-normalised and normalised (to pF2 and 20°C, Q_{10}: 2.58) DT_{50} values of isoflucypram for modelling purposes (modelling endpoints)

Study	Soil	Model fitted	DT_{50} not normalised [days]	DT_{50} normalised [days]
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof	SFO	438	438
	Laacher Hof AXXa	SFO	236	236
	Hoefchen am Hohensch	SFO	348	348
	Dollendorf II	SFO	277	277
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA	SFO	630	570
	Louisville, NE	SFO	224	200
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa	SFO	263	263
Geometric mean:			**323**	**314**

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

BCS-CN88460-carboxylic acid *(M12)* was found as major soil metabolite in a soil metabolism studies with isoflucypram under aerobic conditions.
A summary of the degradation rates of isoflucypram and its major degradation product in soil in the laboratory is given in section CA 7.1.2.1.

- **Kinetic evaluation of the aerobic soil metabolism studies**

Report:	KCA 7.1.2.1.2/01; [redacted], G.; [redacted], W.; 2017; M-608255-01-1
Title:	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under aerobic laboratory conditions
Report No.:	EnSa-17-0102
Document No.:	M-608255-01-1
Guideline(s):	not applicable
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive Summary

The route and rate of degradation of isoflucypram under laboratory aerobic conditions was investigated in three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1). In the current report, a kinetic analysis of residue data was conducted in order to derive kinetic parameters suitable to trigger additional studies (trigger endpoints) and for modelling and environmental risk assessments (modelling endpoints) for the major metabolite BCS-CN88460-carboxylic acid *(M12)*.

The metabolite BCS-CN88460-carboxylic acid was found at relevant amounts (> 5%) in the soils Laacher Hof AXXa and Dollendorf II in [redacted]; [redacted]; 2014; M-486690-01-1, in the soil Louisville, NE in [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1, as well as in the soil Laacher Hof AXXa in [redacted]; [redacted]; 2017; M-599926-01-1. Because residue data of BCS-CN88460-carboxylic acid was at sufficient data points above the limit of detection (LOD) in all soils in [redacted]; [redacted]; 2014; M-486690-01-1, the soil Louisville, NE ([redacted]; [redacted]; [redacted]; 2017; M-588260-01-1), and the soil Laacher Hof AXXa in [redacted]; [redacted]; 2017; M-599926-01-1, degradation of BCS CN88460-carboxylic acid was also included into the pathway. In the soil Sanger, CA ([redacted]; [redacted]; [redacted]; 2017; M-588260-01-1), BCS-CN88460-carboxylic acid was found in concentrations above LOD only in two measurements; consequently, for this soil a parent only fit was conducted. Further metabolites were only observed in minor amounts (< 5%) and were therefore not included in the kinetic evaluation.

The model fit as well as the statistical evaluation of the results were carried out with the in-house developed software KinGUI, version 2.1. The selection of the most appropriate kinetic model was based on a detailed statistical analysis including visual assessment, χ^2err statistics, randomness of residuals, and t-test significance following the FOCUS guidance (2006, 2014).

The resulting DT_{50} values (i.e., modelling and trigger endpoints) of BCS-CN88460-carboxylic acid are given in Table 7.1.2.1.2- 1 and Table 7.1.2.1.2- 2.

Table 7.1.2.1.2- 1: Soil degradation not-normalised DT_{50} values of BCS-CN88460-carboxylic acid *(M12)* to trigger additional studies (trigger endpoints)

Report	Soil origin	Soil type	pH[a)]	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	f.f.	St. (χ^2err) [%]	Method of calc.
[redacted]; 2014; M-486690-01-1	Hanscheider Hof, Germany	loam	5.7	20	53.1	45.4	151	0.330	7.08	SFO
	Laacher Hof AXXa, Germany	loamy sand	6.3	20	53.1	113	377	0.257	5.27	SFO
	Hoefchen am Hohenseh, Germany	silt loam	6.6	20	53.1	114.8	749.1	0.440	8.70	SFO
	Dollendorf II, Germany[b)]	loam	7.4	20	53.1	18.4	61.2	0.500	8.55	SFO
[redacted]; 2017; M-588260-01-1	Sanger, CA, USA	sandy loam	6.3	20.4	64.9[c)]	-[d)]	-[d)]	-[d)]	-[d)]	SFO
	Louisville, NE, USA	silty clay loam	6.3	20.4	70.0	n.r.	n.r.	0.286	20.01	SFO
[redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	20	55.0	n.r.	n.r.	0.255	16.05	SFO
Geometric mean:						**34.4**	**114**			
Arithmetic mean:								**0.345**		

n.r. = not reliable
a) Measured in $CaCl_2$
b) From modified pathway fit excluding the residue data of DAT 104
c) Calculated based on study moisture and MWHC
d) Only two metabolite detect above LOD, only parent only fit conducted

Table 7.1.2.1.2- 2: Soil degradation not-normalised and normalised (to pF2 and 20°C, Q_{10}: 2.58) DT_{50} values of BCS-CN88460-carboxylic acid *(M12)* for modelling purpose (modelling endpoints)

Report	Soil origin	Soil type	pH[a)]	Temp. [°C]	MWHC [%]	DT_{50} [days]	DT_{90} [days]	f.f.	DT_{50} [days] 20°C, pF2/ 10 kPa e)	St. (χ^2err) [%]	Method of calc.
[redacted]; 2014; M-486690-01-1	Han-scheider Hof, Germany	loam	5.7	20	53.1	45.4	151	0.330	45.4	7.08	SFO
	Laacher Hof AXXa, Germany	loamy sand	6.3	20	53.1	113	377	0.257	113	5.27	SFO
	Hoefchen am Hohenseh, Germany	silt loam	6.6	20	53.1	148	491	0.440	148	8.70	SFO
	Dollendorf II, Germany[b)]	loam	7.4	20	53.1	18.4	61.2	0.500	18.4	8.55	SFO
[redacted]; 2017; M-588260-01-1	Sanger, CA, USA	sandy loam	6.3	20.4	64.9[c)]	-[d)]	-[d)]	-[d)]	-[d)]	-[d)]	SFO
	Louisville, NE, USA	silty clay loam	6.3	20.4	70.0[c)]	n.r.	n.r.	0.286	n.r.	20.01	SFO
[redacted]; 2017; M-599926-01-1	Laacher Hof AXXa, Germany	sandy loam	5.8	20	55.0	n.r.	n.r.	0.255	n.r.	16.05	SFO
Geometric mean:						**34.4**	**114**		**34.4**		
Arithmetic mean:								**0.345**			

n.r. = not reliable
a) Measured in $CaCl_2$
b) From modified pathway fit excluding the residue data of DAT 104
c) Calculated based on study moisture and MWHC
d) Metabolite only found in two samples – parent only fit conducted
e) Normalised using a Q_{10} of 2.58 and Walker equation 0.7

I. METHODS

Soil residue data from the aerobic soil degradation studies of isoflucypram ([redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; 2017; M-599926-01-1) were used. In these studies, the degradation of isoflucypram was studied in soil Hanscheider Hof (loam), soil Laacher Hof (loamy sand), soil Höfchen (silt loam), soil Dollendorf (loam), soil Sanger (sandy loam), soil Louisville (silty clay loam) and soil Laacher Hof (sandy loam with a test concentration of 75 g/ha.
Detailed information on the kinetic analysis is given in the corresponding chapter of the parent compound in section CA 7.1.2.1.1.

II. RESULTS AND DISCUSSION

Results of the kinetic evaluation of three different studies ([redacted]; [redacted]; 2014; M-486690-01-1; [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1; [redacted]; [redacted]; 2017; M-599926-01-1) concerning the metabolite BCS-CN88460-carboxylic acid *(M12)* are given below.

Study Hellpointner and Junge (2014):

The kinetic evaluation was started by assuming a single first-order (SFO) degradation.

For the soils Hanscheider Hof, Laacher Hof AXXa, and Hoefchen am Hohenseh, the SFO pathway fit was visually and statistically acceptable as the residuals were well distributed and the error value of the χ^2err test was low.

Inspection of parent and metabolite residue data of the soil Dollendorf II indicates an irregular behaviour at DAT 104. Parent residues at DAT 104 tend to be too low whereas residues of BCS-CN88460-carboxylic acid are significantly too high (approximately factor 2 higher compared to determined residues at DAT 84 and DAT 120). This could be caused by an error in the analytical procedure. Thus, the kinetic evaluations have been conducted in 2 steps, considering all data and excluding DAT 104 residues, respectively (Figure 7.1.2.1.2- 4 and Figure 7.1.2.1.2- 5). The modified fit resulted visually and statistically to a significant improved fit.

The degradation of the metabolite was described with the SFO model following the recommendations of FOCUS (2006, 2014). Kinetic parameters derived from the pathway fit for soils Hanscheider Hof, Laacher Hof AXXa, Hoefchen am Hohenseh, and Dollendorf II (modified fit) were selected as trigger and modelling endpoints and are presented in Table 7.1.2.1.2- 3.

The metabolite fits are for all four soils very robust and lead to a reliable description of the measured residues. Residuals are clearly randomly scattered around the zero line without any systemetic deviation. Computed formation fractions are highly significant with generally low standard deviations (Table 7.1.2.1.2- 3).

Table 7.1.2.1.2- 3: BCS-CN88460-carboxylic acid *(M12)*: kinetic and statistical results of the SFO pathway curve fits

Soil		DT_{50} [days]	DT_{90} [days]	f.f. [c)]	VA	χ^2err [%]	k_1 / α [1/d / -]	t-test k_1 / k_2	MS
Hanscheider Hof	SFO-SFO	**45.4**	151	**0.330±0.044**	+	7.08	0.015280	< 0.001	**T/M**
Laacher Hof AXXa	SFO-SFO	**113**	377	**0.257±0.022**	+	5.27	0.006114	< 0.001	**T/M**
Höfchen am Hohenseh	SFO-SFO	**14.8**	49.1	**0.440±0.081**	o	8.70	0.046870	< 0.001	**T/M**
Dollendorf II	SFO-SFO[a)]	47.9	159	0.307±0.101	o	32.13	0.014467	0.053	
	SFO-SFO[b)]	**18.4**	61.2	**0.500±0.068**	+	8.55	0.037639	< 0.001	**T/M**

a) Initial fit including all residue data
b) Modified fit excluding residue data for DAT 104
c) Kinetic formation fraction f.f. ± Standard Deviation STD
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

Figure 7.1.2.1.2- 1: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Hanscheider Hof from the SFO pathway fit – initial fit

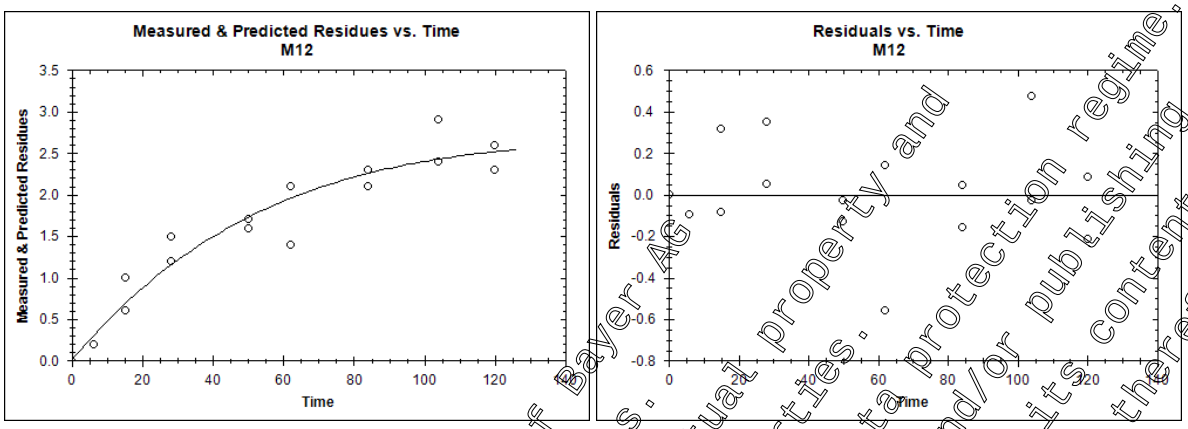


Figure 7.1.2.1.2- 2: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Laacher Hof AXXa from the SFO pathway fit – initial fit

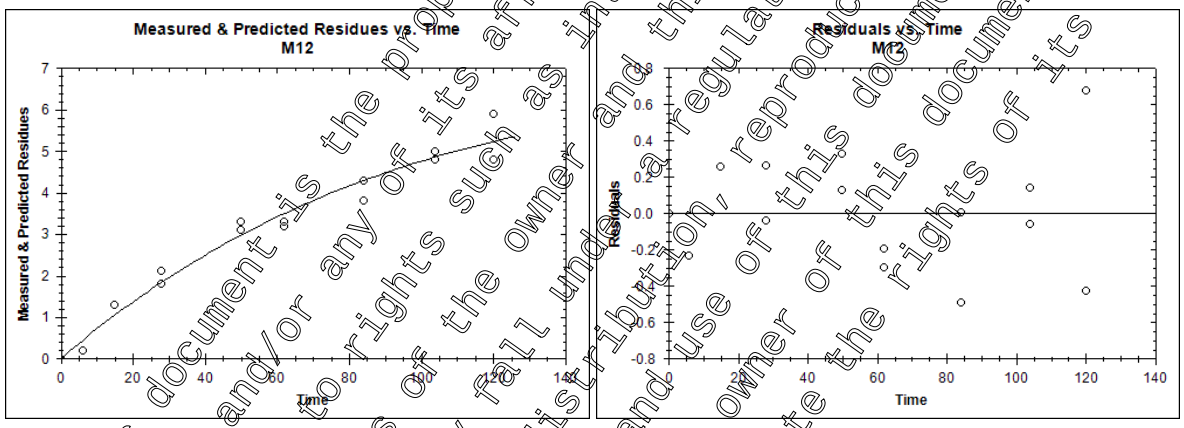


Figure 7.1.2.1.2- 3: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Hoefchen am Hohensch from the SFO pathway fit – initial fit

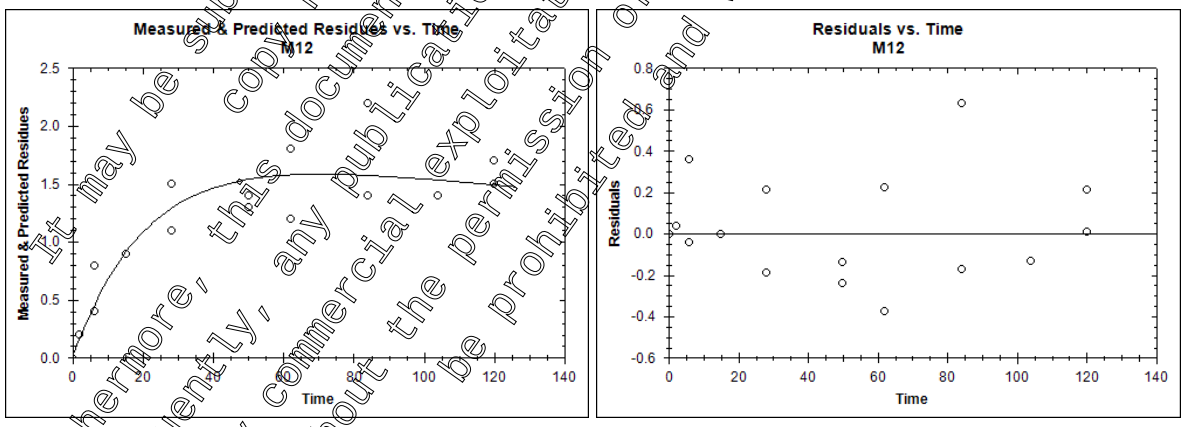


Figure 7.1.2.1.2- 4: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Dollendorf II from the SFO pathway fit – initial fit

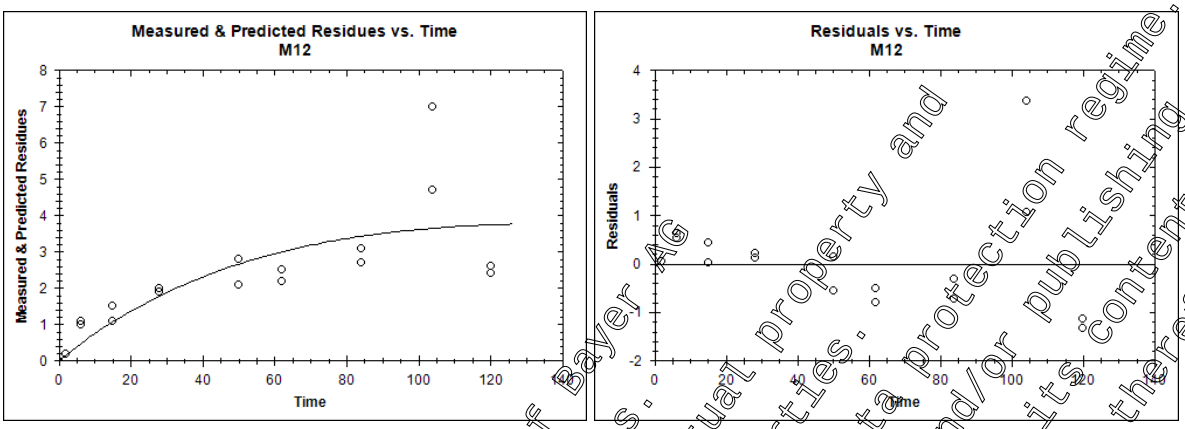
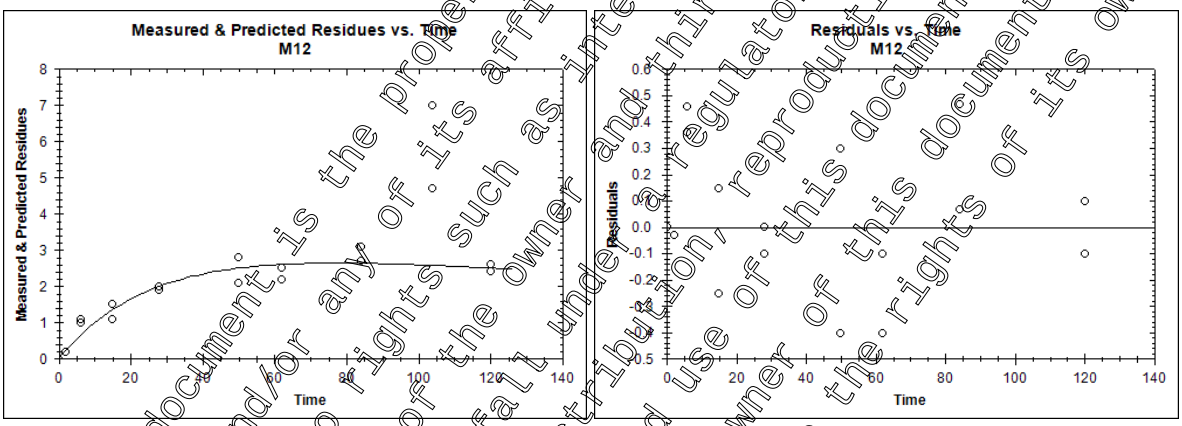


Figure 7.1.2.1.2- 5: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Dollendorf II from the SFO pathway fit – modified fit



Study [redacted]; [redacted]; [redacted]; 2017; M-588260-01-1:

For the soil Sanger, CA, the metabolite BCS-CN88460-carboxylic acid was not considered as it had only been found in concentrations above LOD at two time points.

For the soil Louisville, NE, the kinetic evaluation was started by assuming a single first-order (SFO) degradation. As for the parent substance isoflucypram, an initial fit including all residue data and a modified fit excluding the residue data from DAT 0 (2nd replicate) were conducted. In both cases, the pathway fit was visually acceptable (compare Figure 7.1.2.1.2- 6 and Figure 7.1.2.1.2- 7) but the derived rate constant was statistically not reliable (Table 7.1.2.1.2- 4). Therefore, no reliable degradation parameters could be derived and the DT_{50} was rejected for further evaluation.

The formation fraction of BCS-CN88460-carboxylic acid, however, was considered reliable as the visual fit was acceptable and the standard deviation was small. The steady increase of the metabolite residues is well described with residuals clearly randomly scattered around the zero line without any systemetic deviation. As the modified fit did not lead to clear improvements, the formation fraction from the initial fit was chosen.

Table 7.1.2.1.2- 4: BCS-CN88460-carboxylic acid *(M12)*: kinetic and statistical results of the SFO pathway curve fits

Soil		DT_{50} [days]	DT_{90} [days]	f.f. [c]	VA	χ^2err [%]	k_1 / α [1/d / -]	t-test k_1 / k_2	MS
Sanger, CA	SFO-SFO	n.a.	n.a.	n.a.		n.a.	n.a.	n.a.	
Louisville, NE	SFO-SFO[a]	> 1000	> 1000	**0.286±0.071**	o	20.01	< 0.001	0.500	
	SFO-SFO[b]	> 1000	> 1000	0.274±0.067	o	20.11	< 0.001	0.500	

a) Initial fit including all residue data
b) Modified fit excluding residue data for DAT 104
c) Kinetic formation fraction f.f. ± Standard Deviation STD
n.a. Not available (Parent only fit for soil Sanger, CA)
MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

Figure 7.1.2.1.2- 6: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Louisville, NE from the SFO pathway fit – initial fit

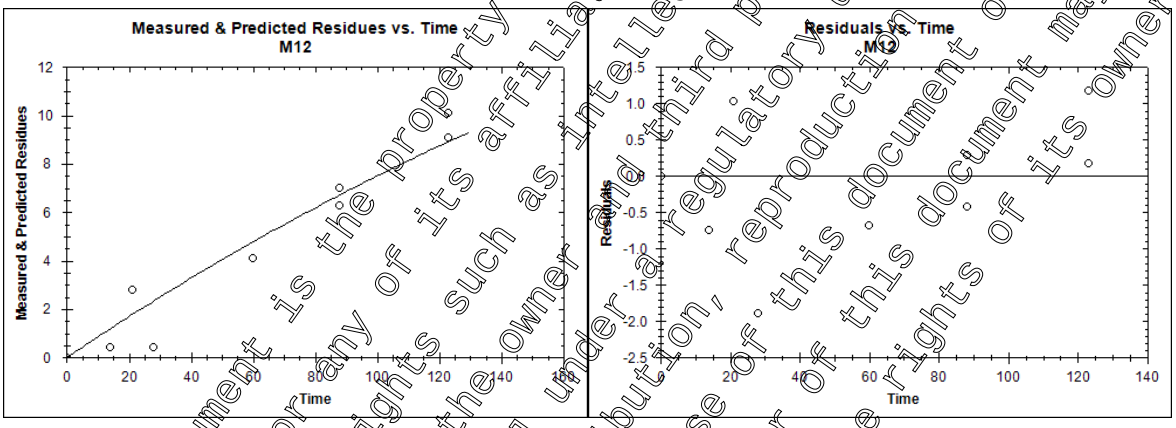
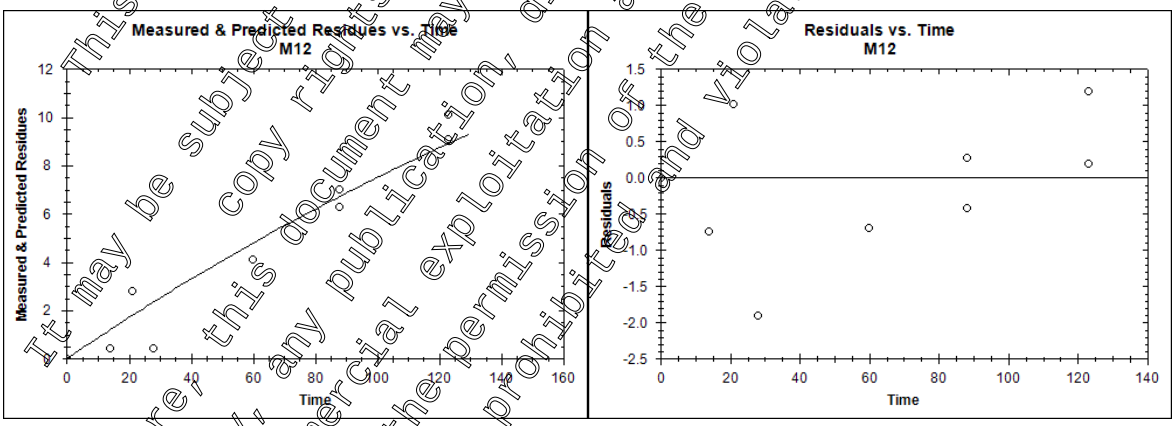


Figure 7.1.2.1.2- 7: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Louisville, NE from the SFO pathway fit – modified fit



Study [redacted]; [redacted]; 2017; M-599926-01-1:

The kinetic evaluation was started by assuming a single first-order (SFO) degradation.

For the soil Laacher Hof AXXa the SFO pathway fit was visually good with well distributed residuals (Figure 7.1.2.1.2- 8) and an error value of the χ^2err test acceptable for metabolites. However, the degradation half-life derived for BCS-CN88460-carboxylic acid is statistically not significant (t-test > 5%).

However, the fit might have been deteriorated by the residue data measured at DAT 85 where concentrations are exceptionally low for the parent substance, while concentrations of the metabolite are increased. If these data are removed from the fit the χ^2err decreases and the formation fraction is still reliable. The visual and statistical assessment, however, is not improved and the derived metabolite half-life is still not significant (Figure 7.1.2.1.2- 9, Table 7.1.2.1.2- 5).

Consequently, the DT_{50} value was rejected for further evaluation. The formation fraction of BCS-CN88460-carboxylic acid, however, was considered reliable as the visual fit was acceptable and the standard deviation was small (Table 7.1.2.1.2- 5). The steady increase of the metabolite residues is well described with residuals clearly randomly scattered around the zero line without any systemetic deviation.

Table 7.1.2.1.2- 5: BCS-CN88460-carboxylic acid *(M12)*: kinetic and statistical results of the SFO pathway curve fits

Soil		DT_{50} [days]	DT_{90} [days]	f.f. [c]	VA	χ^2err [%]	k_1 / k_{fm} [1/d] -	t-test k_1 / k_2	MS
Laacher Hof AXXa II	SFO-SFO[a]	228	759	**0.255±0.048**	+	16.05	0.003035	0.188	
	SFO-SFO[b]	486	>1000	0.243±0.034	+	12.63	0.001427	0.288	

a) Initial fit including all residue data
b) Modified fit excluding residue data for DAT 104
c) Kinetic formation fraction f.f. = Standard Deviation STD.
MS: Model selected (T for trigger evaluation; M for modelling evaluation)

Figure 7.1.2.1.2- 8: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Laacher Hof AXXa from the SFO pathway fit – initial fit

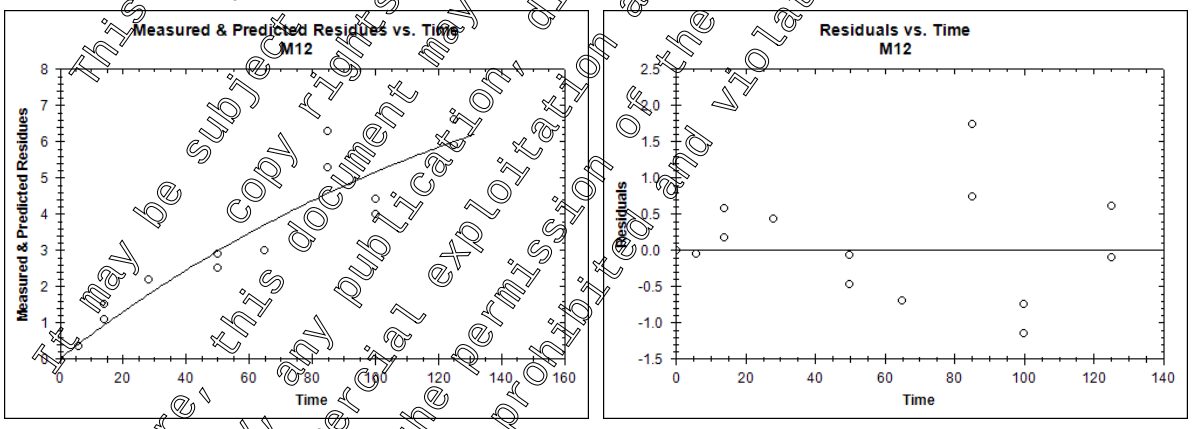
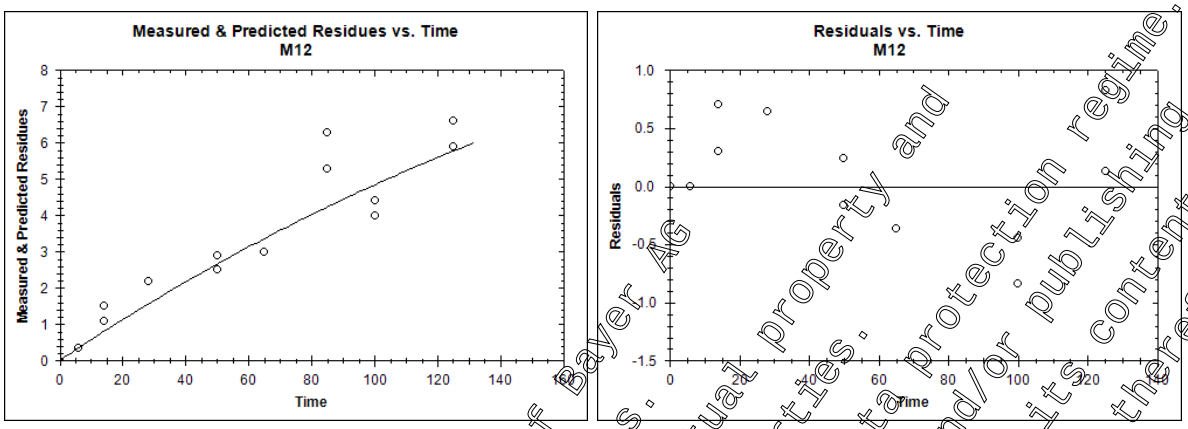


Figure 7.1.2.1.2- 9: KinGUI results for BCS-CN88460-carboxylic acid *(M12)* in soil Laacher Hof AXXa from the SFO pathway fit – modified fit



III. CONCLUSIONS

The trigger and modelling endpoints for the metabolite BCS-CN88460-carboxylic acid *(M12)* are presented in Table 7.1.2.1.2- 6 and Table 7.1.2.1.2- 7, respectively.

Table 7.1.2.1.2- 6: Soil degradation not-normalised DT_{50} values of BCS-CN88460-carboxylic acid *(M12)* to trigger additional studies (trigger endpoints)

Study	Soil	Model fitted	DT_{50} not normalised [days]
[redacted]; [redacted] 2014; M-486690-01-1	Hanscheider Hof	SFO	45.4
	Laacher Hof AXXa	SFO	113
	Hoefchen am Hohensek	SFO	14.8
	Dollendorf II	SFO	18.4
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA, USA	SFO	n.a.
	Louisville, NE, USA	SFO	n.r.
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa	SFO	n.r.
Geometric mean:			**34.4**

n.a. = Not available (only two metabolite detects above LOD, all detects below LOQ)
n.r. = Not reliable

Table 7.1.2.1.2- 7: Soil degradation not-normalised and normalised (to pF2 and 20°C, Q_{10}: 2.58) DT_{50} values (SFO fit) and formation fraction (f.f.) of BCS-CN88460-carboxylic acid *(M12)* for modelling purposes (modelling endpoints)

Study	Soil	Model fitted	DT_{50} not normalised [days]	DT_{50} normalised [days]	f.f.
[redacted]; [redacted]; 2014; M-486690-01-1	Hanscheider Hof	SFO	45.4	45.4	0.330
	Laacher Hof AXXa	SFO	113	113	0.257
	Hoefchen am Hohenseh	SFO	14.8	14.8	0.440
	Dollendorf II	SFO	18.4	18.4	0.500
[redacted]; [redacted]; [redacted]; 2017; M-588260-01-1	Sanger, CA, USA	SFO	n.a.	n.a.	n.a.
	Louisville, NE, USA	SFO	n.r.	n.r.	0.286
[redacted]; [redacted]; 2017; M-599926-01-1	Laacher Hof AXXa	SFO	n.r.	n.r.	0.255
Geometric mean:			**34.4**	**34.4**	
Arithmetic mean					**0.345**

n.a. = Not available (only two metabolite detects above LOD, all detects below LOQ)
n.r. = Not reliable

CA 7.1.2.1.3 Anaerobic degradation of the active substance

Report: KCA 7.1.2.1.3/01; [redacted]; [redacted]; 2015; M-513456-01-1
Title: [Pyrazole-4-14C]BCS-CN88460: Anaerobic degradation / metabolism in one soil
Report No.: EnSa-14-0246
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, 835.4200; Japanese MAFF Test Guidelines 12 Nousan 8147, No. 2-5-3
Guideline deviation(s): none
GLP/GEP: yes

Executive Summary

The degradation data of isoflucypram as reported in [redacted]; [redacted]; 2015; M-513456-01-1 in section CA 7.1.1.2 were kinetically evaluated according to FOCUS Kinetics report (FOCUS, 2006). The experimental data could be described by a single first order (SFO) kinetic model. The DT_{50} value of isoflucypram under anaerobic conditions was > 1000 days.
It is concluded that no significant degradation of isoflucypram will occur in soil under anaerobic conditions following an aerobic incubation phase.

Table 7.1.2.1.3- 1: Degradation kinetics of isoflucypram in soil under anaerobic conditions

Soil	Texture (USDA)	Best fit model[a)]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual assessment[b)]
Laacher Hof AXXa	loamy sand	SFO	> 1000	> 1000	0.7	-

a) SFO: single first order
b) Visual assessment: - = poor

I. MATERIALS AND METHODS

Details on the study conduct and its results are summarised under KCA 7.1.1.2/01, page 42.
The data for the test item were evaluated according to FOCUS kinetics (2006)[1] using the software KinGUI 2. Model input datasets were the residual amounts found in each replicate test system at each sampling interval of the anaerobic phase.
For the evaluation of the data three different kinetic models (Single First Order model (SFO), First Order Multi Compartment model (FOMC) and Double First Order in Parallel model (DFOP)) were tested in order to determine the best-fit kinetic model. The best-fit kinetic model was selected on the basis of the chi^2 scaled-error criterion and on the basis of a visual assessment of the goodness of the fits (diagrams of measured and calculated values vs. time, diagrams of residuals vs. time). DT_{50} and DT_{90} values (time until 50 or 90% degradation) were calculated from the resulting kinetic parameters.

II. RESULTS AND DISCUSSION

The degradation of isoflucypram under anaerobic conditions followed single first order (SFO) kinetics in all soils according to the lowest chi^2 error values and visual assessments of fits.
A summary of all kinetic data is given in Table 7.1.2.1.3- 2, the best fits are highlighted in bold letters there. The half-life for isoflucypram was > 1000 days in the tested soil under anaerobic conditions.

Table 7.1.2.1.3- 2: Summary of the kinetic evaluation (for trigger values according to FOCUS) of the degradation of isoflucypram in soil under anaerobic conditions
(best fits are highlighted in **bold** letters)

Soil (Texture (USDA)	Kinetic model[a]	DT_{50} [days]	DT_{90} [days]	Chi^2 error [%]	Visual assessment[b]
Laacher Hof AXXa (loamy sand)	**SFO**	**> 1000**	**> 1000**	1.8	-
	FOMC	> 1000	> 1000	2.0	-
	DFOP[c]	n.d.	n.d.	n.d.	n.d.

n.d. = not detected
a) SFO: Single first order, FOMC: First order multi compartment, DFOP: Double first order in parallel
b) Visual assessment: + = good, o = moderate, - = poor
c) Could not be calculated

III. CONCLUSIONS

Isoflucypram was not degraded in soil under anaerobic conditions in the laboratory in the dark. The calculated best fit DT_{50} value was > 1000 days in the tested soil.

[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.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

As no major metabolites were found in the anaerobic soil metabolism study of isoflucypram ([redacted]; [redacted]; 2015; M-513456-01-1) no kinetic evaluation was conducted to derive trigger and modelling endpoints according to FOCUS Guidance 2014 for isoflucypram major soil degradation products.

CA 7.1.2.2 Field studies

The trigger and modelling endpoints of isoflucypram and its major soil metabolite BCS-CN88460, carboxylic acid *(M12)* derived from field dissipation trials are summarised in Table 7.1.2.2- 1 to Table 7.1.2.2- 2 and Table 7.1.2.2- 3 to Table 7.1.2.2- 4, respectively.

Table 7.1.2.2- 1: Isoflucypram:
Field degradation endpoints for trigger purpose; not-normalised; European field sites
([redacted]; 2017; M-595964-01-1)

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ actual}$ [days]	$DT_{90\ actual}$ [days]	St. (χ^2 err) [%]	Method of calculation[c)]
Burscheid, Germany	silt loam, bare soil	5.3	60	142	1774	3.2	trigger: DFOP
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	129	1637	5.6	trigger: DFOP
Parcay Meslay, Northern France	loam, bare soil	5.9	60	150	2341	2.4	trigger: DFOP
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	17.0	69.4	4.7	trigger: DFOP
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	72.2	2036	5.8	trigger: DFOP
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	25.1	797	10.3	trigger: DFOP
pH dependence	no						

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) DFOP: double first order in parallel

**Table 7.1.2.2- 2: Isoflucypram:
Field matrix degradation endpoints for modelling purpose;
normalised to 20°C, 100% field capacity, Q_{10} = 2.58; European field sites**
([redacted] [redacted]; 2017; M-595964-01-1)

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ matrix}$ norm[c)] [days]	St. (χ^2err) [%]	Method of calculation[d)]
Burscheid, Germany	silt loam , bare soil	5.3	60	281	3.1	DFOP slow phase
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	489	6.1	DFOP slow phase
Parcay Meslay, Northern France	loam, bare soil	5.9	60	449	2.7	DFOP slow phase
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	137	3.7	HS slow phase
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	649	5.9	DFOP slow phase
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	258	10.3	DFOP slow phase
Geometric mean:				**335**		
pH dependence	no					

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
d) DFOP: double first order in parallel; HS: Hockey stick

**Table 7.1.2.2- 3: BCS-CN88460-carboxylic acid *(M12)*:
Field degradation endpoints for trigger purpose; not-normalised; European field sites**
([redacted] [redacted]; 2017; M-595964-01-1)

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ actual}$ [days]	$DT_{90\ actual}$ [days]	St. (χ^2err) [%]	Method of calculation[c)]
Burscheid, Germany	silt loam , bare soil	5.3	60	389	1291	10.7	trigger: SFO
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	179	595	11.8	trigger: SFO
Parcay Meslay, Northern France	loam, bare soil	5.9	60	696	2311	9.00	trigger: SFO
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	72	239	8.69	trigger: SFO
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	311	1032	21.7	trigger: SFO
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	211	702	24.6	trigger: SFO
pH dependence	no						

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) SFO: single first order

Table 7.1.2.2- 4: BCS-CN88460-carboxylic acid *(M12)*:
Field matrix degradation endpoints for modelling purpose;
normalised to 20°C, 100% field capacity, Q_{10} = 2.58; European field sites
([redacted]; 2017; M-595964-01-1)

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	DT_{50} matrix norm[c)] [days]	St. (χ^2err) [%]	Formation fraction k_f / k_{pd}	Method of calculation[d)]
Burscheid, Germany	silt loam , bare soil	5.3	60	**172**	4.3	0.0386	SFO
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	**67.3**	13.8	0.0563	SFO
Parcay Meslay, Northern France	loam, bare soil	5.9	60	**340**	8.9	0.0345	SFO
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	**82.4**	11.0	0.0651	SFO
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	**228**	23	0.0430	SFO
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	**172**	23.9	0.0183	SFO
Geometric mean:				**153**			
Arithmetic mean:						**0.043**	
pH dependence	no						

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
d) SFO: single first order

CA 7.1.2.2.1 Soil dissipation studies

In Europe a total of six field dissipation trials run with isoflucypram, three conducted in Northern Europe and three conducted in Southern Europe using unlabelled Isoflucypram + Prothioconazole formulated as EC 200.

In addition, kinetic evaluations of the degradation behaviour of isoflucypram and its major soil metabolite BCS-CN88460-carboxylic acid *(M12)* in soil under field conditions have been performed according to FOCUS kinetics (FOCUS 2006, 2014) to derive kinetic parameters suitable for environmental risk assessment and modelling purpose ([redacted], G.; [redacted], B.; 2017; M-608368-01-1 and [redacted], G.; [redacted], B.; 2017; M-608370-01-1).

A summary of the degradation rates of isoflucypram and its major degradation product BCS-CN88460-carboxylic acid *(M12)* in soil in the field is given in section CA 7.1.2.2.

- **Terrestrial field dissipation study**

Report:	KCA 7.1.2.2.1/01; [redacted]; [redacted]; 2017; M-595964-01-1
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
GLP/GEP:	**yes**

Executive 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 BCS CN88460-carboxylic acid *(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 7.1.2.2.1- 1: 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)	7.9	DFOP	77.6	> 1000
Vilobi d'Onyar (Spain) 14-2750-06	loam (0-30 cm) sandy clay loam (30-100 cm)	5.8	DFOP	257	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 BCS-CN88460-carboxylic acid *(M12)*.
The maximum amounts of BCS-CN88460-carboxylic acid 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 7.1.2.2.1- 2), which are 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 have 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. The location, site descriptions, climatic data and properties of the soils are given in the following tables.

Table 7.1.2.2.1- 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	in 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	in 0.1 km distance from the plot	in 0.4 km distance from the plot
Meteorological conditions compared to long-term average within normal levels (yes/no)	yes	yes	yes

Table 7.1.2.2.1- 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	6.9	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. 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 passages from opposite directions with each 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 7.1.2.2.1-4).

Table 7.1.2.2.1- 4: 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	2014-04-04	2014-06-06	2014-06-16
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	2.5
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	2014-05-12	2014-06-20	2014-05-12
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)

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 BCS-CN88460-carboxylic acid in/on soil.
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 BCS-CN88460-carboxylic acid 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 BCS-CN88460-carboxylic acid.

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).
Model input datasets comprised of the residual amounts found in each replicate test system at each sampling interval. The initial total recovery at DAT-0 (2 samplings after incorporation) was included in the parameter optimization procedure, but for optimal goodness of fit, the value was allowed to be estimated by the model. The best-fit kinetic model was selected on the basis of the chi^2 scaled-error criterion and on the basis of a visual assessment of the goodness of the fits. DT_{50} and DT_{90} values were calculated from the resulting kinetic parameters.

[1] [redacted]; 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 MCA 4.1.2

II. RESULTS AND DISCUSSION

A. ANALYTICAL RESULTS

Control samples
Residues of isoflucypram and BCS-CN88460-carboxylic acid 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 (Barcay 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.

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 BCS-CN88460-carboxylic acid

The maximum amounts of BCS-CN88640-carboxylic acid *(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.

Table 7.1.2.2.1- 5: 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 7.1.2.2.1- 6: Analytical results of BCS-CN88460-carboxylic acid preprocessed 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 chi² 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 are summarised in Table 7.1.2.2.1- 7 with best fits highlighted in bold letters. 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. In detail, the results are shown in Table 7.1.2.2.1- 7.

The dissipation of isoflucypram showed a biphasic behaviour. After treatment, isoflucypram dissipated in a first step very fast within a couple of days followed by a second slower step until study termination.

Table 7.1.2.2.1- 7: Isoflucypram: calculation of dissipation times

Location and trial no.	Kinetic model[a),b)]	DT_{50} [d]	DT_{90} [d]	Visual assessment[c)]	Chi^2 error [%]
Burscheid	SFO	241	801	-	16.98
(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	55.3	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

Isoflucypram was moderately to fast degraded in soil under the conditions of field testing at six trial sites in Northern and Southern Europe. The dissipation of isoflucypram could be described by biphasic kinetic models with calculated best fit half-lives between 16.5 and 177 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 BCS-CN88460-carboxylic acid *(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. BCS-CN88460-carboxylic acid was found in the top 0-20 cm only.

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Kinetic evaluations of field dissipation study

Kinetic evaluation for trigger endpoints

Report:	KCA 7.1.2.2.1/02; [redacted], G.; [redacted], B.; 2017; M-608368-01-1
Title:	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under field conditions for trigger purpose
Report No.:	EnSa-17-0634
Document No.:	M-608368-01-1
Guideline(s):	not applicable
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive Summary

Non-normalised (not for temperature or moisture) dissipation DT_{50} values of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* in soil under European field conditions ([redacted]; [redacted]; 2017; M-595964-01-1) were derived for trigger purpose according to FOCUS guidance (2006[1], 2014[2]).

In the experimental field dissipation studies, 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.

In this evaluation, the initial soil concentration was free fitted together with all degradation rates and formation fractions, based on the IRLS error model (Iteratively reweighted least square), using KinGUI 2. The selection of the kinetic model was based on a detailed statistical analysis including visual assessment, χ^2 statistic and significance of a t-test.

In all soils, the best fit for dissipation of isoflucypram (parent and its metabolite BCS-CN88460-carboxylic acid for trigger purpose could be described assuming a DFOP-SFO model for parent and metabolite (Table 7.1.2.2.1- 8, Table 7.1.2.2.1- 9).

[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.

Table 7.1.2.2.1- 8: Isoflucypram: field degradation endpoints for trigger purpose; non-normalised; European field sites

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ actual}$ [days]	$DT_{90\ actual}$ [days]	St. (χ^2err) [%]	Method of calculation[c)]
Burscheid, Germany	silt loam , bare soil	5.3	60	143	1774	3.2	trigger: DFOP
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	129	1637	5.6	trigger: DFOP
Parcay Meslay, Northern France	loam, bare soil	5.9	60	150	2341	2.4	trigger: DFOP
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	17.0	69.4	4.7	trigger: DFOP
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	72.2	2036	5.8	trigger: DFOP
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	25.1	797	10.3	trigger: DFOP
pH dependence	no						

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) DFOP: double first order in parallel

Table 7.1.2.2.1- 9: BCS-CN88460-carboxylic acid *(M12)*: field degradation endpoints for trigger purpose; non-normalised; European field sites

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ actual}$ [days]	$DT_{90\ actual}$ [days]	St. (χ^2err) [%]	Method of calculation[c)]
Burscheid, Germany	silt loam , bare soil	5.3	60	389	1291	10.7	trigger: SFO
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	179	595	11.8	trigger: SFO
Parcay Meslay, Northern France	loam, bare soil	5.9	60	696	2311	9.00	trigger: SFO
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	72	239	8.69	trigger: SFO
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	311	1032	21.7	trigger: SFO
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	211	702	24.6	trigger: SFO
pH dependence	no						

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) SFO: single first order

I. METHODS

The behaviour of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* was investigated in a terrestrial field soil dissipation study designed to determine the dissipation under representative European field conditions ([redacted]; [redacted]; 2017; M-595964-01-1). The study included trials at six sites at six geographic locations in Germany, the UK, France, Italy and Spain (Table 7.1.2.2.1- 10). Each test site received a single application at a nominal application rate of the active substance of 100 g/ in spring. However, the actual amount of isoflucypram applied was 88.1 to 98.2 g/ha (Table 7.1.2.2.1- 11). Application was made on bare soil.

Table 7.1.2.2.1- 10: Geographical data of the field dissipation trials

Site	Trial no.	Latitude[a)]	Longitude[a)]	Elevation
Burscheid, Germany	14-2750-01	51°04.110'N	07°05.690'E	205 m
Great Chishill, UK	14-2750-02	52°03'17.07"N	0°08'33.78"E	146 m
Parcay Meslay, Northern France	14-2750-03	47°27'47.0''N	0°45'11.9''E	110 m
St. Etienne du Gres, Southern France	14-2750-04	43°48'20.0''N	4°43'12.0''E	11 m
Albaro di Ronco Aldige, Italy	14-2750-05	45°34'50.35"N	11°18'97.9"E	23 m
Vilobi d'Onyar, Spain	14-2750-06	41°52`53.43"N	2°44`52,39" E	139 m

a) by Google Earth

Table 7.1.2.2.1- 11: Application rates of isoflucypram in field dissipation trials

Site	Actual application rate [g/ha]
Burscheid, Germany	98.2
Great Chishill, UK	96.8
Parcay Meslay, Northern France	88.1
St. Etienne du Gres, Southern France	90.9
Albaro di Ronco Aldige, Italy	90.1
Vilobi d'Onyar, Spain	88.2

The evaluation is conducted to derive kinetic parameters suitable for trigger purpose according to FOCUS Kinetics (FOCUS 2006, 2014). The kinetic analysis was performed 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).

In case of a trigger evaluation, for a parent substance the best fit model should be evaluated based on a visual assessment, t-test and χ^2-test of SFO, FOMC or DFOP models. The best fit model of the parent is then chosen, and for the metabolite in the total system it is tested, whether SFO yields an appropriate fit based on visual assessment, t-test and χ^2 test. Generally, an error of $\chi^2 < 15\%$ is considered as acceptable.
Otherwise, FOMC or DFOP could be assessed for a metabolite and the best-fit model be chosen. However, bi-phasic models for metabolites bear a complexity in formation and degradation in parallel, and consequently for the interpretation and comparison with effects in ecotoxicological studies. Thus, biphasic models typically are not used in case of metabolites.

II. RESULTS AND DISCUSSION

Results of the simple first-order or bi-phasic kinetic evaluation of 6 field dissipation s including the active substance isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* are given below according to FOCUS kinetics (FOCUS, 2006, 2014).
Dissipation rates are evaluated for the corresponding study conditions at the field site (not temperature

or moisture normalised).
For trigger purpose the best fit model is required (SFO, FOMC, DFOP) for a parent compound, based on the error of the χ^2 test and visual acceptability.

- **Degradation of isoflucypram**

The non-normalised degradation DT_{50} matrix values of isoflucypram are summarised in Table 7.1.2.2.1- 12.
In all soils, the best fit for dissipation of isoflucypram for trigger purpose could be described assuming a DFOP model. The statistical assessment of the best fit model shows good results with relative errors ε of the χ^2 test below 15%. The visual inspection of the fit shows for each soil a good acceptability.

Table 7.1.2.2.1- 12: Estimated field matrix degradation of isoflucypram for trigger purpose, non-normalised (bold = best fit model for trigger purpose**)**

Site	Kinetic model[a)]	$DisT_{50, initial}$ [days]	$DisT_{90, initial}$ [days]	t-test, $k_{initial}$	St. (χ^2err) [%]	Visual fit[b)]
Burscheid, Germany	SFO	269	894	< 0.001	12.1	o
	FOMC	149	7370	-	3.6	+
	DFOP	**143**	**1774**	**< 0.001**	**3.2**	**+**
Great Chishill, UK[c)]	SFO	248	824	< 0.001	13.0	o
	FOMC	182	2308	-	10.4	+
	DFOP	180	1322	0.001	10.9	+
Great Chishill, UK[d)]	SFO	238	789	< 0.001	12.6	o
	FOMC	145	2844	-	6.0	+
	DFOP	**129**	**1637**	**< 0.001**	**5.6**	**+**
Parcay Meslay, Northern France	SFO	437	1450	< 0.001	15.0	o
	FOMC	149	47974	-	2.8	+
	DFOP	**150**	**2341**	**< 0.001**	**2.4**	**+**
St. Etienne du Gres, Southern France	SFO	18.4	61.2	< 0.001	8.8	o
	FOMC	17.9	70.8	-	7.8	o
	DFOP	**17.0**	**69.4**	**< 0.001**	**4.7**	**+**
Albaro, Italy	SFO	313	1041	< 0.001	19.7	o
	FOMC	90.0	8285	-	7.4	+
	DFOP	**72.2**	**2036**	**< 0.001**	**5.8**	**+**
Vilobi, Spain	SFO	144	478	< 0.001	24.1	o
	FOMC	34.6	2373	-	11.6	+
	DFOP	**25.1**	**797**	**< 0.001**	**10.3**	**+**

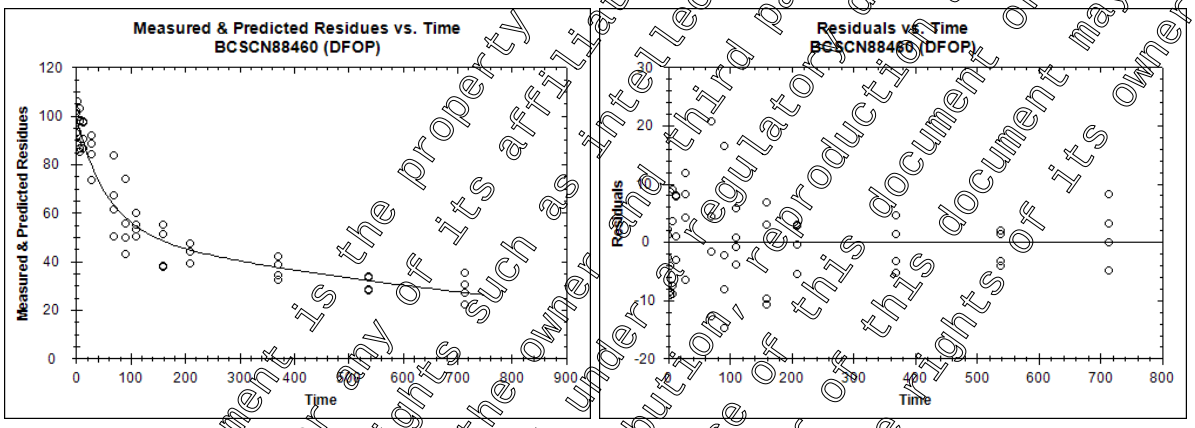
a) SFO: single first order, FOMC: first-order multi-compartment model, DFOP: double first order in parallel
b) Visual fit: + = good, o = moderate, - = poor
c) Initial fit including all residue data
d) Modified fit excluding parent residue data for DAT 111

The selection of the most suitable model for trigger purpose is explained below. It should be noted that the given time is not the true experimental time but the transformed time based on the time step normalisation approach.

Burscheid, Germany

The initial simple SFO fit performed statistically relative poor (χ^2 error of 12.1%). The visual assessment indicates systematic deviations in the residual plot between day 40 until end of study. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 3.2%). The visual assessment of the DFOP fit shows no systematic deviations until end of study (Figure 7.1.2.2.1- 1). Residuals are clearly randomly scattered around the zero line. Determined residue levels at end of study are well predicted by the fitted curve. Following the FOCUS decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for trigger purpose.

Figure 7.1.2.2.1- 1: Burscheid: DFOP kinetics selected for trigger purpose

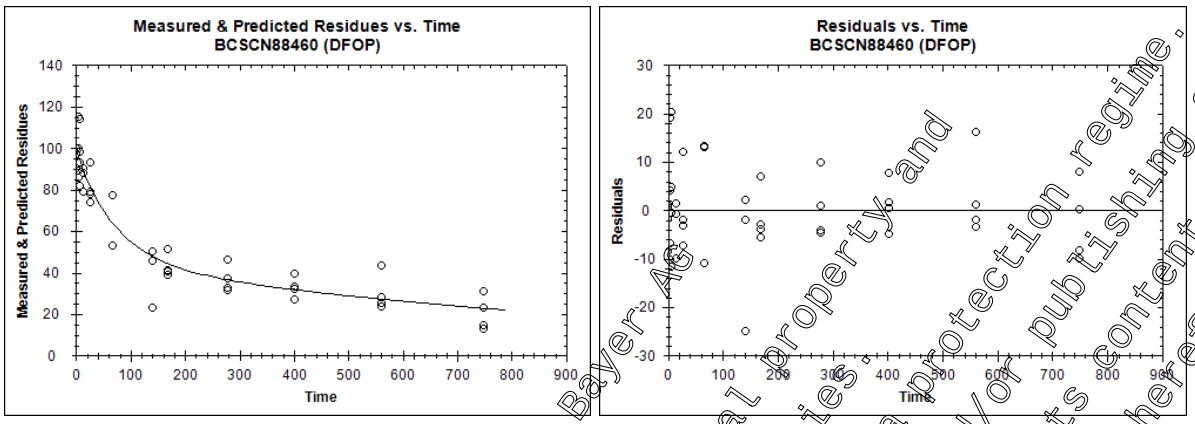


Great Chishill, United Kingdom

The soil residues of the parent showed at day 111 of the Great Chishill trial a sudden unexpected increase not consistent with the steady parent decay (Table 10 in the report) DAT 67 71%, DAT 111 80%, DAT 140 40%). Parent soil residues at DAT 111 are excluded for the presented final kinetic evaluations.

The initial simple SFO fit performed statistically relative poor (χ^2 error of 12.6%). The visual assessment indicates some systematic deviations in the residual plot in the second phase of the study. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 5.6%). The visual assessment of the DFOP fit shows no systematic deviations until end of study (Figure 7.1.2.2.1- 2). Residuals are clearly randomly scattered around the zero line. Determined residue levels at end of study are well predicted by the fitted curve.

Figure 7.1.2.2.1- 2: Great Chishill: DFOP kinetics selected for trigger purpose (modified fit)

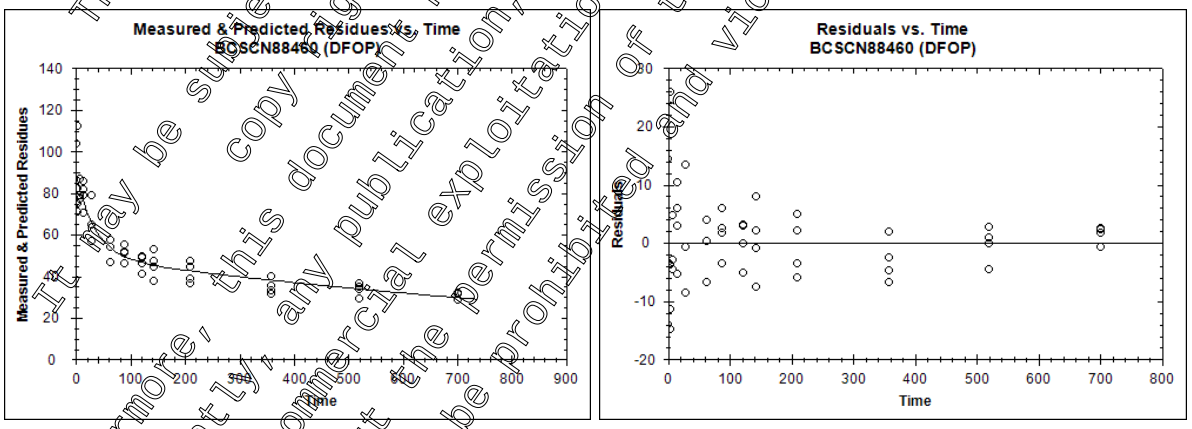


Following the FOCUS decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for trigger purpose.
Additional DFOP evaluations with the full residue data of Great Chishill (incl. DAT 111 of parent) are presented in the report. These fits did not change the principle outcome of the kinetic evaluation but lead to worsened statistics (*e.g.* χ^2 error of 10.9% compared to 5.6%).

Parcay Meslay, Northern France

The initial simple SFO fit performed statistically relative poor (χ^2 error of 15.0%). The visual assessment indicates a systematic deviation in the residual plot during the study period. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 2.4%). The visual assessment of the DFOP fit shows no systematic deviations until end of study (Figure 7.1.2.2.1- 3). Residuals are clearly randomly scattered around the zero line. Determined residue levels at end of study are well predicted by the fitted curve.
Following the FOCUS decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for trigger purpose.

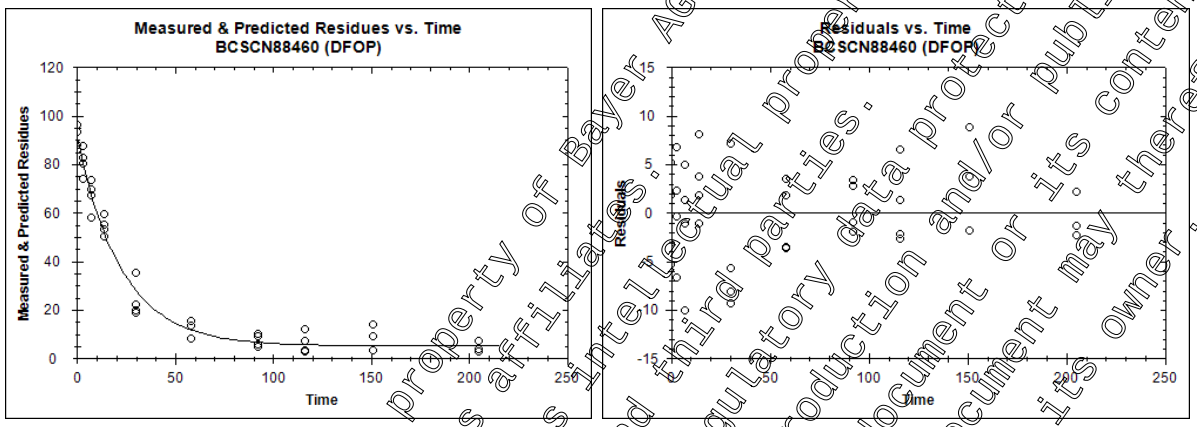
Figure 7.1.2.2.1- 3: Parcay Meslay: DFOP kinetics selected for trigger purpose



St. Etienne du Gres, Southern France

The initial simple SFO fit performed statistically relative poor (χ^2 error of 8.3%). The visual assessment indicates systematic deviations in the residual plot between day 40 until end of study. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 4.7%). The visual assessment of the DFOP fit shows no systematic deviations until end of study.

Figure 7.1.2.2.1- 4: St. Etienne du Gres: DFOP kinetics selected for trigger purpose



Residuals are clearly randomly scattered around the zero line. Determined residue levels at end of study are well predicted by the fitted curve.

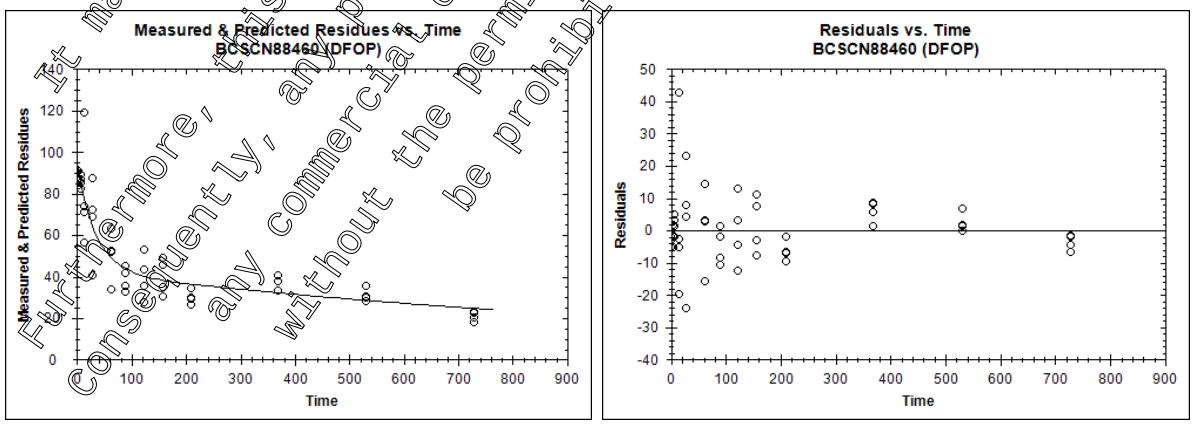
Following the FOCUS decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for trigger purpose.

Albaro, Italy

The initial simple SFO fit performed statistically poor with an χ^2 error of 19.7%. The visual assessment indicates a systematic deviation in the residual plot during the study period. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 5.8%). The visual assessment of the DFOP fit shows low residual level with no systematic deviations until end of study (Figure 7.1.2.2.1- 5). Residuals are clearly randomly scattered around the zero line. Determined residue levels at end of study are well predicted by the fitted curve.

Following the FOCUS decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for trigger purpose.

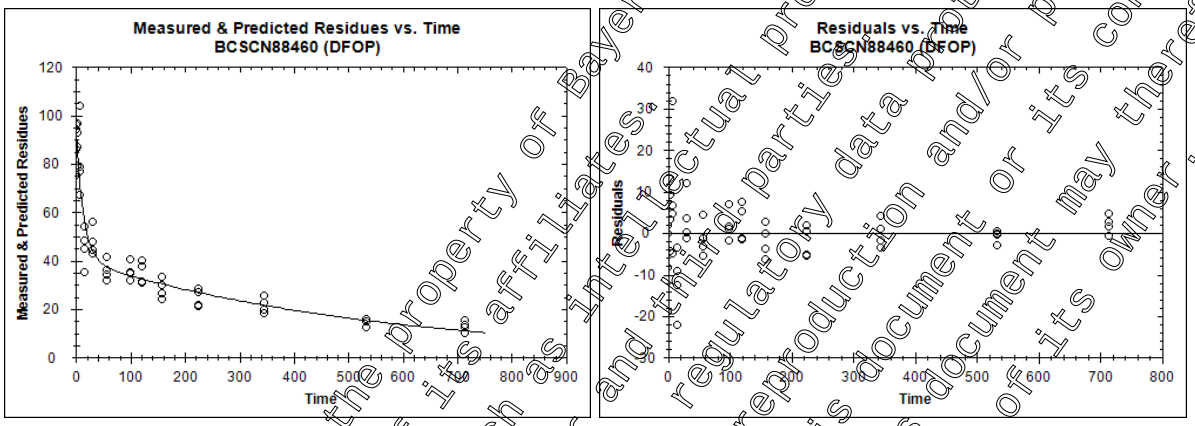
Figure 7.1.2.2.1- 5: Albaro: DFOP kinetics selected for trigger purpose



Vilobi, Spain

The initial simple SFO fit performed statistically very poor with an χ^2 error of 24.1%. The visual assessment indicates a systematic deviation in the residual plot during the study period. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ2 error of 10.3%). The visual assessment of the DFOP fit shows very low residual level with no systematic deviations until end of study (Figure 7.1.2.2.1- 6). Some scatter is visible in the very early residue data points but residuals are overall randomly scattered around the zero line. Determined residue levels at end of study are well predicted by the fitted curve.

Figure 7.1.2.2.1- 6: Vilobi: DFOP kinetics selected for trigger purpose



- **Degradation of BCS-CN88460-carboxylic acid *(M12)***

Non-normalised field degradation parameter of metabolite BCS-CN88460-carboxylic acid *(M12)* are summarised in Table 7.1.2.2.1- 13. The best and reasonable model for trigger purpose for the parent was chosen (bold) and the corresponding degradation rates and formation fractions for the metabolite were selected. For metabolite, only a SFO fit is tested.

Table 7.1.2.2.1- 13: Estimated field matrix degradation of the metabolite BCS-CN88460-carboxylic acid for trigger purpose, non-normalised

Site	Kinetic model[a] parent	$DisT_{50, initial}$ [days]	$DisT_{90, initial}$ [days]	t-test, k	St. (χ^2err) [%]	Formation fraction parent=>met	Visual fit[b]
Burscheid, Germany	SFO	91.2	303	< 0.001	17.6	0.0981	o
	FOMC	382	1267	0.001	11.0	0.0354	o
	DFOP	**389**	**1291**	**< 0.001**	**10.7**	**0.0354**	**o**
Great Chishill, UK[c]	SFO	51	169	< 0.001	13.2	0.1383	o
	FOMC	163	541	0.008	13.3	0.0506	o
	DFOP	**179**	**595**	**< 0.001**	**11.8**	**0.0476**	**o**
Great Chishill, UK[d]	SFO	49	162	< 0.001	12.9	0.1450	o
	FOMC	125	416	< 0.001	13.1	0.0636	o
	DFOP	128	426	< 0.001	11.9	0.0628	o
Parcay Meslay, Northern France	SFO	49.9	166	< 0.001	17.9	0.2416	o
	FOMC	662	2199	0.004	11.7	0.0328	o
	DFOP	**696**	**2311**	**0.001**	**9.0**	**0.0330**	**o**
St. Etienne du Gres, Southern France	SFO	62.3	207	< 0.001	7.8	0.0689	o
	FOMC	63.2	210	0.043	8.9	0.0688	+
	DFOP	**72**	**239**	**< 0.001**	**8.7**	**0.0648**	**+**
Albaro, Italy	SFO	26.7	88.7	< 0.001	23.7	0.3790	o
	FOMC	282	938	0.010	23.9	0.0422	o
	DFOP	**311**	**1032**	**< 0.001**	**21.7**	**0.0412**	**o**
Vilobi, Spain	SFO	17.4	57.7	< 0.001	30.3	0.1532	o
	FOMC	183	605	0.003	25.2	0.0186	+
	DFOP	**211**	**702**	**< 0.001**	**24.6**	**0.0176**	**+**

a) SFO: single first order, FOMC: first-order multi-compartment model, DFOP: double first order in parallel
b) Visual fit: + = good, o = moderate, - = poor
c) Initial fit including all residue data
d) Modified fit excluding parent residue data for DAT 111

Burscheid, Germany

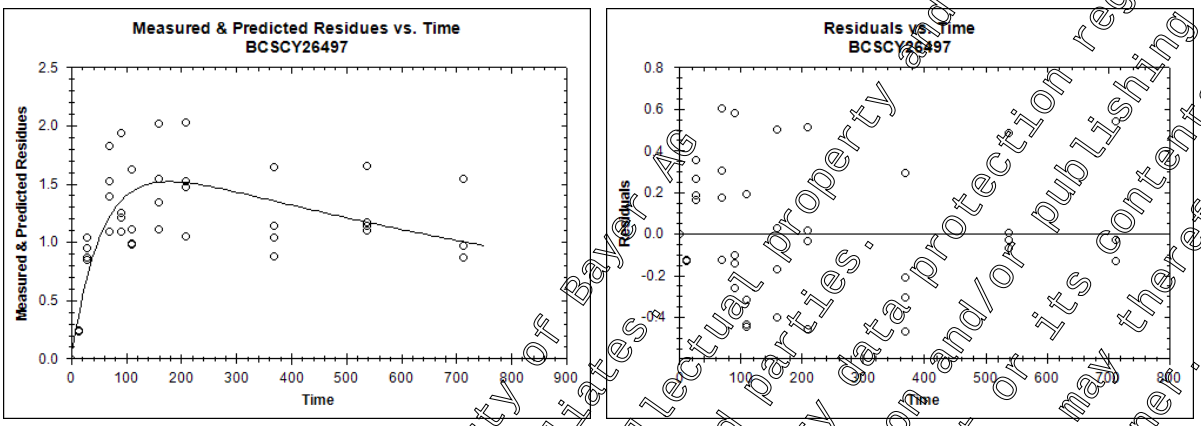
Following the FOCUS decision tree the field degradation of isoflucypram at the Burscheid trial is best described assuming the DFOP model for trigger purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 10.7% with an χ^2 error of 4.4% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 7). The visible scatter in the residual plot is fully randomized and may be caused by the low residue level of the metabolite BCS-CN88460-carboxylic (< 2 g a.s. eq./ha). Determined residue levels at end of study are well predicted by the fitted curve.

Considering the statistical and visual assessment the field degradation of isoflucypram and the

metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for trigger purpose.

Figure 7.1.2.2.1- 7: Burscheid: SFO fits of BCS-CN88460-carboxylic acid



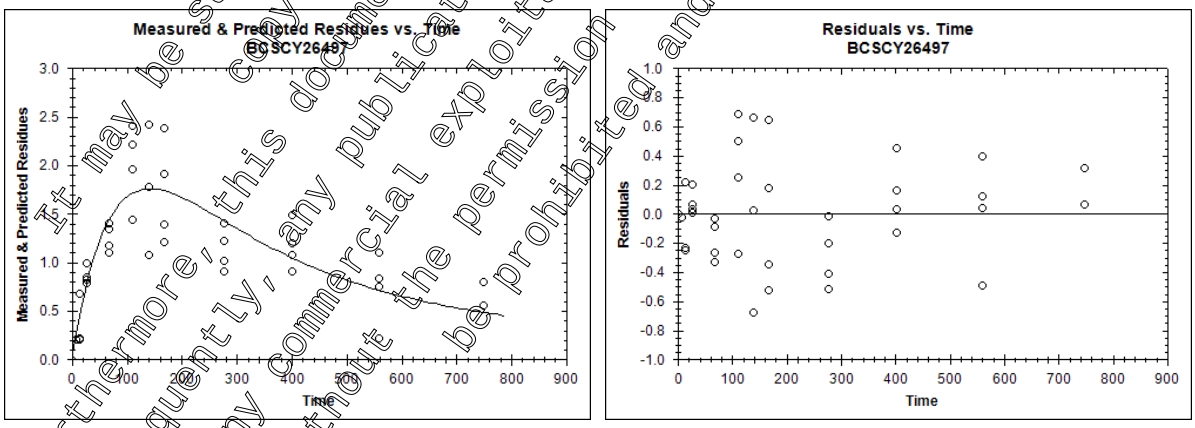
Great Chishill, United Kingdom

Following the EFSA decision tree the field degradation of isoflucypram at the Great Chishill trial is best described assuming the DFOP model for trigger purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 11.8% with an χ^2 error of 7.9% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 8). The visible scatter in the residual plot is fully randomized and may be caused by the low residue level of the metabolite BCS-CN88460-carboxylic (~ 2.4 g a.s. eq/ha). Determined residue levels at end of study are well predicted by the fitted curve.

Considering the statistical and visual assessment the field degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for trigger purpose.

Figure 7.1.2.2.1- 8: Great Chishill: SFO fits of BCS-CN88460-carboxylic acid



Additional metabolite evaluations with the full parent residue data of Great Chishill (incl. DAT 111) are presented in the report. These fits did not show any significant impact on the kinetic evaluation of the metabolite but increased the χ^2 error of the overall fit from 7.9% to 15.1%.

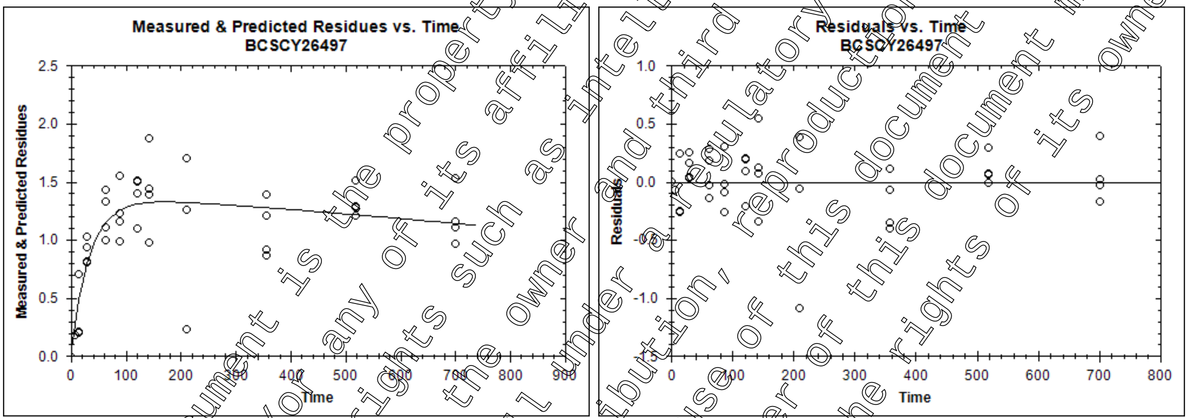
Parcay Meslay, Northern France

Following the FOCUS decision tree the field degradation of isoflucypram at the Parcay Meslay trial is best described assuming the DFOP model for trigger purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 9.0% with an χ^2 error of 3.4% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 9). The visible scatter in the residual plot is fully randomized and may be caused by the low residue level of the metabolite BCS-CN88460-carboxylic (< 1.9 g a.s. eq./ha). Determined residue levels at end of study are well predicted by the fitted curve.

Considering the statistical and visual assessment the field degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for trigger purpose.

Figure 7.1.2.2.1- 9: Parcay Meslay: SFO fits of BCS-CN88460-carboxylic acid



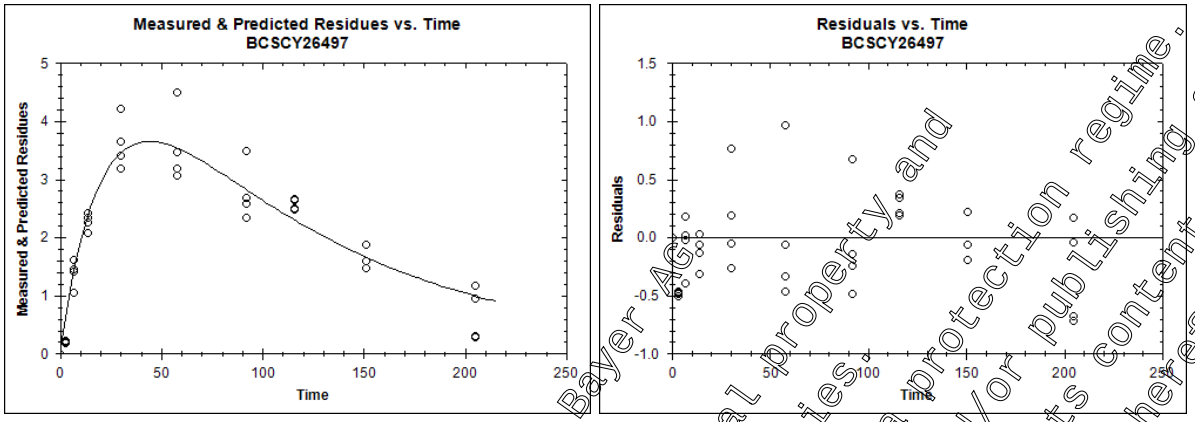
St. Etienne du Gres, Southern France

Following the FOCUS decision tree the field degradation of isoflucypram at the St. Etienne du Gres trial is best described assuming the DFOP model for trigger purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 8.7% with an χ^2 error of 6.4% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 10). The visible scatter in the residual plot is fully randomized. Determined residue levels at end of study are well predicted by the fitted curve. Maximum residue level of the metabolite BCS-CN88460-carboxylic are higher compared to the other trial (< 4.5 g a.s. eq./ha) allowing a more robust fit of the metabolite decline.

Considering the statistical and visual assessment the field degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for trigger purpose.

Figure 7.1.2.2.1- 10: St. Etienne du Gres: SFO fits of BCS-CN88460-carboxylic acid



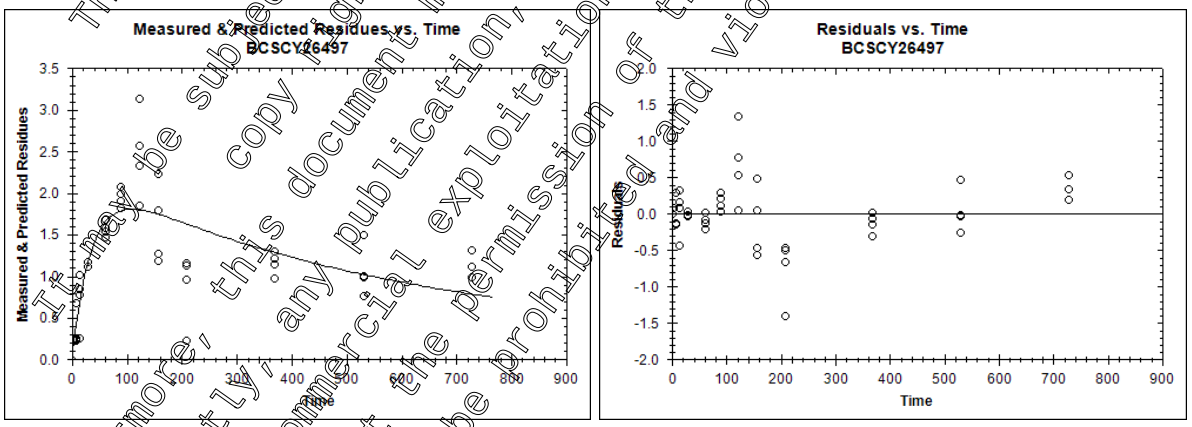
Albaro, Italy

Following the FOCUS decision tree the field degradation of isoflucypram at the Albaro trial is best described assuming the DFOP model for trigger purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ2 error is 21.7% with an χ^2 error of 8.2% for the overall fit (parent and metabolite). The relative high metabolite χ^2 error seems to be caused by some scatter in the metabolite residue data and low metabolite residue levels (< 3.1 % a.s. eq./ha). Thus, the metabolite χ^2 error does also not improve considering other kinetic model for the parent. The visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 11). Determined residue levels at end of study are well predicted by the fitted curve.

Considering the statistical and visual assessment the field degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for trigger purpose.

Figure 7.1.2.2.1- 11: Albaro: SFO fits of BCS-CN88460-carboxylic acid



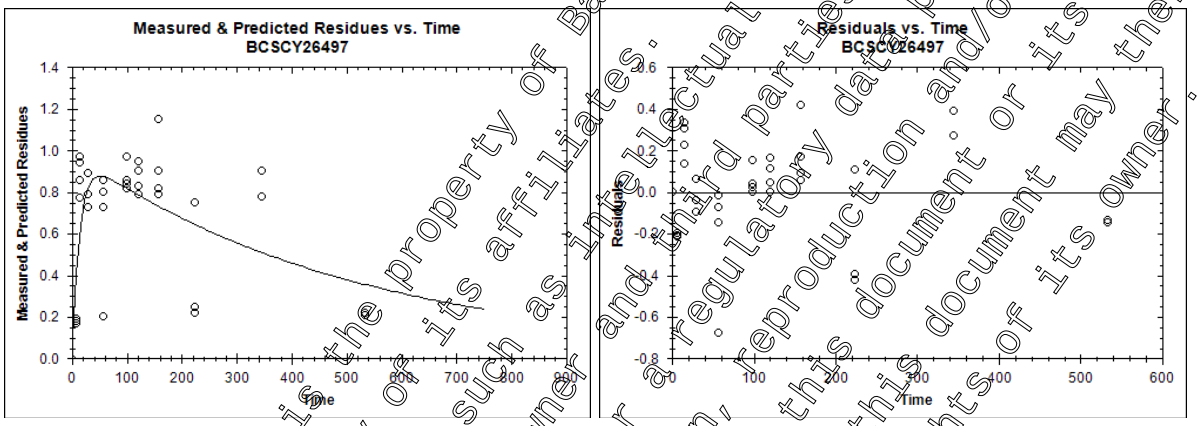
Vilobi, Spain

Following the EFSA decision tree the field degradation of isoflucypram at the Vilobi trial is best described assuming the DFOP model for trigger purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual relative poor fit. Calculated metabolite

χ^2 error is 24.6% with an χ^2 error of 14.1% for the overall fit (parent and metabolite). The relative high metabolite χ^2 error seems to be caused by large scatter in the metabolite residue data and very low metabolite residue levels (< 1.2 g a.s. eq./ha). Thus, the metabolite χ^2 error does also not improve considering other kinetic model for the parent. The visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 12).
Considering the statistical and visual assessment is somewhat questionable if the field degradation of the metabolite BCS-CN88460-carboxylic acid can be sufficiently reliable described to derive a metabolite DT_{50} for trigger purpose. The resulting DT_{50} may be still accepted as it lays clearly within the range of the other trials. As the overall metabolite residue level is acceptable described by the fitted curve, the resulting metabolite formation fraction can be selected as reliable trigger input.

Figure 7.1.2.2.1- 12: Vilobi: SFO fits of BCS-CN88460-carboxylic acid



III. CONCLUSIONS

After the kinetic evaluation of field dissipation trials using simple first-order (SFO) and bi-phasic models in comparison, the best description of isoflucypram could be given using an DFOP fit for 6 European trials (Table 7.1.2.2.1- 12). The overall field degradation parameter for trigger purposes are summarised in Table 7.1.2.2.1- 8 for the parent isoflucypram and in Table 7.1.2.2.1- 9 for the metabolite BCS-CN88460-carboxylic acid.

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Kinetic evaluations for modelling endpoints

The dissipation of isoflucypram in agricultural soils under natural field conditions was investigated in several trials in Europe. Based on these trials, a kinetic evaluation was performed to estimate normalised (20°C, pF 2) dissipation times (DT_{50}) for use in model simulations of environmental exposures (modelling endpoints) for isoflucypram and its major metabolites BCS-CN88460-carboxylic acid *(M12)*.

Report:	KCA 7.1.2.2.1/03; [redacted], G.; [redacted], B.; 2017; M-608370-01-1
Title:	Isoflucypram (ISY) and metabolite - Kinetic evaluation of the degradation in soil under field conditions for modelling purpose
Report No.:	EnSa-17-0533
Document No.:	M-608370-01-1
Guideline(s):	not applicable
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive Summary

Normalised (20°C, 100% field capacity) degradation DT_{50} matrix values of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* in the soil matrix under European field conditions ([redacted]; [redacted]; 2017; M-595964-01-1) were derived for modelling purpose 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, 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.

Simulated (with PEARL) daily soil temperatures and moisture contents were used 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.

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).

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 (Table 7.1.2.2.1- 14). The corresponding DT_{50} and formation fractions (f.f.)

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[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

for the metabolite BCS-CN88460-carboxylic acid *(M12)* are summarised in Table 7.1.2.2.1- 15.

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 335 days and for the metabolite with 153 days (normalised to 20°C, 100% field capacity). The arithmetic mean formation fraction of the metabolite BCS-CN88460-carboxylic acid in soil under field conditions is 0.043.

Table 7.1.2.2.1- 14: Isoflucypram:
Field matrix degradation endpoints for modelling purpose; normalised to 20°C, 100% field capacity, Q_{10} = 2.58; European field sites

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ matrix}$ norm[c)] [days]	St. (χ^2-err) [%]	Method of calculation[d)]
Burscheid, Germany	silt loam , bare soil	5.6	60	281	3.1	DFOP slow phase
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	489	6.1	DFOP slow phase
Parcay Meslay, Northern France	loam, bare soil	5.9	60	449	2.7	DFOP slow phase
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	137	3.7	HS slow phase
Albaro di Ronco all Adige, Italy	clay, bare soil	7.6	60	649	5.9	DFOP slow phase
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	258	10.3	DFOP slow phase
Geometric mean:				**335**		
pH dependence	no					

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
d) DFOP: double first order in parallel; HS: Hockey stick

Table 7.1.2.2.1- 15: BCS-CN88460-carboxylic acid *(M12)*: Field matrix degradation endpoints for modelling purpose; normalised to 20°C, 100% field capacity, Q_{10} = 2.58; European field sites

Location, country	Soil type[a)]	pH ($CaCl_2$)	Depth[b)] [cm]	$DT_{50\ matrix}$ norm[c)] [days]	St. (χ^2err) [%]	Formation fraction k_f / k_{pd}	Method of calculation[d)]
Burscheid, Germany	silt loam , bare soil	5.3	60	**172**	4.3	0.0386	SFO
Great Chishill, United Kingdom	clay loam, bare soil	7.0	60	**67.3**	13.8	0.0563	SFO
Parcay Meslay, Northern France	loam, bare soil	5.9	60	**340**	8.9	0.0345	SFO
St. Etienne du Gres, Southern France	clay loam, bare soil	7.5	60	**82.4**	11.0	0.0651	SFO
Albaro di Ronco all Adige, Italy	clay, bare soil	7.0	60	**228**	23.1	0.0430	SFO
Vilobi d'Onyar, Spain	loam, bare soil	5.8	60	**172**	23.9	0.0183	SFO
Geometric mean:				**153**			
Arithmetic mean:						**0.043**	
pH dependence	no						

a) according to USDA
b) Experimental soil sampling depth considered to derive total residues for kinetic evaluation
c) Normalised using a Q_{10} of 2.58 and Walker equation coefficient of 0.7
d) SFO: single first order

I. METHODS

The behaviour of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* was investigated in a terrestrial field soil dissipation study designed to determine the dissipation under representative European field conditions ([redacted]; [redacted]; 2017; M-595964-01-1)). The study included trials at six sites at six geographic locations in Germany, the UK, France, Italy and Spain (Table 7.1.2.2.1- 16). Each test site received a single application at a nominal application rate of the active substance of 100 g in spring. However, the actual amount of isoflucypram applied was 88.1 to 98.2 g/ha (Table 7.1.2.2.1- 17). Application was made on bare soil.

The evaluation is conducted to derive kinetic parameters suitable for modelling purpose according to FOCUS kinetics (FOCUS 2006, 2014) and EFSA guidance on field dissipation studies (EFSA 2014). It includes a time-step normalisation to standard reference conditions for soil temperature (20°C, Q_{10} of 2.58) and soil moisture (field capacity, pF2), as well as a quality check of the results. Processes potentially occurring at the soil surface, e.g. photo-degradation and volatilisation, during the field study should be eliminated to result finally in a DT_{50} representing the degradation in the soil.

The kinetic analysis was performed according to FOCUS kinetics (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).

Based on local soil and weather data, daily soil moisture and temperature were calculated using a simulation model. The effect of soil moisture and temperature on degradation was included into the evaluation according to FOCUS (2014) using a reference temperature of 20°C and a soil moisture of 100% field capacity (FC).

The kinetic evaluation followed the recent guidance of EFSA (2014) on derivation of so-called $DegT_{50,matrix}$ for use in exposure modelling.

In case a bi-phasic model was selected only the slow phase degradation was considered which leads to conservative kinetic endpoints.

Table 7.1.2.2.1- 16: Geographical data of the field dissipation trials

Site	Trial no.	Latitude[a)]	Longitude[a)]	Elevation
Burscheid, Germany	14-2750-01	51°04.110'N	07°05.690'E	205 m
Great Chishill, UK	14-2750-02	52°03'17.07"N	0°08'33.78"E	146 m
Parcay Meslay, Northern France	14-2750-03	47°27'47.0''N	0°45'11.9''E	110 m
St. Etienne du Gres, Southern France	14-2750-04	43°48'20.0''N	4°43'12.0''E	11 m
Albaro di Ronco Aldige, Italy	14-2750-05	45°34'50.35"N	11°18'97.9" E	23 m
Vilobi d'Onyar, Spain	14-2750-06	41°52`53.43"N	2°44`52,39" E	139 m

a) by Google Earth

Table 7.1.2.2.1- 17: Application rates of isoflucypram in field dissipation trials

Site	Actual application rate [g/ha]
Burscheid, Germany	98.2
Great Chishill, UK	96.8
Parcay Meslay, Northern France	88.1
St. Etienne du Gres, Southern France	90.9
Albaro di Ronco Aldige, Italy	90.1
Vilobi d'Onyar, Spain	88.2

II. RESULTS AND DISCUSSION

Normalised (20°C, 100% field capacity) degradation $DT_{50\ matrix}$ values of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* in the soil matrix under European field conditions were derived for modelling purpose according to FOCUS kinetics (FOCUS 2006, 2014) and the EFSA guidance on field dissipation studies (EFSA 2014). Processes potentially occurring at the soil surface, *e.g.* photo-degradation, volatilisation, during the field study should be eliminated to result finally in a $DT_{50\ matrix}$ representing the degradation in the soil matrix or bulk.

Simulated (with PEARL) daily soil temperatures and moisture contents were used to normalise the evaluated parameters to reference conditions according to FOCUS groundwater assumptions (Arrhenius equation, Q_{10} = 2.58; Walker equation, pF2). The residue data together with the transformed times (transformed time approach, time step normalisation) were kinetically and statistically evaluated, based on the procedure explained by FOCUS kinetics, using KinGUI 2.1.

In the attempt to separate soil surface degradation processes, such as photo-degradation and volatilisation, from bulk soil degradation, an important threshold to start a kinetic evaluation might be the time, when at least 10 mm precipitation (+ irrigation) have been fallen (EFSA 2014). Then, it is assumed that the active substance is sufficiently deep washed into the soil matrix.

- **Degradation of isoflucypram**

According to the EFSA decision tree (EFSA 2014), the kinetic evaluation was started by assuming a simple first-order (SFO) dissipation for the parent compound in soil, excluding the first sample points, until the 10 mm rain criterion has been reached (Table 7.1.2.2.1- 18).

Further on, the bi-phasic models DFOP (double first order in parallel) and HS (hockey stick) were checked with the whole data for the field matrix degradation of isoflucypram in all soils, besides following the decision tree of EFSA (2014) (Table 7.1.2.2.1- 18).

Some main parameters fitted, as degradation rates k, $DT_{50\ matrix}$, switch parameters g or t_b and some statistical parameters are summarised in Table 7.1.2.2.1- 18. Finally selected values to be considered as modelling input are given in bold.

The $DT_{50\ matrix}$ values are reported to be used for environmental fate modelling purposes. Therefore, where needed, recalculated DT_{50} are mentioned as follows: for DFOP and HS, $DT_{50\ 2nd\ phase}$ of slow phase.

Table 7.1.2.2.1- 18: Estimated field matrix degradation of isoflucypram for modelling purpose, normalised to 20°C, 100% field capacity

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]	St. (χ^2err) [%]	Visual fit[b)]
Burscheid, Germany	SFO	-	0.004847	< 0.001	-	-	143	10.25	o
	DFOP	**0.04892**	**0.002471**	**< 0.001 / < 0.001**	**-**	**0.3709**	**281**	**3.12**	+
	HS	0.01259	0.002708	< 0.001 / < 0.001	40.17	-	256	3.33	+
Great Chishill, UK	SFO	-	0.006008	< 2e-16	-	-	115	10.06	o
	DFOP	**0.01492**	**0.001417**	**0.0058 / 0.2186**	**-**	**0.6276**	**489**	**6.10**	+
	HS	0.00842	0.002813	< 0.001 / < 0.001	94.31	-	246	5.43	+
Parcay Meslay, Northern France	SFO	-	0.002949	< 0.001	-	-	235	10.26	o
	DFOP	**0.03813**	**0.001545**	**< 0.001/ < 0.001**	**-**	**0.4128**	**449**	**2.74**	+
	HS	0.01370	0.001834	< 0.001/ < 0.001	38.5	-	378	3.13	+
St. Etienne du Gres, Southern France	SFO	-	0.048852	0.001	-	-	14.2	13.10	o
	DFOP	0.06241	0.001317	< 0.001/ 0.342	-	0.9134	526	3.89	+
	HS	**0.05392**	**0.005058**	**< 0.001 / 0.0368**	**39.0**	**-**	**137**	**3.68**	+
Albaro, Italy	SFO	-	0.003383	< 0.001	-	-	205	15.59	o
	DFOP	**0.02233**	**0.001068**	**< 0.001/ 0.019**	**-**	**0.5324**	**649**	**5.88**	+
	HS	0.00798	0.001134	< 0.001 / 0.004	1001.3	-	611	4.97	+
Vilobi, Spain	SFO	-	0.005510	0.001	-	-	126	23.84	o
	DFOP	**0.14200**	**0.002691**	**< 0.001/ < 0.001**	**-**	**0.5315**	**258**	**10.28**	+
	HS	0.06729	0.003093	< 0.001 / < 0.001	10.64	-	224	8.69	+

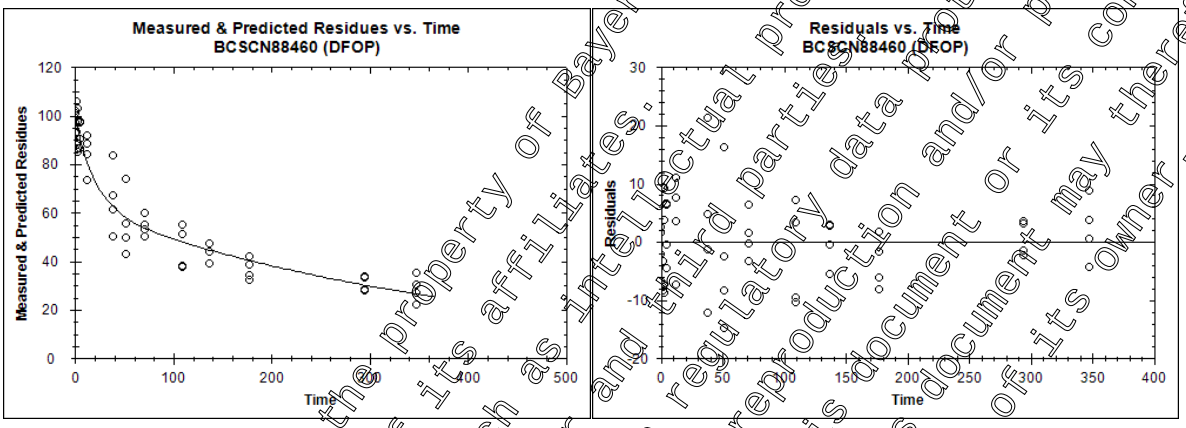
$DT_{50\ matrix}$ half-lives for modelling: DFOP; HS: DT_{50} of slow phase
a) SFO: single first order; DFOP: double first order in parallel; HS: Hockey stick
b) Visual fit: + = good, o = moderate, - = poor

The selection of the most suitable model for modelling purpose is explained below. It should be noted that the given time is not the true experimental time but the transformed time based on the time step normalisation approach.

Burscheid, Germany

The initial simple SFO fit performed statistically relative poor (χ^2 error of 10.3%). The visual assessment indicates systematic deviations in the residual plot between day 40 until end of study. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 3.1%). The visual assessment of the DFOP fit shows no systematic deviations until end of study (Figure 7.1.2.2.1- 13). The bi-phasic HS model could not improve the overall fit. Following the EFSA decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for modelling purpose.

Figure 7.1.2.2.1- 13: Burscheid: DFOP kinetics selected for modelling purpose



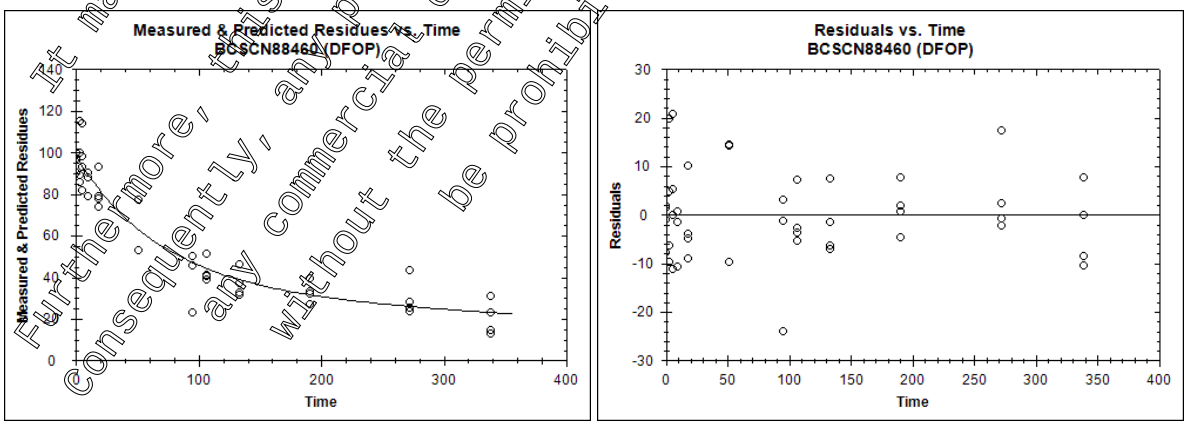
Great Chishill, United Kingdom

The soil residues of the parent showed at day 111 of the Great Chishill trial a sudden unexpected increase not consistent with the steady parent decay (DAT-67: 71%, DAT-111: 80%, DAT-140: 40%). Parent soil residues at DAT-111 are excluded for the presented kinetic evaluations.

The initial simple SFO fit performed statistically relative poor (χ^2 error of 10.1%). The visual assessment indicates some systematic deviations in the residual plot in the second phase of the study. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 6.1%). The visual assessment of the DFOP fit shows no systematic deviations until end of study (Figure 7.1.2.2.1- 14). The bi-phasic HS model could not improve the overall fit especially considering the observed scatter in the residue data.

Following the EFSA decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for modelling purpose.

Figure 7.1.2.2.1- 14: Great Chishill: DFOP kinetics selected for modelling purpose

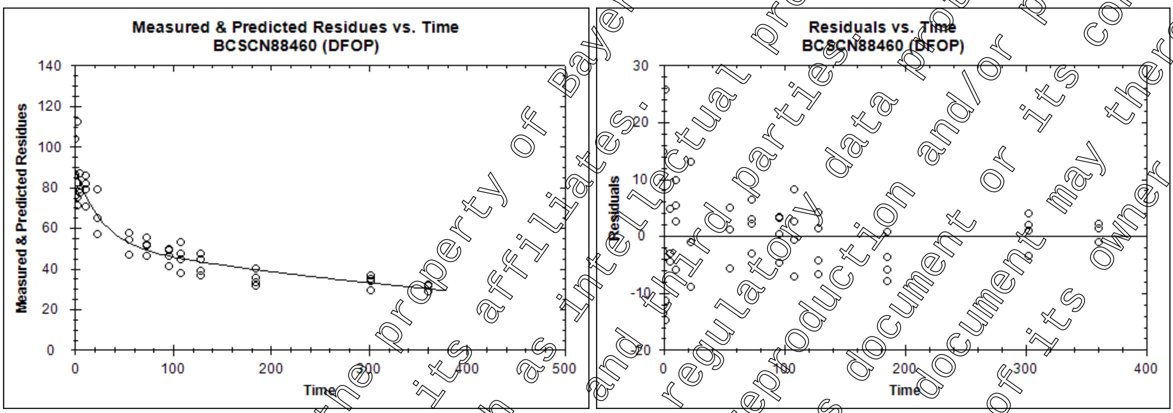


Parcay Meslay, Northern France

The initial simple SFO fit performed statistically relative poor (χ^2 error of 10.3%). The visual assessment indicates a systematic deviation in the residual plot during the study period. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 2.7%). The visual assessment of the DFOP fit shows no systematic deviations until end of study (Figure 7.1.2.2.1- 15). The bi-phasic HS model could not improve the overall fit.

Following the EFSA decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for modelling purpose.

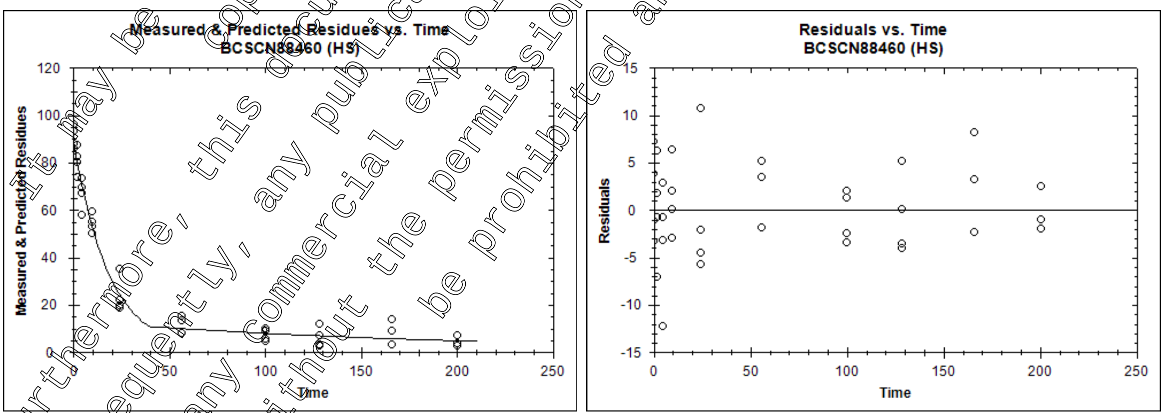
Figure 7.1.2.2.1- 15: Parcay Meslay: DFOP kinetics selected for modelling purpose



St. Etienne du Gres, Southern France

The initial simple SFO fit performed statistically relative poor (χ^2 error of 13.1%). The visual assessment indicates systematic deviations in the residual plot between day 40 until end of study. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 3.9%). The visual assessment of the DFOP fit shows no systematic deviations until end of study. The bi-phasic HS model resulted also to a good description of the residue decline with a slightly lower χ^2 error of 3.7%. The visual assessment of the HS fit is also slightly better compared to DFOP with an overall lower residual level and no systematic deviations until end of study (Figure 7.1.2.2.1- 16).

Figure 7.1.2.2.1- 16: St. Etienne du Gres: HS kinetics selected for modelling purpose

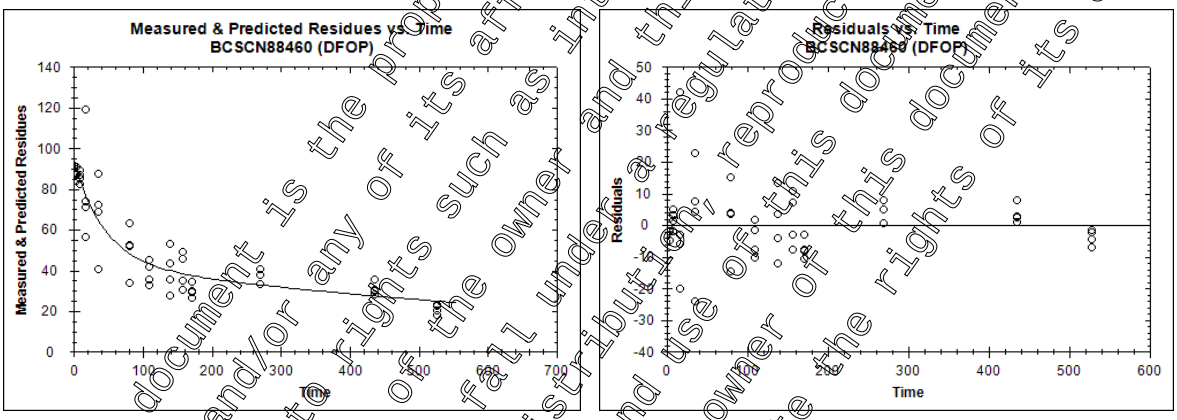


It should be noted that residue levels at end of study are at St. Etienne du Gres clearly below 10% of initial amounts (ca. 3.3%). Thus, the use of the DFOP model should be considered with care especially extrapolating outside the experimental time period. In this situation the HS may be more suitable for modelling purpose. Furthermore the g_{fast} value of 0.91 resulting from the DFOP fit is outside the EFSA

validity range (> 75). Based on the EFSA framework for evaluation of existing field studies not tailored for $DegT_{50\ matrix}$ (EFSA 2014) the HS model is under this circumstance to be chosen to derive modelling input.
Following the EFSA decision tree the matrix degradation of isoflucypram is best described assuming the HS model for modelling purpose.

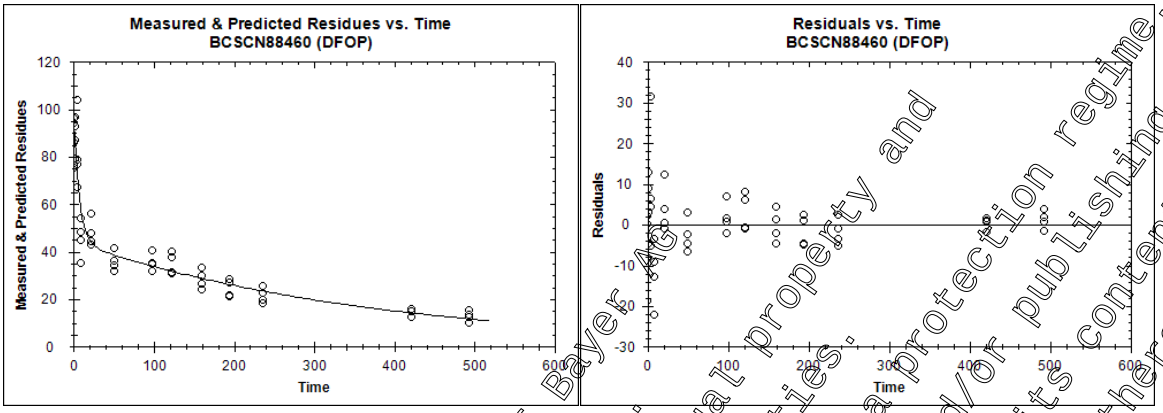
Albaro, Italy
The initial simple SFO fit performed statistically poor with an χ^2 error of 15.6%. The visual assessment indicates a systematic deviation in the residual plot during the study period. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 5.9%). The visual assessment of the DFOP fit shows low residual level with no systematic deviations until end of study (Figure 7.1.2.2.1- 17). The bi-phasic HS model could not improve the overall fit significantly.
Following the EFSA decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for modelling purpose.

Figure 7.1.2.2.1- 17: Albaro: DFOP kinetics selected for modelling purpose



Vilobi, Spain
The initial simple SFO fit performed statistically very poor with an χ^2 error of 23.8%. The visual assessment indicates a systematic deviation in the residual plot during the study period. The decline of the residues could be significantly better described assuming the biphasic DFOP model (χ^2 error of 10.3%). The visual assessment of the DFOP fit shows very low residual level with no systematic deviations until end of study (Figure 7.1.2.2.1- 18). Some scatter is visible in the very early residue data points. The bi-phasic HS model could not improve the overall fit significantly.
Following the EFSA decision tree the matrix degradation of isoflucypram is best described assuming the DFOP model for modelling purpose.

Figure 7.1.2.2.1- 18: Vilobi: DFOP kinetics selected for modelling purpose



- **Degradation of BCS-CN88460-carboxylic acid *(M12)***

Normalised (20°C, 100% field capacity) degradation $DT_{50\ matrix}$ values of metabolite BCS-CN88460-carboxylic acid *(M12)* are summarised in Table 7.1.2.2.1- 19. The best and reasonable model for modelling purpose for the parent was chosen (bold) and the corresponding degradation rates and formation fractions for the metabolite were selected. For metabolite, only a SFO kinetic is tested.
Details of the metabolite fits are discussed further below. It should be noted that the given time is not the true experimental time but the transformed time based on the time step normalisation approach.
Only low residue level of the metabolite BCS-CN88460-carboxylic acid were found in the field trials (< 4.5 g a.s. eq/ha) indicating a generally low metabolite formation under outdoor field conditions. This is a significant difference to the observed formation under standard laboratory conditions.

Table 7.1.2.2.1- 19: Estimated field matrix degradation of the metabolite BCS-CN88460-carboxylic acid for modelling purpose, normalised to 20°C, 100% field capacity

Site	Kinetic model[a)] parent	k [1/d]	t-test, k	$DT_{50\ matrix}$ [d]	St. (χ^2err) [%]	Formation fraction parent=>met	Visual fit[b)]
Burscheid, Germany	SFO	0.00827	< 0.001	83.8	15.92	0.0568	o
	DFOP	**0.00404**	**< 0.001**	**172**	**4.27**	**0.0386**	**o**
	HS	0.00428	< 0.001	162	4.56	0.0405	o
Great Chishill, UK	SFO	0.02097	< 0.001	33.1	14.60	0.0102	o
	DFOP	**0.01031**	**< 0.001**	**67.3**	**13.82**	**0.0563**	**o**
	HS	0.01050	< 0.001	66.0	11.47	0.0569	o
Parcay Meslay, Northern France	SFO	0.00844	0.001	82.2	13.33	0.0967	o
	DFOP	**0.00204**	**0.001**	**340**	**8.89**	**0.0345**	**o**
	HS	0.00217	< 0.001	319	8.08	0.0355	o
St. Etienne du Gres, Southern France	SFO	0.00958	0.001	72.3	10.68	0.0688	o
	DFOP	0.00834	< 0.001	83.1	10.23	0.0645	o
	HS	**0.00841**	**< 0.001**	**82.4**	**10.99**	**0.0651**	**o**
Albaro, Italy	SFO	0.01583	0.00169	43.8	26.33	0.1634	o
	DFOP	**0.00304**	**< 0.001**	**228**	**23.12**	**0.0430**	**o**
	HS	0.00330	0.001	210	22.94	0.0459	o
Vilobi, Spain	SFO	0.01522	0.0204	45.5	27.62	0.0566	o
	DFOP	**0.00403**	**< 0.001**	**172**	**23.94**	**0.0183**	**-**
	HS	0.00404	< 0.001	172	22.62	0.0182	-

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

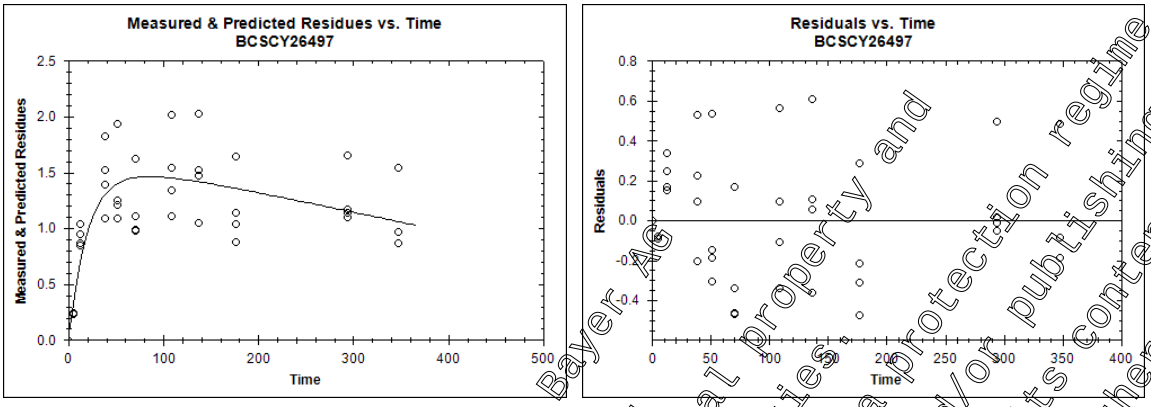
Burscheid, Germany

Following the EFSA decision tree the matrix degradation of isoflucypram at the Burscheid trial is best described assuming the DFOP model for modelling purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 10.2% with an χ^2 error of 4.3% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 19). The visible scatter in the residual plot is fully randomized and may be caused by the low residue level of the metabolite BCS-CN88460-carboxylic acid (< 2 g a.s. eq/ha).

Considering the statistical and visual assessment the matrix degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for modelling purpose.

Figure 7.1.2.2.1- 19: Burscheid: SFO fits of BCS-CN88460-carboxylic acid



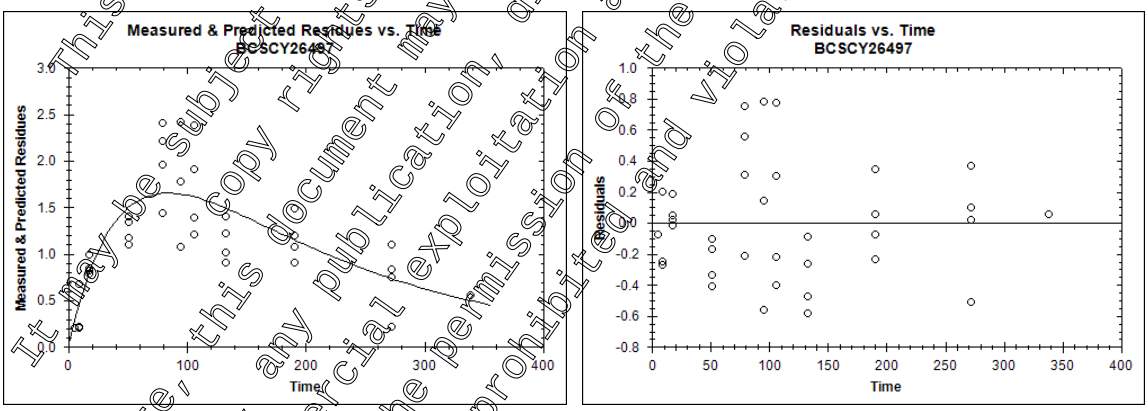
Great Chishill, United Kingdom

Following the EFSA decision tree the matrix degradation of isoflucypram at the Great Chishill trial is best described assuming the DFOP model for modelling purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 14.6% with an χ^2 error of 15.5% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 20). The visible scatter in the residual plot is fully randomized and may be caused by the low residue level of the metabolite BCS-CN88460-carboxylic acid (< 2.4 g a.s. eq/ha).

Considering the statistical and visual assessment the matrix degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for modelling purpose.

Figure 7.1.2.2.1- 20: Great Chishill: SFO fits of BCS-CN88460-carboxylic acid



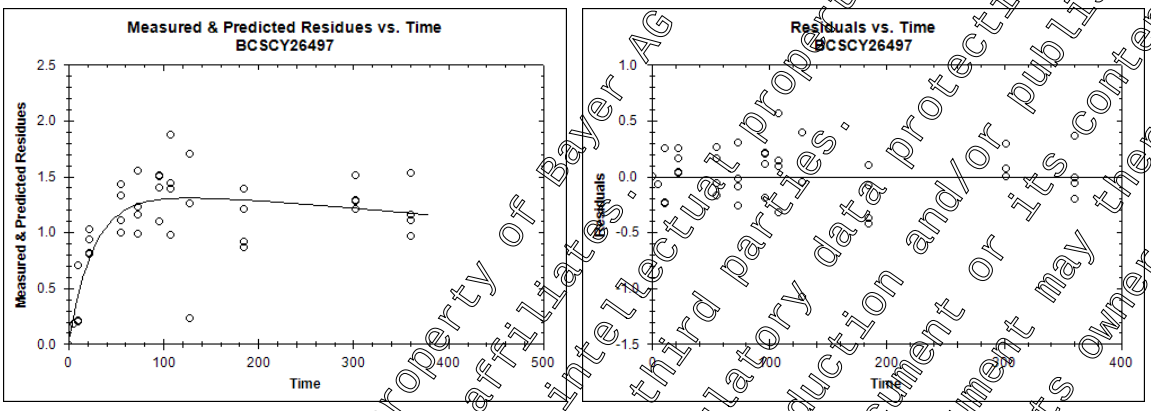
Parcay Meslay, Northern France

Following the EFSA decision tree the matrix degradation of isoflucypram at the Parcay Meslay trial is best described assuming the DFOP model for modelling purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 8.9% with an χ^2 error of 3.8% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 21).

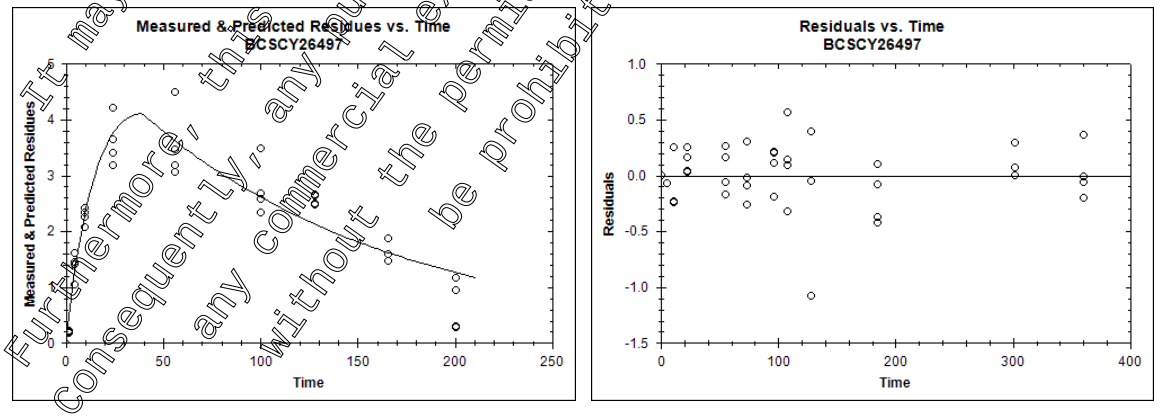
The visible scatter in the residual plot is fully randomized and may be caused by the low residue level of the metabolite BCS-CN88460-carboxylic acid (< 1.9 g a.s. eq/ha).
Considering the statistical and visual assessment the matrix degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for modelling purpose.

Figure 7.1.2.2.1- 21: Parcay Meslay: SFO fits of BCS-CN88460-carboxylic acid



St. Etienne du Gres, Southern France
Following the EFSA decision tree the matrix degradation of isoflucypram at the St. Etienne du Gres trial is best described assuming the HS model for modelling purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with HS kinetics for parent and SFO kinetics for metabolite.
Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 11.0% with an χ^2 error of 5.1% for the overall fit (parent and metabolite). Visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 22). The visible scatter in the residual plot is fully randomized. Maximum residue level of the metabolite BCS-CN88460-carboxylic acid are higher compared to the other trial (< 4.5 g a.s. eq/ha) allowing a more robust fit of the metabolite decline.
Considering the statistical and visual assessment the matrix degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a HS-SFO model to derive input for modelling purpose.

Figure 7.1.2.2.1- 22: St. Etienne du Gres: SFO fits of BCS-CN88460-carboxylic acid



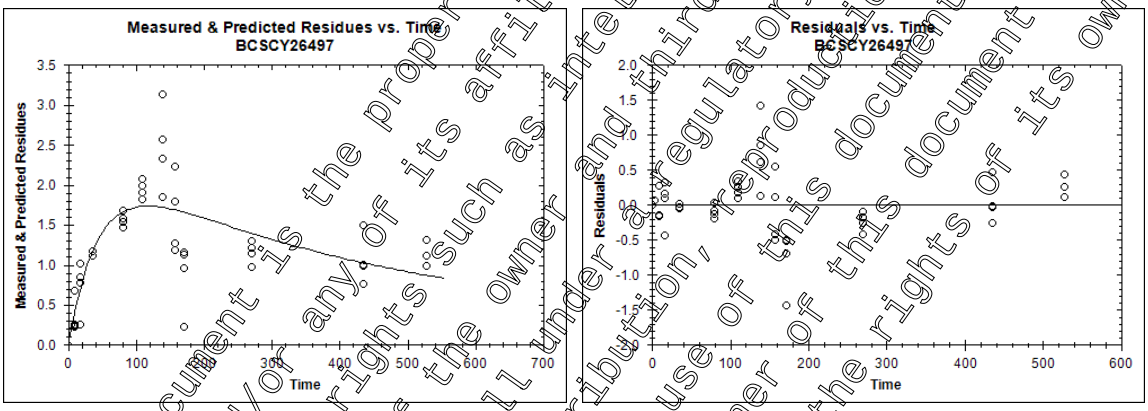
Albaro, Italy

Following the EFSA decision tree the matrix degradation of isoflucypram at the Albaro trial is best described assuming the DFOP model for modelling purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual acceptable fit. Calculated metabolite χ^2 error is 23.1% with an χ^2 error of 8.3% for the overall fit (parent and metabolite). The relative high metabolite χ^2 error seems to be caused by some scatter in the metabolite residue data and low metabolite residue levels (< 3.1 g a.s. eq/ha). Thus, the metabolite χ^2 error does also not improve considering other kinetic model for the parent. The visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 23).

Considering the statistical and visual assessment the matrix degradation of isoflucypram and the metabolite BCS-CN88460-carboxylic acid can be well described assuming a DFOP-SFO model to derive input for modelling purpose.

Figure 7.1.2.2.1- 23: Albaro: SFO fits of BCS-CN88460-carboxylic acid



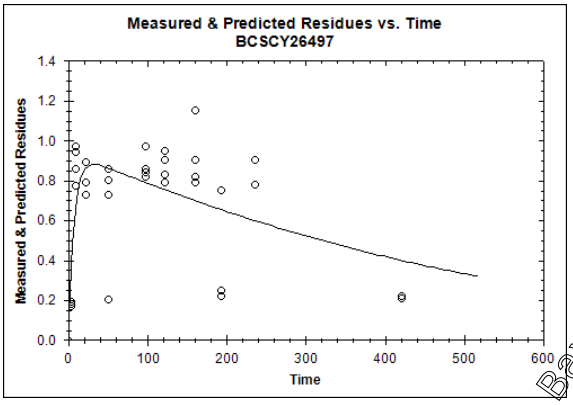
Vilobi, Spain

Following the EFSA decision tree the matrix degradation of isoflucypram at the Vilobi trial is best described assuming the DFOP model for modelling purpose. The kinetic evaluation of the metabolite BCS-CN88460-carboxylic acid is based on parent and metabolite fit with DFOP kinetics for parent and SFO kinetics for metabolite.

Metabolite SFO evaluation leads to a statistical and visual relative poor fit. Calculated metabolite χ^2 error is 23.9% with an χ^2 error of 14.1% for the overall fit (parent and metabolite). The relative high metabolite χ^2 error seems to be caused by large scatter in the metabolite residue data and very low metabolite residue levels (< 1.2 g a.s. eq/ha). Thus, the metabolite χ^2 error does also not improve considering other kinetic model for the parent. The visual assessment indicates no systematic deviation in the residual plot during the study period (Figure 7.1.2.2.1- 24).

Considering the statistical and visual assessment is somewhat questionable if the matrix degradation of the metabolite BCS-CN88460-carboxylic acid can be sufficiently reliable described to derive a metabolite DT50 for modelling purpose. The resulting DT_{50} may be still accepted as it lays clearly within the range of the other trials. As the overall metabolite residue level is acceptable described by the fitted curve, the resulting metabolite formation fraction can be selected as modelling input.

Figure 7.1.2.2.1- 24: Vilobi: SFO fits of BCS-CN88460-carboxylic acid



Formation of the metabolite BCS-CN88460-carboxylic acid in soil under field conditions

The metabolite formation fraction (f.f.) or formation rate (k_f) is an import modelling input factor. According to FOCUS kinetic the acceptability of the fitted formation fraction can be evaluated based on the robustness of the overall fit. The fitted formation fraction may be also compared to the observed maximum metabolite residue level and the computed standard deviation (STD).

Table 7.1.2.2.1- 20 summarises the formation data of BCS-CN88460-carboxylic acid. All given formation fractions are statistically reliable and can be used for modelling purpose.

Depending on the FOCUS exposure model the metabolite formation fraction (f.f.) is directly used as model input (e.g. PEARL). Alternatively the corresponding metabolite formation rate (k_f) from the precursor to the metabolite may be employed as model input (e.g. PELMO). Both ways are identical as the two parameter are directly interlinked based on the equation $k_f = f.f.* k_{dp}$ (with being the modelling degradation rate constant of the precursor).

The arithmetic mean formation fraction of the metabolite BCS-CN88460-carboxylic acid in soil under field conditions is 0.043 (Table 7.1.2.2.1- 20).

Table 7.1.2.2.1- 20: BCS-CN88460-carboxylic acid *(M12)*:
Field matrix degradation endpoints for modelling purpose;
normalised to 20°C, 100% field capacity, Q_{10} = 2.58; European field sites

Location, country	Soil type (USDA)	Parent k_{dp} [1/d]	Max. met. residue level [g a.s.eq/ha]	Met k_f [1/d]	f. f. k_f / k_{dp}	STD[a)]	Method of calculation (p-met)
Burscheid, Germany	silt loam , bare soil	0.00247	2.0	0.00403	0.0386	0.0042	DFOP-SFO
Great Chishill, United Kingdom	cay loam, bare soil	0.00142	2.4	0.01030	0.0563	0.0099	DFOP-SFO
Parcay Meslay, Northern France	loam, bare soil	0.00154	1.9	0.00204	0.0345	0.0038	DFOP-SFO
St. Etienne du Grès, Southern France	clay loam, bare soil	0.00506	4.5	0.00841	0.0651	0.0042	HS-SFO
Albar, Italy	clay, bare soil	0.00107	3.1	0.00304	0.0430	0.0060	DFOP-SFO
Vilobi, Spain	loam, bare soil	0.00269	1.2	0.00403	0.0183	0.0024	DFOP-SFO
Arithmetic mean:					**0.043**		

a) Standard deviation STD of metabolite f.f.

III. CONCLUSIONS

After the kinetic evaluation of field dissipation trials using simple first-order (SFO) and bi-phasic models in comparison, the best description of isoflucypram could be given using a DFOP fit for 5 European trials, and a HS fit for the one remaining trial (Table 7.1.2.2.1- 18).

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 335 days and for the metabolite with 153 days (normalised to 20°C, 100% field capacity) (Table 7.1.2.2.1- 14 and Table 7.1.2.2.1- 15). The arithmetic mean formation fraction of the metabolite BCS-CN88460-carboxylic acid in soil under field conditions is 0.043 (Table 7.1.2.2.1- 20).

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Storage stability in soil

Report:	KCA 7.1.2.2.1/04, [redacted]; 2016; M-574766-01-1
Title:	Determination of the storage stability of BCS-CN88460 and the metabolite BCS-CN88460-carboxylic acid in soil for 24 months
Report No.:	P641 14 1803
Document No.:	M-574766-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, EC DGA: Appendix H - Storage stability of residue samples, 7032/VI/95 rev. 5 of 22 July 1997 US EPA OCSPP 860.1380, Storage Stability Data
Guideline deviation(s):	yes, see report
GLP/GEP:	yes

Executive Summary

The purpose of this study was to determine the stability of isoflucypram and BCS-CN88460-carboxylic acid *(M12)* in soil under freezer storage conditions.
Untreated soil samples of soil Höfchen (silt loam) and soil Dollendorf (clay loam) were fortified with isoflucypram and BCS-CN88460-carboxylic acid respectively.
The fortified concentrations of isoflucypram and BCS-CN88460-carboxylic acid were about 10 µg/kg.
Soil samples were analysed for isoflucypram and BCS-CN88460-carboxylic acid on day 0 and after 93, 194, 308, 363, 539, and 720 days of storage below -18°C. Due to a technical error on day 235, the temperature rose to an average of -12.2°C for 5 hours and 55 minutes. Never the less this had no impact on the stability of the compounds.
Soil samples were analysed for isoflucypram and BCS-CN88460-carboxylic acid by means of HPLC-MS/MS according to method 01432. Soil samples of 20 g were extracted in a microwave extractor with 40 mL of a mixture of acetonitrile/water/acetic acid (4000+1000+30, v/v/v), respectively.
Possible matrix effects of isoflucypram and BCS-CN88460-carboxylic acid are eliminated by using an internal standard solution of isotopically labeled reference items. This solution is added to the sample solutions after extraction. Then a subsample was centrifuged to remove fine particles of the soil. Identification and quantitation of the test item and its degradation products 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 µg/kg in soil. The limit of determination (LOD) for each single analyte was 0.3 µg/kg.
Mean concurrent recoveries for each soil typ were within the range of 100% to 102%. The relative standard deviations were within the range of 2.13% to 5.24%
The recovered amounts of isoflucypram and BCS-CN88460-carboxylic acid on day 0 and after

720 days were compared to the standardized recovery rates (day 0 = 100%).
Residue results were not corrected for the concurrent recoveries at the respective day of analysis, since the mean concurrent recoveries were within a range of 70 - 110% and thus fulfilled the requested analytical guideline requirements.
At all sampling intervals on average more than 70% of the fortified amount of isoflucypram and BCS-CN88460-carboxylic acid were recovered from the stored samples (not corrected for concurrent recoveries).
The mean recovered amount in the two soils after 720 days of freezer storage ranged from 104% to 105% for isoflucypram and BCS-CN88460-carboxylic acid. Altogether, the study results demonstrate that isoflucypram and BCS-CN88460-carboxylic acid residues are stable in soil for at least 720 days of storage under frozen conditions.
The concentrations in the control samples (at each storage interval at least two control samples per soil were analysed), were always below the LOQ of 1 µg/kg.

I. MATERIALS AND METHODS

A. MATERIALS

1. Reference Items and Internal Standards

Reference items:

Isoflucypram					
Batch code:	BCS-CN88460-01-02				
	BCS-CN88460-PU-01				
Cerificate of analysis:	AZ 1867	AZ 19352	AZ 20542		
Purity:	98.4%	99.4%	99.1%		
BCS-CN88460-carboxylic acid *(M12)* (BCS-CY26497)					
Batch code:	BCS-CY26497-01-01				
	BCS-CY26497-01-02				
Certificate of analysis:	MZ 00913	MZ 00984	MZ 01057	MZ 01102	MZ 01188
Purity:	97.0%	98.8%	98.8%	98.8%	98.8%

Internal standards:

[d_5] BCS-CN88460 (BCN-CN88460 ISTD)	
Batch number:	MXM 7255-1-4
Purity:	99%
[d_5] BCS-CY26497 (BCN- CY26497 ISTD)	
Batch number:	MXM 7360-1-6
Purity:	> 98%

2. Test Soils

The control materials used for the storage stability experiments originated from two German soils Höfchen and Dollendorf. Two different soils were used for each analyte in order to assess a possible influence of different soil characteristics. The soil samples were classified according to DIN- and/or USDA specifications. Soil characteristics of the used soils are summarised in Table 7.1.2.2.1- 21.

Table 7.1.2.2.1- 21: Soil characteristics

	Soil Höfchen	Soil Dollendorf
Description	Plot 4011; 0-30 cm soil layer	Plot 20100423; 0-20 cm soil layer
Textural description according to USDA [fraction %]		
clay (<0.002 mm)	19.4	31
silt (0.002-0.050 mm)	76.3	38
sand (0.050-2.000 mm)	4.3	31
Soil type	silt loam	clay loam
pH (in $CaCl_2$ solution)	6.7	7.3
pH (in H_2O)	7.4	7.4
Organic carbon [%]	0.92	5
Organic matter [%][a)]	1.58	8.6
Cation exchange capacity[meq /100 g dry soil]	12.4	20.6
Max. water holding capacity[g /100 g dry soil]	39.9	79.1

a) organic matter = organic carbon x 1.72

B. STUDY DESIGN

1. Preparation of samples

To determine the storage stability of isoflucypram and BCS-CN88460-carboxylic acid untreated soil samples of 20 g each were weighed into 100 mL glas bottles with screw caps. Each of the reference items was dissolved in acetonitrile to obtain fortification standard solutions with concentrations of about 1 mg/L of each compound. For the preparation of the storage stability samples 0.2 mL of the 1 mg/L fortification standard solution dissolved in acetonitrile was added to each of the corresponding sets of soil samples (A and B-samples).

For the preparation of the samples used for the concurrent recovery experiments, 0.2 mL of the 1 mg/L fortification standard solution was added to each of the corresponding sets of soil samples. The resulting concentration in all soil samples was approximately 10 µg/kg soil.

2. Storage of bulk control material

Except for the time during subsampling and subsequent analysis, the homogeneous control matrices were maintained in deep-frozen storage (-18°C or below).

3. Sampling

Soil samples were analysed for isoflucypram and BCS-CN88460-carboxylic acid on day 0 and after 93, 194, 308, 363, 539, and 720 days of storage below -18°C. Due to a technical error on day 235, the temperature rose to an average of -12.2°C for 5 hours and 55 minutes. Never the less this had no impact on the stability of the compounds.

3. Analytical procedures

The analytical method 01432 ([redacted]; 2014; M-499794-01-1, KCA 4.1.2) was developed for the determination of isoflucypram and the metabolite BCS-CN88460-carboxylic acid residues in/on soil and sediment.

Soil and sediment samples of 20 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 BCS-CN88460-carboxylic acid are eliminated by using an internal standard solution of isotopically labeled 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 method was validated using three different soils "Höfchen", "Laacher Hof" "Dollendorf" and sediment "OECD 218/219".

The limit of quantitation (LOQ) for each single analyte was 1 µg/kg in soil. The limit of determination (LOD) for each single analyte was 0.3 µg/kg.

II. RESULTS AND DISCUSSION

A. CONCURRENT RECOVERIES

In order to assess the accuracy of the residue analyses, concurrent recoveries were determined by analysing freshly fortified samples alongside with the stored fortified samples. At all storage intervals concurrent recoveries were determined at the 10 µg/kg fortification level.

Table 7.1.2.2.1- 22: Isoflucypram, concurrent recoveries

Soil	Fortification level [µg/kg]	Recoveries (single values) [%]				Mean [%]	RSD [%]
Höfchen	10	101	102	103	103	101	2.42
		103	104	100	102		
		100	99	99	98		
		101	104	104	99		
		104	104	104	101		
		101	100	101	101		
		102	93	99	100		
Dollendorf	10	102	99	98	99	100	2.13
		101	104	105	104		
		100	100	100	99		
		100	100	100	99		
		99	104	100	100		
		101	102	99	100		
		102	101	98	95		

Table 7.1.2.2.1- 23: BCS-CN88460-carboxylic acid *(M12)*, concurrent recoveries

Soil	Fortification level [µg/kg]	Recoveries (single values) [%]				Mean [%]	RSD [%]
Höfchen	10	104	100	102	100	101	3.90
		105	99	106	107		
		103	104	104	102		
		91	96	98	105		
		102	100	100	94		
		98	97	95	101		
		106	100	104	103		
Dollendorf	10	105	100	96	100	102	5.24
		100	106	100	109		
		105	108	99	104		
		101	91	92	100		
		99	102	99	108		
		101	100	102	104		
		119	103	103	105		

B. STORAGE STABILITY

The following tables show the levels for isoflucypram and BCS-CN88460-carboxylic acid measured in the stored spiked samples of soil. In each table the recoveries determined in the stored spiked samples are uncorrected. In further columns the results are normalised to day 0. Mean values were calculated with unrounded values. Therefore minor deviations may occur when the values given in the table are used.

Table 7.1.2.2.1- 24: Recovered amounts of isoflucypram in percent of nominal added amount in soil

Soil	Day	Mean recovery Mean [%]	Standardised to day 0 [%]
Höfchen	0	101	100
	93	100	99
	194	100	99
	308	103	102
	363	103	102
	539	103	102
	720	104	103
Dollendorf	0	99	100
	93	102	103
	194	100	101
	308	104	105
	363	105	106
	539	103	104
	720	104	105

Table 7.1.2.2.1- 25: Recovered amounts of BCS-CN88460-carboxylic acid *(M12)* in percent of nominal added amount in soil

Soil	Day	Mean recovery Mean [%]	Standardised to day 0 [%]
Höfchen	0	98	100
	93	101	103
	194	102	104
	308	101	103
	363	99	101
	539	98	100
	720	104	106
Dollendorf	0	98	100
	93	104	106
	194	102	104
	308	101	103
	363	100	102
	539	99	101
	720	105	107

The recovered amounts of isoflucypram and BCS-CN88460-carboxylic acid on day 0 and after 720 days were compared to the standardized recovery rates (day 0 = 100%).
Residue results were not corrected for the concurrent recoveries at the respective day of analysis, since the mean concurrent recoveries were within a range of 70 - 110% and thus fulfilled the requested analytical guideline requirements.
At all sampling intervals on average more than 70% of the fortified amount of isoflucypram and BCS-CN88460-carboxylic acid were recovered from the stored samples (not corrected for concurrent recoveries).
The mean recovered amount in the two soils after 720 days of freezer storage ranged from 104% to 105% for isoflucypram and BCS-CN88460-carboxylic acid. Altogether, the study results demonstrate that isoflucypram and BCS-CN88460-carboxylic acid residues in soil for at least 720 days of storage under frozen conditions.

III. CONCLUSIONS

The mean recovered amount in the two soils after 720 days of freezer storage ranged from 104% to 105% for isoflucypram and BCS-CN88460-carboxylic acid. Altogether, the study results demonstrate that isoflucypram and BCS-CN88460-carboxylic acid residues are stable in soil for at least 720 days of storage under frozen conditions.

CA 7.1.2.2.2 Soil accumulation studies

Due to the use pattern and the degradation rates of isoflucypram no significant accumulation in soil would be expected.
The accumulation potential of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* after long term use was assessed in the PEC_{soil} calculation below.

Report:	KCA 7.1.2.2.2/01; [redacted], G.; [redacted], W.; 2017; M-608723-01-1
Title:	Isoflucypram (ISY): Core PECsoil EUR - Modelling core info document for soil risk assessment in Europe
Report No.:	EnSa-17-0654
Document No.:	M-608723-01-1
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive Summary

The accumulation potential of isoflucypram and its metabolite BCS-CN88460-carboxylic acid *(M12)* after long term use was also assessed based on the maximum $PEC_{soil,max}$ concentration of the compounds. The resulting soil accumulation factors (calculated as ration between $PEC_{soil, total}$ and $PEC_{soil,max}$) are 1.5 for isoflucypram and 1.03 for BCS-CN88460-carboxylic acid and are independent from the evaluated use pattern. The results are summarised in the following tables:

Table 7.1.2.2.2- 1: PEC_{soil} of isoflucypram and its major degradation product BCS-CN88460-carboxylic acid *(M12)* for the uses assessed, taking the effect of accumulation into account

Use pattern		PEC_{soil} [mg/kg]	
		Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Cereals, early 1×75 g a.s./ha	Plateauconcentration (20 cm) after year 6	0.010	0.00007
	$PEC_{accumulation}$ (PEC_{act} + PEC_{soil} plateau	0.030	0.00224
Cereals, late 1×75 g a.s./ha	Plateauconcentration (20 cm) after year 6	0.005	0.00003
	$PEC_{accumulation}$ (PEC_{act} + PEC_{soil} plateau	0.015	0.00112

Table 7.1.2.2.2- 2: Soil accumulation factor of isoflucypram and BCS-CN88460-carboxylic acid *(M12)* for the uses assessed

Use pattern	Soil accumulation factor	
	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Cereals, early 1×75 g a.s./ha	1.50	1.03
Cereals, late 1×75 g a.s./ha	1.50	1.03

I. METHODS

Application and GAP

The use of isoflucypram in cereals in Europe was assessed according to the Good Agricultural Practice (GAP) as summarised in Table 7.1.2.2.2- 3.

Table 7.1.2.2.2- 3: Application data of isoflucypram according to the use pattern in Europe

Individual crop	FOCUS crop	Rate [g a.s./ha]	Interval [days]	Plant interception [%]	BBCH stage	Amount reaching soil [g a.s./ha]
Cereals, early	cereals	1 × 75	-	80	30 - 39	1 × 15
Cereals, late	cereals	1 × 75	-	90	40 - 69	1 × 7.5

The calculations were based on the maximum intended application rate together with the maximum intended number of applications per season and (for multi-application sequences) the minimum interval between the applications. Crop interception was taken into account according to the BBCH growth stage, as recommended by FOCUS (2014)[1].

For the metabolite, the (pseudo) application rate is calculated based on the maximum amount of the metabolite observed in soil degradation studies and the molar mass correction (Table 7.1.2.2.2- 4, Table 7.1.2.2.2- 5).

Table 7.1.2.2.2- 4: Summary of properties for metabolite rate calculation

Parameter	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Molar mass [g/mol]	399.85	429.8
Correction factor	1	1.0749
Maximum occurrence in soil [%]	100	9.6

Table 7.1.2.2.2- 5: Calculation of metabolite application rates

Crop, rate	Application no.	Application rate [g/ha]	
		Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Cereals, early, 1×75 g a.s./ha	1	15	1.55
Cereals, late, 1×75 g a.s./ha	1	7.5	0.775

Calculation

All calculations and assumptions are done in accordance to the Guidance Document on Persistence of Soil (EU Commission 2000[2] and according to FOCUS (1997)[3].

Potential accumulation after long term use is assessed, based on the maximum $PEC_{soil,max}$ concentration of the respective compound.

[1] FOCUS 2014: Generic Guidance for Tier 1 FOCUS Groundwater Assessments, Version 2.2
[2] EU Commission, 2000: Guidance Document on Persistence in Soil (Working Document) 9188/VI/97 rev 8
[3] FOCUS, 1997: Soil persistence models and EU registration. Final report of the work off the Soil Modelling Work group of FOCUS

The compound and scenario input parameters as used for the calculation are given in Table 7.1.2.2.2- 6 and Table 7.1.2.2.2- 7. A plateau mixing depth of 20 cm was used.

Table 7.1.2.2.2- 6: Compound input parameters

Compound	Molar mass [g/mol]	Molar mass correction factor	Maximum occurrence in soil [%]	DT_{50} a) [days]
Isoflucypram	399.85	1	100	630
BCS-CN88460-carboxylic acid *(M12)*	429.8	1.0749	9.6	113

a) worst case DT_{50} lab, not-normalised ([redacted], G.; [redacted], W., 2017; M-608255-01-1)

Table 7.1.2.2.2- 7: Scenario input parameters

Parameter	
Soil bulk density [kg/L]	1.5
Soil mixing depth [cm]	5
Tillage depth for plateau [cm]	20

II. RESULTS AND DISCUSSION

Overview of maximum PEC_{soil} values of isoflucypram and its metabolite for all use patterns under consideration is shown in Table 7.1.2.2.2-8.

The accumulation potential of isoflucypram and its metabolite BCS-CN88460-carboxylic acid after long term use was also assessed, employing the larger soil depth for the calculation of the background concentration in cases where tillage is relevant. The results are presented in Table 7.1.2.2.2- 9 and Table 7.1.2.2.2- 10.

Table 7.1.2.2.2- 8: Maximum PEC_{soil} of isoflucypram and its metabolite for the uses assessed

Use pattern	Max. PEC_{soil} [mg/kg]	
	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Cereals, early, 1×75 g a.s./ha	0.020	0.00217
Cereals, late, 1×75 g a.s./ha	0.010	0.00109

Table 7.1.2.2.2- 9: PEC_{soil} of isoflucypram and its metabolite for the uses assessed, taking the effect of accumulation into account

Use pattern		PEC_{soil} [mg/kg]	
		Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Cereals, early, 1×75 g a.s./ha	plateau (20 cm)	0.010	0.00007
	total	0.030	0.00224
Cereals, late, 1×75 g a.s./ha	plateau (20 cm)	0.005	0.00003
	total	0.015	0.00112

Table 7.1.2.2.2- 10: Soil accumulation factor of isoflucypram and BCS-CN88460-carboxylic acid *(M12)* for the uses assessed

Use pattern	Soil accumulation factor	
	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*
Cereals, early, 1×75 g a.s./ha	1.50	1.03
Cereals, late, 1×75 g a.s./ha	1.50	1.03

CONCLUSIONS

The worst case accumulation factor calculated for the different use pattern is 1.50 for the parent isoflucypram and 1.03 for the metabolite BCS-CN88460-carboxylic acid

CA 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

The mobility of isoflucypram and the major soil metabolite BCS-CN88460-carboxylic acid *(M12)* was assessed in batch-equilibrium adsorption/desorption.
An overall summary is given in the following Modelling core info documents:

Report: KCA 7.1.3.1/01; [redacted], G.; [redacted], W.; 2017; M-608724-02-1
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): not applicable
Guideline deviation(s): none
GLP/GEP: **no**

Report: KCA 7.1.3.1/02; [redacted], G.; [redacted], W.; 2017; M-608725-02-1
Title: Isoflucypram (ISY): Core PECsw EUR - Modelling core info document for surface water risk assessment in Europe
Report No.: EnSa-17-0656
Document No.: M-608725-02-1
Guideline(s): not applicable
Guideline deviation(s): none
GLP/GEP: **no**

Executive Summary
The adsorption data of isoflucypram and its major metabolite BCS-CN88460-carboxylic acid *(M12)* which are used in the **PEC$_{gw}$** and **PEC$_{sw/sed}$** calculations are summarised in the following table:

Table 7.1.3.1- 1: Overall summary of adsorption constants $K_{oc(ads)}$ in soils of isoflucypram and its major degradation product BCS-CN88460-carboxylic acid *(M12)*

Parameter	Compound		Value in accordance with EU endpoint y/n / Reference
	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*	
K_{foc} / K_{fom} [mL/g]	1580 / 916.3 geometric mean, n=7	37.1 / 21.5 geometric mean, pH 7.5, n=2	n.a.
1/n	0.9142 arithmetic mean, n=7	0.9424 arithmetic mean, pH 7.5, n=2	n.a.

n.a. = not applicable for a new active substance submission

- **Isoflucypram**

The adsorption of isoflucypram to soil was investigated in two batch equilibrium studies with seven soils by [redacted]; [redacted]; 2014; M-499024-01-1 and [redacted]; 2015; M-518345-01-1. The K_{foc} values ranged from 1151.2 to 3594.0 mL/g, with Freundlich exponents between 0.869 and 1.015 (Table 7.1.3.1- 2). For the leaching calculations, the adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1580 mL/g (K_{fom} = 916.3 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.9142.

Table 7.1.3.1- 2: Summary of soil adsorption/desorption for isoflucypram

Soil name	Soil type	OC [%]	pH (CaCl₂)	K_f [mL/g]	K_{foc} [mL/g]	1/n	Evaluated on EU level y/n / Reference
Laacher Hof AXXa, GER	loamy sand	2.1	6.0	29.184	1389.7	0.8904	n / [redacted]; [redacted]; 2014; M-499024-01-1
Hoefchen am Hohenseh 4a, GER	silt loam	1.9	6.3	29.812	1569.1	0.8788	
Hanscheider Hof, GER	loam	2.3	5.4	32.430	1410.0	0.8972	
Dollendorf II, GER	loam	5.1	7.2	58.711	1151.2	0.8690	
Sanger, CA, USA	sandy loam	0.9	6.2	13	1394	1.0150	n / [redacted]; 2015; M-518345-01-1
Louisville, NE, USA	silt loam	1.8	6.5	25	1384	0.9101	
Lawrence, KS, USA	silty clay loam	0.34	7.5	12	3594	0.9387	
Geometric mean (n=7)					**1580**		
Arithmetic mean (n=7)						**0.9142**	
pH-dependency					**no**		

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

The adsorption of BCS-CN88460-carboxylic acid to soil was investigated in two batch equilibrium studies, one with five soils by [redacted]; 2014; M-499692-01-1 and one with four soils by [redacted]; 2017; M-589856-01-1. The K_{foc} values ranged from 28.1 to 289.8 mL/g, with Freundlich exponents between 0.8914 and 0.9604 (Table 7.1.3.1- 3).

The adsorption of BCS-CN88460-carboxylic acid to soil is pH dependent following an S shaped curve which is typical for many ionic substances with a single ionisable functional group (Figure 7.1.3.1- 1). A sigmoid correlation analysis of pH and K_{foc} values with the Input Decision Tool version 3.3 resulted into a $K_{foc\ a}$ of the dissociated BCS-CN88460-carboxylic acid of 37.7 mL/g considering an apparent pKa of 5.6 in soil. 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.
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 7.1.3.1- 5). 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 7.1.3.1- 4). 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 is in this case 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 BCS-CN88460-carboxylic acid 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).

For the leaching assessment and for the $PEC_{sw/sed}$ assessment, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.

Table 7.1.3.1- 3: Summary of soil adsorption/desorption for BCA-CN88460-carboxylic acid *(M12)*

Soil name	Soil type	OC [%]	pH ($CaCl_2$)	K_f [mL/g]	K_{foc} [mL/g]	1/n	Evaluated on EU level y/n / Reference
Wurmwiese, GER	sandy loam	1.9	7.3	2.0094	105.8	0.9297	n / [redacted]; 2014; M-499692-01-1
Höfchen am Hohenseh 4a, GER	silt loam	2.0	6.3	0.7575	37.9	0.8952	
Dollendorf II, GER	loam	4.5	7.3	1.2635	28.1	0.9243	
Guadalupe, CA, USA	sandy loam	0.7	6.7	0.2689	38.4	0.9311	
Springfield, NE, USA	silt loam	1.7	6.6	1.2018	70.7	0.9185	
Northwood, North Dakota, USA	loamy sand	0.94	4.9	2.724	289.8	0.9497	n / [redacted]; 2017; M-589856-01-1
Morris, Minnesota, USA	clay loam	2.40	7.7	1.178	49.1	0.9604	
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=9)					**75.3**		
Arithmetic mean (n=9)						**0.9219**	
pH-dependency					**yes**		

Figure 7.1.3.1- 1: K_{oc}-pH_{water} correlation for BCS-CN88460-carboxylic acid *(M12)* evaluated with Input Decision Tool version 3.3

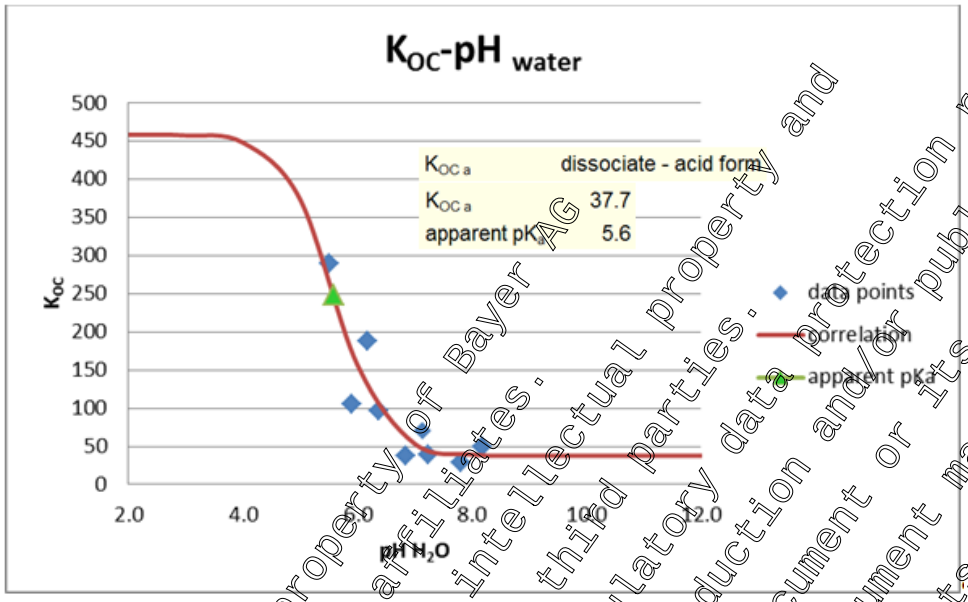

Table 7.1.3.1- 4: Soil adsorption/desorption for BCS-CN88460-carboxylic acid *(M12)* at weak acidic soil conditions (around pH 5.4)

Soil name	Soil type	OC [%]	pH ($CaCl_2$)	K_f [mL/g]	K_{foc} [mL/g]	1/n	Evaluated on EU level y/n / Reference
Wurmwiese, GER	sandy loam	1.9	5.3	2.0094	105.8	0.9297	n / [redacted]; 2014; M-499692-01-1
Northwood, North Dakota, USA	loamy sand	0.94	4.9	2.724	289.8	0.9497	n / [redacted]; 2017; M-589856-01-1
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 7.1.3.1- 5: Soil adsorption/desorption for BCS-CN88460-carboxylic acid *(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	Evaluated on EU level y/n / Reference
Dollendorf II, GER	loam	4.5	7.3	1.2635	28.1	0.9243	n / [redacted] 2014; M-499692-01-1
Morris, Minnesota, USA	clay loam	2.40	7.7	1.178	49.1	0.9604	n / [redacted] 2017; M-589856-01-1
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 1580 mL/g (K_{fom} = 916.3 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.9142.
For the leaching assessment of BCS-CN88460-carboxylic acid *(M12)*, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.

CA 7.1.3.1.1 Adsorption and desorption of the active substance

The adsorption and desorption behaviour of isoflucypram was assessed in two batch equilibrium studies using the pyrazole labelled compound.

Report: KCA 7.1.3.1.1/01; [redacted]; 2024; M-499024-01-1
Title: [Pyrazole-14C]BCS-CN88460: Adsorption/desorption on four European soils
Report No.: En8a-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): not specified
GLP/GEP: **yes**

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

Table 7.1.3.1.1- 1: 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 7.1.3.1.1- 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.

[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.

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.

2. Test Soils

The study was carried out using four different soils (see Table 7.1.3.1.1- 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 7.1.3.1.1- 3: Physico-chemical properties of test soils

Parameter	Results	
Soil designation	Lacher 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 /100 g DW] at 1/3 bar (pF 2.0) [%]	 52.5 15.6	 60.4 26.4
Bulk density (disturbed) [g/cm³]	1.22	1.09

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

cont.

Table 7.1.3.1.1- 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]	9.4	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/cm³]	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. 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), which were equilibrated for 72 hours.

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 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 7.1.3.1.1- 4.
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.
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 7.1.3.1.1- 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 item was stable under the test conditions (parental mass balance: > 103% AR). Therefore, the sorption behaviour was calculated based on the radioactivity measured in the supernatant only.
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 7.1.3.1.1- 5.

Table 7.1.3.1.1- 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 results according to the Freundlich equation is presented in Table 7.1.3.1.1- 6.

Table 7.1.3.1.1- 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, 3.4, 3.8, 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 7.1.3.1.1- 7.

Table 7.1.3.1.1- 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 7.1.3.1.1- 8.

Table 7.1.3.1.1- 8: Desorption constants of isoflucypram in soils

Soil	$K_{f(des)}$ [mL/g]	1/n	$K_{oc(des)}$ [mL/g]	r²
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.

The results are included in the summary of the adsorption and desorption behaviours of isoflucypram and its major degradation product in soil given in section CA 7.1.3.1.

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Report:	KCA 7.1.3.1.1/02; [redacted]; 2015; M-518345-01-1
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):	not specified
GLP/GEP:	**yes**

Executive Summary

The adsorption/desorption characteristics of pyrazole-labelled isoflucypram was examined using two North American soils and one North American sediment:

Table 7.1.3.1.1-9: 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. HPLC analysis of the adsorption aqueous fraction showed no degradation in all test systems. 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 showed that isoflucypram was stable at the 24-hour time point.
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 7.1.3.1.1- 10: 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 7.1.3.1.1- 11: 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 7.1.3.1.1- 12: Physico-chemical properties of test soils and sediment

Parameter	Results		
Soil	EFS-487 (soil)	EFS-488 (soil)	EFS-511 (sediment)
Geographic location City State Country	 Sanger California USA	 Louisville Nebraska USA	 Lawrence Kansas USA
Latitude and longitude	N 36.70232 W 119.46355	N 41.03654 W 096.14983	39.0471331, -95.1965618
Soil taxonomic classification (USDA)	no information available		
Soil series	no information available		
Textural class (USDA) Sand [%] Silt [%] Clay [%]	sandy loam 68.5 28.4 3.1	silt loam 15.5 63.0 21.5	silty clay loam 6.8 63.5 29.7
pH - in 0.01 M $CaCl_2$ (1:1) - 1:1 soil:water ration - in saturated paste	6.2 6.7 6.6	6.5 7.0 6.8	7.5 7.9 7.7
Organic carbon [% OC] Organic matter [% OM]	0.90 1.5	1.8 3.2	0.34 0.58
Cation exchange capacity [meq/100 g]	6.7	16.6	20.1
Water holding capacity (gm/100 gm) % moisture at 1/10 bar % moisture at 2.0 pF units % moisture at 1/3 bar % moisture at 2.5 pF units % moisture at 15 bar	27.6 23.4 10.9 4.1	64.4 38.6 38.6 27.8 27.8 17.7	51.7 44.1 34.9 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.

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.

Table 7.1.3.1.1- 13: 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 7.1.3.1.1- 14: 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 for each soil can be found in the table below. 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.

Table 7.1.3.1.1- 15: Adsorption 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)	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		

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 7.1.3.1.1- 16: Average percent desorbed of isoflucypramon 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 7.1.3.1.1- 17: 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 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).
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.

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.

The results are included in the summary of the adsorption and desorption behaviours of isoflucypram and its major degradation product in soil given in section CA 7.1.3.1.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

The adsorption and desorption behaviour of the major soil degradation product BCS-CN88460-carboxylic acid *(M12)* was assessed in two batch equilibrium studies using the pyrazolyl-labelled compound.

Report:	KCA 7.1.3.1.2/01; [redacted]; 2014; M-499692-01-1
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):	not specified
GLP/GEP:	**yes**

Executive Summary

The adsorption behaviour of BCS-CN88460-carboxylic acid *(M12)* was studied in five soils in batch equilibrium experiments in the dark at 20.2°C.

Table 7.1.3.1.2- 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 BCS-CN88460-carboxylic acid *(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 and Guadalupe, CA 1:1.
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 BCS-CN88460-carboxylic acid 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 BCS-CN88460-carboxylic acid 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:

Table 7.1.3.1.2- 2: Summary of the adsorption data of BCS-CN88460-carboxylic acid *(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 BCS-CN88460-carboxylic acid 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

[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.

Reference item

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

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 7.1.3.1.2- 3: Physico-chemical properties of test soils

Parameter	Results		
Soil designation	Wurmwiese	Hoefchen am Hohensen 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° 04.814' 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 6.7 6.8	6.6 7.2	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 Hohensch 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 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 mg/L. 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 Hohensch 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 ≥ 16 hours, 2 mL of the respective application solution were spiked in.
The batches were equilibrated. Following the determined shaking period, 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. 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 7.1.3.1.2- 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 7.1.3.1.2- 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 7.1.3.1.2- 5.

Table 7.1.3.1.2- 5: Concentration of BCS-CN88460-carboxylic acid in the solid and liquid phases at the end of adsorption period (mean of duplicates)

Concentration [mg/L]	Soil [mg/kg]	Solution [mg/L]	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 BCS-CN88460-carboxylic acid *(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 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 7.1.3.1.2- 6.

Table 7.1.3.1.2- 6: Adsorption constants of BCS-CN88460-carboxylic aicd 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 7.1.3.1.2- 7: Concentration of BCS-CN88460-carboxylic acid 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	0.082	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 7.1.3.1.2- 8.

Table 7.1.3.1.2- 8: Desorption constants of BCS-CN88460-carboxylic acid 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

The adsorption coefficients K_{fads} of BCS-CN88460-carboxylic acid *(M12)* in five test soils were determined 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 BCS-CN88460-carboxylic acid were found to be in the same range as the respective adsorption coefficients (30.6 mL/g – 116.3 mL/g).

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 [BCS-CN88460-carboxylic acid can be classified as mobile to intermediate mobile in the tested soils.

The results are included in the summary of the adsorption and desorption behaviours of isoflucypram and its major degradation product in soil given in section CA 7.1.3.1.

Report:	KCA 7.1.3.1.2/02; [redacted]; 2017; M-589856-01-1
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 US EPA Fate, Transport and Transformation Test Guidelines, OCSPP 835.1230, Adsorption/Desorption (Batch Equilibrium), 2008
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The adsorption behaviour of BCS-CN88460-carboxylic acid *(M12)* was studied in four US agricultural soils in batch equilibrium experiments in the dark at 20°C:

Table 7.1.3.1.2- 9: 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 pretest 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, 1:2 for MMN soil, 1:1 for SCA soil, and 1:2 for SKS soil were used. The nominal test concentrations of BCS-CN88460-carboxylic acid 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 behavior 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 BCS-CN88460-carboxylic acid, 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 BCS-CN88460-carboxylic acid can be classified as 'Low' for two soils (END and SCA), 'Intermediate' for one soil (SKS) and 'Mobile' for one soil (MMN).

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 BCS-CN88460-carboxylic acid:

Table 7.1.3.1.2- 10: Summary of the adsorption/desorption data of BCS-CN88460-carboxylic acid *(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

[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.

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 7.1.3.1.2- 11: 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 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 ± 2.9% AR, which demonstrates the effectiveness of the extraction method. The recovery of radioactivity for all soils is presented in the following table.

Table 7.1.3.1.2- 12: Recovery of radioactivity
(as percentage of applied radioactivity, mean of two replicates)

Concentration [µg/mL]	Soil			
	END	MMN	SCA	SKS
1.07	96.35	96.4	96.3	96.2
0.29	97.9	98.2	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 7.1.3.1.2- 13: Concentration of BCS-CN88460-carboxylic acid in the solid and liquid phases at the end of adsorption period

Concentration [mg/L]	Soil [mg/kg]	Solution [mg/L]	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 BCS-CN88460-carboxylic acid *(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 BCS-CN88460-carboxylic acid *(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 7.1.3.1.2- 14: Adsorption constants of BCS-CN88460-carboxylic acid 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 7.1.3.1.2- 15: Concentration of BCS-CN88460-carboxylic acid 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 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 7.1.3.1.2- 16: Desorption constants of BCS-CN88460-carboxylic aicd in soils

Soil	$K_{f(des)}$ [mL/g]	1/n	$K_{oc(des)}$ [mL/g]	r²
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 BCS-CN88460-carboxylic acid *(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} 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 BCS-CN88460-carboxylic acid 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.

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, BCS-CN88460-carboxylic acid can be classified as mobile to low mobility for adsorption, and once sorbed will remain strongly sorbed.

The results are included in the summary of the adsorption and desorption behaviours of isoflucypram and its major degradation product in soil given in section CA 7.1.3.1.

CA 7.1.3.2 Aged sorption

Studies are not required under Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009.

CA 7.1.4 Mobility in soil

The plant uptake factor and the transpiration stream concentration factor of isoflucypram in wheat were determined – default value and Briggs estimate (KCA 7.1.4/01).
In addition studies on the plant uptake factor and the transpiration stream concentration factor of isoflucypram and BCS-CN88460-carboxylic-acid *(M12)* were performed (KCA 7.1.4/02 and KCA 7.1.1.1/03, respectively).

Report:	KCA 7.1.4/01; [redacted], G.; [redacted], W.; 2017; M-608724-02-1
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**

Executive Summary

The plant uptake factors of isoflucypram and its major metabolite BCS-CN88460-carboxylic acid *(M12)* which are used in the **PEC$_{gw}$** calculations are summarised in the following table.

Table 7.1.4- 1: Overall summary of the plant uptake factors of isoflucypram and its major degradation product BCS-CN88460-carboxylic acid *(M12)*

Parameter	Compound		Value in accordance with EU endpoint y/n / Reference
	Isoflucypram	BCS-CN88460-carboxylic acid *(M12)*	
Plant uptake factore	Default: 0 (Tier 1a, Tier 2) Briggs estimate: 0.10 (Tier 1b)	Default: 0	n.a.

n.a. = not applicable for a new active substance submission

Plant uptake factor (PUF/TSCF)

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 7.1.4- 2). This is also in line with the approach recommended by FOCUS (2014). It is also possible to consider experimentally determined TSCF.

[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*

Table 7.1.4- 2: Plant uptake factors for isoflucypram and its metabolite 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	[redacted]; [redacted]; 2014; M-484636-01-1 (summarised in MCA section 2; CA 2.7)
BCS-CN88460-carboxylic acid	at 23°C: 2.11 at pH 5 0.22 at pH 7 -1.1 at pH 9	0.75 0.29 0.03	[redacted]; [redacted]; 2015; M-519996-01-1 (summarised in MCA section 2; CA 2.7)

a) Used for estimation of TSCF

The Briggs estimation leads for isoflucypram to a TSCF of 0.10 (Table 7.1.4- 2). The plant uptake of isoflucypram has been also demonstrated experimentally ([redacted] 2017; M-587420-02-1). The experimental mean TSCF is 0.14. Consequently, the Briggs estimated value of 0.10 was used as refined input for the leaching assessment.
The Briggs estimated TSCF of BCS-CN88460-carboxylic acid is pH dependent. Thus, the default PUF of zero was considered as input for the leaching assessment.

Tiered approach
For PEC_{gw} assessments a tiered approach concerning the DT_{50} values of isoflucypram and its metabolite BCS-CN88460-carboxylic acid, the formation fraction of BCS-CN88460-carboxylic acid, and the PUF values should be considered. In Tier 1 only laboratory data are considered for DT_{50} values and formation fractions, which can be modified by the PUF values. Field data are included in Tier 2 where a further modification by PUF values is not possible. A detailed description is presented in Table 7.1.4- 3.

Table 7.1.4- 3: Tiered approach for isoflucypram and its metabolite used for modelling

Compound	Tier 1a			Tier 1b			Tier 2		
	DT_{50}[a)] [days]	ff[a)]	PUF[d)]	DT_{50}[a)] [days]	ff[a)]	PUF[e)]	DT_{50}[b)] [days]	ff[c)]	PUF[d)]
Isoflucypram	314	n.a.	0.0	314	n.a.	**0.1**	323	n.a.	0.0
BCS-CN88460-carboxylic acid	34.4	0.345	0.0	34.4	0.345	**0.0**	84.1	0.043	0.0

a) From laboratory data
b) From laboratory and field data
c) From field data
d) PUF representing worst case default
e) PUF based on Briggs equation

CONCLUSIONS

The Briggs estimation leads for isoflucypram to a TSCF of 0.10. The experimental plant uptake factor mean TSCF is 0.14. Consequently, the Briggs estimated value of **0.10** was used as refined input for the leaching assessment.
The Briggs estimated TSCF of BCS-CN88460-carboxylic acid *(M12)* is pH dependent. Thus, the default PUF of **zero** was considered as input for the leaching assessment.

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- **Isoflucypram**

Report:	KCA 7.1.4/02; [redacted]; 2017; M-587420-02-1
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
Guideline(s):	Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC The OECD Principles of Good Laboratory Practice (OECD 1998) and respective national regulations
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

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. 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).

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. About 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 below).

Table 7.1.4- 4: 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 poured with water on Perlite substrate before exposed to constant illumination using LED lights and pouring with nutrient solution (NS). The characteristics of the Perlite substrate, illumination and the composition of untreated nutrient solution are given in the table below.

Table 7.1.4- 5: Test conditions

Perlite characteristics		Illumination caracteristics		Nutrient solution
Granule size d [mm]	Mass ρ_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 (300 mL) 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 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 were conducted at 0 (t_{start}), 2 (t_0), 4 (t_1) and 10 days (t_{end}) after treatment. Samplings were performed at t_1 and t_{end}. 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 glas 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, radioactivity formerly attached to the root surfaces was quantified after external treatment with ACN/H_2O (4/1; *v/v*). 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.

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, 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 100.9% AR) and 92.2% AR for t_{end} (range from 88.7 to 95.4% AR) (see Table 7.1.4- 6). 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 7.1.4- 7).

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 7.1.4- 8).

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 7.1.4- 9).

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 7.1.4- 6).

Table 7.1.4- 6: Mass balance (in percentage of applied)

	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 7.1.4- 7: Water uptake

Sample	sample ID	V t_{start} initial [mL]	V t_{end} initial [mL]	ΔV $t_{start} \rightarrow t_{end}$ [mL]	net ΔV $t_{start} \rightarrow t_{end}$ [mL]	ΔV $t_{start} \rightarrow t_{end}$ [%]
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

Table 7.1.4- 8: 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 7.1.4- 9: 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 7.1.4- 10).

Table 7.1.4- 10: 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

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 7.1.4- 11).

Table 7.1.4- 11: 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 good plant health indicated by intense biomass increase and water consumption throughout the testing period demonstrated a reliable and robust test system for the determination of the Plant Uptake Factor (PUF) and the Transpiration Stream Concentration Factor (TSCF) of the test item. This was supported by a pH of the test solutions between 6.49 and 6.96 for the treated test systems and continuous aerobic conditions during the experimental period. Deviations between the values of individual test replicates were low indicated by an overall coefficient of variation of 21.94% (PUF) and 14.25% (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 92.2%.

The mean PUF for pyrazole-labelled isoflucypram in wheat plants 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. For all plants analysed, more than 37.9% of the test item taken up by the roots was allocated to the shoots.

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

Report:	KCA 7.1.4/03; [redacted]; 2017; M-588284-02-1
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):	Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC The OECD Principles of Good Laboratory Practice (OECD 1998) and respective national regulations
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

The uptake of pyrazolyl-labelled BCS-CN88460-carboxylic acid *(M12)* 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).
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 BCS-CN88460-carboxylic acid 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.

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 BCS-CN88460-carboxylic acid 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 below).

Table 7.1.4- 12: PUF and TSCF values of BCS-CN88460-carboxylic acid 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 and early growth, plants were poured with water on Perlite substrate before exposed to constant illumination using LED lights and pouring with nutrient solution (NS). The characteristics of the Perlite substrate, illumination and the composition of untreated nutrient solution are given in the table below.

Table 7.1.4- 13: 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 (300 mL) 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 BCS-CN88460-carboxylic acid 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 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, radioactivity formerly attached to the root surfaces was quantified after external treatment with ACN/H_2O (4/1; *v/v*). 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.

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 7.1.4- 14). 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 7.1.4- 15).
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 BCS-CN88460-carboxylic acid 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 7.1.4- 16).
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 7.1.4- 17).
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 7.1.4- 14).

Table 7.1.4- 14: Mass balance (in percentage of applied)

	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 7.1.4- 15: 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

Table 7.1.4- 16: Concentrations of BCS-CN88460-carboxylic acid *(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 7.1.4- 17: Concentrations of BCS-CN88460-carboxylic acid *(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	0.126	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 BCS-CN88460-carboxylic acid 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 7.1.4- 18).

Table 7.1.4- 18: Plant uptake factor (PUF) for BCS-CN88460-carboxylic acid *(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 BCS-CN88460-carboxylic acid 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 7.1.4- 19).
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 7.1.4- 19: Transpiration stream concentration factor (TSCF) for BCS-CN88460-carboxylic acid *(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 good plant health indicated by intense biomass increase and water consumption throughout the testing period demonstrated a reliable and robust test system for the determination of the Plant Uptake Factor (PUF) and the Transpiration Stream Concentration Factor (TSCF) of the test item. This was supported by a pH of the test solutions between 6.53 and 6.99 for the treated test systems and continuous aerobic conditions during the experimental period. 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 for pyrazolyl-labelled BCS-CN88460-carboxylic acid 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.

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 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 CA 7.1.3.1.1.

CA 7.1.4.1.2 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 CA 7.1.3.1.2.

CA 7.1.4.2 Lysimeter studies

The leaching behaviour of isoflucypram and its major soil metabolite BCS-CN88460-carboxylic acid *(M12)* are addressed by standard Focus groundwater modelling. Therefore, lysimeter studies were not conducted.

CA 7.1.4.3 Field leaching studies

Field leaching studies have not been conducted for the active substance as sufficient information can be derived from the existing studies.

CA 7.2 Fate and behaviour in water and sediment

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.
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: BCS-CN88460-carboxylic acid *(M12)* with a maximum occurrence in the total system of 6.6% AR (water layer 5.4%; sediment 1.3%, respectively). 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 7.2- 1.

A summary of maximum occurrences of the major metabolite BCS-CN88460-carboxylic acid *(M12)*, CO_2 and non-extractable residues in aquatic systems is given in Table 7.2- 1.

Summaries of half-lives for trigger evaluation and modelling purpose are shown in Table 7.2- 2 and Table 7.2- 3, respectively.

Table 7.2- 1: Summary of maximum occurrences of the major metabolite BCS-CN88460-carboxylic acid *(M12)*, carbon dioxide and non-extractable residues in aquatic systems (in percent of applied radioactivity)

Compound	Hydrolysis [%]	Photolysis [%]	Aerobic mineralisation [%]	Water-sediment, aerobic [%]	
				water layer	sediment
BCS-CN88460-carboxylic acid *(M12)*	-	-	-	5.4	1.3
Carbon dioxide	a)	0.1	0.9	0.3	
Non-extractable residues				6.4	

a) Carbon dioxide and volatile organic compounds were not collected

Table 7.2- 2: Summary of DT_{50} values in aquatic studies of isoflucypram and its major aquatic metabolite BCS-CN88460-carboxylic acid *(M12)* for trigger evaluation

Compound		DT_{50} [days] range	arith. mean	geo. mean
Hydrolysis				
Isoflucypram		Isoflucypram was stable at all pH values, therefore no DT_{50} and DT_{90} values were calculated		
Aquatic photolysis study				
Isoflucypram		150		
	environmental days	*484 (Phoenix) 750 (Athens)*		
BCS-CN88460-carboxylic acid *(M12)*		-		
	environmental days			
Aerobic mineralisation in surface water (pelagic test)				
Isoflucypram	low concentration	> 1000		
	high concentration	> 1000		
BCS-CN88460-carboxylic acid *(M12)*		-		
Water-sediment study, aerobic				
Isoflucypram	water	2.79 – 2.06		2.40
	sediment	282 – n.r.		282
	total system	211 – 593		354
BCS-CN88460-carboxylic acid *(M12)*	water	n.e.		n.e.
	sediment	n.e.		n.e.
	total system	n.r.		n.r.

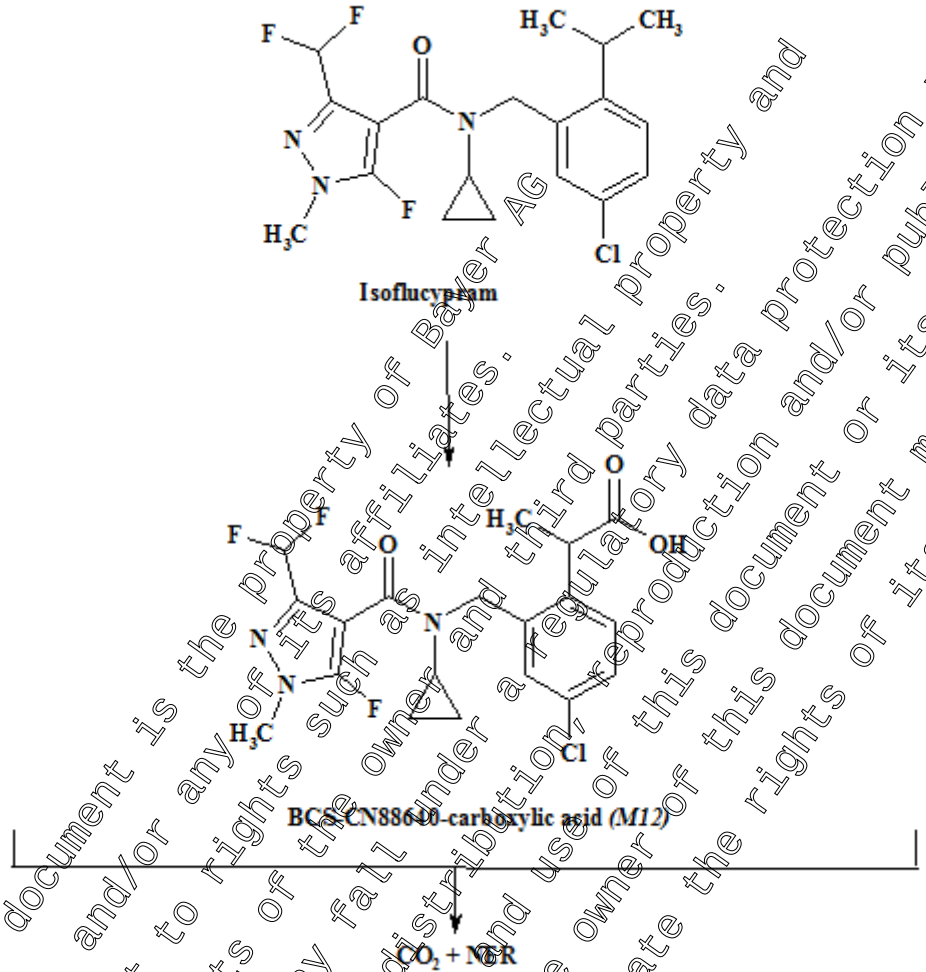
n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
n.e. = Not evaluable, not sufficient data points

Table 7.2- 3: Summary of input parameters of isoflucypram and its major soil metabolite BCS-CN88460-carboxylic acid *(M12)* in water and sediment for modelling purpose (PEC_{sw})

Compound	Risk assessment	Compartment	DT_{50} [days]	remark	Max. occurrence observed[c]
Isoflucypram	PEC_{sw} Step 1	total system	354	geo. mean of total system	100
	Step 2	water	354	geo. mean of total system	100
		sediment	354	geo. mean of total system	83.0
	Step 3	water	354[a)]	geo. mean of total system	100
			1000[b)]	default value	
		sediment	354[a)]	geo. mean of total system	83.0
			1000[b)]	default value	
BCS-CN88460-carboxylic acid *(M12)*	Step 1 & 2	water	1000	default value	5.4
		sediment	1000	default value	1.3
		total system	1000	default value	6.6

According to FOCUS (2015) for substances with a K_{oc} between 100 and 2000 mL/g two options should be tested:
a) $DegT_{50}$system used for degradation in water, default DT_{50} of 1000 days used for degradation in sediment
b) $DegT_{50}$system used for degradation in sediment, default DT_{50} of 1000 days used for degradation in water
c) % molar basis with respect to parent

Figure 7.2- 1: Proposed degradation of isoflucypram in aerobic aquatic water/sediment systems



CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

Statement on water treatment processes

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.

CA 7.2.1.1 Hydrolytic degradation

Report:	KCA 7.2.1.1/01; [redacted]; [redacted]; 2015; M-510623-01-1
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**

Executive 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 7.2.1.1- 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 nitrogen through the buffer solutions.
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 7.2.1.1- 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 7.2.1.1- 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 7.2.1.1- 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 7.2.1.1- 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 7.2.1.1- 4.

The hydrolytic route of degradation of isoflucypram at three pH values is summarised in Table 7.2.1.1- 5 (mean values).

Table 7.2.1.1- 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 7.2.1.1- 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 7.2.1.1- 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. No degradation products of isoflucypram were observed.

Hydrolytic degradation is unlikely to contribute to the degradation of isoflucypram under typical conditions of the environment.

CA 7.2.1.2 Direct photochemical degradation

Report:	KCA 7.2.1.2/01; [redacted]; 2013; M-461939-01-1
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):	not specified
GLP/GEP:	**yes**

Executive Summary

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 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 estimates based on the two modelling concepts (Zepp & Cline, Frank & Kloepffer) were well 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 7.2.1.2- 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 7.2.1.2- 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 x 10^{-7}	55	120	530
February	0.144 x 10^{-6}	27	56	240
March	0.283 x 10^{-6}	15	28	120
April	0.475x 10^{-6}	**9.4**	17	68
May	0.605 x 10^{-6}	**8.3**	13	53
June	0.678 x 10^{-6}	**7.9**	12	47
July	0.604x 10^{-6}	**8.8**	13	44
August	0.590 x 10^{-6}	**9.1**	14	45
September	0.342x 10^{-6}	**14**	23	87
October	0.188x 10^{-6}	**22**	43	190
November	0.831 x 10^{-7}	42	97	482
December	0.436 x 10^{-7}	84	180	920

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50th 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.

Reference item:

Non-labelled isoflucypram
Certificate-ID: AZ 18080
Batch code: BCS-CN88460-01-02
Certified assay: 98.4% w/w (^{1}H-NMR spectroscopy)

2. Test Systems

The study was carried out using three different buffer solutions of pH 4.0, pH 7.0 and pH 9.0.

Table 7.2.1.2- 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 25°C ± 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-radiodection during the maximum irradiation period of 500 minutes. For respective data see Table 7.2.1.2- 4, for evaluation of data see Table 7.2.1.2- 5.

Table 7.2.1.2- 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 7.2.1.2- 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 Φ = 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 7.2.1.2- 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 Φ = 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 7.2.1.2- 7: Frank & Klöpfer modelling

Month	Photolysis constant [1/sec] pH 7	Environmental DT50 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. 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.
A comparison of the estimates derived from models of Zepp & Cline and Frank & Kloepffer shows that both approaches are well 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. This assessment does not consider other potential mechanisms which may enhance the degradation in natural water, e.g. by indirect photolytic processes.

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Report:	KCA 7.2.1.2/02; [redacted]; [redacted]; 2015; M-510627-01-1
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**

Executive 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 7.2.1.2- 8: 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. 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. 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.

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/m², 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 7.2.1.2- 9).
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 7.2.1.2- 9: 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 7.2.1.2- 10.
The route of degradation of isoflucypram in aqueous buffer solution at pH 7 under aerobic irradiated and dark conditions is summarised in Table 7.2.1.2- 11.

Table 7.2.1.2- 10: 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 7.2.1.2- 11).

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 7.2.1.2- 11). No degradation products of isoflucypram > 10% AR were observed.

Table 7.2.1.2- 11: 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 7.2.1.2- 12: 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).
Photodegradation is unlikely to contribute to the degradation of isoflucypram under typical light conditions of the environment.

CA 7.2.1.3 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.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "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 CA 7.2.2.3.

CA 7.2.2.2 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 CA 7.2 and Figure 7.2- 1.

Report:	KCA 7.2.2.2/01; [redacted]; [redacted]; 2017; M-582106-01-1
Title:	[Pyrazole-4-14C]BCS-CN88460: Aerobic mineralization in surface water
Report No.:	MEL-NN017
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**

Executive 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 101.7% of the applied radioactivity (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).
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 7.2.2.2- 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.

Phenyl-UL-^{14}C- and carboxyl-^{14}C-labelled benzoic acid
Sample ID: C-1189
Specific activity: 125.00 mCi/mMole
Radiochemical purity: 100%

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 7.2.2.2- 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.

[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 7.2.2.2- 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 7.2.2.2- 4 and Table 7.2.2.2- 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 7.2.2.2- 6 and Table 7.2.2.2- 7. No degradation products were observed in the surface water.

Table 7.2.2.2- 4: Material balance of radioactivity in surface water under aerobic conditions at low 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.	0.6	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 7.2.2.2- 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 7.2.2.2- 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 7.2.2.2- 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 7.2.2.2- 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).

In total, isoflucypram does not degrade in surface water under aerobic conditions.

CA 7.2.2.3 Water/sediment study

The route and rate of degradation of isoflucypram in water/sediment systems under aerobic conditions were investigated using the pyrazole-label. A summary of the route and rate of degradation of isoflucypram in water and sediment is given in section CA 7.2 and Figure 7.2- 1.
A summary of the data of the water/sediment study of [redacted]; [redacted]; 2017; M-580411-01-1 which are used in $PEC_{sw/sed}$ calculations is given in the following Modelling core info document:

- **PEC_{sw} Modelling Core Info**

Report:	KCA 7.2.2.3/01; [redacted], G.; [redacted], W.; 2017; M-608725-02-1
Title:	Isoflucypram (ISY): Core PEC_{sw} EUR - Modelling core info document for surface water risk assessment in Europe
Report No.:	EnSa-17-0656
Document No.:	M-608725-02-1
Guideline(s):	not applicable
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive Summary

The input parameters of the water/sediment study of [redacted]; [redacted]; 2017; M-580411-01-1 which are used in the **$PEC_{sw/sed}$** calculations are summarised in the following table:

Table 7.2.2.3- 1: Input parameters related to active substance isoflucypram and metabolite for $PEC_{sw/sed}$ calculations

Parameter	Compound		Value in accordance with EU endpoint y/n / Reference
	Isoflucypram	**BCS-CN88460-carboxylic acid *(M12)***	
DT_{50} in water [days]	354 (Step 2) 354[a]/1000[b] (Step 3)	1000	n.a.
DT_{50} in sediment [days]	354 (Step 2) 354[a]/1000[b] (Step 3)	1000	n.a.
DT_{50} in total system [days]	354 (Step 1)	1000	n.a.
Maximum occurrence observed (% molar basis with respect to parent)	water: 100 sediment: 83.0 total system: 100	water: 5.4 sediment: 1.3 total system: 6.6	n.a.

According to FOCUS (2015) for substances with a K_{oc} between 100 and 2000 mL/g two options should be tested:
a) $DegT_{50}$system used for degradation in water, default DT_{50} of 1000 days used for degradation in sediment
b) $DegT_{50}$system used for degradation in sediment, default DT_{50} of 1000 days used for degradation in water
n.a. = not applicable for a new active substance submission

Degradation in aquatic system

- **Isoflucypram**

The degradation and dissipation behaviour of isoflucypram was characterised by data from a laboratory aerobic water-sediment study by [redacted]; [redacted]; 2017; M-580411-01-1 including two water-sediment systems. The kinetic evaluation was conducted by [redacted], G.; [redacted], W.; 2017; M-608356-02-1 according to FOCUS (2006, 2014). Resulting simple first order DT_{50} values are shown in Table 7.2.2.3- 2.

The geometric mean total system degradation DT_{50} of 354 days for isoflucypram was used for all phases in the Steps 1-2 calculations. For FOCUS Step 3 degradation DT_{50} for the single phases are required. Since these are not available the geometric mean total system DT_{50} of 354 days was taken into account. According to FOCUS (2015), for substances with a K_{foc} value between 100 and 2000 mL/g such as isoflucypram two options have to be tested to derive the worst case PEC_{sw} values:

1. The geometric mean total system DT_{50} of 354 days was used for the water phase and a default DT_{50} value of 1000 days was used for the sediment phase.
2. The geometric mean total system DT_{50} of 354 days was used for the sediment phase and a default DT_{50} value of 1000 days was used for the water phase.

Table 7.2.2.3- 2: Summary of degradation in water / sediment of the active substance isoflucypram

Isoflucypram Water / sediment system	Distribution: Max. in sediment 83.0%) after 51 d (Wiehltalsperre) pH water / sed	$DegT_{50}$ whole sys. [days]	$DegT_{90}$ whole sys. [days]	Kinetic fit	$DisT_{50}$ water [days]	$DisT_{90}$ water [days]	Kinetic fit	$DisT_{50}$ sed [days]	$DisT_{90}$ sed [days]	Kinetic fit	Evaluated on EU level y/n / Reference
Anglersee, sand[a)]	7.1 / 6.6	211	702	SFO	22.3	74.2	FOMC recalc	282	938	SFO	n / [redacted], G.; [redacted]
Wiehltalsperre, loam[a)]	7.3 / 5.1	593	> 1000	SFO	11.9	39.6	FOMC recalc	n.r.	n.r.	-	[redacted], W.; 2017; M-608356-02-1
Geometric mean at 20°C		**354**			16.3			282			

n.r. Not fully reliable, mathematically not significantly different from 0, not usable
a) [redacted]; [redacted]; 2017; M-580411-01-1

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

The degradation and dissipation behaviour of BCS-CN88460-carboxylic acid was investigated in the laboratory aerobic water-sediment study with the parent substance isoflucypram ([redacted]; [redacted]; 2017; M-580411-01-1). The kinetic evaluation was conducted by [redacted], G.; [redacted], W.; 2017; M-608356-02-1 according to FOCUS (2006, 2014). However, due to its limited formation in aquatic systems, no reliable degradation half-lives for BCS-CN88460-carboxylic acid could be derived. Consequently, the DT_{50} in water, sediment, and total system was set to a conservative value of 1000 days for the use in FOCUS Steps 1-2 calculations.

The maximum occurrence of BCS-CN88460-carboxylic acid in water/sediment systems was 6.6% AR observed after 100 days in the system Anglersee ([redacted]; [redacted]; 2017; M-580411-01-1).

CONCLUSIONS

The geometric mean total water/sediment system degradation DT_{50} of 354 days for isoflucypram was used for all phases in the Steps 1-2 calculations. For FOCUS Step 3 degradation DT_{50} for the single phases are required. Since these are not available the geometric mean total system DT_{50} of 354 days was taken into account. According to FOCUS (2015), for substances with a K_{foc} value between 100 and 2000 mL/g such as isoflucypram two options have to be tested to derive the worst case PEC_{sw} values:

1. The geometric mean total system DT_{50} of 354 days was used for the water phase and a default DT_{50} value of 1000 days was used for the sediment phase.
2. The geometric mean total system DT_{50} of 354 days was used for the sediment phase and a default DT_{50} value of 1000 days was used for the water phase.

For BCS-CN88460-carboxylic acid *(M12)* no reliable degradation half-lives could derive from the aerobic water-sediment study with the parent isoflucypram, due to limited formation in aquatic systems. Therefore, the DT_{50} in water, sediment, and total system was set to a conservative value of 1000 days for the use in FOCUS Steps 1-2 calculations. The maximum occurrence of BCS-CN88460-carboxylic acid in water/sediment systems was 6.6%.

***

- **Study water/sediment, aerobic**

Report:	KCA 7.2.2.3/02; [redacted]; [redacted]; 2017; M-580411-01-1
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 US EPA OCSPP Test Guideline No. 835.4300 / 835.4400
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive 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:

Table 7.2.2.3- 3: Water-sediment systems used

Water-sediment system	System ID	Source	Texture[a)] (USDA)	pH water[b)]	pH sediment[c)]	TOC water[d)] [mg/L]	TOC 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.

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 BCS-CN88460-carboxylic acid *(M12)* with a maximum occurrence of 6.6% AR at DAT-100 in system Anglersee (see Table 7.2.2.3- 4). 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 7.2.2.3- 4).

Table 7.2.2.3- 4: Identified degradation product (maximum occurrence in total system)

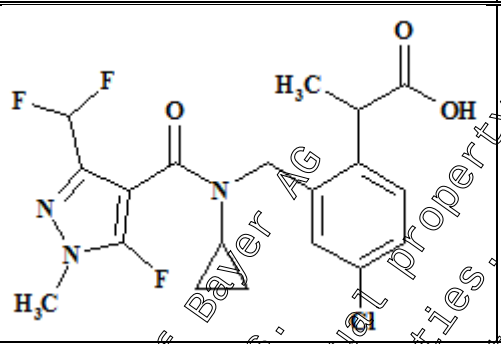
Compound	Chemical structure	Maximum occurrence in total system [%]
BCS-CN88460-carboxylic acid *(M12)*		6.6

Table 7.2.2.3- 5: 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]	Chi² error [%]	Visual assessment[b)]
Anglersee	water layer	FOMC	29	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 7.2.2.3- 6. The sediment microbial activity was determined at start of equilibration as well as start and end of the study (Table 7.2.2.3- 7).

Table 7.2.2.3- 6: 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 7.2.2.3- 7: 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.
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.
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.

4. Determination of Degradation Kinetics

The degradation kinetics of the test item was determined according to FOCUS kinetics (2006[1]) using the software KinGUI 2. Model input datasets were the residual amounts found in each replicate test system at each sampling interval. The initial recovery (material balance) at DAT-0 was included in the parameter optimization procedure, but for optimal goodness of fit, the value was allowed to be estimated by the model. DT_{50} and DT_{90} values were calculated from the resulting kinetic parameters.

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

[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.

+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 clearly positive values for the redox potentials and the oxygen contents indicate 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 7.2.2.3- 8 and Table 7.2.2.3- 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 7.2.2.3- 8 and Table 7.2.2.3- 9.
The route of degradation of isoflucypram in Anglersee and Wiehltalsperre water-sediment systems under aerobic conditions is summarised in Table 7.2.2.3- 8 and Table 7.2.2.3- 9. The proposed degradation pathway is presented in Figure 7.2.2.3- 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 7.2.2.3- 8 and Table 7.2.2.3- 9).

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 BCS-CN88460-carboxylic acid *(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 7.2.2.3- 8 and Table 7.2.2.3- 9).

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 BCS-CN88460-carboxylic acid *(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 7.2.2.3- 8 and Table 7.2.2.3- 9).

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 BCS-CN88460-carboxylic acid *(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 7.2.2.3- 8 and Table 7.2.2.3- 9).

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 7.2.2.3- 8 and Table 7.2.2.3- 9).

Table 7.2.2.3- 8: 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	82.3	40.4	32.4	24.9	14.3	12.5	10.3	8.2
	sediment	15.2	51.9	60.8	68.7	76.2	65.3	65.7	63.2
	entire system[d)]	97.5	92.3	93.1	93.6	90.6	77.8	76.0	71.4
BCS-CN88460-carboxylic acid *(M12)*	water	n.d.	n.d.	n.d.	n.d.	1.9	2.4	3.2	5.4
	sediment	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	1.2	1.3
	entire system[d)]	n.d.	n.d.	n.d.	n.d.	1.9	3.0	4.4	6.6
Sum of unid./diff. residues[a)]	water	n.d.	n.d.	< LOD	n.d.	< LOD	5.7	5.6	7.1
	sediment	n.d.	n.d.	< LOD	n.d.	n.d.	2.0	2.3	5.3
	entire system[d)]	n.d.	n.d.	< LOD	n.d.	< LOD	7.7	7.9	12.4
Total extractable residues[b)]	water	82.3	40.4	32.4	24.9	16.8	20.7	19.1	20.6
	sediment	15.2	51.9	60.8	68.7	76.2	67.8	69.2	69.8
	entire system[d)]	97.5	92.3	93.1	93.6	93.0	88.5	88.3	90.4
Carbon dioxide[c)]		n.a.	0.1	0.1	< 0.1	< 0.1	0.3	0.1	0.1
Volatile organic compounds[c)]		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
Non-extractable residues[c)]		0.4	0.9	1.5	1.9	2.8	3.6	4.6	6.4
Total recovery[b)]		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, SD: standard deviation,
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

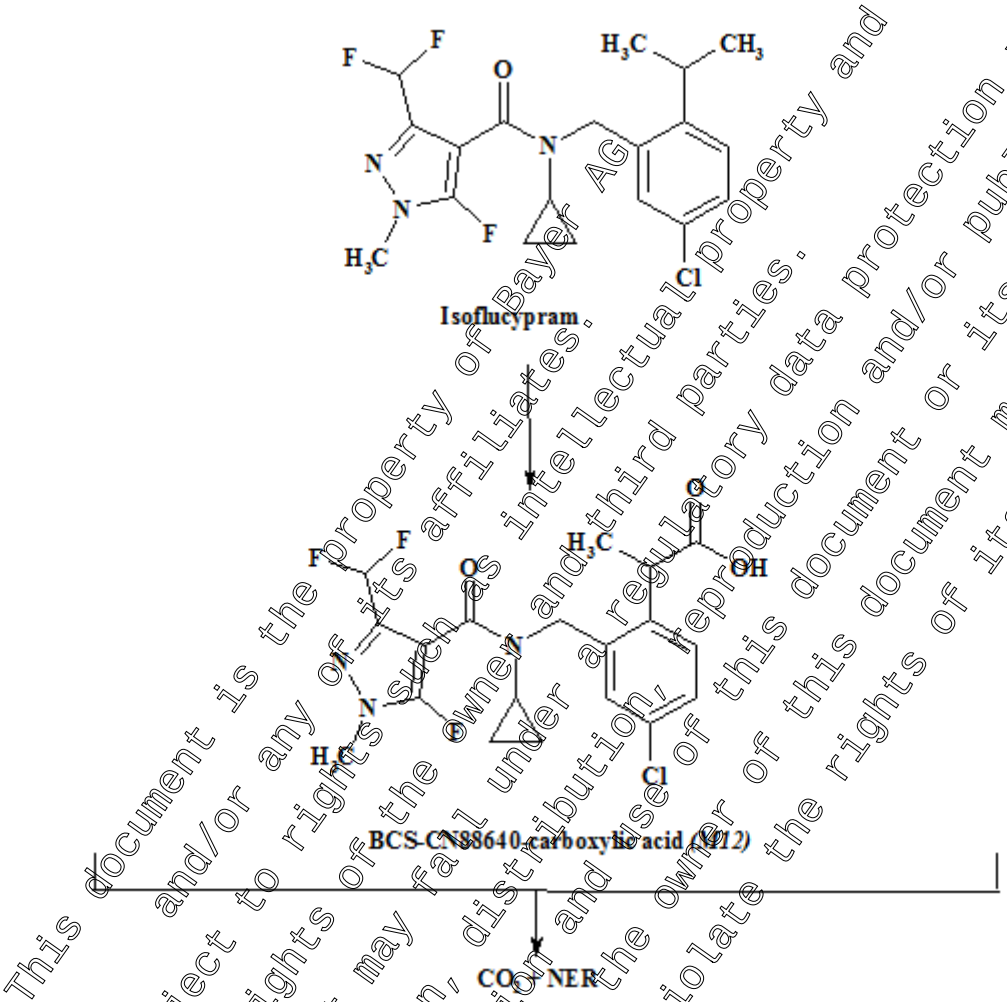
Table 7.2.2.3- 9: Degradation of isoflucypram in system Wiehltalsperre under aerobic conditions (expressed as percentage of applied radioactivity)

Compound	Source	DAT							
		0	3	7	14	29	51	72	100
Isoflucypram	water	**78.4**	**37.7**	**26.7**	**17.4**	**12.2**	**8.5**	**7.5**	**4.4**
	sediment	**17.6**	**53.5**	**67.2**	**75.2**	**80.2**	**83.0**	**80.5**	**80.1**
	entire system[d)]	**96.0**	**91.2**	**93.9**	**92.6**	**92.4**	**91.4**	**88.0**	**84.5**
BCS-CN88460-carboxylic acid *(M12)*	water	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD	< LOD
	sediment	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD
	entire system[d)]	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	1.2	< LOD
Sum of unid./diff. residues[a)]	water	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	LOD	1.7
	sediment	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD
	entire system[d)]	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD	2.2
Total extractable residues[b)]	water	78.4	37.7	26.7	17.4	12.8	8.5	8.1	6.0
	sediment	17.6	53.5	67.2	75.2	80.2	83.0	81.1	80.6
	entire system[d)]	96.0	91.2	93.9	92.6	93.0	91.4	89.2	86.6
Carbon dioxide[c)]		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1
Volatile organic compounds[c)]		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	n.d.	< 0.1
Non-extractable residues[c)]		0.7	1.7	2.2	3.4	4.1	3.8	4.5	6.2
Total recovery[b)]		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, SD: standard deviation, 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

Figure 7.2.2.3- 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 7.2.2.3- 10: 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 7.2.2.3- 11: 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

Isoflucypram dissipated rapidly from the water in water/sediment systems under aerobic conditions in the laboratory in the dark. The calculated best fit DT_{50} values for the dissipation of isoflucypram from water were 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.

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: BCS-CN88460-carboxylic acid *(M12)* with a maximum occurrence of 6.6% AR in the total system.

***

- **Kinetic evaluation of the aerobic water/sediment study**

Report: KCA 7.2.2.3/03; [redacted], G.; [redacted], W.; 2017; M-608356-02-1
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: **no**

Executive 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 ([redacted]; [redacted]; 2017; M-580411-01-1).

The objective of this study was to obtain degradation or dissipation half-lives of and its aquatic metabolite BCS-CN88460-carboxylic acid *(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). 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 7.2.2.3- 12 to Table 7.2.2.3- 16.

For metabolite BCS-CN88460-carboxylic acid, 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 for the total system could be derived for the system Anglersee.

Table 7.2.2.3- 12: Degradation and dissipation in water / sediment systems: trigger 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. [°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[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 20°C				**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) [redacted]; 2017; M-580414-01-1

[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

Table 7.2.2.3- 13: Degradation and dissipation in water / sediment systems: modelling 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. [°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[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 20°C				**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) [redacted]; [redacted]; 2017; M-580411-01-1

Table 7.2.2.3- 14: Degradation in water and sediment: modelling 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) [redacted]; [redacted]; 2017; M-580411-01-1

Table 7.2.2.3- 15: Degradation and dissipation in water / sediment systems: trigger endpoints of BCS-CN88460-carboxylic acid *(M12)*, Level M-I

Metabolite BCS-CN88460-carboxylic acid *(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) [redacted]; [redacted]; 2017; M-580411-01-1

Table 7.2.2.3- 16: Degradation and dissipation in water / sediment systems: modelling endpoints of BCS-CN88460-carboxylic acid *(M12)*, Level M-I (pathway fit)

Metabolite BCS-CN88460-carboxylic acid *(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) [redacted]; [redacted]; 2017; M-580411-01-1

I. METHODS

The objective of this study was to obtain degradation or dissipation half-lives of isoflucypram and its aquatic metabolite BCS-CN88460-carboxylic acid *(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 BCS-CN88460-carboxylic acid 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 ([redacted]; [redacted]; 2017; M-580411-01-1). 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 7.2.2.3- 17.

Table 7.2.2.3- 17: General information on aerobic aquatic laboratory studies with isoflucypram

Water-sediment system	Texture of sediment	Radioactive label	Duration [days]	Metabolites observed	Reference
Anglersee, GER	sand	pyrazole-label	100	BCS-CN88460-carboxylic acid (M12)	[redacted]; [redacted]; 2017; M-580411-01-1)
Wiehltalsperre, GER	loam				

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 (page 242). The most appropriate kinetic parameters are summarised per water-sediment system, for modelling and trigger purpose, for parent isoflucypram and its metabolite BCS-CN88460-carboxylic acid.

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. So, for modelling purpose, $DT_{50\ mod}$ from biphasic models is estimated by DT_{90} / 3.32.

Table 7.2.2.3- 18: 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

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. The statistics (chi² err) were improved for most of the fits (compare Figure 7.2.2.3- 2 and Figure 7.2.2.3- 3 for system Anglersee; Figure 7.2.2.3- 4 and Figure 7.2.2.3- 5 for system Wiehltalsperre). For derivation of modelling and trigger endpoints, the modified fits were taken into account.
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 7.2.2.3- 2: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Anglersee (initial fit)

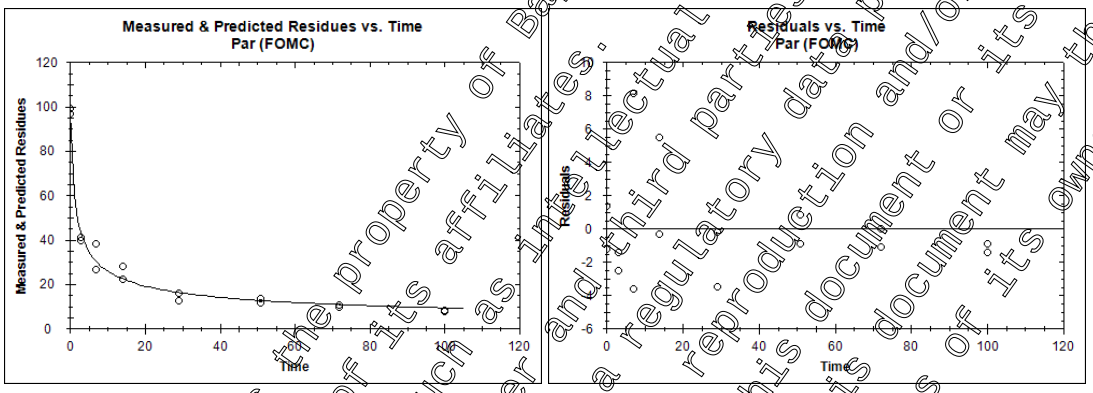


Figure 7.2.2.3- 3: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Anglersee (modified fit)

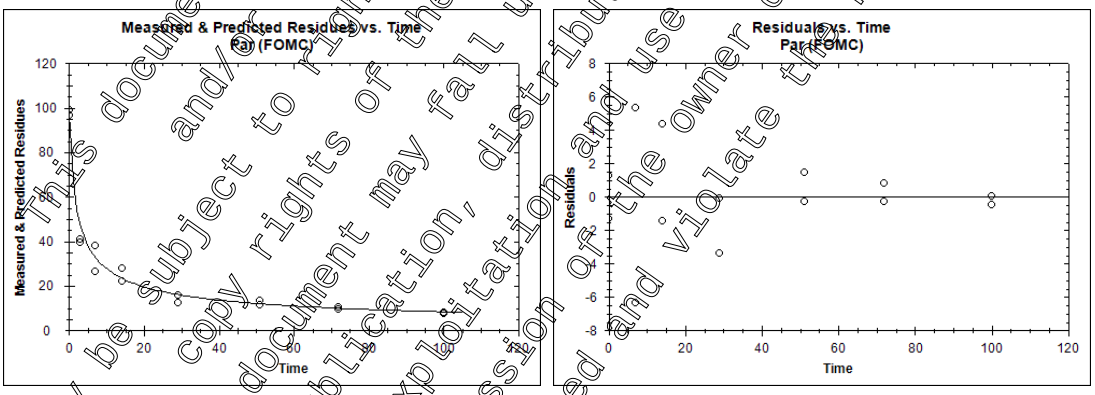


Figure 7.2.2.3- 4: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Wiehltalsperre (initial fit)

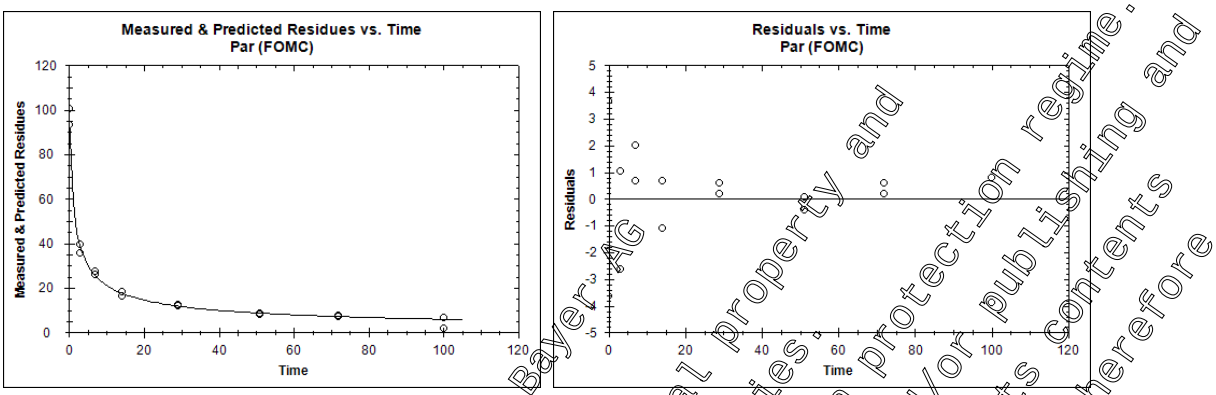
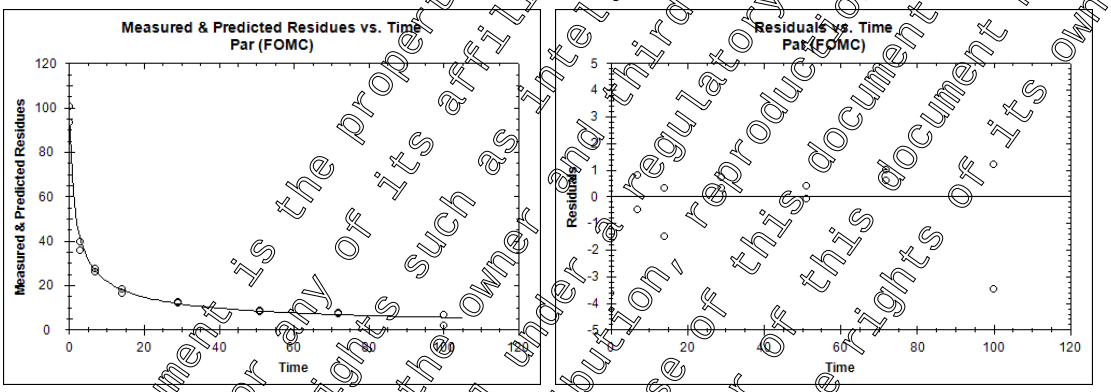


Figure 7.2.2.3- 5: KinGUI results for dissipation from water of isoflucypram at level P-I (FOMC parent only fit), system Wiehltalsperre (modified fit)



Dissipation from sediment, isoflucypram (level P-I)

Table 7.2.2.3- 19: Isoflucypram: kinetic and statistical results of dissipation from water

Kinetic model	DT_{50} trigger [days]	DT_{90} trigger [days]	DT_{50} mod* [days]	fVA	χ^2-err [%]	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	0.623		0.000069		0.0623	
FOMC	>1000	> 1000	>1000	o	n.e.	0.005904	0.131686			

* $DT_{50\ mod}$ = 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)

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.

Dissipation from water or sediment, BCS-CN88460-carboxylic acid, decline (level M-I)
The dissipation of the metabolite BCS-CN88460-carboxylic acid in water or sediment phase was tried to evaluate 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 BCS-CN88460-carboxylic acid was only detected in three (water) or one (sediment) samples. Based on these few data points a kinetic evaluation was not possible.

Degradation kinetics

Degradation in total system, isoflucypram (level P-I)

Table 7.2.2.3- 20: 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	
FOMC[a)]	222	738	222	o	2.41	390.8	124900			
SFO[b)]	**211**	**702**	**211**	o	2.09	0.003281			<0.001	**M/T**
FOMC[b)]	211	702	211	o	2.31	12850	3916000			
Wiehltalsperre										
SFO[a)]	681	>1000	681	o	1.43	0.001018			<0.001	
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	**M/T**
FOMC[b)]	593	>1000	-	o	0.97	3.663E+6	3.737E+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

For the systems Anglersee and Wiehltalsperre a pathway fit of isoflucypram with BCS-CN88460-carboxylic acid 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. For derivation of modelling and trigger endpoints, the modified fits were taken into account as the statistical significance (chi²err) was clearly improved. 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 7.2.2.3- 6: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Anglersee (initial fit)

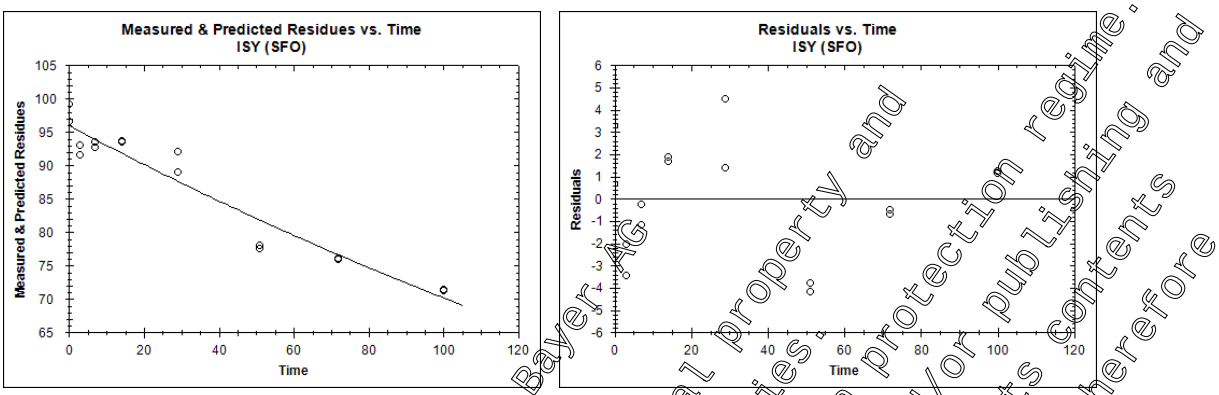


Figure 7.2.2.3- 7: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Anglersee (modified fit)

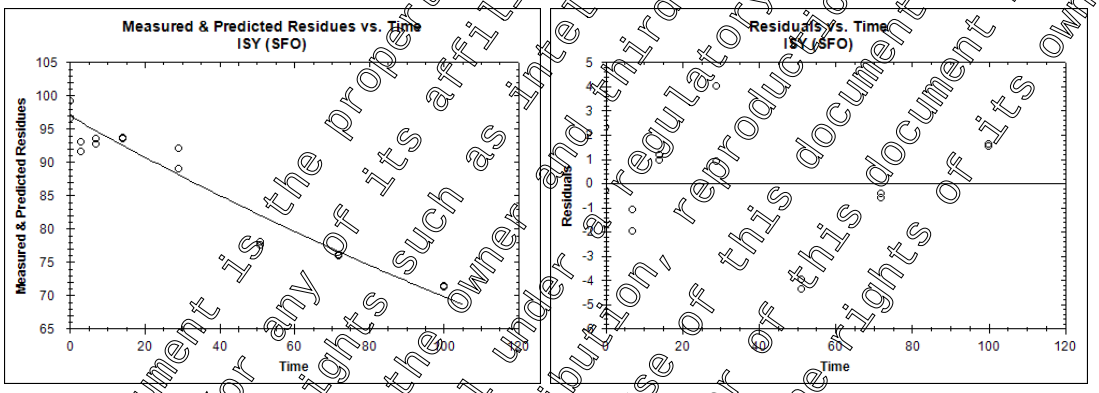


Figure 7.2.2.3- 8: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Wichtalsperre (initial fit)

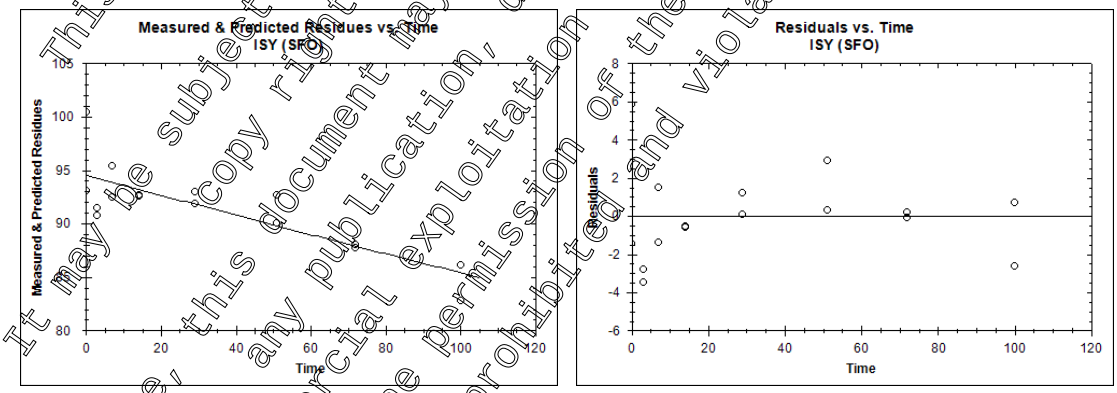
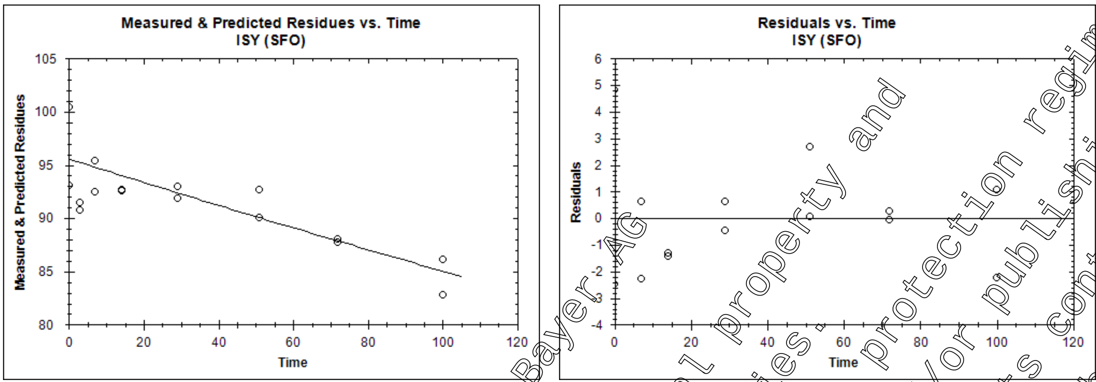


Figure 7.2.2.3- 9: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Wiehltalsperre (modified fit)



Degradation in total system, BCS-CN88460-carboxylic acid *(M12)*, pathway (level M-I)

In general, the kinetic evaluation of total system degradation of the metabolite BCS-CN88460-carboxylic acid 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 7.2.2.3- 21: BCS-CN88460-carboxylic acid: 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^2 err [%]	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

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 BCS-CN88460-carboxylic acid were included in the pathway fit even though the metabolite had only been detected in three samples. However, it was not possible to derive reliable degradation half-lives or formation fractions from these data.

Degradation in water and sediment phase (level P-II)

A 2-compartmental approach was taken into account to estimate the degradation of isoflucypram in water and sediment compartment, in parallel, including partitioning processes via reaction rates. A simple first-order (SFO) kinetics was used to describe degradation separately in the water and sediment phase, as well as reversible transfer or partitioning between these compartments.

Table 7.2.2.3- 22: Estimated (SFO) parameters for degradation and partitioning of isoflucypram in water and sediment, separately (level P-II)

Test system	Compartment	M_0	DT_{50} [days]	DT_{90} [days]	VA	χ^2err [%]	k [1/d/-]	t-test of k
Anglersee[a)]	water	97.90	63.8 n.r.	212 n.r.	+	14.93	0.01086	0.327
	sediment	0	> 1000 n.r.	> 1000 n.r.	+	5.31	< 0.001	0.500
Anglersee[b)]	water	97.90	59.9 n.r.	199 n.r.	+	8.30	0.01157	0.160
	sediment	0	756 n.r.	> 1000 n.r.	+	3.56	0.0009	0.362
Anglersee[c)]	water	97.90	> 1000 n.r.	> 1000 n.r.	+	14.37	n.e.	n.e.
	sediment	0	267	888	+	5.41	0.0025942	< 0.001
Anglersee[d)]	water	97.90	> 1000 n.r.	> 1000 n.r.	+	7.88	n.e.	n.e.
	sediment	0	194	644	+	3.91	0.003578	< 0.001
Wiehltalsperre[a)]	water	96.75	> 1000 n.r.	> 1000 n.r.	+	15.99	< 0.001	0.500
	sediment	0	> 1000 n.r.	> 1000 n.r.	+	3.35	3.012E-14	0.500
Wiehltalsperre[b)]	water	96.75	169 n.r.	> 1000 n.r.	+	10.01	0.004101	0.323
	sediment	0	> 1000 n.r.	> 1000 n.r.	+	2.20	< 0.001	0.500

Test system	Partitioning from compartment	$k_{wat\text{-}sed}$ [1/d/-]	$k_{sed\text{-}wat}$ [1/d/-]	t-test of k	$F_{sed\ modelling}$	$F_{sed\ theoretical}$
Anglersee[a)]	water	0.2638		< 0.001	0.810	0.775
	sediment		0.06203	< 0.001		
Anglersee[b)]	water	0.1616		< 0.001	0.836	0.775
	sediment		0.03163	< 0.001		
Anglersee[c)]	water	0.2677		< 0.001	0.812	0.775
	sediment		0.06185	< 0.001		
Anglersee[d)]	water	0.1658		< 0.001	0.843	0.775
	sediment		0.03083	< 0.001		
Wiehltalsperre[a)]	water	0.2849		< 0.001	0.874	0.909
	sediment		0.0410	< 0.001		
Wiehltalsperre[b)]	water	0.1956		< 0.001	0.893	0.909
	sediment		0.02341	< 0.001		

n.r. = Not fully reliable, mathematically not significantly different from 0; not usable
n.e. = Not evaluated (pathway water -> sink deleted from kinetic model)
a) Initial approach including all residue data and with all parameters free
b) Modified approach excluding the residue data from DAT 3 and with all parameters free
c Modified approach including all residue data and fixing the transformation fraction from water to sediment to 1 (pathway water -> sink excluded)
d) Modified approach excluding the residue data from DAT 3 and fixing the transformation fraction from water to sediment to 1 (pathway water -> sink excluded)

For the system Anglersee, the initial approach (all parameters free) did not result in reliable degradation parameters as the t-test indicated that the degradation rates were not significantly different from zero. This is also valid if the residue data of DAT 3 are excluded from the fit, even though the chi²err is significantly decreased by this procedure. In a modified approach (fixing the transformation fraction from water to sediment to 1) a statistically reliable half-life for the sediment could be derived, independent of the inclusion or exclusion of DAT 3 residue data.
Additionally, the F_{sed} test according to FOCUS kinetics was carried out to assess the reliability of the modelled parameters. The fraction of parent compound that transfers into the sediment at equilibrium (F_{sed}) is calculated using: 1. fitted Level P-II model parameters ($F_{sed,\ modelling}$) as well as 2. conditions of the study or tabled values ($F_{sed,\ theoretical}$). Theoretical and modelled F_{sed} values show a moderate to

excellent conformity, which might confirm the reliability of estimated partitioning rates (Table 7.2.2.3- 22).
For the system Wiehltalsperre, the degradation rates were not significantly different from zero and statistically not reliable.

As no separate degradation rates for water and sediment could be derived, a further attempt was made to describe the degradation of isoflucypram occurring in water-sediment systems. Therefore the initial degradation rate constants for sediment were fixed to the $DegT_{50}$ values estimated for the degradation in total system (level P-I) while the initial degradation in water was fixed to 1000 days. This resulted in a good visual fit with reliable transition rates between water and sediment (Figure 7.2.2.3- 10 and Figure 7.2.2.3- 11 for systems Anglersee and Wiehltalsperre, respectively). It is hereby shown that significant degradation occurs in the sediment phase which is at least as fast as given by the total system DT_{50} values. Residue data in the water phase show a higher degradation as estimated by using a DT_{50} value of 1000 days. While it is not possible to derive degradation parameters from these fits, it is nevertheless possible to conclude that significant degradation of isoflucypram occurs in the water and sediment phases.

Figure 7.2.2.3- 10: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Wiehltalsperre (modified fit)

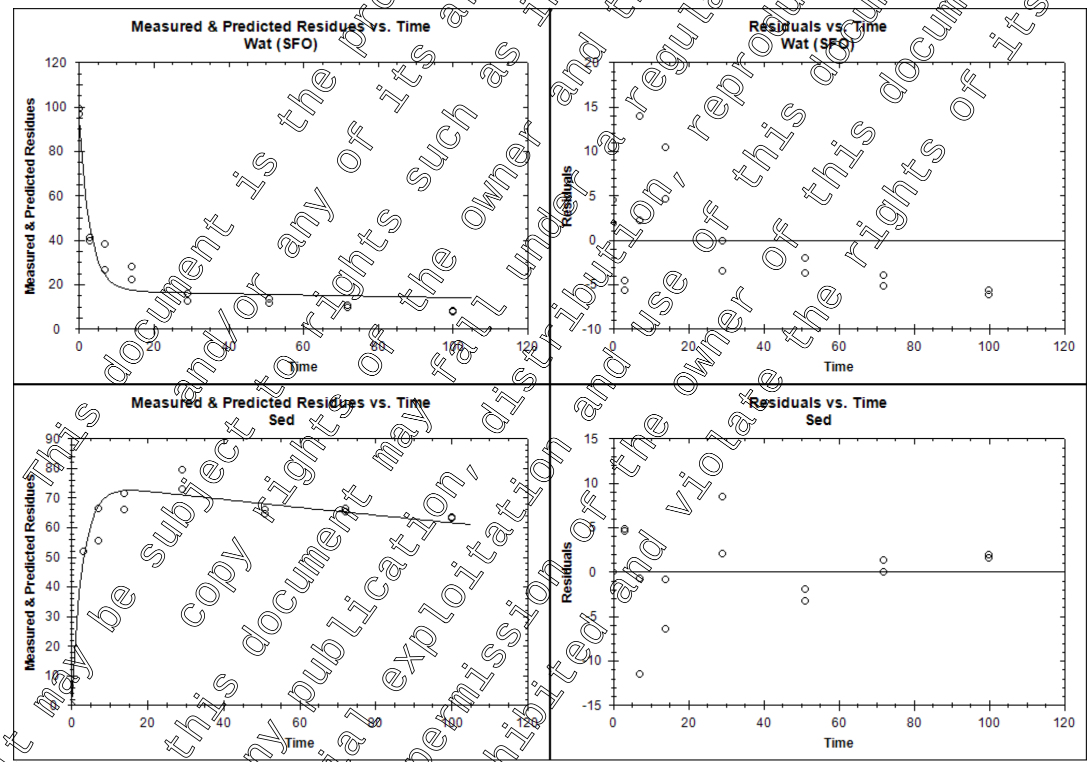
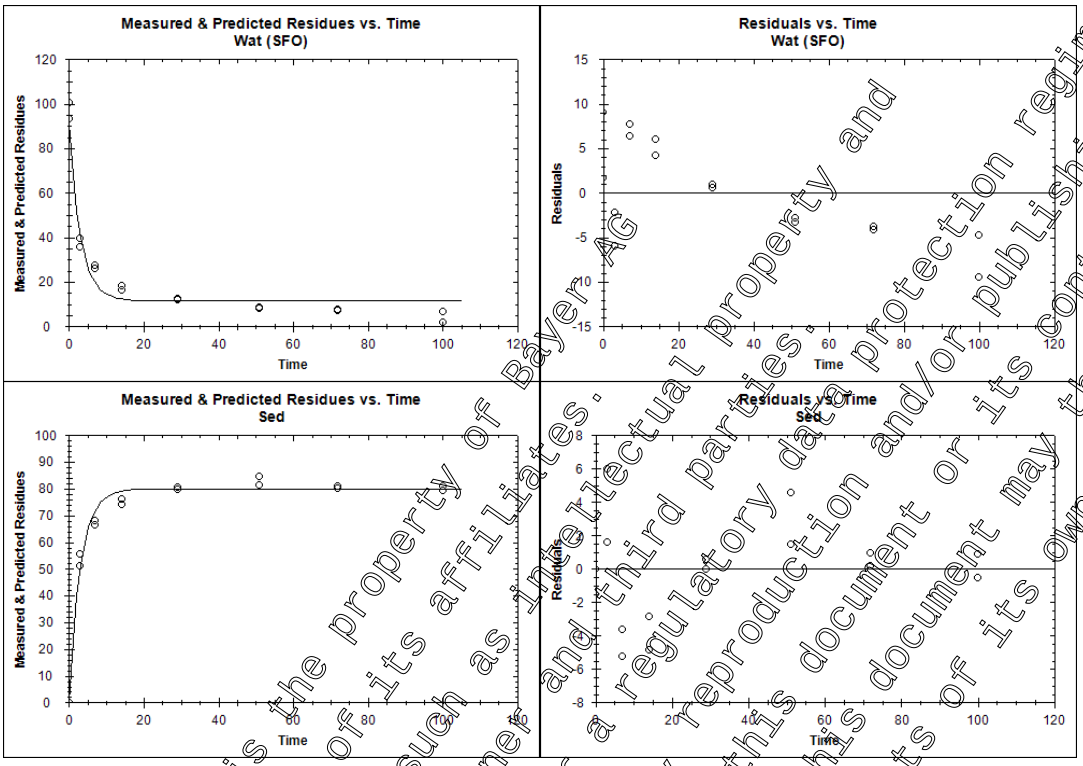


Figure 7.2.2.3- 11: KinGUI results for dissipation from water of isoflucypram at level P-I (SFO pathway fit), system Wiehltalsperre (modified fit)



III. CONCLUSIONS

The trigger and modelling endpoints for isoflucypram are presented in Table 7.2.2.3- 23 and Table 7.2.2.3- 24 to Table 7.2.2.3- 25, respectively.

For metabolite BCS-CN88460-carboxylic acid 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 = 0.228) could be derived for the system Anglersee.

Table 7.2.2.3- 23: 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	214	702	SFO	2.79	74.2	FOMC recalc.	282	938	SFO
Wiehltalsperre	593	1000	SFO	2.06	39.6	FOMC recalc.	n.r.	n.r.	-
Geometric mean	**354**			**2.40**			**282**		

Table 7.2.2.3- 24: Degradation and dissipation in water / sediment systems: modelling 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	211	702	SFO	22.3	74.2	FOMC recalc.	282	938	SFO
Wiehltalsperre	593	> 1000	SFO	11.9	39.6	FOMC recalc.	n.r.	n.r.	-
Geometric mean	**354**			**16.3**			**282**		

Table 7.2.2.3- 25: Degradation and dissipation in water / sediment systems: modelling endpoints of isoflucypram, Level P-II

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	n.r.	n.r.	-	n.r.	n.r.	-	n.r.	n.r.	-
Wiehltalsperre	n.r.	n.r.	-	n.r.	n.r.	-	n.r.	n.r.	-

CA 7.2.2.4 Irradiated water/sediment study

The route and rate of degradation of isoflucypram in water and sediment were comprehensively studied in sections CA 7.2.1, CA 7.2.2.2 and CA 7.2.2.3. Therefore, the route and rate of degradation of isoflucypram in irradiated water/sediment systems were not studied. A summary of the route and rate of degradation of isoflucypram in water and sediment is given in section CA 7.2 and Figure 7.2- 1.

CA 7.2.3 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 CA 7.2 and Figure 7.2- 1.

CA 7.3 Fate and behaviour in air

Isoflucypram has a very low vapour pressure of 1.2×10^{-7} Pa ([redacted]; 2014; M-488244-01-1, summarised in MCA section 2, CA 2.2). 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.

CA 7.3.1 Route and rate of degradation in air

Report:	KCA 7.3.1/01; [redacted]; 2015; M-544687-01-1
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):	Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline: N/A
Guideline deviation(s):	none
GLP/GEP:	**no**

Executive 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^{TM} (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 x 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 x 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^{TM} 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 ($K_{total,\ indirect\ photoreaction} \approx k_{OH}$) 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

Isoflucypram is considered to be susceptible to reactions with hydroxyl radicals which contribute to the overall degradation of the substance in the atmosphere. Two parts of the molecule were identified as potential targets for radical reactions. An attack by hydroxyl radicals should result in the formation of multiple primary radicals. Their formation may be followed by secondary oxidation products that can be eliminated from the atmosphere by wet and/or dry deposition.

CA 7.3.2 Transport via air

The transport via air of isoflucypram was not studied since its vapour pressure is below the trigger value of 10^{-5} Pa.

CA 7.3.3 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.

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

The proposed residue definitions relevant for risk assessment for each compartment are summarised in the following table:

Table 7.4.1- 1: Proposed residue definitions relevant for risk assessment

Compartment	Residue definition for risk assessment
Soil	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Groundwater	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Surface water	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Sediment	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Air	Isoflucypram

CA 7.4.2 Definition of the residue for monitoring

The enforcement method for soil includes the active substance only.

The enforcement method for water includes the active substance only.

The relevant residue with regard to quantification in air is the parent compound only.

CA 7.5 Monitoring data

No formal monitoring program is requested or required to address this point.