Document Title

Summary of the fate and behaviour in the environment for fluopicolide

Part 2

Data Requirement(s)

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

Date

2020-08-11

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on behalf of

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[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 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

The adsorption and desorption of fluopicolide has been investigated in five studies (KCA 7.1.3.1.1/01 to KCA 7.1.3.1.1/05). Studies KCA 7.1.3.1.1/01 and KCA 7.1.3.1.1/02 were evaluated during the previous EU review. Studies KCA 7.1.3.1.1/03 to KCA 7.1.3.1.1/05 are new studies for Annex I renewal submission.

Report reference	Author, Year	Comment
KCA 7.1.3.1.1/01 M-241228-02-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
KCA 7.1.3.1.1/02 M-233840-01-1	[redacted] 2003	
KCA 7.1.3.1.1/03 M-544194-02-1	[redacted] 2015	New data not yet reviewed
KCA 7.1.3.1.1/04 M-550735-01-1	[redacted] 2016	
KCA 7.1.3.1.1/05 M-595721-01-1	[redacted] 2017	

Summary of sorption parameters derived for fluopicolide

Report reference	Soil	Soil Code	Texture	pH	OC (%)	K_f (mL/g)	K_{OC} (mL/g)	1/n
KCA 7.1.3.1.1/01 M-241228-02-1	Pikeville Sediment	EFS-54	Loam	4.5	2.07	7.73	373*	0.926*
	Pikeville, North Carolina	EFS-65	Sand	4.7	0.5	1.42	283	0.924
	Abington	EFS-86	Sandy loam	7.5	2.21	3.36	152	0.884
	Sarotti	EFS-88	Silty clay loam	7.4	0.9	3.2	356	0.905
	Münster	EFS-93	Loamy sand	5.7	1.3	4.54	349	0.929
	Münster	EFS-94	Loamy sand	6.2	0.2	0.21	106*	0.931*
	Münster	EFS-95	Loamy sand	6.2	0.2	0.17	83*	0.951*
KCA 7.1.3.1.1/02 M-233840-01-1	Philippsburg	03/02	Sandy loam	6.3	0.6	1.49	248	0.841
	Senas	03/03	Clay loam	7.6	1.5	3.59	239	0.882
	Huntlosen	03/04	Loamy sand	7.3	1.6	9.27	580	0.953
	Rodelsee	03/05	Clay	7	1.5	2.59	172	0.859
KCA 7.1.3.1.1/03 M-544194-02-1	[redacted]	[redacted]	Loam	5	1.8	4.65	258.6	0.9258
	[redacted]	[redacted]	Silt loam	6.1	1.9	6.22	327.5	0.8741
	Dollendorf II,	Doll	Clay loam	7.3	4.8	11.71	244.1	0.8596
	[redacted]	[redacted]	Sandy loam	6.5	1.5	4.04	269.3	0.8723
KCA 7.1.3.1.1/04 M-550735-01-1	Burscheid	OVG08	Silt loam	6.1	0.7	2.12	303.3	0.8868
	Great Chishill	ENG2	Clay	7.3	2.1	5.40	257.0	0.9076
	Parcay Meslay	FR09B	Loam	6.7	1.3	3.35	257.4	0.8992
	Tarascon Le Cavades	FR08	Clay loam	7.6	0.9	1.84	204.9	0.8668
	Valerie Tomelim	LT09	Silty clay	7.2	2.1	3.93	187.0	0.9110
	Vilobi D'Onyar	SPA01	Sandy loam	6.3	0.8	2.34	292.0	0.8818
KCA 7.1.3.1.1/05 M-595721-01-1	Abington	AB	Sandy loam	7.3	2.6	5.6	214.7	0.868
	Lamberton	LB	Loam	5.6	2.6	8.6	331.9	0.844
	Lignières	LN	Sandy loam	5.7	0.8	2.9	363.1	0.888
	Muenster	MS	Loamy sand	5.6	1.2	3.4	282.6	0.916
	Pikeville	PV	Loamy sand	4.5	1.8	6.2	342.6	0.873
	Sarotti	SR	Silty clay loam	6.9	1.4	2.6	185.6	0.851
Arithmetic mean							-	**0.888**
Geometric mean							**267.7**	-

*excluded from calculation of mean values

Data Point:	KCA 7.1.3.1.1/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Amendment no. 1 to final report - The adsorption/desorption of AE C638206 in U.S. and European soils
Report No:	CU99E512-01
Document No:	M-241228-02-1
Guideline(s) followed in study:	OECD: No. 106; USEPA (=EPA): PAG-N 163-1
Deviations from current test guideline:	The studied followed the OECD Proposal for Updating Guideline 106, Revised Draft Document, Oct. 1997. The soils were tested over a range of four concentrations (0.01, 0.05, 0.1, and 1.0 ug/mL) whereas the current guideline requires five test substance concentrations. There is insufficient data in the report to confirm parental mass balance.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Tests to investigate the adsorption/desorption characteristics of [phenyl-U-^{14}C]-fluopicolide were performed under conditions of batch equilibrium experiments in four soils, one sediment and two sub-soils in the laboratory in the dark at 25 ± 1 °C.

Soil	Soil Code	Texture (USDA)	pH (CaCl$_2$)	OC (%)
Pikeville Sediment	EFS-54	loam	4.5	2.07
Pikeville, North Carolina	EFS-65	sand	4.7	0.5
Abington	EFS-86	sandy loam	7.5	2.21
Sarotti	EFS-88	silty clay loam	7.4	0.9
Münster	EFS-93	loamy sand	5.7	1.3
Münster	EFS-94	loamy sand	6.2	0.2
Münster	EFS-95	loamy sand	6.2	0.2

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1:5 (all soils). Test concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L of [phenyl-U-^{14}C]-fluopicolide were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. Desorption phase of the study was carried out by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ solution for two desorption cycles of 24 hours.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and the amount of test item in the supernatants was analyzed by liquid scintillation counting (LSC). The sorption parameters were calculated using Freundlich isotherms. The mass balance of the soils in the definitive test was determined by LSC of the supernatants after adsorption, desorption and by combustion of the remaining soils. Mean material balances were 99.1, 106, 104, 106, 101, 108 and 109% AR for soil Pikeville Sediment, Pikeville North Carolina, Abington, Sarotti, Münster (0-30 cm), Münster (30-85 cm) and Münster (> 85 cm), respectively. The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

The stability of the test item was investigated in a preliminary test. The majority of the radioactivity in the calcium chloride supernatants was fluopicolide with small amounts of the metabolite M-01 (called AE C653711 in the report) observed in Pikeville North Carolina, Sarotti and Abington soils. After 24 hours adsorption the amount of fluopicolide in supernatant solution was 98, 94, 93, 88 and 92% ROI in soils Münster (0-30 cm), Pikeville sediment, Pikeville North Carolina, Sarotti and Abington, respectively. After 48 h the amount of fluopicolide in supernatant solution was 99, 96, 89, 78 and 84% ROI in soils Münster (0-30 cm), Pikeville sediment, Pikeville North Carolina, Sarotti and Abington, respectively. Only fluopicolide (100% ROI) was detected in soil extracts at 24, 48 and 72 hours.

At the end of the adsorption phase 68.4-72.7% AR was adsorbed to Pikeville sediment, 29.3-36.1% AR was adsorbed to soil Pikeville North Carolina, 41.7-62.3% AR was adsorbed to soil Abington, 49.5-63.6% AR was adsorbed to soil Sarotti, 41.2-60.0% AR was adsorbed to soil Münster (0-30 cm), 10.5-13.4% AR was adsorbed to soil Münster (30-85 cm) and 9.5-10.4% AR was adsorbed to soil Münster (>85 cm).

The adsorption constant $K_{F(ads)}$ of fluopicolide was between 1.42 and 7.73 mL/g for the tested top-soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 152 to 373 mL/g. The Freundlich exponent 1/n was between 0.884 and 0.929, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

For sub-soils, $K_{F(ads)}$ of fluopicolide was 0.21 and 0.17 mL/g for depth 30-85 and > 85, respectively; the respective normalized $K_{OC(ads)}$ was106 and 83 mL/g. The Freundlich exponent 1/n was 0.931 and 0.951, respectively.

Soil origin	Pikeville Sediment	Pikeville, North Carolina	Abington, United Kingdom	Sarotti, Hattersheim, Germany
Soil ID	EFS-54	EFS-65	EFS-86	EFS-88
Soil type (USDA)	Loam	Sand	Sandy loam	Silty clay loam
pH (0.01M $CaCl_2$)	4.5	4.7	7.5	7.4
Organic carbon [%]	2.07	0.5	2.21	0.9
$K_F^{(ads)}$ [mL/g]	7.73	1.42	3.36 [A]	3.20
1/n	0.926	0.924	0.884 [A]	0.905
$K_{F,OC}^{(ads)}$ [mL/g]	373	283	152 [A]	356
$K_F^{(des1)}$ [mL/g]	9.53	2.03	3.92	3.84
1/n	0.940	0.905	0.890	0.904
$K_{F,OC}^{(des1`)}$ [mL/g]	460	406	177	427

Soil origin	Munster (0-30 cm)	Munster (30-85 cm)	Munster (>85 cm)
Soil ID	EFS-93	EFS-94	EFS-95
Soil type (USDA)	Loamy sand	Loamy sand	Loamy sand
pH (0.01M $CaCl_2$)	5.7	6.2	6.2
Organic carbon [%]	1.3	0.2	0.2
$K_F^{(ads)}$ [mL/g]	4.54	0.21	0.17
1/n	0.929	0.931	0.951
$K_{F,OC}^{(ads)}$ [mL/g]	349	106	83
$K_F^{(des1)}$ [mL/g]	5.42	1.02	0.94
1/n	0.942	1.042	0.965
$K_{F,OC}^{(des1`)}$ [mL/g]	417	508	471

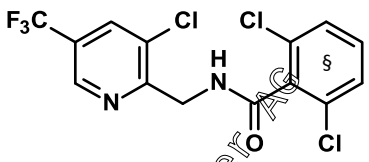
[A] Taken from report amendment

I. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-fluopicolide (referred to as [U-^{14}C-phenyl]-AE C638206 in the report)



§ = position of radiolabel.

Batch number:	ESR-245
Specific radioactivity:	5.33 MBq/mg (144 µCi/mg)
Radiochemical purity:	100.0% (HPLC)
Stability of test compound:	Generally stable during the equilibrium periods (up to 48 hours). However, some degradation did occur (maximum of 22%) of the solution phase at 48 hours.

2. Test Soil

Sorption tests were performed with four topsoils covering a wide range of pH, organic carbon content and texture. Additionally, the test was performed using one sediment and two sub-soils of different depth. After collection, soils were homogenized, air-dried, and passed through a 2 mm sieve. The characteristics of soils originating from Europe and the US are summarised in Table 7.1.3.1.1-1.

Table 7.1.3.1.1- 1: Physico-chemical properties of test soils

Parameter	Soil						
Soil Designation	**EFS-54**	**EFS-65**	**EFS-86**	**EFS-88**	**EFS-93**	**EFS-94**	**EFS-95**
Geographic Location							
City	Pikeville Sediment	Pikeville, North Carolina	Abington	Sarotti	Münster	Münster	Münster
Country	NC, USA	NC, USA	England	Germany	Germany	Germany	Germany
Horizon (cm)	0 - 30	0 - 30	0 - 30	0 - 30	0 - 30	30 - 85	> 85
Textural Classification	loam [1]	sand [1]	sandy loam [1]	silty clay loam [1]	loamy sand [2]	loamy sand [2]	loamy sand [2]
Sand (%)	48.5	93.8	67.0	n.a.	79.5	88.5	79.2
Silt (%)	31.7	1.8	22.0	n.a.	17.4	10.6	18.2
Clay (%)	19.8	4.4	11.0	n.a.	3.1	0.9	2.6
pH							
in H_2O (1:1) or (1:5)	5.1	5.3	7.4	n.a.	6	6.8	6.9
in $CaCl_2$ (1:1) or (1:5)	4.5	4.7	7.5	7.4	5.7	6.2	6.2
Organic Matter (%)	3.56	0.9	3.80	4.6	2.24	0.34	0.3
Organic carbon (%) *	2.07	0.5	2.24	0.9	1.3	0.2	0.2
Cation Exchange Capacity (meq/100 g)	10.61	2.85	18	13.3	6.0	2.0	1.4
Water Holding Capacity (%)							
maximum	39.04	22.66	56.1	62.2	-	-	-
at 1/10 bar	n.a.	n.a.	18.8	28.3	-	-	-
at 1/3 bar	26.71	3.33	14.6	n.a.	-	-	-

n.a.: not analysed;
n.r.: not reported
1 According to USDA (sand: 0.05 - 2.0 mm, silt: 0.002 - 0.05 mm, clay: <0.002 mm)
2 According to ADAS (sand: 0.063 - 2.0 mm, silt: 0.002 - 0.063 mm, clay: <0.002 mm)
* Calculated by dividing organic matter content by 1.72

B. Study Design

1. Experimental Conditions

The test system for adsorption and desorption in batch equilibrium experiments consisted of Pyrex® tubes closed with Teflon® lined caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption and desorption equilibration times and the stability of the test item were determined.

The main test was performed in duplicate. The adsorption phase was carried out using air-dried soil pre-equilibrated (12-18 hours) in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:5 (10 g soil dry weight/ 50 mL solution). Fluopicolide was applied at nominal concentrations of 1.0, 0.1, 0.05 and 0.01 mg/L in acetonitrile. The amount of organic solvent in samples did not exceed 1%. The desorption phase was performed twice by supplying pre-adsorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution for all test concentrations. The adsorption and desorption steps were carried out each for 24 hours in the dark at 25 ± 1 °C under continuous agitation.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		10 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ 12-18 hours overnight
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 0.01, 0.05, 0.1 and 1.0 µg/mL
	Analytically (LSC) measured concentrations	Concentrations in test solution: Not reported.
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile Study media – calcium chloride
Soil: Solution ratio		1:5 i.e. 10 g soil dry weight equivalent to 50 mL solution
Number of replicates	Control	Not stated
	Treatments	Duplicate
Equilibration conditions	Time	24 hours
	Temperature	25±1°C
	Dark	In the dark
	Shaking method	End-over-end shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	10 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from negative values to 72% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		2
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 43-48 mL was used as equilibration solution.
Soil: Solution ratio		1:5 i.e. 10 g soil dry weight equivalent to 50 mL solution
Number of replicates	Control	Not stated
	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hours
	Temperature	25±1°C
	Dark	In the dark
	Shaking method	End-over-end shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	10 minutes
	Method of separating supernatant	Supernatant was carefully decanted.
Desorption cycle 2	Method	Same as above

2. Analytical Procedures

Radioactivity in supernatants and soil extracts was determined by LSC. Radioactivity in the extracted soil was determined by combustion.

In a preliminary test to establish the stability of the test item, soil samples were extracted with acetonitrile. Triplicate samples of each soil were treated at concentration of 5 µg/mL. After 24- and 48-hours equilibration, the amount of fluopicolide in the aqueous supernatant and acetonitrile soil extract was measured by LSC and HPLC in a single replicate after separation of the phases by centrifugation. After 72 hours equilibration the final replicate was extracted with 50 mL acetonitrile which was analysed by HPLC. The radioactivity remaining in the soil was quantified by combustion.

In the definitive test, after each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of fluopicolide in the supernatants analysed by liquid scintillation counting (LSC). The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only. After the desorption steps, the soil was air-dried and the radioactivity content determined by combustion/LSC to establish the material balance.

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. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption. A soil-to-solution ratio of 1:5 was regarded adequate for all experiments as the levels of fluopicolide adsorbed to all top soils were within the acceptable range of 20 to 80%. An equilibration time of 24 hours was needed to reach adsorption equilibrium and was also used for the desorption cycles.

B. Transformation of test substance

Following an adsorption phase of 24, 48 or 72 hours to soil, HPLC analysis of water and soil extracts showed the majority of the radioactivity in the calcium chloride supernatants was fluopicolide with small amounts of the metabolite M-01 (called AE C653741 in the report) observed particularly in soils Pikeville North Carolina, Sarotti and Abington in the preliminary measurements. After 24 hours adsorption the amount of fluopicolide in supernatant solution was 98, 94, 93, 88 and 92% ROI in soils Münster (0-30 cm, different batch than the adsorption test), Pikeville sediment, Pikeville North Carolina, Sarotti and Abington, respectively. After 48 hours the amount of fluopicolide in supernatant solution was 99, 96, 89, 78 and 84% ROI in soils Münster (0-30 cm, different batch than the adsorption test), Pikeville sediment, Pikeville North Carolina, Sarotti and Abington respectively. Only fluopicolide (100% ROI) was detected in soil extracts for all soils at 24, 48 or 72 hours.

C. Findings

Mean material balances were 99.1, 105.3, 103.8, 106.5, 101.2, 108.3 and 109.1% AR for soil Pikeville Sediment, Pikeville North Carolina, Abington, Sarotti, Münster (0-30 cm), Münster (30-85 cm) and Münster (> 85 cm), respectively (summarised in Table 7.1.3.1.1- 2). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.1- 2: Definitive test: Mass balance (% AR) of [phenyl-U-^{14}C]-fluopicolide

Test concentration (mg/L)	Pikeville Sediment	Pikeville, North Carolina	Abington	Sarotti	Münster 0-30 cm	Münster 30-85 cm	Münster >85 cm
	EFS-54	EFS-65	EFS-86	EFS-88	EFS-93	EFS-94	EFS-95
1.0 mg/L	97.6	107.3	102.9	109.7*	98.0	110.8	110.1
0.1 mg/L	98.1	105.4	101.7	103.5	103.4	109.3	110.0
0.05 mg/L	99.4	100.3*	102.5	104.5	103.4	108.8	109.8
0.01 mg/L	101.4	108.3	108.2	108.5	100.0	104.4	106.8
Mean	99.1	105.3	103.8	106.5	101.2	108.3	109.1
SD	1.68	3.55	2.97	3.03	2.65	2.75	1.56

Note: Mass balances were virtually quantitative. Values derived from mean values of duplicate samples in terms of percentages of AR. SD = standard deviation. *Only one value available.

The results of adsorption tests of [phenyl-U-^{14}C]-fluopicolide onto four soils, one sediment and two sub-soils are summarised in Table 7.1.3.1.1- 3, Table 7.1.3.1.1- 4 and Table 7.1.3.1.1- 5. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.1- 1 to Figure 7.1.3.1.1- 7 .

At the end of the adsorption phase 68.4-72.7% AR was adsorbed to Pikeville sediment, 29.5-36.1% AR was adsorbed to soil Pikeville North Carolina, 41.7-62.3% AR was adsorbed to soil Abington, 49.5-63.6% AR was adsorbed to soil Sarotti, 41.2-60.0% AR was adsorbed to soil Münster (0-30 cm), 10.5-13.4% AR was adsorbed to soil Münster (30-85 cm) and 9.5-10.4% AR was adsorbed to soil Münster (>85 cm).

The adsorption constant $K_{F(ads)}$ of fluopicolide was between 1.42 and 7.73 mL/g for the tested top-soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 152 to 373 mL/g. The Freundlich exponent 1/n was between 0.884 and 0.929, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

For sub-soils, $K_{F(ads)}$ of fluopicolide was 0.21 and 0.17 mL/g for depth 30-85 and >85, respectively; the respective normalized $K_{OC(ads)}$ was106 and 83 mL/g. The Freundlich exponent 1/n was 0.931 and 0.951, respectively.

Table 7.1.3.1.1- 3 Definitive test: Percentage of adsorbed and desorbed [phenyl-U-^{14}C]-fluopicolide in soil

Phase	Test Conc [mg/L]	Pikeville Sediment			Pikeville			Abington			Sarotti		
		Replicate			Replicate			Replicate			Replicate		
		1	2	mean	1	2	mean	1	2	mean	1	2	mean
Adsorption [1]	1	*67.2*	*69.5*	*68.4*	*29.7*	*29.8*	*29.8*	*51.1*	*52.2*	*51.7*	*49.9*	*49*	*49.5*
	0.1	*67.9*	*71.4*	*69.7*	*29.5*	*n.a.*	*29.5*	*55*	*56*	*55.5*	*54.3*	*53.8*	*54.1*
	0.05	*68.7*	*71.7*	*70.2*	*32.6*	*33.8*	*33.2*	*61.8*	*62.8*	*62.3*	*55.8*	*55.9*	*55.9*
	0.01	*72.6*	*72.7*	*72.7*	*38.3*	*33.8*	*36.1*	*61.8*	*62.8*	*62.3*	*n.a.*	*63.6*	*63.6*
1st Desorption [2]	1	19.5	19.1	*19.3*	18.5	19.1	18.8	24.3	23.9	24.1	23.7	23.6	23.7
	0.1	18.2	17.8	*18.0*	17.8	26.8	22.3	22.6	22.4	22.5	23	22.7	22.9
	0.05	17.3	17.2	*17.3*	19	19.9	19.5	22.3	22.1	22.2	22.3	22.2	22.3
	0.01	16.8	16.9	*16.9*	18.9	19.1	19.0	21.7	22.4	22.4	22	22.3	22.2
2nd Desorption [3]	1	12.9	12.6	*12.8*	5.5	6.4	5.5	11.4	11.8	11.6	10.9	10.3	10.6
	0.1	12.4	12.1	*12.3*	5.6	n.a.	5.6	12.2	12.1	12.2	11.5	11.2	11.4
	0.05	11.9	11.8	*11.9*	6.3	6.6	6.5	12.1	12.2	12.2	11.8	11.8	11.8
	0.01	9.9	11.8	*10.9*	6.3	6.4	6.4	12.6	12.7	12.7	n.a.	12.3	12.3

Phase	Test Conc [mg/L]	Münster (0-30 cm)			Münster (30-85 cm)			Münster (>85 cm)		
		Replicate			Replicate			Replicate		
		1	2	mean	1	2	mean	1	2	mean
Adsorption [1]	1	*50.5*	*54*	*52.3*	*9.9*	*11.1*	*10.5*	*9.4*	*9.5*	*9.5*
	0.1	*60.1*	*59.9*	*60*	*12.6*	*12.2*	*12.4*	*10.1*	*10*	*10.1*
	0.05	*58.5*	*59.7*	*59.1*	*11.6*	*13.9*	*12.8*	*8.9*	*11.6*	*10.3*
	0.01	*58.1*	*59.4*	*58.8*	*13.3*	*13.4*	*13.4*	*10.6*	*10.1*	*10.4*
1st Desorption [2]	1	22.4	22.6	22.5	7.9	8.9	8.4	7.5	7.6	7.6
	0.1	22.6	22.4	22.5	10.0	9.7	9.9	7.7	7.4	7.6
	0.05	22.5	23	22.8	9.7	10.7	10.2	7.9	8.9	8.0
	0.01	21.8	21.9	21.9	10.5	11.4	11.0	8.0	8.0	8.0
2nd Desorption [3]	1	14.9	12.1	12.9	1.5	1.6	1.6	1.6	1.6	1.6
	0.1	12.8	12.7	12.8	2.0	2.0	2.0	2.1	2.3	2.2
	0.05	12.9	13.4	13.2	1.4	2.6	2.0	1.6	2.4	2.0
	0.01	12.9	12.8	12.9	2.2	1.9	2.1	2.6	2.1	2.4

n.a.: not analysed
[1] end of adsorption phase, mean values expressed as percentage of applied radioactivity
[2] end of 1st desorption phase, mean values expressed as percentage of applied radioactivity
[3] end of 2nd desorption phase, mean values expressed as percentage of applied radioactivity
Values calculated based on data of the report are given in italics. Percentage of adsorbed calculated by subtracting residues in supernatant after adsorption (percentage) from total mass balance (percentage)

Table 7.1.3.1.1- 4: Definitive test: Concentration of [phenyl-U-^{14}C]-fluopicolide in aqueous and solid phase following 24 hours of adsorption.

Concentration (µg/mL)	Pikeville Sediment		Pikeville, North Carolina		Abington		Sarotti	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.010	0.0345	0.0029	0.0169	0.0076	0.0285	0.0044	0.0237	0.0052
0.010	0.0346	0.0028	0.0146	0.0076	0.0289	0.0046	0.0290	0.0053
0.050	0.1617	0.0161	0.0690	0.0377	0.1275	0.0249	0.1253	0.0266
0.050	0.1692	0.0156	0.0713	0.0392	0.1287	0.0253	0.1235	0.0264
0.100	0.3172	0.0343	0.1236	0.0753	0.2465	0.0528	0.2424	0.0556
0.100	0.3359	0.0332	n.a.	n.a.	0.2521	0.0518	0.2396	0.0561
1.000	3.1142	0.3829	1.2262	0.8409	2.2159	0.6221	2.1521	0.6500
1.000	3.2414	0.3691	1.2020	0.8345	2.2625	0.6405	2.0958	0.6614

Concentration (µg/mL)	Münster (0-30 cm)		Münster (30-85 cm)		Münster (> 85 cm)	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.010	0.0276	0.0041	0.0030	0.0107	0.0105	0.0025
0.010	0.0281	0.0044	0.0031	0.0100	0.0106	0.0020
0.050	0.1367	0.0237	0.0116	0.0515	0.0528	0.0072
0.050	0.1398	0.0241	0.0153	0.0522	0.0527	0.0136
0.100	0.2591	0.0427	0.0259	0.0950	0.0971	0.0189
0.100	0.2595	0.0427	0.0234	0.0955	0.0977	0.0174
1.000	2.3266	0.5142	0.1882	0.9955	1.0512	0.1572
1.000	2.4871	0.5167	0.2381	1.0018	1.0116	0.1906

n.a.: not analysed

Table 7.1.3.1.1- 5: Summary of adsorption/desorption constants and correlation coefficients of [phenyl-U-^{14}C]-fluopicolide in soil at 25 °C

Phase	Parameter	Units	Top-soils					Sub-soils	
			Pikeville Sediment	Pikeville	Abington[A]	Sarotti	Münster (0-30 cm)	Münster (30-85 cm)	Münster (> 85 cm)
Adsorption	$K_{F,ads}$	[mL/g]	7.73	1.42	3.36	3.2	4.54	0.21	0.17
	1/n	-	0.926	0.924	0.884	0.905	0.929	0.931	0.951
	R^2	-	0.999	0.999	1	0.998	0.999	0.996	0.987
	$K_{OC,ads}$	[mL/g]	373	283	152	356	349	106	83
1st Desorption	$K_{F,des1}$	[mL/g]	9.55	2.03	3.92	3.84	5.42	1.02	0.94
	1/n	-	0.94	0.905	0.89	0.904	0.942	1.042	0.965
	R^2		0.999	0.996	1	0.997	0.999	0.984	0.995
	$K_{OC,des1}$	[mL/g]	460	406	177	427	417	508	471
2nd Desorption	$K_{F,des2}$	[mL/g]	9.75	1.23	4.39	4.49	6.04	4.27	8.57
	1/n		0.917	0.755	0.889	0.888	0.944	1.245	1.532
	R^2	-	0.998	0.993	1	0.999	0.997	0.919	0.919
	$K_{OC,des2}$	[mL/g]	471	245	199	499	464	2134	4284

[A] K_F, K_{OC}, and 1/n values for Abington soil taken from report amendment, replacing original reported values of K_F 7.53, K_{OC} 341 and 1/n 0.929 following discovery of an error in the calculation.

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.1- 6**)**. The concentrations in the supernatant and the soil as given in the report were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

In the evaluation of the data according to the EFSA evaluators checklist an error in the original reported values of K_F, K_{OC}, and 1/n values for Abington soil was discovered. Correct values are provided in an amendment to the report.

Table 7.1.3.1.1- 6 Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Pikeville Sediment	Pikeville, North Carolina	Abington	Sarotti	Münster (0-30 cm)	Münster (30-85 cm)	Münster (> 85 cm)
Code	-	-	EFS 54	EFS 65	EFS 86	EFS 88	EFS 93	EFS 94	EFS 95
Adsorption method	-	-	indirect	indirect	indirect	indirect	indirect	indirect	indirect
Soil solution ratio	g/mL		1:5	1:5	1:5	1:5	1:5	1:5	1:5
Mass balance of ^{14}C	%	>90%	97-101.9	100.3-109.6	101.0-109.6	103.3-109.7	95.9-104.4	103.6-113.8	105.1-111.0
f – due to loss processes (estimated)	%	-	10	10	10	10	10	10	10
Adsorbed percentage (δ)	%	>20%	61.7–72.0	15.9-24.7	36.0-56.0	33.9-48.0	48.3-59.0	Low [includes -ve values]	Low [includes -ve values]
K_D x soil:solution ratio		>0.3	1.63–2.47	0.29–0.44	0.71–1.30	0.63–1.12	0.90–1.35	0.04 – 0.06	0.03 – 0.05
$^{\#}K_{fE}$ / K_f†	-	<1.2	1.16-1.19	1.68-2.69	1.22-1.39	1.26-1.42	1.20-1.26	-1.0-0.41	-0.41-0.38
ads K_F	L/kg	-	7.736	1.416	3.351	3.201	4.546	0.213	0.166
95% confidence interval	-	-	7.069-8.466	1.292-1.553	3.255-3.449	2.815-3.640	4.181-4.942	0.177-0.255	0.120-0.230
ads 1/n	-	-	0.926	0.924	0.882	0.908	0.930	0.930	0.950
95% confidence interval	-	-	0.903-0.948	0.896-0.952	0.874-0.890	0.868-0.941	0.907-0.952	0.867-0.991	0.840-1.060
ads R^2	-	>0.975	0.999	0.999	1.000	0.998	0.999	0.996	0.987
ads $K_{F,OC}$	L/kg	-	373.7	283.3	151.6	355.7	349.7	106.3	83.2
Visual fit to Freundlich isotherm	-	-	Good	Good	Good	Accept.	Accept.	Accept.	Accept.
Residual plots randomly distributed	-	-	Good	Good	Good	Accept.	Accept.	Good	Accept.

†There is insufficient data in the report to confirm parental mass balance (and thus determine precise "f" value for evaluation). Assuming an "f" value of 10 leads to acceptable Kfe / Kf values (approx. 1.3) in four of the five soils but is less good in the fifth soil ((ca 2.5 at top concentration).

Relevant quality checks were performed to evaluate the acceptability of the study. For soils: EFS 54, EFS 65, EFS 86, EFS 88 and EFS 93 these checks confirmed that the mass balance was acceptable (95.9 - 109.7%) and that the % adsorption was also generally acceptable (15.9 - 72.0%; only <20% at one concentration in one soil). The reported LOQ of 0.19 ng of the analytical method (LSC) is assumed to be acceptable. The validity of using the indirect method, based on a Kd * soil/solution ratio > 0.3, could not be confirmed. The degree of sorption was sufficient, however there were signs of degradation the effect of which could not be fully assessed with the limited data available in the report. The R^2 of the standard linear regressions ranged from 0.998 to 1.000 and the visual fit of both the standard regression and the residual plots were either acceptable or good.

Overall the study has been conducted to a reasonable standard, although certain criteria that might be expected in a modern study have not been reported. In particular there is insufficient data in the report to confirm parental mass balance (and thus determine appropriate "f" value for evaluation). Samples in adsorption equilibrium experiment (24, 48 and 72 hours) have been solvent extracted and the adsorption supernatants and solvent extracts analysed by HPLC. For analysis of all solvent extracts 100% ROI (regions of interest) is reported as parent. Aqueous supernatants show some degradation with fluopicolide ranging from 89 to 94% ROI after 24 hours equilibration (but as low as 78% after longer equilibration). It was not possible to generate parental mass balance figures from the reported data. However, assuming an "f" value of 10 (based on the extent of degradation observed at 24 hours) leads to acceptable K_{fe} / K_f values (approximately 1.3) in four of the five soils but is less good in the fifth soil (*ca.* 2.5 at top concentration).

For the subsoils EFS 94 & EFS 95, due to the very low sorption in these soils, additional quality criteria as set out in evaluator's guidelines are largely not met.

The results of the evaluation are summarised in the tables below.

Figure 7.1.3.1.1- 1: Freundlich Isotherms of fluopicolide in Pikeville Sediment (EFS 54) at 25°C

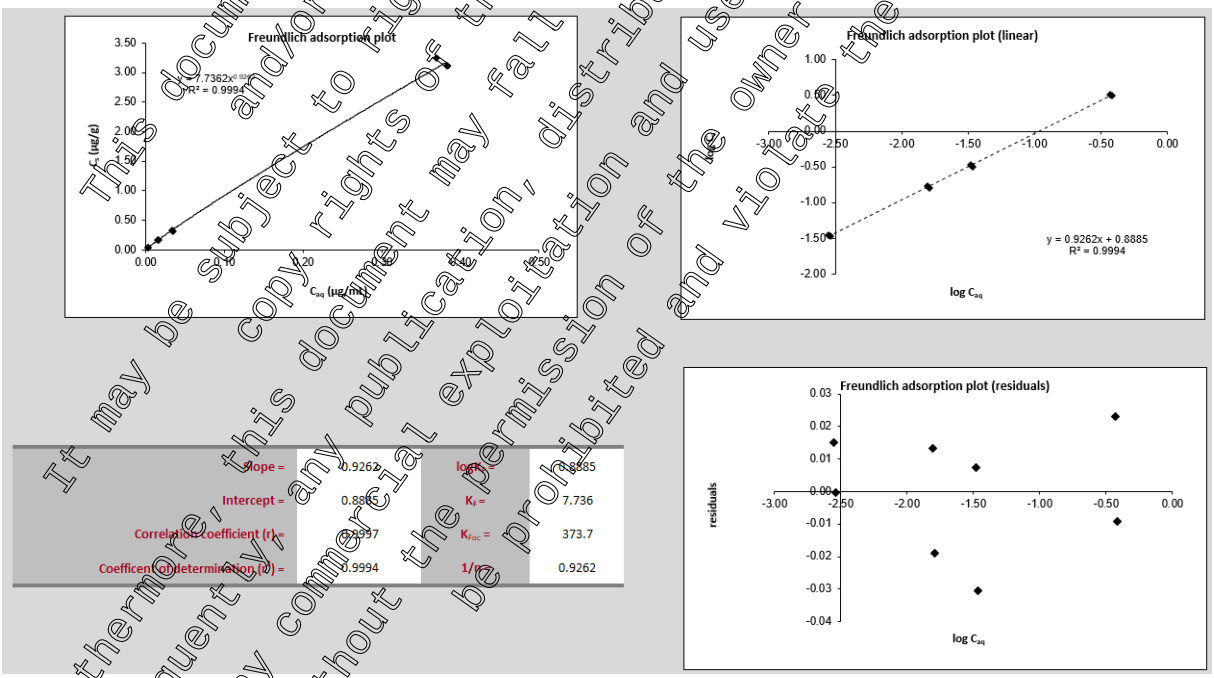
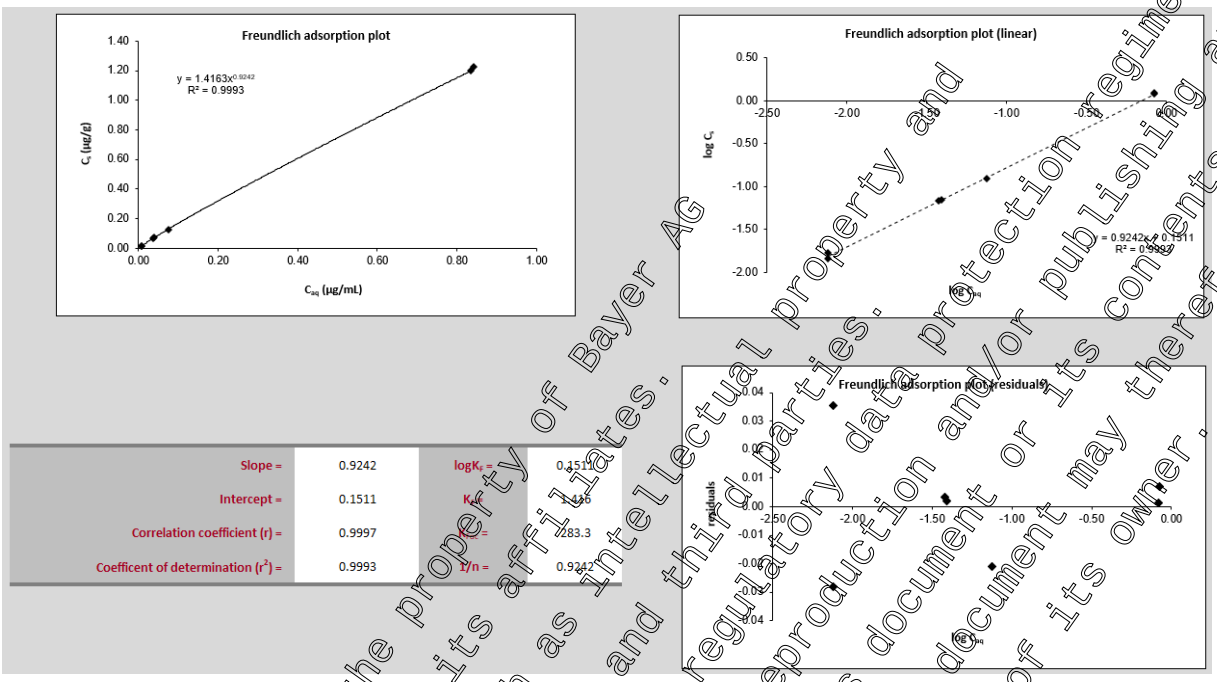
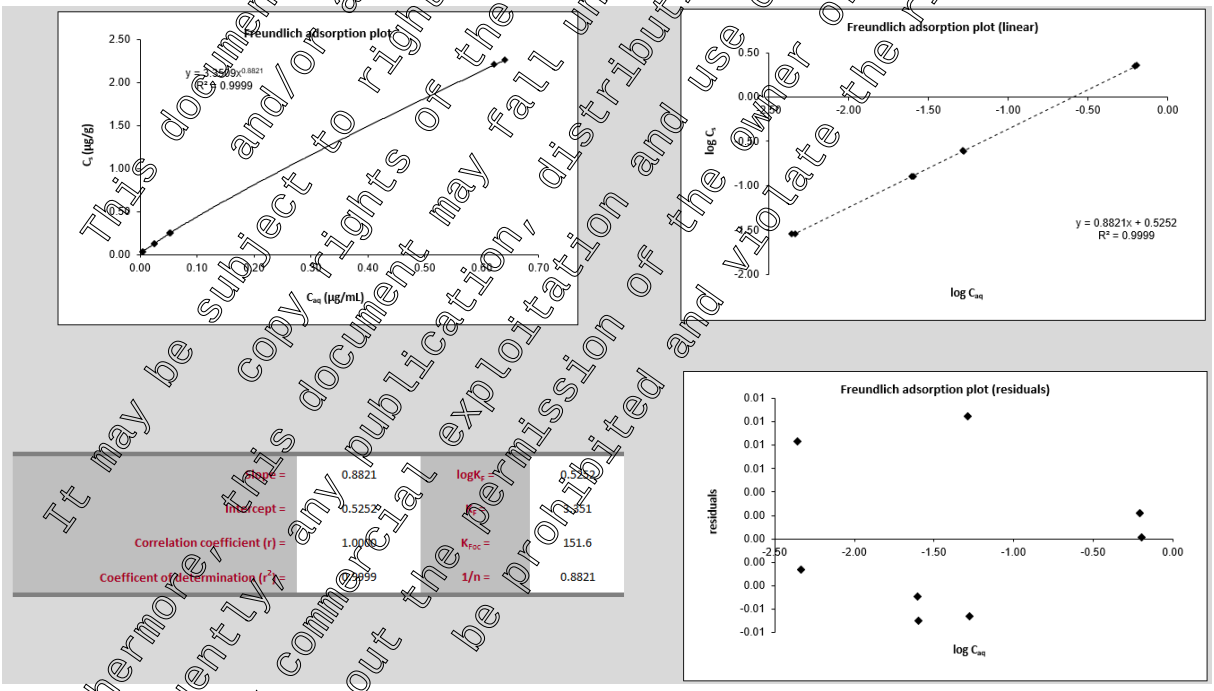


Figure 7.1.3.1.1- 2: Freundlich Isotherms of fluopicolide in Soil Pikeville, North Carolina (EFS 65) at 25 °C



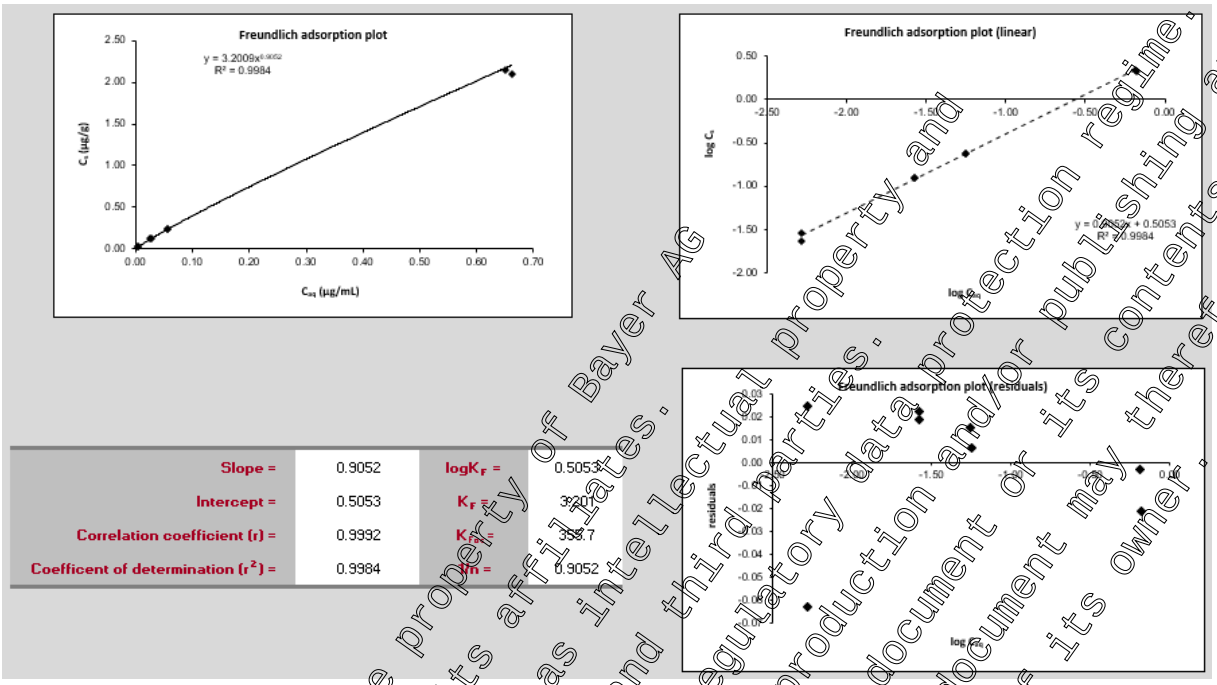
Slope =	0.9242	$\log K_f$ =	0.1511
Intercept =	0.1511	K_f =	1.416
Correlation coefficient (r) =	0.9997	K_{foc} =	283.3
Coefficent of determination (r^2) =	0.9993	1/n =	0.9242

Figure 7.1.3.1.1- 3: Freundlich Isotherms of fluopicolide in Soil Abington (EFS 86) at 25 °C



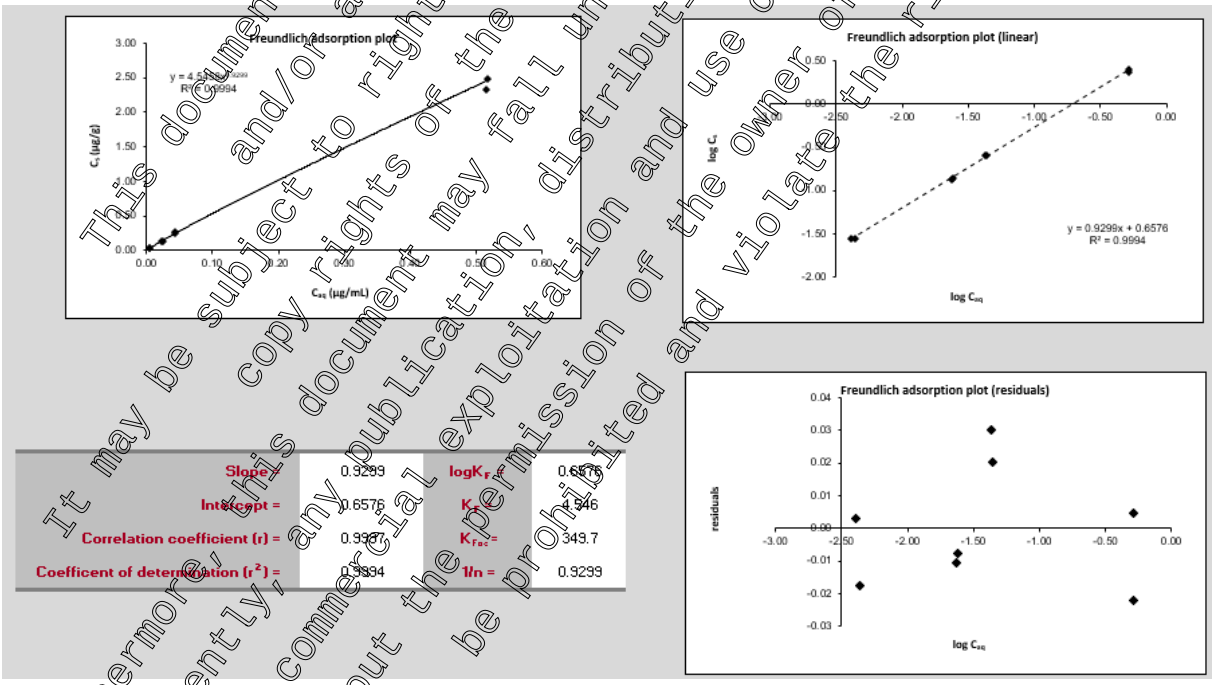
Slope =	0.8821	$\log K_f$ =	0.5252
Intercept =	0.5252	K_f =	3.351
Correlation coefficient (r) =	1.0000	K_{foc} =	151.6
Coefficent of determination (r^2) =	0.9999	1/n =	0.8821

Figure 7.1.3.1.1- 4: Freundlich Isotherms of fluopicolide in Soil Sarotti (EFS 88) at 25 °C



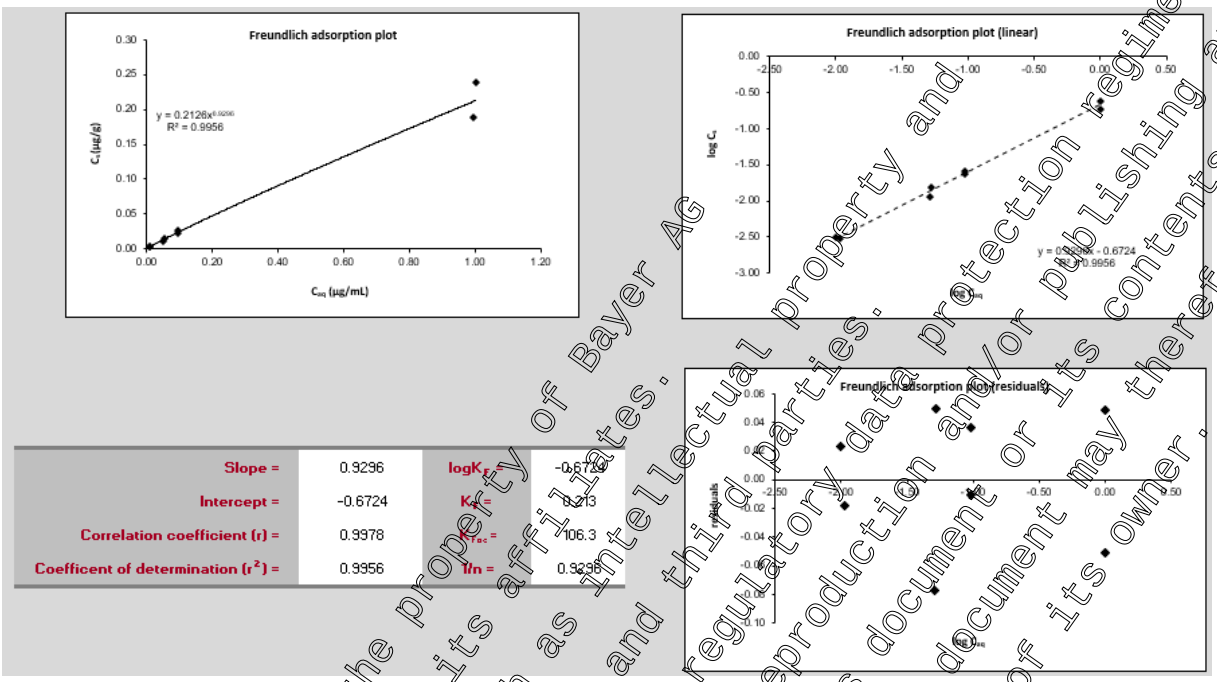
Slope =	0.9052	$logK_F$ =	0.5053
Intercept =	0.5053	K_F =	3.201
Correlation coefficient (r) =	0.9992	K_{Foc} =	358.7
Coefficent of determination (r^2) =	0.9984	1/n =	0.9052

Figure 7.1.3.1.1- 5: Freundlich Isotherms of fluopicolide in Soil Münster (0-30 cm) (EFS 93) at 25 °C



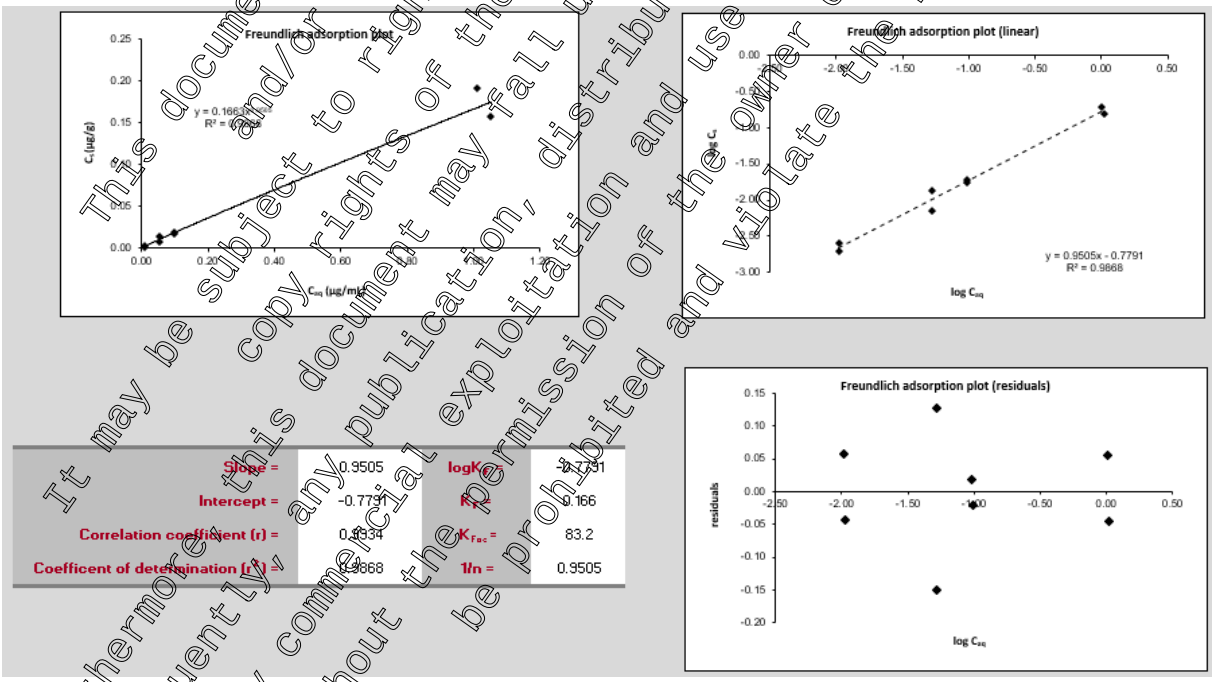
Slope =	0.9299	$logK_F$ =	0.6576
Intercept =	0.6576	K_F =	4.546
Correlation coefficient (r) =	0.9997	K_{Foc} =	343.7
Coefficent of determination (r^2) =	0.9994	1/n =	0.9299

Figure 7.1.3.1.1- 6: Freundlich Isotherms of fluopicolide in Soil Münster (30-85 cm) (EFS 94) at 25 °C



Slope =	0.9296	$\log K_F$ =	-0.6724
Intercept =	-0.6724	K_F =	0.213
Correlation coefficient (r) =	0.9978	K_{Foc} =	106.3
Coefficent of determination (r^2) =	0.9956	1/n =	0.9296

Figure 7.1.3.1.1- 7: Freundlich Isotherms of fluopicolide in Soil Münster (> 85 cm) (EFS 95) at 25 °C



Slope =	0.9505	$\log K_F$ =	-0.7791
Intercept =	-0.7791	K_F =	0.166
Correlation coefficient (r) =	0.9934	K_{Foc} =	83.2
Coefficent of determination (r^2) =	0.9868	1/n =	0.9505

A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.1- 7. The impact on reported endpoints is summarised in Table 7.1.3.1.1- 8.

Table 7.1.3.1.1- 7: Summary of Quality Criteria and Regulatory Interpretation

Fluopicolide			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Pikeville Sediment	loam	EFS-54	9	0	0
Pikeville, North Carolina	sand	EFS-65	6	2	1
Abington	sandy loam	EFS-86	8	0	1
Sarotti	silty clay loam	EFS-88	8	0	1
Münster (0-30 cm)	loamy sand	EFS-93	8	0	1
Münster (30-85 cm)	loamy sand	EFS-94	5	1	3
Münster (>85 cm)	loamy sand	EFS-95	5	1	3

Table 7.1.3.1.1- 8: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Pikeville Sediment	loam	EFS-54	373	373.7	0.926	0.926
Pikeville, North Carolina	sand	EFS-65	283	283.3	0.924	0.924
Abington	sandy loam	EFS-86	152 [A]	151.6	0.884 [A]	0.882
Sarotti	silty clay loam	EFS-88	356	355.7	0.905	0.905
Münster (0-30 cm)	loamy sand	EFS-93	349	349.7	0.929	0.93
Münster (30-85 cm)	loamy sand	EFS-94	106	106.3	0.931	0.93
Münster (>85 cm)	loamy sand	EFS-95	83	83.2	0.951	0.95

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.
[A] Value taken from report amendment

III. Conclusion

The adsorption constant $K_{F(ads)}$ of fluopicolide was between 1.42 and 7.73 mL/g for the tested top-soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 152 to 373 mL/g. The Freundlich exponent 1/n was between 0.884 and 0.929, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

For sub-soils, $K_{F(ads)}$ of fluopicolide was 0.21 and 0.17 mL/g for depth 30-85 cm and > 85 cm, respectively; the respective normalized $K_{OC(ads)}$ was106 and 83 mL/g. The Freundlich exponent 1/n was 0.931 and 0.951, respectively.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the values derived in four topsoils and one sediment were acceptable according to the quality criteria and therefore acceptable for regulatory use.

Assessment and conclusion by applicant:

Although the study has a number of deviations from the current version of OECD 106 (2002), it is considered valid to assess the adsorption and desorption characteristics of fluopicolide in soil.

Data Point:	KCA 7.1.3.1.1/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE C638206: Adsorption to and desorption from four european soils collected from field dissipation trial sites
Report No:	C033799
Document No:	M-233840-01-1
Guideline(s) followed in study:	EU (=EEC): 91/414/EEC, Section 7.1.2
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption behaviour of [phenyl-UL-^{14}C]-fluopicolide were studied in four soils in batch equilibrium experiments in the laboratory in the dark at 20 °C.

Soil	Soil Code	Source	Texture (USDA)	pH ($CaCl_2$)	OC [%]
Philippsburg	03/02	Germany	Sandy loam	6.3	0.6
Senas	03/03	France	Clay loam	7.6	1.5
Huntlosen	03/04	Germany	Loamy sand	5.3	1.6
Rodelsee	03/05	Germany	Clay	7	1.5

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1/3 (all soils). [Phenyl-UL-^{14}C]-fluopicolide was dissolved in 0.01M calcium chloride solution at nominal concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L. The desorption phases were performed by supplying pre-adsorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution. A total of three desorption cycles were conducted for each soil. An adsorption equilibrium time of 48 hours and a desorption equilibrium time of 2 hours were selected for each soil. The test was performed in glass flasks with screw caps.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and the amount of test item in the supernatants was analyzed by liquid scintillation counting (LSC). The sorption parameters were calculated using Freundlich isotherms.

For all soils, the recovery of radioactivity was quantitative with recoveries ranging from 99.7% to 102.8% of applied radioactivity. The stability of [phenyl-U-^{14}C]-fluopicolide was confirmed in adsorption supernatants, desorption supernatants and soil extracts.

In the definitive adsorption test, 41.15 to 58.42% AR, 26.19 to 37.90% AR, 17.74 to 21.81% AR and 27.90 to 40.36% AR were adsorbed in Philippsburg, Senas, Huntlosen and Rödelsee soils, respectively.

The calculated Freundlich adsorption coefficients (K_f) ranged from 1.49 to 9.27. When corrected for organic carbon content of the soil, the K_{oc} values obtained ranged from 172 to 580. The Freundlich exponents ranged from 0.841 to 0.953 indicating that the concentration of the test item affected the adsorption behaviour in the examined concentration range in three out of four soils.

The study results were evaluated with the EFSA OECD 106 checklist. This confirmed all of soils tested were acceptable according to the quality checks and thus it can be concluded that these 4 soils are appropriate for use in regulatory modelling.

Following the adsorption phase, 18.92 to 20.55%, 17.37 to 21.21%, 13.68 to 15.05% and 18.84 to 23.54% of the initially adsorbed amount were desorbed in Philippsburg, Senas, Huntlosen and Rödelsee soils after 2 hours, respectively.

According to Briggs, [phenyl-UL-^{14}C]-fluopicolide can be classified as low for adsorption and desorption.

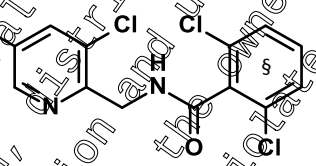
Soil origin	Philippsburg	Senas	Huntlosen	Rödelsee
Soil ID	03/02	03/03	03/04	03/05
Soil type (USDA)	Sandy loam	Clay loam	Loamy sand	Clay
pH (0.01M $CaCl_2$)	6.3	7.6	5.3	7.0
Organic carbon [%]	0.6	1.5	1.6	1.5
$K_F^{(ads)}$ [mL/g]	1.49	2.59	9.27	2.59
1/n	0.841	0.882	0.953	0.859
$K_{F,OC}^{(ads)}$ [mL/g]	248	239	580	172
$K_F^{(des1)}$ [mL/g]	2.25	4.72	11.77	2.93
1/n	0.844	0.893	0.970	0.848
$K_{F,OC}^{(des1`)}$ [mL/g]	374	315	736	195

1. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-fluopicolide (referred to as [U-^{14}C-phenyl]-AE C638206 in the report)



§ = position of radiolabel.

Batch number: SEL/1200 (9R1935)

Specific radioactivity: 5.5 MBq/mg (148.6 µCi/mg)

Radiochemical purity: 99.8 % (TLC and autoradiography)

Stability of test compound: Generally stable during the equilibrium periods (up to 48 hours).

2. Test Soil

The adsorption/desorption behaviour of [phenyl-U-^{14}C]-fluopicolide was characterised in four soils using the batch equilibrium method. The four soils, taken from field dissipation test sites, were categorised under the ADAS and USDA classifications as a sandy loam (Philippsburg), a clay loam (Senas), a loamy sand (Huntlosen) and a clay (Rödelsee). The soil characteristics are given below in Table 7.1.3.1.1- 9.

Table 7.1.3.1.1- 9: Physico-chemical characteristics of test soils

Characteristic / Code	Units	Soil			
Soil Batch No.	-	03/02	03/03	03/04	03/05
Location	City or Township	Philippsburg	Senas	Huntlosen	Rodelsee
Origin	State, Country	Germany	France	Germany	Germany
Textural Class	USDA	Sandy Loam	Clay Loam	Loamy Sand	Clay
Particle Size Analysis, ADAS:					
Total Sand	(0.063 - 2.00 mm)	79%	19%	78%	37%
Silt	(0.002 - 0.063 mm)	10%	49%	16%	19%
Clay	(< 0.002 mm)	11%	31%	6%	44%
Textural Class	ADAS	Sandy Loam	Clay Loam	Loamy Sand	Clay
pH	Water (1:5)	7.1	8.3	6.3	7.9
	1M KCl	6.5	7.5	5.2	7.1
	0.01 M $CaCl_2$	6.3	7.6	5.3	7.0
Organic Carbon	%	0.6	1.5	1.6	1.5
$Ca_{exchangeable}$	mg/kg	638	6848	489	2356
$Mg_{exchangeable}$	mg/kg	18.5	95.3	37.9	462
$Na_{exchangeable}$	mg/kg	62.9	29.8	20.0	8.9
$K_{exchangeable}$	mg/kg	228	347	141	862
$Mn_{exchangeable}$	mg/kg	0.97	1.26	27.92	0.72
$CaCO_3$	%	0	41.0	0	0
Phosphorus total	mg/kg	513	1084	774	1214
Nitrogen total	%w/w	0.057	0.150	0.102	0.160
Maximum Water Holding Capacity	g/100 g dry matter	33.0	49.9	39.6	52.4
Water Holding Capacity	% at Saturation	35.8	49.7	39.8	54.4
	% at 0.05 bar	14.7	31.2	22.2	29.7
	% at 0.1 bar	11.6	26.3	14.9	26.5
	% at 0.33 bar	7.1	19.3	8.8	19.7
	% at 2 bar	5.8	16.1	7.0	17.0
	% at 15 bar	3.5	11.8	3.7	13.7

B. Study Design

1. Experimental Conditions

The test system for adsorption and desorption in batch equilibrium experiments consisted of thick walled borosilicate glass tubes with an external plastic coating. The tubes were of approximately 125 mL capacity and sealed with a screw cap which was lined with a Teflon seal.

Preliminary tests checked for adsorption to the tubes, determined any background radioactivity in the soil, determined the appropriate soil:solution ratio to be used, and determined the time required for the compound to equilibrate between soil and water under both adsorption and desorption conditions.

The main test was performed in duplicate. The adsorption phase was carried out using air-dried soil pre-equilibrated (12 hours) in aqueous 0.01 M $CaCl_2$ solution with a soil-to-solution ratio of 1:3 (20 g soil dry weight/ 60 mL solution). Fluopicolide was applied at nominal concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L (dosing stock contained 2 mL of acetonitrile, diluting to approximately 15 mL with de-ionised water). The amount of organic solvent in samples did not exceed 1%. The desorption phase was performed three times by supplying pre-adsorbed soil samples with fresh aqueous 0.01 M $CaCl_2$

solution for all test concentrations. The adsorption step was carried out for 48 hours in the dark at 20 ± 2 °C under continuous agitation. The desorption steps were carried out each for 2 hours in the dark at 20 ± 2 °C under continuous agitation.

[Phenyl-U-^{14}C]-fluopicolide was dissolved in 0.01M calcium chloride solution at concentrations of 0.0098, 0.048, 0.097, 0.493 and 0.948 mg/L in Philippsburg and Senas soils and 0.0096, 0.046, 0.097, 0.465 and 0.563 mg/L in Huntlosen and Rodelsee soils. In Huntlosen and Rodelsee soils samples at the highest concentration rate were treated at a lower concentration than intended (0.56 mg/mL versus 0.95 mg/mL for the Philippsburg and Senas soils). It was concluded that the highest concentration treatment solution exceeded the aqueous solubility of [phenyl-U-^{14}C]-fluopicolide, even in the presence of acetonitrile and did not remain totally in solution for the time period between treatments. Once applied to the samples [phenyl-U-^{14}C]-fluopicolide was diluted in calcium chloride solution and no longer exceeded the aqueous solubility. This dilution combined with the 48 hour equilibration period ensured that the test compound was fully dissolved. It was concluded that this did not have any impact on the validity of the results of the study.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		20 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ 12 hours
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 0.01, 0.05, 0.1, 0.5 and 1.0 µg/mL
	Analytically (LSC) measured concentrations	Concentrations in test solution: 0.010, 0.048, 0.097, 0.493 and 0.948 µg/mL
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile and de-ionised water Study media – calcium chloride
Soil: Solution ratio		1:3 i.e. 20 g soil dry weight equivalent to 60 mL solution
Number of replicates	Control	Not stated
	Treatments	Duplicate
Equilibration conditions	Time	48 hrs
	Temperature	20±2°C
	Dark	In the dark
	Shaking method	End-over-end shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	2000 rpm
	Duration	10 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 36 to 81% AR. See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		3
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 60 mL was used as equilibration solution.
Soil: Solution ratio		1:3 i.e. 20 g soil dry weight equivalent to 60 mL solution
Number of replicates	Control	Not stated
	Treatments	Duplicate
Desorption Equilibration conditions	Time	2 hours
	Temperature	20±2°C
	Dark	In the dark
	Shaking method	End-over-end shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	2000 rpm
	Duration	10 minutes
	Method of separating supernatant	Supernatant was carefully decanted.
Desorption cycle 2 & 3	Method	Same as above

2. Analytical Procedures

After each adsorption and desorption step the aqueous supernatant was separated from the soil by centrifugation and the amount of fluopicolide in the supernatants was analysed by liquid scintillation counting (LSC).

The partition of the test item in the adsorption and desorption batch equilibrium experiment was determined based on the radioactivity content in the supernatant only. After the desorption steps, all soils were extracted with acetonitrile. The radioactivity remaining in soil after extraction was quantified by combustion to establish the material balance.

For the parental mass balance, adsorption supernatants, desorption supernatants and soil extracts from samples treated at the highest two concentrations were analysed by HPLC. No significant degradation of [phenyl-U-^{14}C]-fluopicolide occurred over the duration of the test (<1%).

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. Mass balance and results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption. A soil-to-solution ratio of 1:3 was regarded adequate for all experiments as the levels of fluopicolide adsorbed to all top soils were within the acceptable range of 20 to 80%. An equilibration time of 48 h was needed to reach adsorption equilibrium. An equilibration time of 2 h was used for desorption.

For the definitive test the overall mean mass balance of each soil investigated ranged from 99.7 to 102.8% AR (Table 7.1.3.1.1- 10).

Table 7.1.3.1.1- 10: Definitive test: Total recovery (% AR) of [phenyl-U-^{14}C]-fluopicolide in samples after adsorption and desorption phases

Soil	Philippsburg	Senas	Huntlosen	Rodelsee
	03/02	03/03	03/04	03/05
	Sandy Loam	Clay Loam	Loamy Sand	Clay
Adsorption	50.8	32.0	19.5	33.9
Desorption 1	19.7	19.4	14.2	21.4
Desorption 2	8.5	11.5	10.4	12.5
Desorption 3	4.3	7.2	8.2	8.0
Solvent Extract	14.4	25.5	44.0	21.1
Combustion	3.6	4.1	6.5	5.5
Total (%)	101.4	99.7	102.8	102.4

B. Transformation of test substance

Following adsorption phase of 48 hours to soil and radio-HPLC analysis, the majority of the radioactivity in the calcium chloride supernatants was fluopicolide with small amounts of an unidentified metabolite observed in the definitive study at a maximum of 0.9% AR. Only fluopicolide was detected in desorption supernatants and soil solvent extracts for all soils.

C. Findings

The calculated Freundlich adsorption coefficients (K_f) ranged from 1.49 to 9.27. In the Huntlosen loamy sand soil, the 1/n value was 0.953, with a linear relationship between the concentration in soil and solution for this soil, with the amount absorbed being independent of concentration. The relationship between the soil and solution concentration for the three remaining soils was non linear with 1/n values ranging from 0.841 to 0.882, which may be may be indicative of increased competition for binding sites in these soils and consequently greater adsorption at lower concentrations. When corrected for organic carbon content of the soil, the K_{oc} values obtained ranged from 172 to 580 (mean value 310).

The first desorption $K_{oc\ des}$ values were slightly higher than the adsorption values for all soils and $K_{oc\ des}$ values for the three desorption cycles demonstrated a gradual increase with successive desorption cycles. These results showed that adsorption would be expected to be only partially reversible.

Table 7.1.3.1.1- 11: Definitive test: Concentration of [phenyl-U-^{14}C]-fluopicolide in aqueous and solid phase following 48 hours of adsorption.

Soil ID	Philippsburg		Senas		Soil ID	Huntlosen		Rodelsee	
Concentration (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Concentration (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.010	0.0158	0.0044	0.0202	0.0029	0.010	0.0226	0.0019	0.0182	0.0032
0.010	0.0152	0.0046	0.0201	0.0029	0.010	0.0229	0.0018	0.0182	0.0032
0.048	0.0693	0.0246	0.0952	0.0156	0.046	0.1083	0.0092	0.0805	0.0137
0.048	0.0684	0.0247	0.0953	0.0155	0.046	0.1083	0.0093	0.0828	0.0170
0.097	0.1251	0.0531	0.1812	0.0342	0.097	0.2288	0.0198	0.1650	0.0392
0.097	0.1249	0.0532	0.1789	0.0348	0.097	0.2278	0.0201	0.1651	0.0392
0.493	0.5468	0.3004	0.9591	0.1969	0.465	1.0708	0.1034	0.7085	0.2137
0.493	0.5201	0.3083	0.8442	0.1989	0.465	1.0756	0.1022	0.7127	0.2128
0.948	0.9963	0.5961	1.5588	0.4053	0.563	1.2798	0.1309	0.8140	0.2743
0.948	0.9703	0.6056	1.5482	0.4079	0.563	1.3331	0.1328	0.7986	0.2776

Table 7.1.3.1.1- 12: Summary of adsorption/desorption constants and correlation coefficients of [phenyl-U-^{14}C]-fluopicolide in soil

Cycle	Soil	03/02	03/03	03/04	03/05
		Philippsburg	Senas	Huntlosen	Rödelsee
	Textural class	Sandy Loam	Clay Loam	Loamy Sand	Clay
Adsorption	K_f	1.49	3.59	9.27	2.59
	1/n	0.841	0.882	0.953	0.859
	K_{oc}	248	239	580	172
	Correlation	1.000	0.999	1.000	0.999
Desorption 1	K_{des1}	2.25	4.72	11.77	2.93
	1/n	0.844	0.893	0.970	0.848
	$K_{oc\ des1}$	374	315	736	195
	Correlation	0.999	0.999	1.000	0.999
Desorption 2	K_{des2}	3.46	5.96	13.43	3.39
	1/n	0.855	0.899	0.970	0.850
	$K_{oc\ des2}$	577	397	840	226
	Correlation	0.998	0.999	1.000	0.997
Desorption 3	K_{des3}	5.36	7.72	15.35	3.93
	1/n	0.868	0.911	0.972	0.850
	$K_{oc\ des3}$	893	515	960	262
	Correlation	0.997	0.999	1.000	0.996

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet tool provided by EFSA (summarised in Table 7.1.3.1.1- 13). The concentrations in the supernatant and the soil as given in the report were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.1- 13 Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Philippsburg	Senas	Huntlosen	Rodelsee
Code	-	-	03/02	03/03	03/04	03/05
Adsorption method	-	-	indirect	indirect	indirect	indirect
Soil solution ratio	g/mL		1:3	1:3	1:3	1:3
Mass balance of ^{14}C	%	>90%	98.9 - 108.5	98.7 - 101.5	100.5 - 107.0	100.1 - 104.7
f – due to loss processes	%	-	3.1	4.3	1.8	0.2
Adsorbed percentage (δ)	%	>20%	36.1 - 55.6	57.0 - 70.6	76.4 - 80.9	50.7 - 66.4
K_D x soil:solution ratio		>0.3	0.53 - 1.20	1.27 - 2.33	3.26 - 4.16	0.96 - 1.88
$^{\#}K_{fE}$ / K_f	-	<1.2	1.1	1.1	1.0	1.0
ads K_F	L/kg		1.488	3.586	9.273	2.587
95% confidence interval		*	1.413 - 1.567	3.306 - 3.890	8.795 - 9.777	2.401 - 2.787
ads 1/n	-		0.841	0.882	0.953	0.859
95% confidence interval	-	*	0.825 - 0.856	0.860 - 0.904	0.940 - 0.965	0.838 - 0.880
ads R^2		>0.975	0.999	0.999	1.000	0.999
ads $K_{F,OC}$	L/kg		248	239	580	173
Visual fit to Freundlich isotherm	-	-	Good	Accept.	Accept.	Accept.
Residual plots randomly distributed	-	-	Good	Accept.	Accept.	Accept.

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance was acceptable (98.9 - 108.5%) and that the % adsorption was also acceptable (50.7 - 80.9%). The acceptability of the analytical method (LSC) was confirmed over the entire range of concentrations measured (reported LOQ of 0.09 mg/L represents <1% at lowest test concentration). The validity of using the indirect method, based on a Kd * soil/solution ratio > 0.3 was confirmed in all soils. The R^2 of the standard linear regressions ranged from 0.999 to 1.000 and the visual fit of both the standard regression and the residual plots were good.

Overall the study looks to have been conducted to a good standard. Although analytical data is reported for adsorption supernatant, desorption supernatant and solvent extracts at the top two concentrations in all soils, there is no reporting of parental mass balance. Sufficient data is however available to do this calculation if it can be assumed that the [phenyl-U-^{14}C]-fluopicolide content in the desorption 2 and desorption 3 supernatants is 100% (as found for the preceding desorption 1 supernatant and subsequent solvent extract). Fluopicolide was essentially stable (lowest HPLC purity 97.7% in an adsorption supernatant; 100% in all other sample types). Using the resultant "f" values leads to acceptable K_{fe} / K_f values (< 1.2) in all of the soils.

The evaluation confirmed all of soils tested were acceptable according to the quality checks and thus it can be concluded that these 4 soils are appropriate for use in regulatory modelling. The results of the evaluation are summarised in the tables below.

Figure 7.1.3.1.1- 8: Freundlich Isotherms of fluopicolide in Philippsburg soil (03/02) at 20°C

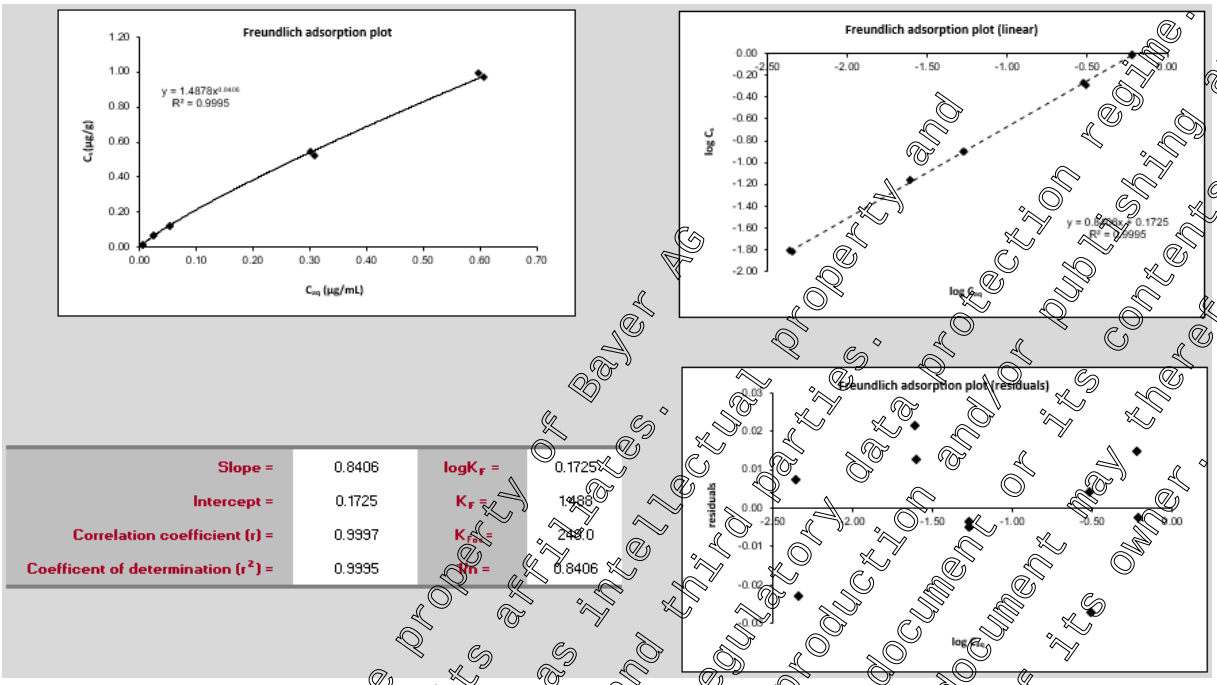


Figure 7.1.3.1.1- 9: Freundlich Isotherms of fluopicolide in Senas Soil (03/03) at 20°C

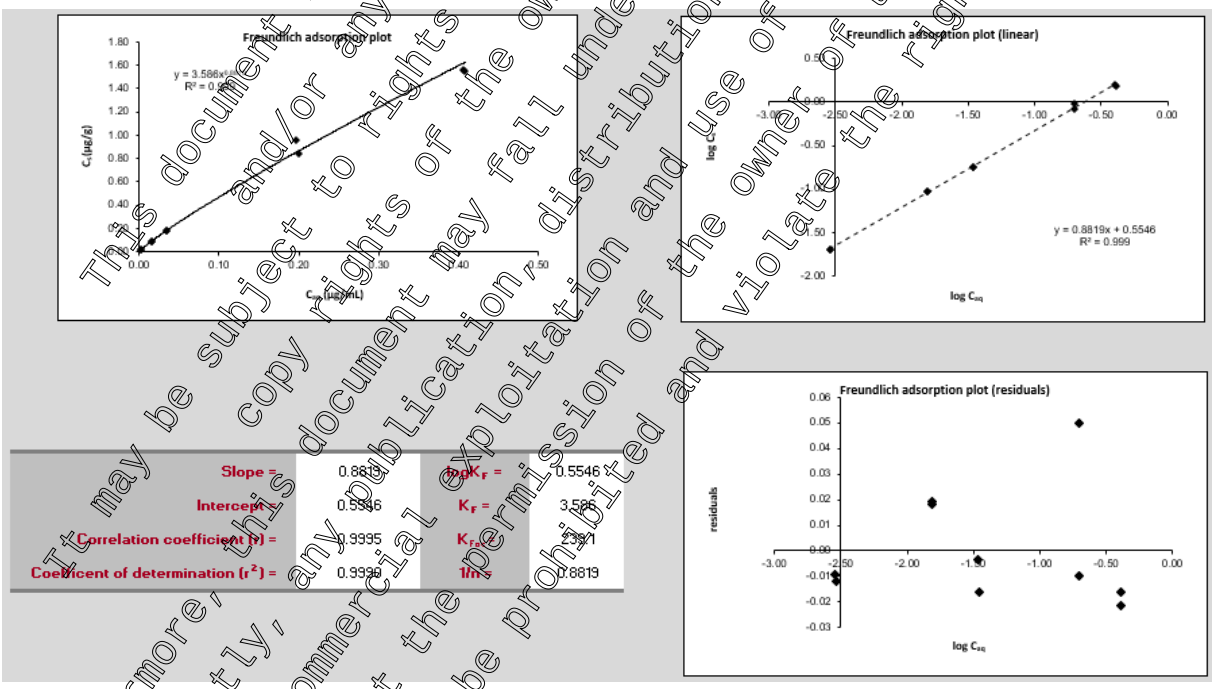


Figure 7.1.3.1.1- 10: Freundlich Isotherms of fluopicolide in Huntlosen Soil (03/04) at 20°C

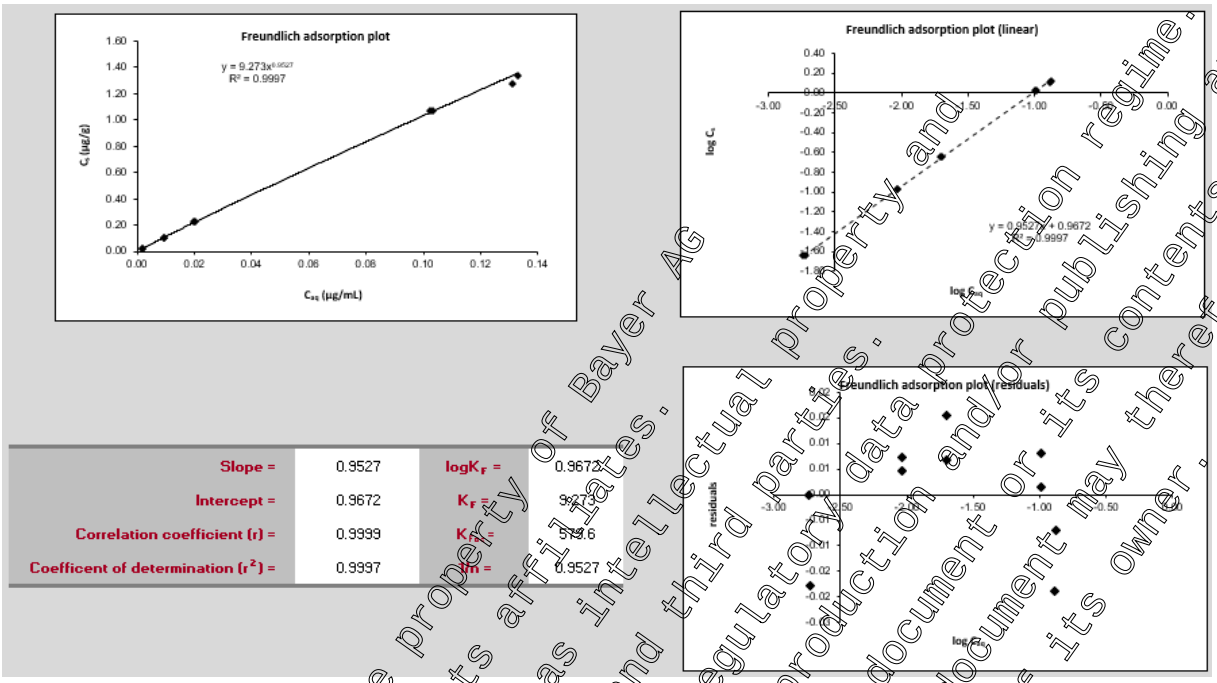
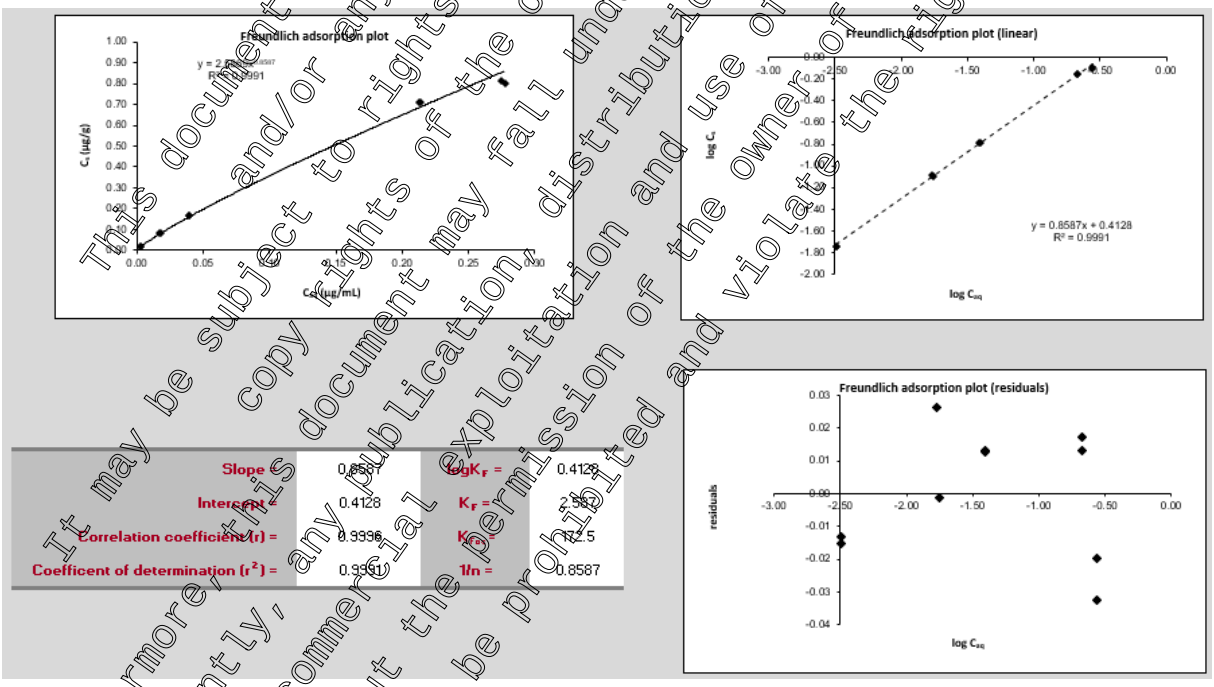


Figure 7.1.3.1.1- 11: Freundlich Isotherms of fluopicolide in Rodelsee Soil (03/05) at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.1- 14. Where applicable, the impact on reported endpoints is summarised in Table 7.1.3.1.1- 15.

Table 7.1.3.1.1- 14: Summary of Quality Criteria and Regulatory Interpretation

Fluopicolide			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Philippsburg	Sandy Loam	03/02	9	0	0
Senas	Clay Loam	03/03	9	0	0
Huntlosen	Loamy Sand	03/04	9	0	0
Rodelsee	Clay	03/05	9	0	0

Table 7.1.3.1.1- 15: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Philippsburg	Sandy Loam	03/02	248	248	0.841	0.841
Senas	Clay Loam	03/03	239	239	0.882	0.882
Huntlosen	Loamy Sand	03/04	580	580	0.953	0.953
Rodelsee	Clay	03/05	172	173	0.859	0.859

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

These results indicate that fluopicolide is moderately absorbed to soil. The adsorption constant $K_{F(ads)}$ of fluopicolide ranged from 1.49 to 9.27 mL/g in the tested soils; the normalised adsorption constant $K_{OC(ads)}$ ranged from 172 to 580 mL/g. The Freundlich exponent 1/n was between 0.841 and 0.953, indicating that the concentration of the test item effects its adsorption behaviour in the concentration range examined.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the four soils were acceptable according to the quality criteria and therefore suitable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of fluopicolide in soil.

Data Point:	KCA 7.1.3.1.1/03
Report Author:	[redacted]
Report Year:	2015
Report Title:	[pyridyl-2,6-14C] fluopicolide: Adsorption/desorption in four different soils - Final report amendment 01 -
Report No:	AS447
Document No:	M-544194-02-1
Guideline(s) followed in study:	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 Adsorption/Desorption (Batch Equilibrium), October 2008
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption characteristics of [2,6-pyridyl-^{14}C]-fluopicolide were studied in four soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 1 °C.

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
[redacted]	Monheim, Germany	Loam	5.0	1.8
H[redacted]	Burscheid, Germany	Silt loam	6.1	1.9
Dollendorf II	Blankenheim, Germany	Clay loam	7.3	4.8
L[redacted]	Monheim, Germany	Sandy loam	6.5	1.5

The adsorption phase of the study was carried out using pre-equilibrated air-dried soil with [2,6-pyridyl-^{14}C]-fluopicolide concentrations of nominal 1.00, 0.30, 0.10, 0.03, and 0.01 mg/L for 24 hours. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

The adsorption 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 48 h showed that >90% of applied [2,6-pyridyl-^{14}C]-fluopicolide could be recovered.

The mass balance was determined by LSC of the supernatants after adsorption and by combustion of the remaining soils. The total radioactivity recovery with respect to the individual vessel ranged from 97.5% to 116.6% of the applied radioactivity in the four tested soils.

In the definitive adsorption test 50.2-66.0%, 61.9-76.2%, 54.5-71.3% and 50.5-67.0% of the applied test material was adsorbed in soils [redacted], L[redacted] Dollendorf II and L[redacted] respectively.

The calculated adsorption constants $K_F^{(ads)}$ of the Freundlich isotherms for the four soils ranged from 4.04 mL/g to 11.71 mL/g. The Freundlich exponents 1/n were in the range of 0.8596 to 0.9258, indicating that the concentration of the test item did affect the adsorption behaviour. The measured $K_{F,OC}^{(ads)}$ values ranged between 244.1 mL/g and 327.5 mL/g. According to BRIGGS depending on the soil type the mobility of [2,6-pyridyl-^{14}C]-fluopicolide can be classified as low mobile in the tested soils. The following table summarizes the key soil properties and results from the study:

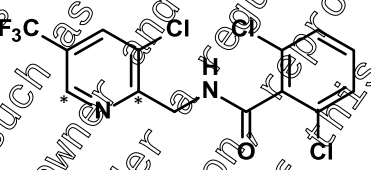
Soil	[redacted]	[redacted]	Dollendorf II	L[redacted]
Soil type (USDA)	Loam	Silt loam	Clay loam	Sandy loam
pH (0.01M $CaCl_2$)	5.0	6.1	7.3	6.5
Organic carbon [%]	1.8	1.9	4.8	1.5
$K_F^{(ads)}$ [mL/g]	4.65	6.22	11.71	4.04
1/n	0.9258	0.8741	0.8596	0.8726
$K_{F,OC}^{(ads)}$ [mL/g]	258.6	327.5	244.1	269.3
$K_F^{(des)}$ [mL/g]	5.15	7.21	13.15	4.48
1/n	0.9016	0.8671	0.8527	0.8561
$K_{F,OC}^{(des)}$ [mL/g]	286.1	379.5	273.9	298.9

I. Material and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-fluopicolide



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

Batch Number: KML-9984

Specific Activity: 5.98 MBq/mg

Radiochemical purity: >99% (HPLC)

Stability of test compound: Stable during the equilibrium periods (up to 48 hours)

2. Test Soil

Sorption tests were performed with four soils covering a range of pH, organic carbon content and texture. The same batches of soils were used in a time dependent sorption study run concurrently with fluopicolide (KCA 7.1.2.1.1/07). The characteristics of the European soils are summarised in Table 7.1.3.1.1- 16.

Table 7.1.3.1.1- 16: Physico-chemical properties of test soils

Soil Designation	[redacted]	**H[redacted]**	**Dollen-dorf II**	**L[redacted]**
Abbreviation	[redacted]	[redacted]	Doll	[redacted]
Soil Batch No.	20140828	20140828	20140827	20140828
Latitude and longitude	[redacted]	[redacted]	[redacted]	[redacted]
Textural class [USDA]	Loam	Silt Loam	Clay Loam	Sandy loam
Textural analysis [USDA]				
Sand [2000-50 µm]	49	15	25	75
Silt [50-2 µm]	32	68	44	16
Clay [<2µm]	19	17	31	9
pH value:				
Water	5.3	6.4	7.6	6.8
$CaCl_2$	5.0	6.1	7.5	6.5
Organic carbon (%)	1.8	1.9	4.8	1.5
Organic matter (%)*	3.10	3.27	8.26	2.58
CEC (meq/100 g soil)	9.7	10.5	18.8	8.2

* Calculated using the following conversion factor: % Organic matter =% Organic carbon x 1.72
CEC: Cation exchange capacity

B. Study design

1. Experimental Conditions:

For the definitive test 5 g for soils [redacted] H[redacted] and L[redacted] and 2 g of soil Dollendorf H were weighed into centrifuge tubes, and 19.98 mL of aqueous 0.01 M $CaCl_2$ stock solution were added. After pre-equilibration for ≥16 hours, 20 µL of the respective application solution (solvent acetonitrile) were spiked in. The concentration of acetonitrile within the test systems was 0.1% by volume. Initial nominal concentrations of the ^{14}C-test substance in the aqueous phase were 1.0, 0.3, 0.1, 0.03 and 0.01 mg/L, thus covering two orders of magnitude. Each determination was performed in duplicate by shaking with an overhead shaker in the dark at 20±2°C.

Preliminary tests included the determination of a suitable soil-to-solution ratio over 24 hours, the time to reach adsorption equilibrium at a soil-to-solution ratio of 1:4 which included an assessment of the stability of the test item in the presence of soil over 72 hours. The stability of fluopicolide in all soils was established in a further pre-test after continuous shaking for 48 hours at a soil-to-solution ratio of 1:4.

In the definitive test an adsorption step of 24 hours was performed for all soils followed by one desorption step also of 24 hours. For work-up the aqueous supernatant was separated from soil by decantation and centrifugation. Radioactivity in water and soil extracts was determined by liquid scintillation counting (LSC). Non-extractable radioactivity in soil was determined by combustion followed by LSC to establish a full material balance.

Finally, the adsorption parameters were calculated using the Freundlich adsorption isotherm. Desorption parameters were also reported but as not required for the fluopicolide risk assessment are not summarised here.

Adsorption phase

Parameters		Description (for all soils)
Condition of the soil and equilibrium solution		Before application soils were pre-equilibrated with 19.98 mL of 0.01 M aqueous $CaCl_2$ for at least 16 hours.
Have these soils been used for other laboratory studies		Yes. The same batches of soils have been used previously in a time-dependent sorption study with fluopicolide (KCA 7.1.2.1.1/07).
Soil (weight/replicate)		2 g (dry weight) per replicate for Dollendorf II. 5 g (dry weight) per replicate for [redacted], H[redacted] and L[redacted]. Each test was performed in duplicate.
Control used		No
Test material concentrations	Nominal application rates	Nominal concentration in test solution: 1.00 mg/L, 0.30 mg/L, 0.10 mg/L, 0.03 mg/L and 0.01 mg/L.
	Analytically measured concentrations	1.08 mg/L, 0.34 mg/L, 0.11 mg/L, 0.03 mg/L and 0.01 mg/L
Identity and concentration of co-solvent		Acetonitrile ≤ 0.1% by volume
Soil : solution ratio		1:10 for soil Dollendorf II i.e. 2 g soil to 20 mL solution 1:4 for soils [redacted], H[redacted] and L[redacted] i.e. 5 g soil to 20 mL solution
pH of the adsorption solution	Initial	Without soil: 6.48 - 6.52
	Final	With soil and test item after 24 hours: range 5.6- 7.2
Number of replications	Treatments	Duplicate
Equilibration	Time	Pre-equilibration: 16 hours
	Temperature	20 ± 2 °C
	Dark	Yes
	Shaking method	Mechanical overhead shaker
	Shaking time	24 hours adsorption phase
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	3000 rpm
	Duration	10 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

Desorption phase

Parameters		Description (for all soils)
Where the soil residues from the adsorption phase used?		Yes
Amount of test material present in the adsorbed state/adsorbed amount		The amount of test item adsorbed to soil after the adsorption step was in the range of 50.2 – 76.2% of the applied radioactivity.
Number of desorption cycles		For all soils one desorption cycle was performed for each soil and each concentration.
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh 0.01 M aqueous $CaCl_2$ solution (Stock Solution I). The total volume of 20 mL was used as equilibrium solution.
Soil : solution ratio		2 g dry matter soil corrected for residual humidity for soil Dollendorf II 5 g dry matter soil corrected for residual humidity for soils [redacted], H[redacted] and L[redacted] Each test was performed in duplicate.
Number of replications	Treatments	Duplicate
Equilibration	Temperature	20 ± 2 °C
	Dark	Yes
	Shaking method	Mechanical overhead shaker
	Shaking time	24 hours desorption phase
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	5000 rpm
	Duration	10 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

2. Analytical procedures:

The purity of the test item was investigated by reverse phase radio-HPLC analysis using ^{14}C-flow-through detection techniques and its identity confirmed by co-chromatography with an analytical standard.

The parental mass balance was established in pre-tests. Supernatants were analysed by LSC and HPLC. Soil samples were exhaustively extracted with acetonitrile (up to 5 times). Soil extracts were analysed by LSC and HPLC. Finally, radioactivity remaining in the extracted soil residue was combusted to determine a complete mass balance.

II. Results and Discussion

A. Mass balance and results of preliminary tests

In pre-tests fluopicolide was shown to be stable at a soil-to-solution ratio of 1:4 with parental mass balances for each soil ranging from 90.9 and 92.2% AR over 48 hours.

For the definitive tests the overall mean material balance for all concentrations of each soil investigated with respect to the individual vessels ranged from 97.5% to 116.6% of the applied radioactivity (data not shown). Mean recovery rates above 110% of applied were only measured for the 0.01 mg/L-batch of soil [redacted] (Table 7.1.3.1.1- 17).

Table 7.1.3.1.1- 17: Definitive test: Mass balance of [2,6-pyridyl-^{14}C]-fluopicolide (% AR)

Test concentration (mg/L)	[redacted]	H[redacted]	Dollendorf II	L[redacted]
1.08	106.95	105.3	104.5	102.5
0.34	103.1	101.6	103.3	101.5
0.11	105.4	106.0	105.5	100.7
0.03	108.6	104.7	101.8	99.2
0.01	106.9	107.8	105.2	103.4
Mean	106.2	105.1	104.1	103.5
SD	±2.1	±2.3	±1.5	±5.7

Calculated from data in the report. Values derived from mean values of duplicate samples in terms of percentages of AR.
SD = standard deviation.

B. Transformation of test substance:

The stability of the test substance in contact with soil under the conditions of the definitive test was confirmed by HPLC analysis in a pre-test and determined to be higher than 99% after 72 hours.

C. Findings:

The adsorption behaviour of [2,6-pyridyl-^{14}C]-fluopicolide was investigated in soil/water slurries based on five different nominal concentrations ranging from approximately 0.01 mg/L to 1.00 mg/L (two orders of magnitude). Based on the outcome of the preliminary tests a soil/solution ratio of 1:4 for soils [redacted], H[redacted] and L[redacted] was used for the dfinitive test. For Dollendorf II soil a soil/solution ratio of 1:10 was used for the definitive test.

Equilibrium of the test item was established after 24 hours which was selected for the adsorption time in the definitive test followed by a desorption step of 24 hours.

Within definitive tests, the amount of [2,6-pyridyl-^{14}C]-fluopicolide adsorbed to soil after 24 hours ranged from 50.2 to 66.0% AR for soil [redacted], 61.9 to 76.2% AR for soil H[redacted], 54.5 to 71.3% AR for soil Dollendorf II and 50.5 to 67.0% AR for soil L[redacted]
The adsorption behaviour of fluopicolide was accurately measured using a nominal concentration range of 0.01 mg/L to 1.0 mg/L by the Freundlich equation for all soils (Table 7.1.3.1.1- 19). The adsorption constants $K_{F, ads}$ of the Freundlich isotherms ranged from 4.03 mL/g to 11.71 mL/g with associated Freundlich exponents 1/n ranging from 0.8596 to 0.9258. The adsorption of the test item to soil was thus affected to some extent by the concentration. The corresponding correlation coefficients for the adsorption isotherms ranged from 0.9965 to 0.9996 indicating a linear fit to the measured data. When normalized for the organic carbon content of soil $K_{OC, ads}$ values ranged from 244.1 mL/g (soil Dollendorf II) to 327.5 mL/g (soil H[redacted]).

Table 7.1.3.1.1- 18: Definitive test: Concentration of [2,6-pyridyl-^{14}C]-fluopicolide in aqueous and solid phase following 24 hours of adsorption (mean ± s.d.).

Concentration	Soil	Solution	Percentage adsorbed
	(mg/kg)	(mg/L)	
Soil ID		[redacted]	
0.011 mg/L	0.029	0.004	65.0 ± 1.0
0.034 mg/L	0.078	0.014	57.6 ± 7.4
0.112 mg/L	0.273	0.044	60.9 ± 0.2
0.336 mg/L	0.802	0.136	59.6 ± 0.4
1.074 mg/L	2.309	0.499	53.6 ± 1.1
Soil ID		H[redacted]	
0.011 mg/L	0.034	0.003	76.0 ± 0.2
0.034 mg/L	0.100	0.009	74.0 ± 0.3
0.112 mg/L	0.318	0.033	70.9 ± 0.6
0.336 mg/L	0.923	0.106	68.6 ± 0.2
1.074 mg/L	2.681	0.406	62.2 ± 0.3
Soil ID		Dollendorf II	
0.011 mg/L	0.079	0.003	70.9 ± 0.3
0.034 mg/L	0.238	0.010	70.6 ± 0.4
0.112 mg/L	0.731	0.039	65.2 ± 0.3
0.336 mg/L	2.076	0.129	61.7 ± 0.2
1.075 mg/L	5.956	0.484	55.3 ± 0.7
Soil ID		L[redacted]	
0.011 mg/L	0.030	0.004	66.7 ± 0.4
0.034 mg/L	0.087	0.012	64.3 ± 0.5
0.112 mg/L	0.271	0.044	60.5 ± 0.3
0.336 mg/L	0.770	0.144	57.2 ± 0.2
1.076 mg/L	2.211	0.524	51.3 ± 0.8

Table 7.1.3.1.1- 19: Adsorption constants of fluopicolide in soil

Phase	Parameter	Units	[redacted]	H[redacted]	Dollendorf II	L[redacted]
			Loam	Silt loam	Clay loam	Sandy loam
Adsorption	$K_{F,ads}$	[mL/g]	4.6545	6.2226	11.7145	4.0391
	1/n	-	0.9258	0.8741	0.8596	0.8723
	R^2	-	0.9965	0.9994	0.9989	0.9996
	$K_{OC,ads}$	[mL/g]	258.6	327.5	244.1	269.3
Desorption	$K_{F,des}$	[mL/g]	5.1493	7.2114	13.1494	4.4829

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the report (see Table 7.1.3.1.1- 20) were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet tool provided by EFSA (summarised in Table 7.1.3.1.1- 21).

Table 7.1.3.1.1- 20: Definitive test: Concentration of fluopicolide in aqueous and solid phase following 48 hours of adsorption used as inputs in checklist

Concentration	[redacted]		[redacted]		Dollendorf II		L[redacted]	
	Soil (mg/kg)	Solution (mg/L)	Soil (mg/kg)	Solution (mg/L)	Soil (mg/kg)	Solution (mg/L)	Soil (mg/kg)	Solution (mg/L)
0.011 mg/L	0.0284	0.0040	0.0337	0.0027	0.0784	0.0033	0.0298	0.0037
	0.0293	0.0038	0.0339	0.0026	0.0792	0.0032	0.0295	0.0037
0.034 mg/L	0.0878	0.0118	0.1003	0.0087	0.2398	0.0098	0.0861	0.0122
	0.0679	0.0168	0.0997	0.0088	0.2368	0.0101	0.0876	0.0119
0.112 mg/L	0.2728	0.0439	0.3154	0.0333	0.7273	0.0394	0.2727	0.0440
	0.2742	0.0435	0.3208	0.0319	0.7351	0.0386	0.2701	0.0446
0.336 mg/L	0.7962	0.1370	0.9193	0.1064	2.0683	0.1294	0.7678	0.1443
	0.8076	0.1341	0.9259	0.1047	2.0835	0.1278	0.7723	0.1432
1.074 mg/L	2.3549	0.4871	2.6676	0.4094	6.0360	0.4728	2.2459	0.5151
	2.2633	0.5099	2.6954	0.4336	5.8752	0.4888	2.1759	0.5325

Table 7.1.3.1.1- 21: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	[redacted]	[redacted]	Dollendorf II	L[redacted]
Adsorption method (direct/indirect)	-		indirect	indirect	indirect	indirect
Soil : solution ratio	g/mL		1:4	1:4	1:10	1:4
Mass balance of ^{14}C (at all tested concentrations)	%	>90%	100.6-116.6	98.9-108.5	101.4-106.0	97.5-115.2
f – due to loss processes			7.9	8.2	7.8	9.1
Adsorbed percentage (δ)	%	>20%	52.8-62.3	59.8-73.6	54.7-68.1	50.7-63.4
K_d x (soil:solution ratio)		>0.3	1.01-1.95	1.55-3.21	1.20-2.48	1.02-2.04
K_{fE} / K_f	-	<1.2	1.17 & 1.18	1.15 & 1.16	1.16 & 1.17	1.21 & 1.22
$^{ads}K_F$	L/kg	*	4.602	6.067	11.713	4.039
(95% confidence interval)			3.491-6.067	5.542-6.641	10.664-12.865	3.812-4.280
$^{ads}1/n$	-		0.922	0.869	0.860	0.872
(95% confidence interval)			0.844-0.999	0.845-0.892	0.834-0.885	0.856-0.888
$^{Ads}R^2$	-	>0.975	0.989	0.999	0.999	0.999
$^{ads}K_{F,OC}$	L/kg		255.7	319.3	244.0	269.3
Visual fit to Freundlich isotherm			Accept.	Accept.	Accept.	Accept.
Residual plots randomly distributed			Accept.	Good	Good	Good

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balances were acceptable (97.5-116.6%). The % adsorption of 50.7-73.6% were all acceptable. The use of the indirect method was appropriate based on a Kd * soil/solution ratio > 0.3 in all soils. The calculated K_{fE} / K_f ratio was slightly greater than 1.2 in just one soil where the parental mass balance was the lowest. The graphical fits of the Freundlich equation based on the standard linear regression form using log-log transformed data alongside the associated residual plots was good with R^2 of the standard linear regressions ranging from 0.989 to 0.999 and the visual fit of both the standard regression and the residual plots being acceptable.

Figure 7.1.3.1.1- 12 Freundlich Isotherms of fluopicolide in [redacted] at 20°C

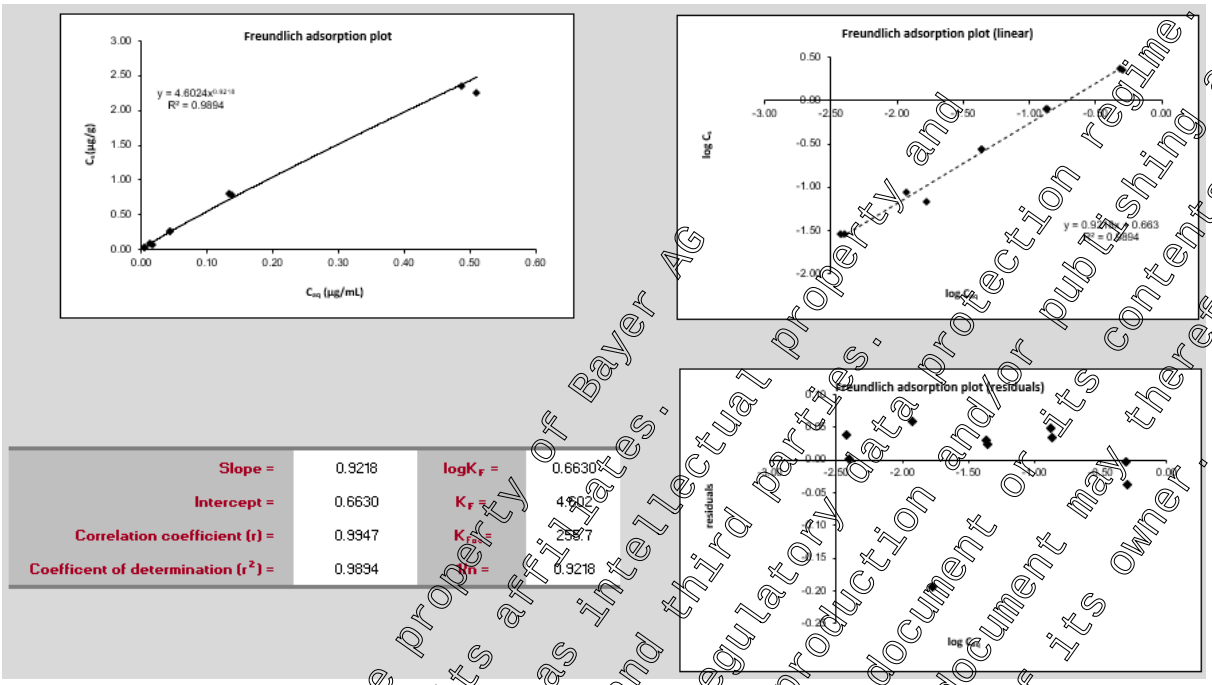


Figure 7.1.3.1.1- 13 Freundlich Isotherms of fluopicolide in P[redacted] at 20°C

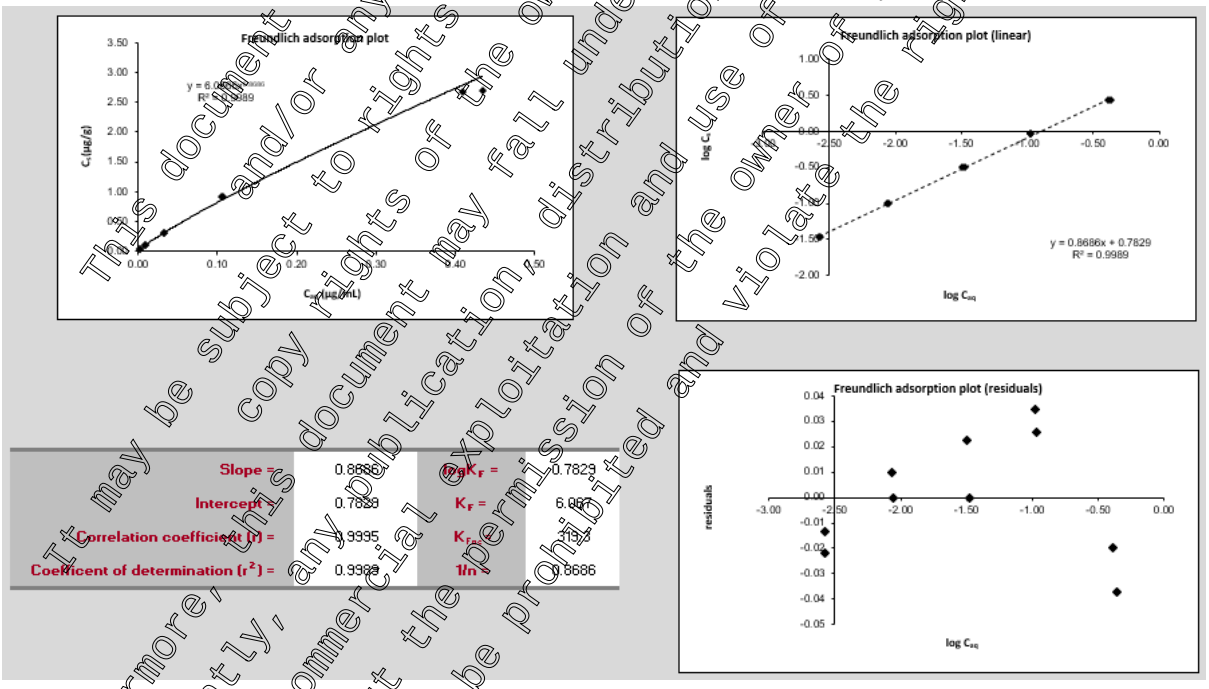


Figure 7.1.3.1.1- 14 Freundlich Isotherms of fluopicolide in Dollendorf II at 20°C

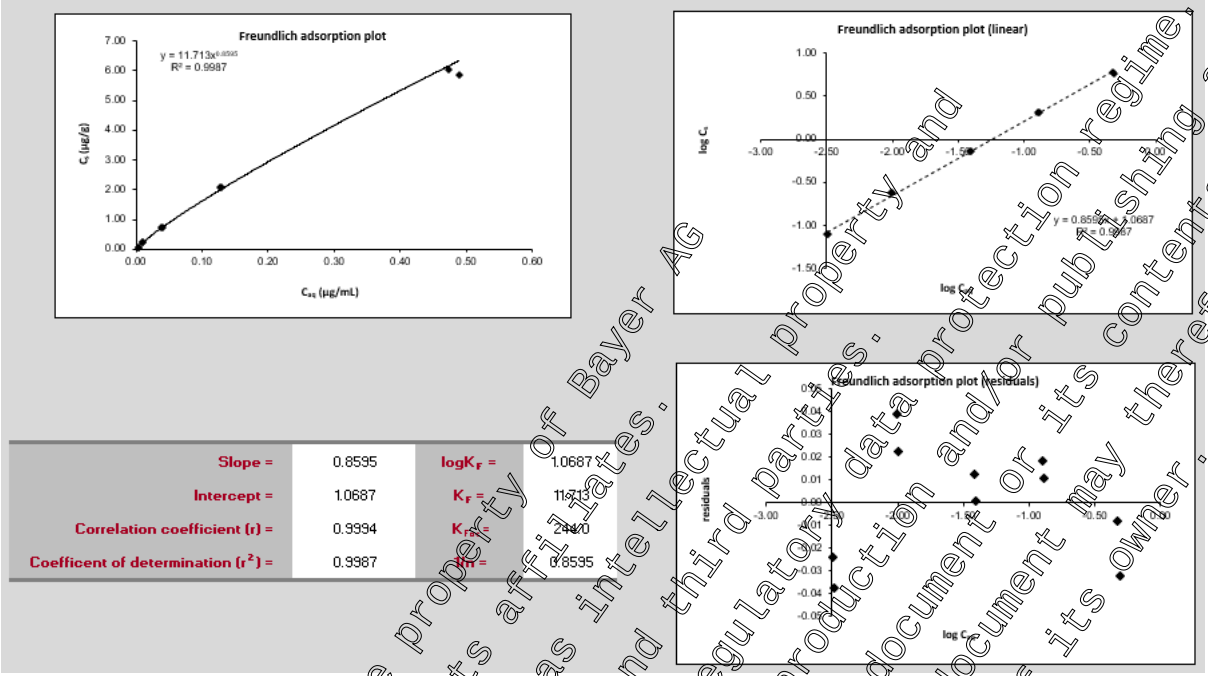
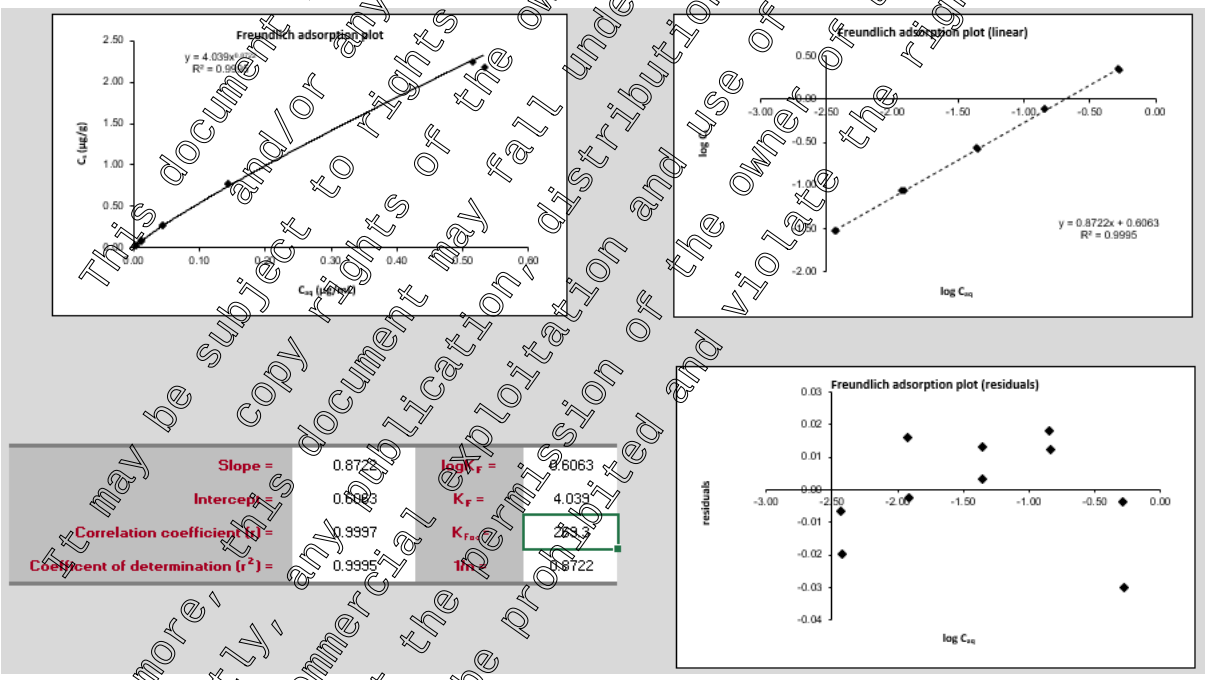


Figure 7.1.3.1.1- 15 Freundlich Isotherms of fluopicolide in [redacted] at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.1- 22. The impact on reported endpoints is summarised in Table 7.1.3.1.1- 23.

Table 7.1.3.1.1- 22: Summary of Quality Criteria and Regulatory Interpretation

Fluopicolide			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
[redacted]	Loam	[redacted]	8	1	0
H[redacted]	Silt loam	[redacted]	9	0	0
Dollendorf II	Clay loam	Doll	9	0	0
[redacted]	Sandy loam	[redacted]	9	1	1

Table 7.1.3.1.1- 23: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
[redacted]	Loam	[redacted]	258.6	255.7	0.9258	0.922
H[redacted]	Silt loam	[redacted]	327.5	319.3	0.8741	0.869
Dollendorf II	Clay loam	Doll	244.1	244	0.8596	0.86
L[redacted]	Sandy loam	[redacted]	269.3	269.3	0.8723	0.872

Note: The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

These results indicate that fluopicolide is moderately absorbed to soil. The adsorption constant $K_{F(ads)}$ of fluopicolide ranged from 4.04 to 11.71 mL/g in the tested soils; the normalised adsorption constant $K_{OC(ads)}$ ranged from 244.1 to 327.5 mL/g. The Freundlich exponent 1/n was between 0.8596 and 0.9258, indicating that the concentration of the test item effects its adsorption behaviour in the concentration range examined.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the four soils were acceptable according to the quality criteria and therefore suitable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of fluopicolide in soil.

Data Point:	KCA 7.1.3.1.1/04
Report Author:	[redacted]
Report Year:	2016
Report Title:	[pyridyl-2,6-14C] Fluopicolide: Adsorption/desorption in six different soils
Report No:	AS449
Document No:	M-550735-01-1
Guideline(s) followed in study:	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, Adsorption/Desorption (Batch Equilibrium), October 2008
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption characteristics of [2,6-pyridyl-^{14}C]-fluopicolide were studied in six soils from sites used for field dissipation studies in batch equilibrium experiments in the laboratory in the dark at 20 ± 1 °C.

Soil	Soil Code	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
Burscheid	VG08	Burscheid, Germany	Silt loam	6.1	0.7
Great Chishill	ENG2	Great Chishill, England	Clay	7.3	2.1
Parcay Meslay	FR09B	Parcay Meslay, France	Loam	6.7	1.3
Tarascon Le Cayades	FR08	Mas du Coq, France	Clay loam	7.6	0.9
Valerio Tomelini	IT09	Albaro di Ronco Atladige	Silty clay	7.2	2.1
Vilobi D'Onyar	SPA01	Vilobi D'Onyar, Spain	Sandy loam	6.3	0.8

The adsorption phase of the study was carried out using pre-equilibrated air-dried soil with [2,6-pyridyl-^{14}C]-fluopicolide concentrations of nominal 1.00, 0.30, 0.10, 0.03, and 0.01 mg/L for 24 hours. The equilibration solution used was 0.01 M aqueous $CaCl_2$.

A soil to solution ratio of 1:4 was used for all soils. The aqueous supernatant after adsorption was separated by centrifugation and the [2,6-pyridyl-^{14}C]-fluopicolide residues in the supernatant were analysed by liquid scintillation counting (LSC). One desorption cycle was conducted for 24 hours.

The adsorption parameters were calculated using the Freundlich adsorption isotherm. For each soil the parental mass balance after 48 h showed that >90% of fluopicolide was recovered.

The mass balance was determined by LSC of the supernatants after adsorption, desorption and by combustion of the remaining soils. The total radioactivity recovery with respect to the individual vessel ranged from 94.1% to 112.1% of the applied radioactivity in the six tested soils.

In the definitive adsorption test 35.2-49.6%, 57.8-69.3%, 45.6-59.1%, 31.6-48.7, 49.7-61.4 and 38.0-54.0% of the applied test material was adsorbed in soils VG08, ENG2, FR09B, FR08, IT09 and SPA1, respectively.

The calculated adsorption constants $K_F^{(ads)}$ of the Freundlich isotherms for the six soils ranged from 1.84 mL/g to 5.40 mL/g. The Freundlich exponents 1/n were in the range of 0.8668 to 0.9110, indicating that the concentration of the test item did affect the adsorption behaviour.

The following table summarizes the key soil properties and results from the study:

Soil origin	**VG08, Burscheid Germany**	**ENG2, Great Chishill, England**	**FR09B, Parcay Meslay, France**	**FR08, Tarascon Le Cayades, France**
Soil type (USDA)	Silt loam	Clay	Loam	Clay loam
pH (0.01M $CaCl_2$)	6.1	7.3	6.7	7.6
Organic carbon [%]	0.7	2.1	1.3	0.9
$K_F^{(ads)}$ [mL/g]	2.12	5.40	3.35	1.84
1/n	0.8868	0.9076	0.8992	0.8668
$K_{F,OC}^{(ads)}$ [mL/g]	303.3	257.0	257.4	204.9
$K_F^{(des)}$ [mL/g]	2.38	6.45	3.96	2.14
1/n	0.8835	0.9035	0.9027	0.8502
$K_{F,OC}^{(des)}$ [mL/g]	340.6	307.1	304.4	238.3

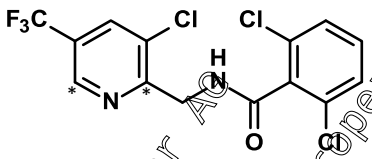
Soil origin	IT09, Valerio Tomelini, Italy	SPA1 Vilobi D'Onyar, Spain
Soil type (USDA)	Silty clay	Sandy loam
pH (0.01M $CaCl_2$)	7.2	6.3
Organic carbon [%]	2.1	0.8
$K_F^{(ads)}$ [mL/g]	3.93	2.34
1/n	0.9110	0.8818
$K_{F,OC}^{(ads)}$ [mL/g]	187.0	292.0
$K_F^{(des)}$ [mL/g]	4.59	2.57
1/n	0.9201	0.8796
$K_{F,OC}^{(des)}$ [mL/g]	218.6	320.8

I. Material and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-fluopicolide (referred to as [pyridyl-2,6-^{14}C]-fluopicolide in the report)



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

Batch Number:	KML 9984
Specific Activity:	5.98 MBq/mg
Radiochemical purity:	>99% (HPLC)
Stability of test compound:	Stable during the equilibrium periods (up to 48 hours)

2. Test Soil

Sorption tests were performed with six soils covering a range of pH, organic carbon content and texture. The soils were taken from European field sites used in terrestrial field dissipation studies with fluopicolide (five sites) and its metabolite M-01 (all six sites). In addition, the same batches of all six soils were used in a time dependent sorption study run concurrently with fluopicolide (KCA 7.1.2.1.1/08). The characteristics of the European soils are summarised in Table 7.1.3.1.1- 24.

Table 7.1.3.1.1- 24: Characteristics of test soils

Soil	Burscheid VG08	Great Chishill ENG2	Parcay Meslay FR09B
Geographic Location	Burscheid [redacted] Westfalia	Great Chishill, England	Parcay Meslay, France
Soil Batch No.	20141121	20141125	20141124
Latitude and longitude	[redacted]	[redacted]	[redacted]
Soil preparation	Air-dried and sieved to 2 mm		
Textural Class (USDA)	Silt loam	Clay	Loam
Sand (%) [A] Silt (%)[A] Clay (%)[A]	19 57 24	35 23 42	31 49 20
pH (0.01 M $CaCl_2$) pH (Water)	6.1 6.2	7.3 7.5	6.7 7.0
Org. Matter [B] (%)	1.20	3.61	2.24
Org. Carbon (%)	0.7	2.1	1.3
CEC (meq/100 g)	11.7	27.2	10.7

Soil	Tarascon Le Cayades FR08	Valerio Tomelini IT09	Vilobi D'Onyar SPA01
Geographic Location	Tarascon Le Cayades, St. Etienne du Gres Mas du Coq, France	Valerio Tomelini, Albaro di Ronco Alladige	Vilobi D'Onyar, Spain
Soil Batch No.	20141121	20141124	20141125
GPS coordinates	[redacted]	[redacted]	[redacted]
Soil preparation	Air-dried and sieved to 2 mm		
Textural Class (USDA)	Clay loam	Silty clay	Sandy loam
Sand (%) [A] Silt (%)[A] Clay (%)[A]	25 43 32	17 41 42	57 31 12
pH (0.01 M $CaCl_2$) pH (Water)	7.6 7.8	7.2 7.4	6.3 6.5
Org. Matter [B] (%)	1.55	3.61	1.38
Org. Carbon (%)	0.9	2.1	0.8
CEC (meq/100 g)	11.2	20.3	8.7

[A] According to USDA classification; [B] % Organic matter =% organic carbon x 1.72
CEC: Cation exchange capacity
[A] Coordinates taken from KCA 7.1.2.2.1/13, M-651179-01-1.

B. Study design

1. Experimental Conditions:

For the definitive test samples aliquots of 5 g dry weight of soil were weighed into centrifuge tubes. Following pre-equilibration, a solution of the test item in 0.01 M aqueous calcium chloride (20 mL) was added to each sample.

Initial nominal concentrations of the ^{14}C-test substance in the aqueous phase were 1.0, 0.3, 0.1, 0.03 and 0.01 mg/L, thus covering two orders of magnitude.

The co-solvent (acetonitrile) did not exceed 0.1% of the total volume of aqueous phase during the tests. Each determination was performed in duplicate by shaking with an overhead shaker in the dark at 20±2°C.

Preliminary tests included the determination of a suitable soil-to-solution ratio over 24 hours, the time to reach adsorption equilibrium at a soil-to-solution ratio of 1:4 which included an assessment of the stability of the test item in the presence of soil over 72 hours. The stability of fluopicolide in all soils was conclusively established in a further pre-test after continuous shaking for 48 hours at a soil-to-solution ratio of 1:4.

For the definitive test an adsorption step of 24 hours was performed for all soils followed by one desorption step also of 24 hours.

For work-up the aqueous supernatant was separated from soil by decantation and centrifugation. Radioactivity in water and soil extracts was determined by liquid scintillation counting (LSC). Non-extractable radioactivity in soil was determined by combustion followed by LSC to establish a full material balance.

Finally, the adsorption parameters were calculated using the Freundlich adsorption isotherm. Desorption parameters were also reported but as not required for the fluopicolide risk assessment are not summarised here.

Adsorption phase

Parameters		Description (for all soils)
Condition of the soil and equilibrium solution		Before application soils were pre-equilibrated with 19.98 mL of 0.01 M aqueous $CaCl_2$ for at least 16 hours.
Have these soils been used for other laboratory studies		Yes. The same batches of all six soils have been used previously in a time-dependent sorption study (KCA 7.1.2.1.1/08) and in field dissipation studies (KCA 7.1.2.1.2/12, KCA 7.1.2.1.2/13 and KCA 7.1.2.1.2/14) with fluopicolide.
Soil (weight/replicate)		5 g (dry weight) per replicate for all soils Each test was performed in duplicate.
Control used		No
Test material concentrations	Nominal application rates	Nominal concentration in test solution: 1.00 mg/L, 0.30 mg/L, 0.10 mg/L, 0.03 mg/L and 0.01 mg/L.
	Analytically measured concentrations	0.887 mg/L, 0.267 mg/L, 0.086 mg/L, 0.025 mg/L and 0.008 mg/L
Identity and concentration of co-solvent		Acetonitrile ≤ 0.1% by volume
Soil : solution ratio		1:4 for all soils, i.e. 5 g soil to 20 mL solution
pH of the adsorption solution	Initial	Without soil: 6.91
	Final	With soil and test item after 24 hours: range 6.4- 7.5
Number of replications	Treatments	Duplicate
Equilibration	Time	Pre-equilibration ≥ 16 hours
	Temperature	20 ± 2 °C
	Dark	Yes
	Shaking method	Mechanical overhead shaker
	Shaking time	24 hours adsorption phase
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	5000 rpm
	Duration	10 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

Desorption phase

Parameters		Description (for all soils)
Where the soil residues from the adsorption phase used?		Yes
Amount of test material present in the adsorbed state/adsorbed amount		The amount of test item adsorbed to soil after the adsorption step was in the range of 31.6 – 69.3% of the applied radioactivity.
Number of desorption cycles		For all soils one desorption cycle was performed for each soil and each concentration.
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh 0.01 M aqueous $CaCl_2$ solution (Stock Solution I). The total volume of 20 mL was used as equilibrium solution.
Soil : solution ratio		5 g dry-matter soil corrected for residual humidity for all soils Each test was performed in duplicate.
Number of replications	Treatments	Duplicate
Equilibration	Temperature	20 ± 2 °C
	Dark	Yes
	Shaking method	Mechanical overhead shaker
	Shaking time	24 hours desorption phase
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	5000 rpm
	Duration	10 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

2. Analytical procedures:

The purity of the test item was investigated by reverse phase radio-HPLC analysis using ^{14}C-flow-through detection techniques and its identity confirmed by co-chromatography with an analytical standard.

The parental mass balance was established in pre-tests. Supernatants were analysed by LSC and HPLC. Soil samples were exhaustively extracted with acetonitrile (up to 5 times) and selected samples additionally extracted with acetone and a mixture of acetonitrile / 0.1 M HCl (9:1, v/v). Soil extracts were analysed by LSC and HPLC. Finally, radioactivity remaining in the extracted soil residue was combusted to determine a complete mass balance.

II. Results and Discussion

A. Mass balance and results of preliminary tests

In pre-tests [2,6-pyridyl-^{14}C]-fluopicolide was shown to be stable at a soil-to-solution ratio of 1+4 with parental mass balances for each soil ranging from 90.8 to 104.0% AR over 48 hours.
For the definitive tests the overall mean mass balance for all concentrations of each soil investigated ranged from 99.4 to 107.1% AR (Table 7.1.3.1.1- 25).

Table 7.1.3.1.1- 25: Definitive test: Mass balance of [2,6-pyridyl-^{14}C]-fluopicolide (% AR)

Test concentration (mg/L)	Burscheid VG08	Great Chishill ENG2	Parcay Meslay FR09B	Tarascon Le Cayades FR08	Valerio Tomelini IT09	Vilobi D'Onyar SPA01
0.89	104.2	96.2	104.2	104.7	101.2	103.1
0.27	104.6	98.4	101.6	103.7	101.0	103.9
0.09	105.7	97.0	104.8	104.4	101.3	102.8
0.03	110.4	100.6	106.9	108.2	105.7	109.4
0.01	110.5	105.1	108.7	108.8	108.1	110.2
Mean	107.1	99.4	105.2	105.9	103.5	105.9
SD	± 3.1	± 4.2	± 2.6	± 2.7	± 3.3	± 3.5

Values derived from mean values of duplicate samples in terms of percentage of AR, SD = standard deviation

B. Transformation of test substance:

The stability of the test substance in contact with soil under the conditions of the definitive test was confirmed by HPLC analysis in a pre-test to be more than 90% AR for all soils over 48 hours (from 90.77 to 104.01% AR).

C. Findings:

The definitive test was performed at a soil-to-solution ratio of 1+4 for all six soils. Equilibrium of the test item was established after 24 hours shaking at this soil solution ratio, thus an adsorption time of 24 hours was chosen for the definitive test followed by a desorption step of 24 hours.

Within definitive tests the amount of [2,6-pyridyl-^{14}C]-fluopicolide adsorbed to soil after 24 hours ranged from 35.2 to 49.6% AR for soil Burscheid, 57.8 to 69.3% AR for soil Great Chishill, 45.6 to 59.1% AR for soil Parcay Meslay, 31.4 to 48.7% AR for soil Tarascon Le Cayades, 49.7 to 61.4% AR for soil Valerio Tomelini and 38.0 to 54.0% AR for soil Vilobi D'Onyar (Table 7.1.3.1.1- 26).
The adsorption behaviour of fluopicolide was accurately measured using a nominal concentration range of 0.01 mg/L to 1.0 mg/L by the Freundlich equation for all soils (Table 7.1.3.1.1- 27). The adsorption constants $K_{F,\ ads}$ of the Freundlich isotherms ranged from 1.84 mL/g to 5.40 mL/g with associated Freundlich exponents 1/n ranging from 0.8668 to 0.9110. The adsorption of the test item to soil was thus affected to some extent by the concentration. The corresponding correlation coefficients for the adsorption isotherms ranged from 0.9992 to 0.9996 indicating a linear fit to the measured data. When normalized for the organic carbon content of soil $K_{OC,\ ads}$ values ranged from 187.0 mL/g (soil Valerio Tomelini, Italy) to 303.5 mL/g (soil Burscheid, Germany).

Table 7.1.3.1.1- 26: Definitive test: Concentration of [2,6-pyridyl-^{14}C]-fluopicolide in aqueous and solid phase following 24 hours of adsorption (mean ± s.d.).

Concentration (mg/L)	Soil (mg/kg)	Solution (mg/L)	% adsorbed (% AR)
Soil ID	**Burscheid (VG08)**		
0.884	1.251	0.572	35.3 ± 0.0
0.266	0.427	0.160	40.0 ± 0.8
0.086	0.151	0.048	43.8 ± 0.2
0.025	0.046	0.014	45.6 ± 0.4
0.008	0.016	0.004	49.5 ± 0.1
Soil ID	**Great Chishill (ENG2)**		
0.878	2.061	0.768	58.1 ± 0.3
0.264	0.675	0.097	63.2 ± 0.1
0.085	0.229	0.029	66.3 ± 0.2
0.025	0.068	0.008	67.6 ± 0.1
0.008	0.022	0.002	69.2 ± 0.1
Soil ID	**Parcay Meslay (FR09B)**		
0.883	1.628	0.478	45.9 ± 0.3
0.266	0.554	0.128	51.8 ± 0.5
0.086	0.189	0.039	54.8 ± 0.3
0.025	0.057	0.011	56.6 ± 0.1
0.008	0.019	0.003	59.0 ± 0.1
Soil ID	**Tarascon Le Cayades (FR08)**		
0.885	1.124	0.604	31.7 ± 0.1
0.266	0.404	0.166	37.9 ± 0.9
0.086	0.145	0.050	42.0 ± 0.1
0.025	0.046	0.014	44.9 ± 0.1
0.008	0.015	0.004	47.9 ± 0.8
Soil ID	**Valerio Tomelini (IT09)**		
0.881	1.786	0.437	50.3 ± 0.7
0.265	0.580	0.121	54.3 ± 0.1
0.086	0.201	0.036	58.2 ± 0.1
0.025	0.060	0.010	59.3 ± 0.0
0.008	0.019	0.003	61.4 ± 0.0
Soil ID	**Vilobi D'Onyar (SPA01)**		
0.884	1.359	0.545	38.3 ± 0.3
0.266	0.446	0.155	41.7 ± 1.2
0.086	0.162	0.046	46.9 ± 0.6
0.025	0.049	0.013	48.4 ± 0.8
0.008	0.017	0.004	53.2 ± 0.8

Table 7.1.3.1.1- 27: Adsorption constants of [2,6-pyridyl-^{14}C]-fluopicolide in soil

Phase	Parameter	Units	Burscheid	Great Chishill	Parcay Meslay	Tarascon Le Cayades	Valerio Tomelini	Vilobi D'Onyar
			Silt loam	Clay	Loam	Clay loam	Silty clay	Sandy loam
Adsorption	$K_{F,ads}$	[mL/g]	2.1231	5.3968	3.3458	1.8445	3.9279	2.336
	1/n	-	0.8868	0.9076	0.8992	0.8668	0.911	0.8818
	R^2	-	0.9996	0.9993	0.9993	0.9992	0.9996	0.9996
	$K_{OC,ads}$	[mL/g]	303.3	257.0	257.4	204.9	187.0	292.0
Desorption	$K_{F,des}$	[mL/g]	2.3842	6.4499	3.9573	2.1448	4.3897	2.566
	1/n	-	0.8835	0.9035	0.9027	0.8502	0.9201	0.8796
	R^2	-	0.9991	0.9986	0.999	0.9972	0.9993	0.9989
	$K_{OC,des}$	[mL/g]	340.6	307.1	304.4	238.3	218.6	320.8

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the study report were used as input data (Table 7.1.3.1.1- 28). Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet tool provided by EFSA (summarised in Table 7.1.3.1.1- 29).

Table 7.1.3.1.1- 28: Definitive test: Concentration of fluopicolide in aqueous and solid phase following 48 hours of adsorption used as inputs in checklist

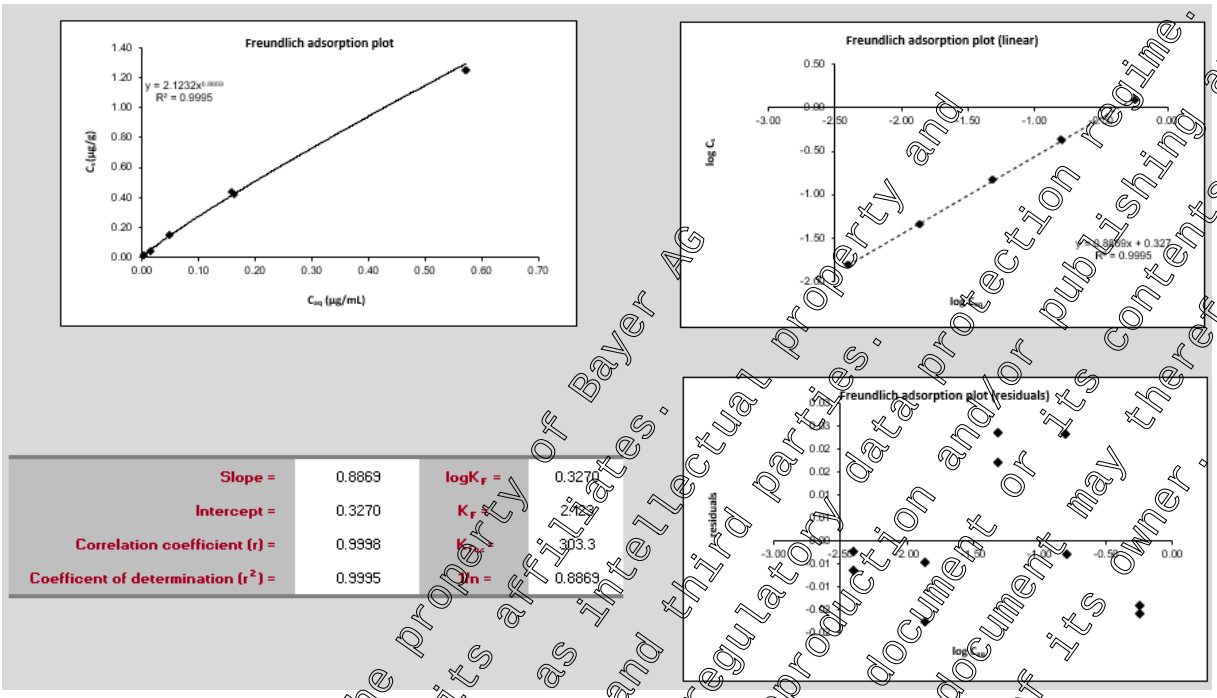
Soil ID	Burscheid		Great Chishill		Parcay Meslay		Tarascon Le Cayades		Valerio Tomelini		Vilobi D'Onyar	
Conc.	Soil	Sol.	Soil	Sol.	Soil	Sol.	Soil	Sol.	Soil	Sol.	Soil	Sol.
	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L
0.008 mg/L	0.016	0.004	0.022	0.002	0.019	0.003	0.015	0.004	0.019	0.003	0.017	0.004
	0.016	0.004	0.022	0.002	0.019	0.003	0.015	0.004	0.019	0.003	0.017	0.004
0.025 mg/L	0.047	0.014	0.068	0.008	0.057	0.011	0.045	0.014	0.060	0.010	0.048	0.013
	0.046	0.014	0.069	0.008	0.057	0.011	0.046	0.014	0.060	0.010	0.050	0.013
0.086 mg/L	0.151	0.048	0.229	0.029	0.188	0.039	0.145	0.050	0.200	0.036	0.160	0.046
	0.152	0.048	0.228	0.029	0.190	0.039	0.144	0.050	0.201	0.036	0.164	0.045
0.267 mg/L	0.419	0.162	0.674	0.098	0.548	0.129	0.394	0.168	0.581	0.121	0.433	0.158
	0.435	0.158	0.676	0.097	0.559	0.127	0.414	0.163	0.579	0.122	0.458	0.152
0.887 mg/L	1.252	0.572	2.051	0.371	1.617	0.481	1.129	0.603	1.762	0.443	1.347	0.548
	1.249	0.573	2.071	0.366	1.638	0.475	1.120	0.605	1.810	0.431	1.372	0.542

Table 7.1.3.1.1- 29: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Burscheid	Great Chishill	Parcay Meslay	Tarascon Le Cayades	Valerio Tomelini	Vilobi D'Onyar
Adsorption method (direct/indirect)	-		indirect	indirect	indirect	indirect	indirect	indirect
Soil : solution ratio	g/mL		1:4	1:4	1:4	1:4	1:4	1:4
Mass balance of ^{14}C (at all tested concentrations)	%	>90%	104.0-110.8	94.1-108.5	101.4-108.8	100.8-109.9	99.8-108.8	102.3-111.4
f – due to loss processes			0.6	1.6	0.8	1.0	0.8	9.2
Adsorbed percentage (δ)	%	>20%	35.4-50.6	58.2-70.0	45.8-60.0	31.8-49.7	50.0-62.3	38.2-54.8
K_d x (soil:solution ratio)		>0.3	0.55-0.99	1.38-2.28	0.84-1.45	0.46-0.95	0.99-1.60	0.61-1.18
K_{fE} / K_f	-	<1.2	1.02	1.03	1.02	1.03	1.01	1.31
$^{ads}K_F$	L/kg	*	2.128	5.396	3.346	1.844	3.927	2.335
(95% confidence interval)			(2.008-2.245)	(4.979-5.848)	(3.108-3.602)	(1.704-1.996)	(3.692-4.178)	(2.174-2.507)
$^{ads}1/n$	-	*	0.887	0.908	0.899	0.867	0.911	0.882
(95% confidence interval)			(0.871-0.903)	(0.887-0.928)	(0.879-0.919)	(0.844-0.889)	(0.895-0.927)	(0.862-0.902)
$^{Ads}R^2$		>0.975	1.000	0.999	0.999	0.999	1.000	0.999
$^{ads}K_{F,OC}$	L/kg		303.3	257.0	257.4	204.9	187.0	291.8
Visual fit to Freundlich isotherm			Accept.	Accept.	Accept.	Accept.	Accept.	Accept.
Residual plots randomly distributed			Good	Good	Good	Good	Good	Good

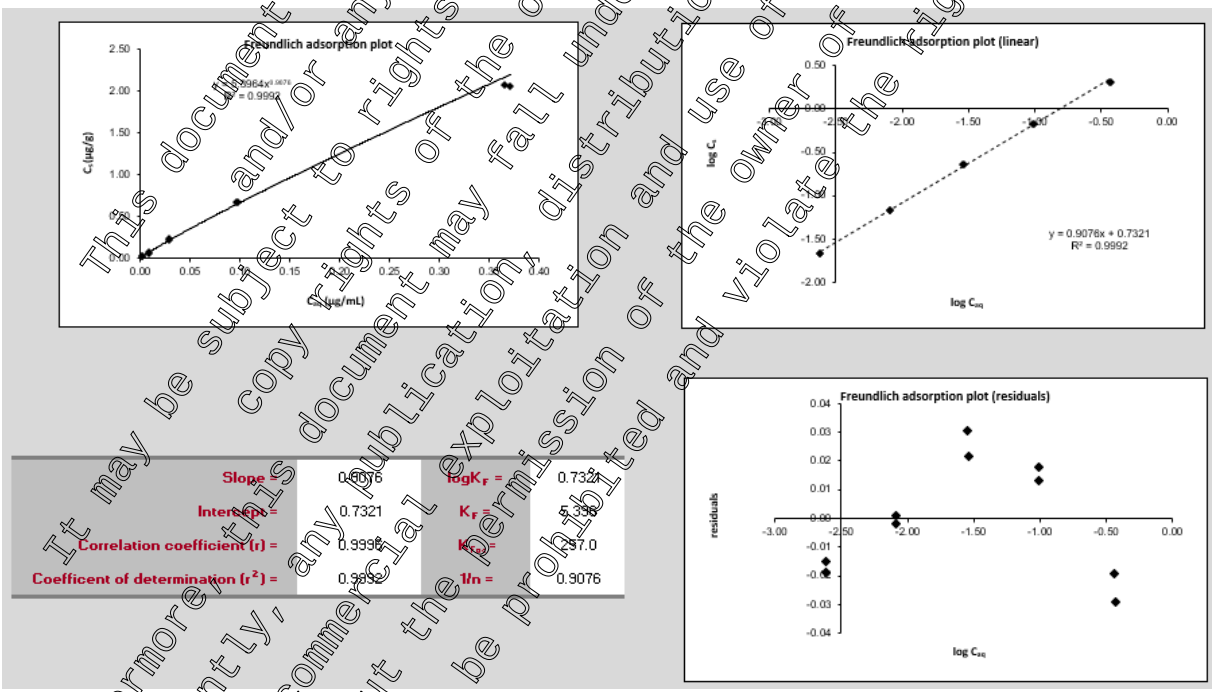
Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance of 94.1-111.4% were acceptable. The % adsorption of 31.8-70.0% were all acceptable. The use of the indirect method was appropriate based on a Kd * soil/solution ratio > 0.3 in all soils. The calculated K_{fE} / K_f ratio was slightly greater than 1.2 in just one soil where the parental mass balance and recovery was the lowest. The graphical fits of the Freundlich equation based on the standard linear regression form using log-log transformed data alongside the associated residual plots was good with R^2 of the standard linear regressions ranging from 0.999 to 1.000 and the visual fit of both the standard regression and the residual plots being acceptable.

Figure 7.1.3.1.1- 16 Freundlich Isotherms of fluopicolide in VG08 at 20°C



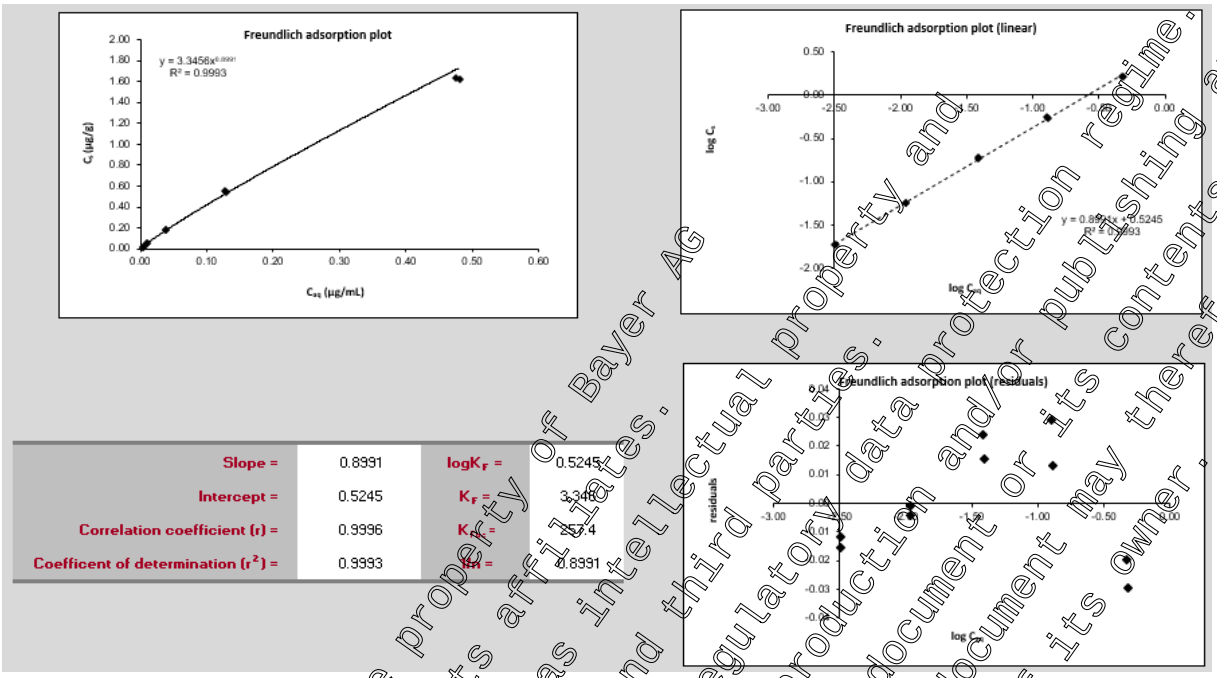
Slope =	0.8869	$logK_F$ =	0.3270
Intercept =	0.3270	K_F =	2.123
Correlation coefficient (r) =	0.9998	K_{oc} =	303.3
Coefficent of determination (r^2) =	0.9995	1/n =	0.8869

Figure 7.1.3.1.1- 17 Freundlich Isotherms of fluopicolide in ENG2 at 20°C



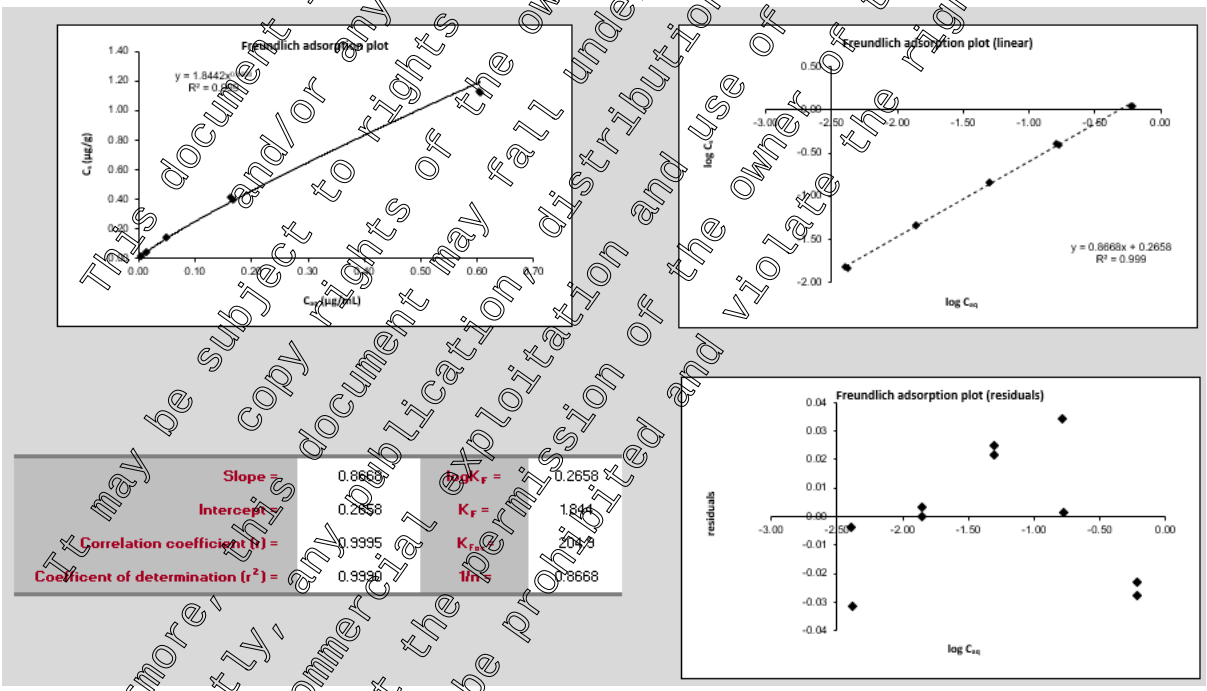
Slope =	0.9076	$logK_F$ =	0.7321
Intercept =	0.7321	K_F =	5.396
Correlation coefficient (r) =	0.9996	K_{oc} =	297.0
Coefficent of determination (r^2) =	0.9992	1/n =	0.9076

Figure 7.1.3.1.1- 18 Freundlich Isotherms of fluopicolide in FR09B at 20°C



Slope =	0.8991	$\log K_F$ =	0.5245
Intercept =	0.5245	K_F =	3.348
Correlation coefficient (r) =	0.9996	K_{Foc} =	257.4
Coefficent of determination (r^2) =	0.9993	1/n =	0.8991

Figure 7.1.3.1.1- 19 Freundlich Isotherms of fluopicolide in FR08 at 20°C



Slope =	0.8668	$\log K_F$ =	0.2658
Intercept =	0.2658	K_F =	1.844
Correlation coefficient (r) =	0.9995	K_{Foc} =	204.9
Coefficent of determination (r^2) =	0.9990	1/n =	0.8668

Figure 7.1.3.1.1- 20 Freundlich Isotherms of fluopicolide in IT09 at 20°C

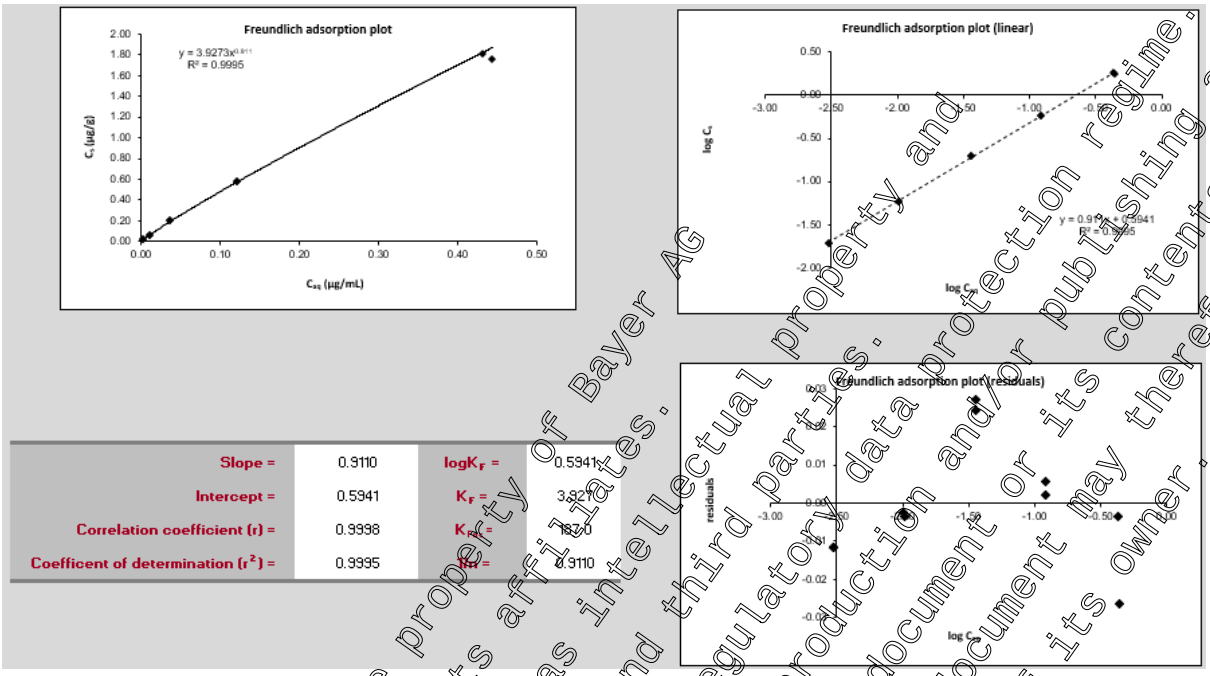
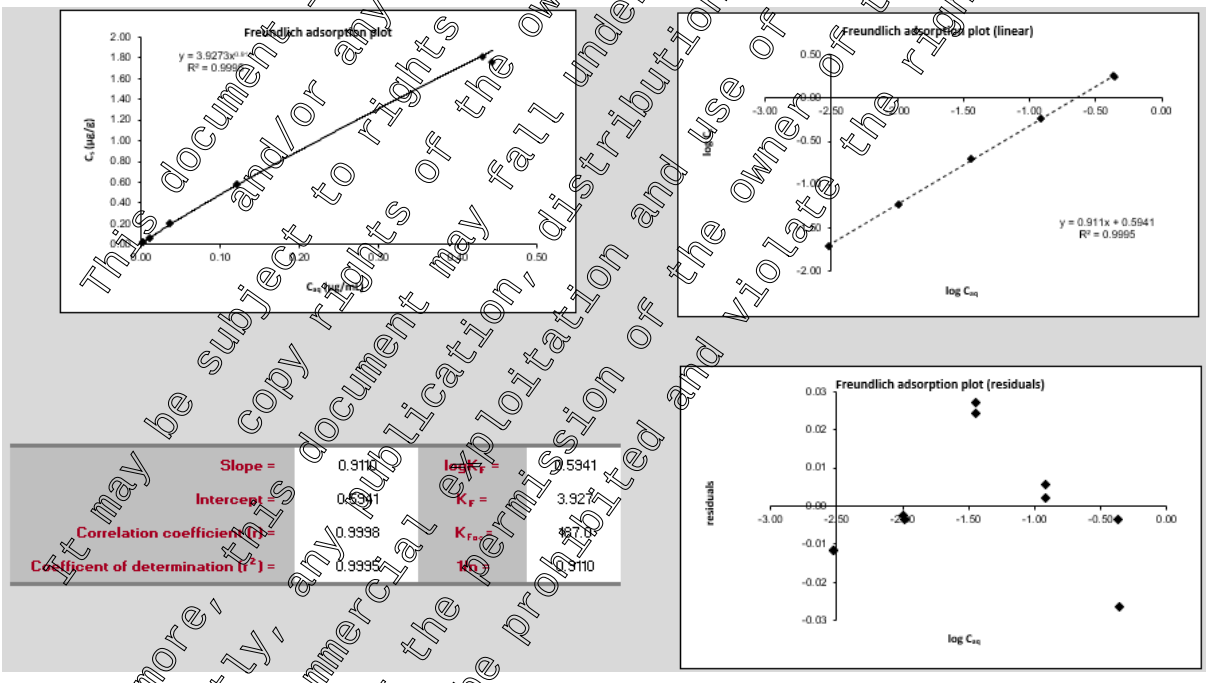


Figure 7.1.3.1.1- 21 Freundlich Isotherms of fluopicolide in SPA1 at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.1- 30. The impact on reported endpoints is summarised in Table 7.1.3.1.1- 31.

Table 7.1.3.1.1- 30: Summary of Quality Criteria and Regulatory Interpretation

Fluopicolide			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Burscheid	Silt loam	VG08	8	1	0
Great Chishill	Clay	ENG2	9	0	0
Parcay Meslay	Loam	FR09B	9	0	0
Tarascon Le Cayades	Clay loam	FR08	9	0	0
Valerio Tomelini	Silty clay	IT09	9	0	0
Vilobi D'Onyar	Sandy loam	SPA01	7	1	1

Table 7.1.3.1.1- 31: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Burscheid	Silt loam	VG08	303.3	303.3	0.8873	0.887
Great Chishill	Clay	ENG2	257	257	0.9035	0.908
Parcay Meslay	Loam	FR09B	257.4	257.4	0.9027	0.899
Tarascon Le Cayades	Clay loam	FR08	204.9	204.9	0.8502	0.867
Valerio Tomelini	Silty clay	IT09	187	187	0.9201	0.911
Vilobi D'Onyar	Sandy loam	SPA01	292	291.8	0.8796	0.882

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

These results indicate that fluopicolide is moderately absorbed to soil. The adsorption constant $K_{F(ads)}$ of fluopicolide ranged from 1.84 to 5.40 mL/g in the tested soils; the normalised adsorption constant $K_{OC(ads)}$ ranged from 187 to 303 mL/g. The Freundlich exponent 1/n was between 0.8502 and 0.9207, indicating that the concentration of the test item effects its adsorption behaviour in the concentration range examined.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the soils were acceptable according to the quality criteria and therefore suitable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of fluopicolide in soil.

Data Point:	KCA 7.1.3.1.1/05
Report Author:	[redacted]
Report Year:	2017
Report Title:	[phenyl-UL-14C] Fluopicolide: Adsorption/desorption on six soils
Report No:	M-595721-01-1
Document No:	M-595721-01-1
Guideline(s) followed in study:	OECD Guideline No. 106, 2000 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009, 2013 US EPA OCSPP Guideline No. 835.1230, 2008
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption behaviour of [phenyl-UL-^{14}C]-fluopicolide were studied in six soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
Abington	AB	Abington, United Kingdom	sandy loam	7.3	2.6
Lamberton	LB	Lamberton, MN, USA	loam	5.6	2.6
Lignieres	LN	Lignieres de Touraine, France	sandy loam	5.7	0.8
Muenster	MS	Muenster-Handorf, Germany	loamy sand	5.6	1.2
Pikeville	PV	Pikeville, NC, USA	loamy sand	4.5	1.8
Sarotti	SR	Hattersheim, Germany	silty clay loam	6.9	1.4

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1/5 (all soils). Test concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L of [phenyl-UL-^{14}C]-fluopicolide were applied in aqueous 0.01 M $CaCl_2$ solution. The desorption phase was performed by supplying pre-adsorbed soil samples with fresh aqueous 0.01 M $CaCl_2$ solution. Adsorption and desorption took place for 24 hours each. The test was performed in glass flasks with screw caps.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and the amount of test item in the supernatants was analyzed by liquid scintillation counting (LSC). The sorption parameters were calculated using Freundlich isotherms.

The test item was stable throughout the study. Mean parental mass balances were 106.6, 96.2, 95.7, 99.9, 104.7 and 103.9% AR after 48 hours of adsorption for soil AB, LB, LN, MS, PV and SR, respectively. Mean material balances were 106.6% AR for soil AB (range from 103.7 to 109.5% AR), 96.2% AR for soil LB (range from 95.9 to 96.6% AR), 95.7% AR for soil LN (range from 93.5 to 97.9% AR), 99.9% AR for soil MS (range from 97.9 to 101.8% AR), 104.7% AR for soil PV (range from 103.0 to 106.5% AR) and 103.9% AR for soil SR (range from 102.5 to 105.2% AR) after 48 hours of adsorption.

In the definitive adsorption test 55.8 to 70.4% AR, 65.9 to 80.6% AR, 37.4 to 50.4% AR, 40.2 to 50.9% AR, 57.0 to 72.0% AR and 36.8 to 53.5% AR were adsorbed in soil AB, LB, LN, MS, PV and SR, respectively.

The calculated adsorption constants K_F of the Freundlich isotherms ranged from 2.6 to 8.6 mL/g for the tested soils. The Freundlich exponents 1/n ranged from 0.844 to 0.916 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 were correlated with the organic carbon content of the soil to get a comparability of the adsorption behaviour in different soils. For [phenyl-UL-14C]-fluopicolide the K_{Foc} values ranged from 185.6 to 363.1 mL/g.

Following the adsorption phase, 20.4 to 31.6%, 11.3 to 24.7%, 36.4 to 44.4%, 37.6 to 48.2%, 20.9 to 33.5% and 32.2 to 47.1% of the initially adsorbed amount were found desorbed in soil AB, LB, LN, MS, PV and SR after 24 hours, respectively.

The desorption $K_{F(des)}$ and the normalized $K_{Foc(des)}$ values were not significantly higher than those obtained for adsorption.

There is no correlation between pH and adsorption for the investigated soils.

According to Briggs, [phenyl-UL-^{14}C]-fluopicolide can be classified as low for adsorption and desorption.

Soil origin	**Abington, United Kingdom**	**Lamberton, MN, USA**	**Lignières de Touraine, France**	**Muenster Handorf, Germany**
Soil ID	AB	LB	LN	MS
Soil type (USDA)	Sandy loam	Loam	Sandy loam	Loamy sand
pH (0.01M $CaCl_2$)	7.3	5.6	5.7	5.6
Organic carbon [%]	2.6	2.6	0.8	1.2
$K_F^{(ads)}$ [mL/g]	5.6	8.6	2.9	3.4
1/n	0.868	0.844	0.888	0.916
$K_{F,OC}^{(ads)}$ [mL/g]	214.7	331.9	363.1	282.6
$K_F^{(des)}$ [mL/g]	8.7	12.4	5.6	5.5
1/n	0.874	0.818	0.920	0.932
$K_{F,OC}^{(des)}$ [mL/g]	335.5	476.8	696.0	460.9

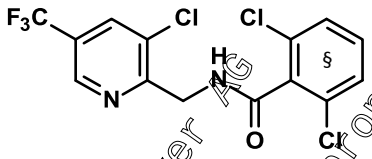
Soil origin	**Pikeville, NC, USA**	**Sarotti, Hattersheim, Germany**
Soil ID	PV	SR
Soil type (USDA)	Loamy sand	Silty clay loam
pH (0.01M $CaCl_2$)	4.5	6.9
Organic carbon [%]	1.8	1.4
$K_F^{(ads)}$ [mL/g]	6.2	2.6
1/n	0.873	0.851
$K_{F,OC}^{(ads)}$ [mL/g]	342.6	185.6
$K_F^{(des)}$ [mL/g]	8.6	4.6
1/n	0.877	0.867
$K_{F,OC}^{(des)}$ [mL/g]	477.0	328.1

I. Materials and Methods

A. Materials

1. Test Item

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



§ = position of radiolabel.

Batch number:	KML 10216
Specific radioactivity:	5.90 MBq/mg
Radiochemical purity:	>99% (HPLC)
Stability of test compound:	Stable during the equilibrium periods (up to 48 hours).

2. Test Soil

Sorption tests were performed with six soils covering a range of pH, organic carbon content and texture. Five of these soils were collected from the same sites as earlier laboratory aerobic soil studies (Lamberton, Sarotti, Münster, Pikeville and Abington soils) and one soil was from a terrestrial field dissipation site used for fluopicolide and its metabolite M-01 (Lignieres soil). In addition, the same batches of all six soils were used in a time dependent sorption study run concurrently with fluopicolide (KCA 7.1.2.1.1/09). The characteristics of the soils are summarised in Table 7.1.3.1.1- 32.

Table 7.1.3.1.1- 32: Characteristics of test soils

Parameter	Soils					
Soil Designation	**Abington**	**Lamberton**	**Lignieres**	**Muenster**	**Pikeville**	**Sarotti**
Abbreviation	AB	LB	LN	MS	PV	SR
Soil Batch ID	16/069	100416-S	20160912	20161014	100516-S	20161109
Textural Classification (USDA)	sandy loam	loam	sandy loam	loamy sand	loamy sand	silty clay loam
Sand [50 - 2000 µm] (%)	66	51	72	79	73	14
Silt [2 – 50 µm] (%)	20	28	16	20	26	54
Clay [< 2 µm] (%)	14	21	12	1	1	32
pH						
in $CaCl_2$ (1:1)	7.3	5.6	5.7	5.6	4.5	6.9
in H_2O (1:1)	7.4	5.8	6.0	6.0	4.9	7.0
Saturated paste	7.3	5.7	6.0	6.0	4.8	6.9
in KCl (1:1)	7.0	5.2	5.2	5.4	4.3	6.4
Organic Carbon (%)	2.6	2.6	0.8	1.2	1.8	1.4
Organic Matter (%)*	4.5	4.5	1.4	2.1	3.1	2.4
CEC (meq/100 g)	19.2	20.7	11.8	6.6	6.0	16.2g
Water Holding Capacity	59.7	59.4	41.6	32.9	44.5	54.8
Max (g H_2O 100 g DW)	59.7	59.4	41.6	32.9	44.5	54.8
at 1/10 bar (%)	20.3	42.3	20.3	27.4	31.0	35.1
Bulk Density (disturbed) (g/cm^3)	1.11	1.09	1.23	1.37	1.20	1.08

* Calculated using the following conversion factor: % Organic matter = % organic carbon x 1.724
CEC: Cation exchange capacity

B. Study design

1. Experimental conditions:

For the definitive test 4 g (dw) soils Abington, Lamberton, Lignieres, Muenster, Pikeville and Sarotti were weighed into centrifuge tubes, and total volumes of 20 mL of aqueous 0.01 M $CaCl_2$ solution were added. After pre-equilibration for ≥ 16 hours, 20 or 38 µL of the respective application solution (solvent water/methanol 1 : 1 v/v) were spiked in. The concentration of methanol within the test systems was 0.1% by volume. Initial nominal concentrations of the ^{14}C-test substance in the aqueous phase were 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L, thus covering two orders of magnitude. Each determination was performed in duplicate by shaking with an overhead shaker in the dark at 20±2°C.

Preliminary tests included the determination of a suitable soil-to-solution ratio over 24 hours, the time to reach adsorption equilibrium at a soil-to-solution ratio of 1:5 which included an assessment of the stability of the test item in the presence of soil over 24 hours.

In the definitive test an adsorption step of 24 hours was performed for all soils followed by one desorption step also of 24 hours. For work-up the aqueous supernatant was separated from soil by decantation and centrifugation. Radioactivity in water and soil extracts was determined by liquid scintillation counting (LSC). Non-extractable radioactivity in soil was determined by combustion followed by LSC to establish a full material balance.

Finally, the adsorption parameters were calculated using the Freundlich adsorption isotherm.

Adsorption phase

Parameter		**Description**
Soil Condition		Soils were equilibrated to study conditions for at least 16 hours with 20 mL aqueous 0.01 M $CaCl_2$ solution (corrected for soil moisture).
Have these soils been used for other laboratory studies?		Yes. The same batches of all six soils have been used previously in a time-dependent sorption study (KCA 7.1.2.1.1/09) and one soil in field dissipation study (KCA 7.1.2.1.2/12 and KCA 7.1.2.1.2/14) with fluopicolide
Soil sample weight		4 g dry weight equivalents per replicate.
solution used for equilibration		aqueous 0.01 M $CaCl_2$ solution.
Control used		$CaCl_2$ solution without soil
Test item concentrations	Nominal application rates	Nominal concentrations in test solution: 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, and 1.0 mg/L.
	Analytically measured concentrations	Concentrations in test solution: 0.0109 mg/L, 0.0577 mg/L, 0.108 mg/L, 0.489 mg/L, and 0.947 mg/L.
Identity and concentration of solvent		methanol / water (1/1, v/v)
Soil-to-solution ratio		1/5, i.e. 4 g soil dry weight to 20 mL solution (corrected for soil moisture).
pH of the equilibration solution (from preliminary test)	Initial	pH of aqueous 0.01 M $CaCl_2$ solution without soil: 5.81
	Final	pH with soil and test item after adsorption equilibrium: range 4.37 – 6.76
Number of replicats	Controls	Duplicate
	Treatments	Duplicate
Equilibration	Time	24 hours
	Temperature	20.3 °C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 rpm
	Shaking time	24 hours
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	1295 x *g*
	Duration	4 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Were the soil residues from the adsorption phase used?		Yes
Amount of test item present in the adsorbed state / adsorbed amount		The amounts of test item adsorbed to soil after asorption ranged from 36.8 to 80.6% AR
Number of desorption cycles		One desorption cycle was performed for each concentration.
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. A total volume of 20 mL was used as equilibration solution.
Soil-to-solution ratio		1:5 i.e. 4 g soil dry weight to 20 mL solution (corrected for soil moisture)
Number of replications	Controls	Duplicate
	Treatments	Duplicate
Desorption equilibration	Time	24 hours
	Mean Temperature	20.3 °C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 ± 2 rpm
	Shaking time	24 hours
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	1295 x *g*
	Duration	4 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

2. Analytical procedures:

The purity of the test item was investigated by radio-HPLC analysis using ^{14}C-flow-through detection techniques and its identity confirmed by co-chromatography with an analytical standard.

The parental mass balance was established in pre-tests. Supernatants were analysed by LSC and HPLC. Soil samples were exhaustively extracted with acetonitrile (3 times). Soil extracts were analysed by LSC and HPLC. Finally, radioactivity remaining in the extracted soil residue was combusted to determine a complete mass balance.

II. Results and Discussion

A. Mass balance and results of preliminary tests

In pre-tests fluopicolide was shown to be stable at a soil-to-solution ratio of 1:5 with mean parental mass balances of the test item were 106.6, 96.2, 95.7, 99.9, 104.7 and 103.9% AR after 48 hours of adsorption for soils AB, LB, LN, MS, PV and SR, respectively.

B. Transformation of test substance:

The stability of the test substance in contact with soil under the conditions of the definitive test was confirmed by HPLC analysis in a pre-test to be and determined to be higher than 95% after 48 hours.

C. Findings:

The adsorption behaviour of [phenyl-UL-^{14}C]-fluopicolide was investigated in six soils based on five different nominal concentrations ranging from approximately 0.01 mg/L to 1.00 mg/L (two orders of magnitude). Based on the outcome of the preliminary tests a soil/solution ratio of 1:5 for all the soils was used for the Definitive Test.

Equilibrium of the test item was established after 24 hours shaking at these soil solution ratios, thus an adsorption time of 24 hours was chosen for the definitive test followed by a desorption step of 24 hours.

Within definitive tests, the amount of [phenyl-UL-^{14}C]-fluopicolide adsorbed to soil after 24 hours ranged from 55.8 to 70.4% AR, 65.9 to 80.6% AR, 37.4 to 50.4% AR, 40.2 to 50.9% AR, 57.0 to 72.0% AR and 36.8 to 53.5% AR were adsorbed in soils AR, LB, LN, MS, PV and SR, respectively.

The adsorption behaviour of fluopicolide was accurately measured using a nominal concentration range of 0.01 mg/L to 1.0 mg/L by the Freundlich equation for all soils (Table 7.1.3.1.1-34). The adsorption constants $K_{F,\ ads}$ of the Freundlich isotherms ranged from 2.6 to 8.6 mL/g for the tested soils. The Freundlich exponents 1/n ranged from 0.844 to 0.916, indicating that the concentration of the test item affected the adsorption behaviour in the examined concentration range. The corresponding correlation coefficients for the adsorption isotherms ranged from 0.9952 to 0.9998, indicating a linear fit to the measured data. When normalized for the organic carbon content of soil $K_{OC,\ ads}$ values ranged from 185.6 to 363.1 mL/g.

Table 7.1.3.1.1- 33: Definitive test: Concentration of [phenyl-UL-^{14}C]-fluopicolide in aqueous and solid phase following 24 hours of adsorption (mean ± s.d.).

Concentration of Test Item	Soil [mg/kg]	Solution [mg/L]	Percentage Adsorbed [% AR]
Soil	**Abington (Soil ID: AB)**		
0.011 mg/L	0.04	0.003	70.4 ± 0.13
0.058 mg/L	0.19	0.020	64.5 ± 0.39
0.108 mg/L	0.35	0.039	64.0 ± 0.63
0.489 mg/L	1.40	0.208	57.4 ± 0.42
0.947 mg/L	2.64	0.419	55.8 ± 1.04
Soil	**Lamberton (Soil ID: LB)**		
0.011 mg/L	0.04	0.002	80.6 ± 0.33
0.058 mg/L	0.23	0.013	78.3 ± 0.06
0.108 mg/L	0.42	0.024	77.8 ± 0.62
0.489 mg/L	1.61	0.167	65.9 ± 6.29
0.947 mg/L	3.26	0.298	68.8 ± 0.30
Soil	**Lignieres (Soil ID: LN)**		
0.011 mg/L	0.03	0.005	50.4 ± 1.83
0.058 mg/L	0.14	0.030	47.2 ± 1.99
0.108 mg/L	0.24	0.060	44.4 ± 0.81
0.489 mg/L	0.99	0.291	40.5 ± 1.13
0.947 mg/L	1.77	0.593	37.4 ± 0.13
Soil	**Muenster (Soil ID: MS)**		
0.011 mg/L	0.03	0.005	50.9 ± 1.19
0.058 mg/L	0.14	0.030	48.0 ± 1.44
0.108 mg/L	0.25	0.059	45.8 ± 1.42
0.489 mg/L	1.10	0.269	44.9 ± 2.93
0.947 mg/L	1.90	0.567	40.2 ± 1.78
Soil	**Pikeville (Soil ID: PV)**		
0.011 mg/L	0.04	0.003	72.0 ± 0.23
0.058 mg/L	0.19	0.019	66.2 ± 0.56
0.108 mg/L	0.36	0.037	65.7 ± 0.35
0.489 mg/L	1.50	0.188	61.5 ± 0.92
0.947 mg/L	2.70	0.407	57.0 ± 2.12
Soil	**Sarotti (Soil ID: SR)**		
0.011 mg/L	0.03	0.005	53.5 ± 0.71
0.058 mg/L	0.13	0.031	45.7 ± 0.03
0.108 mg/L	0.24	0.060	44.9 ± 0.38
0.489 mg/L	0.91	0.308	37.1 ± 1.46
0.947 mg/L	1.74	0.598	36.8 ± 1.51

Table 7.1.3.1.1- 34: Adsorption constants of [phenyl-UL-^{14}C]-fluopicolide in soil

Phase	Parameter	Units	Abington	Lamberton	Lignieres	Muenster	Pikeville	Sarotti
			sandy loam	loam	sandy loam	loamy sand	loamy sand	silty clay loam
Adsorption	$K_{F,ads}$	[mL/g]	5.6	8.6	2.9	3.4	6.2	2.6
	1/n	-	0.868	0.844	0.888	0.916	0.873	0.851
	R^2	-	0.9998	0.9952	0.9997	0.9992	0.9995	0.9994
	$K_{OC,ads}$	[mL/g]	214.7	331.9	363.1	282.6	342.6	185.6
Desorption	$K_{F,des}$	[mL/g]	8.7	12.4	5.6	5.5	8.6	4.6
	1/n	-	0.874	0.818	0.92	0.932	0.877	0.867
	R^2	-	0.9985	0.9908	0.9989	0.9945	0.9986	0.9967
	$K_{OC,des}$	[mL/g]	335.5	476.8	696.0	460.9	477.0	328.1

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the report were used as input data (Table 7.1.3.1.1- 35). Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet tool provided by EFSA (summarised in Table 7.1.3.1.1- 36).

Table 7.1.3.1.1- 35: Definitive test: Concentration of fluopicolide in aqueous and solid phase following 48 hours of adsorption used as inputs in checklist

Soil ID	Abington		Lamberton		Lignieres		Muenster		Pikeville		Sarotti	
Conc.	Soil	Sol.	Soil	Sol.	Soil	Sol.	Soil	Sol.	Soil	Sol.	Soil	Sol.
	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg	mg/L
0.011 mg/L	0.04	0.003	0.04	0.002	0.03	0.006	0.03	0.006	0.0395	0.003	0.03	0.005
	0.04	0.003	0.04	0.002	0.03	0.005	0.03	0.005	0.0393	0.0031	0.03	0.005
0.058 mg/L	0.19	0.02	0.23	0.012	0.13	0.032	0.13	0.031	0.19	0.0198	0.13	0.031
	0.19	0.021	0.23	0.013	0.14	0.029	0.14	0.029	0.193	0.0192	0.13	0.031
0.108 mg/L	0.35	0.038	0.42	0.025	0.25	0.059	0.26	0.057	0.358	0.0367	0.24	0.06
	0.34	0.04	0.42	0.023	0.24	0.061	0.24	0.06	0.354	0.0375	0.25	0.059
0.489 mg/L	1.39	0.21	1.46	0.198	1.02	0.285	1.03	0.284	1.48	0.193	0.94	0.3
	1.41	0.206	1.76	0.136	0.96	0.296	1.17	0.255	1.53	0.184	0.87	0.315
0.947 mg/L	2.69	0.409	3.27	0.293	1.78	0.592	1.99	0.55	2.6	0.427	1.82	0.584
	2.59	0.429	3.24	0.298	1.76	0.594	1.82	0.583	2.8	0.387	1.67	0.613

Sol. = Solution

Table 7.1.3.1.1- 36: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Abington	Lamberton	Lignieres	Muenster	Pikeville	Sarotti
Adsorption method (direct/indirect)	-		indirect	indirect	indirect	indirect	indirect	indirect
Soil : solution ratio	g/mL		1:5	1:5	1:5	1:5	1:5	1:5
Mass balance of ^{14}C (at all tested concentrations)	%	>90%	106.6 (103.7-109.5)	96.2 (95.9-96.6)	95.7 (93.5-97.9)	99.9 (97.9-101.8)	104.7 (103.0-106.5)	103.9 (102.5-105.2)
f – due to loss processes			1.6	7.2	4.9	0.6	1.6	4.3
Adsorbed percentage (δ)	%	>20%	54.7-72.5	68.5-81.6	37.3-54.1	38.4-54.1	54.9-72.1	35.3-54.1
K_d x (soil:solution ratio)		>0.3	1.2-2.7	1.5-4.0	0.59-1.2	0.62-1.2	1.2-2.6	0.54-1.2
K_{fE} / K_f	-	<1.2	1.03	1.12	1.15	1.02	1.03	1.04
$^{ads}K_F$	L/kg	*	5.375	8.687	2.844	3.329	6.156	2.570
(95% confidence interval)			(5.049-5.723)	(6.524-11.567)	(2.600-3.112)	(2.881-3.846)	(5.628-6.733)	(2.353-2.806)
$^{ads}1/n$	-	*	0.848	0.848	0.873	0.906	0.873	0.844
(95% confidence interval)			(0.830-0.866)	(0.775-0.921)	(0.844-0.902)	(0.860-0.952)	(0.848-0.898)	(0.816-0.872)
$^{Ads}R^2$	-	>0.975	0.999	0.989	0.998	0.996	0.999	0.998
$^{ads}K_{F,OC}$	L/kg		206.7	334.1	355.5	277.4	342.0	183.6
Visual fit to Freundlich isotherm			Accept.	Accept.	Accept.	Accept.	Accept.	Accept.
Residual plots randomly distributed			Good	Good	Good	Good	Good	Good

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance of 95.7-106.6% was acceptable. The % adsorption of 37.3-81.6% were all acceptable. The acceptability of the analytical method was confirmed over the entire range of concentrations measured (reported LOQ of 0.4 ng/L for LSC). The use of the indirect method was appropriate based on a Kd* soil:solution ratio > 0.3 in all soils. The calculated K_{fE} / K_f ratio was < 1.2 in all soils. The graphical fits of the Freundlich equation based on the standard linear regression form using log-log transformed data alongside the associated residual plots was good with R^2 of the standard linear regressions ranging from 0.989 to 0.999 and the visual fit of both the standard regression and the residual plots being acceptable.

Figure 7.1.3.1.1- 22 Freundlich Isotherms of fluopicolide in Abington at 20°C

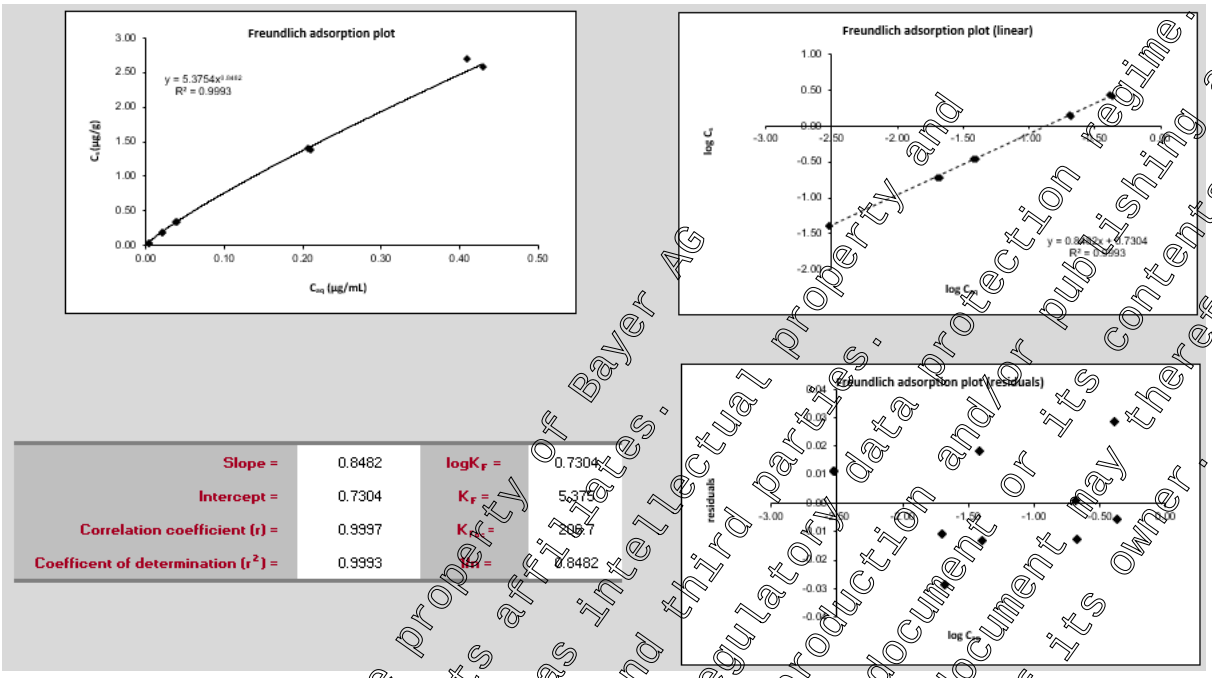


Figure 7.1.3.1.1- 23 Freundlich Isotherms of fluopicolide in Lamberton at 20°C

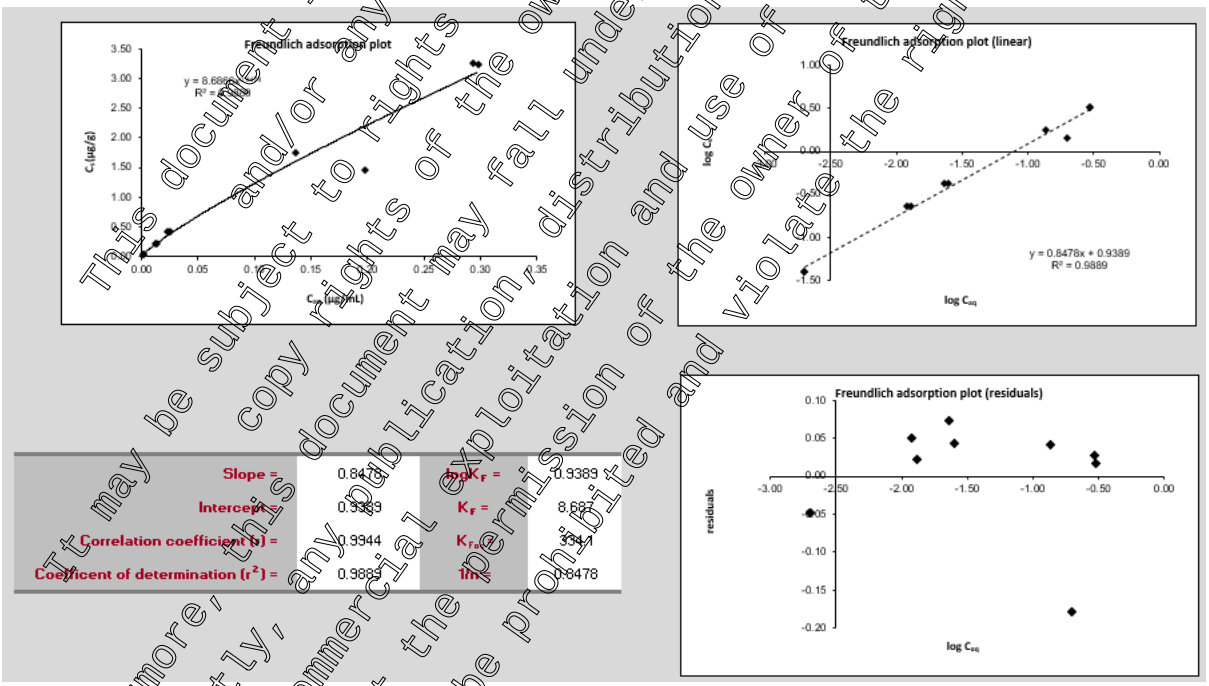


Figure 7.1.3.1.1- 24 Freundlich Isotherms of fluopicolide in Lignieres at 20°C

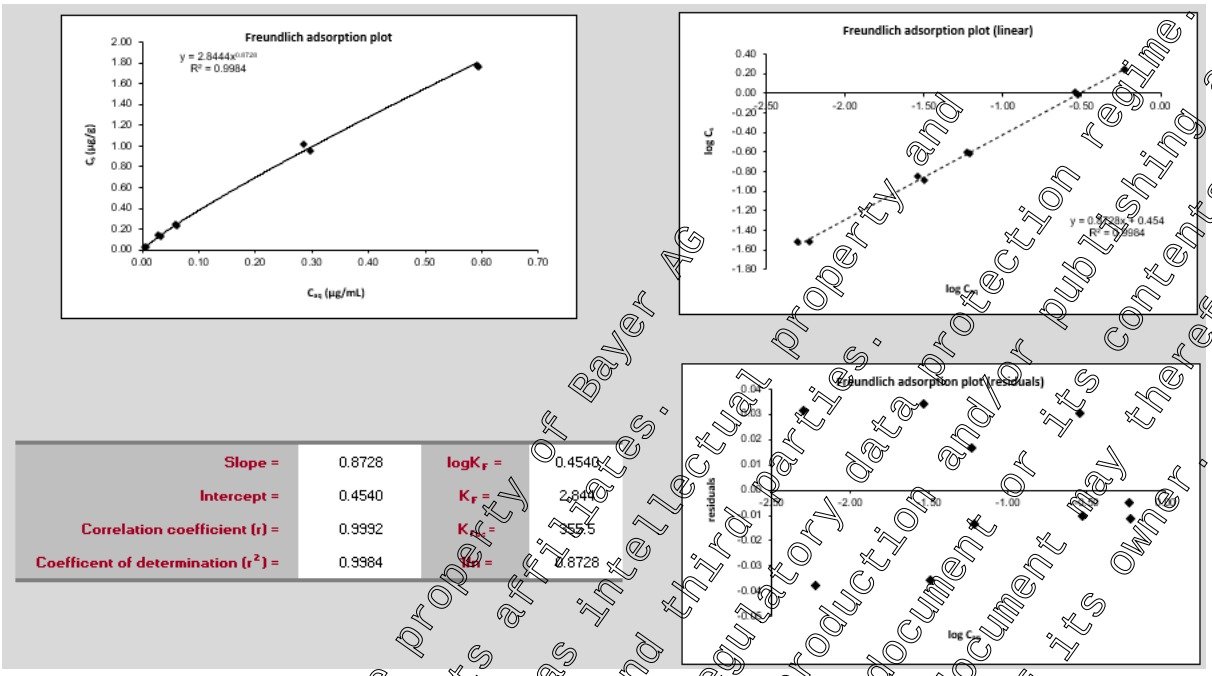


Figure 7.1.3.1.1- 25 Freundlich Isotherms of fluopicolide in Muenster at 20°C

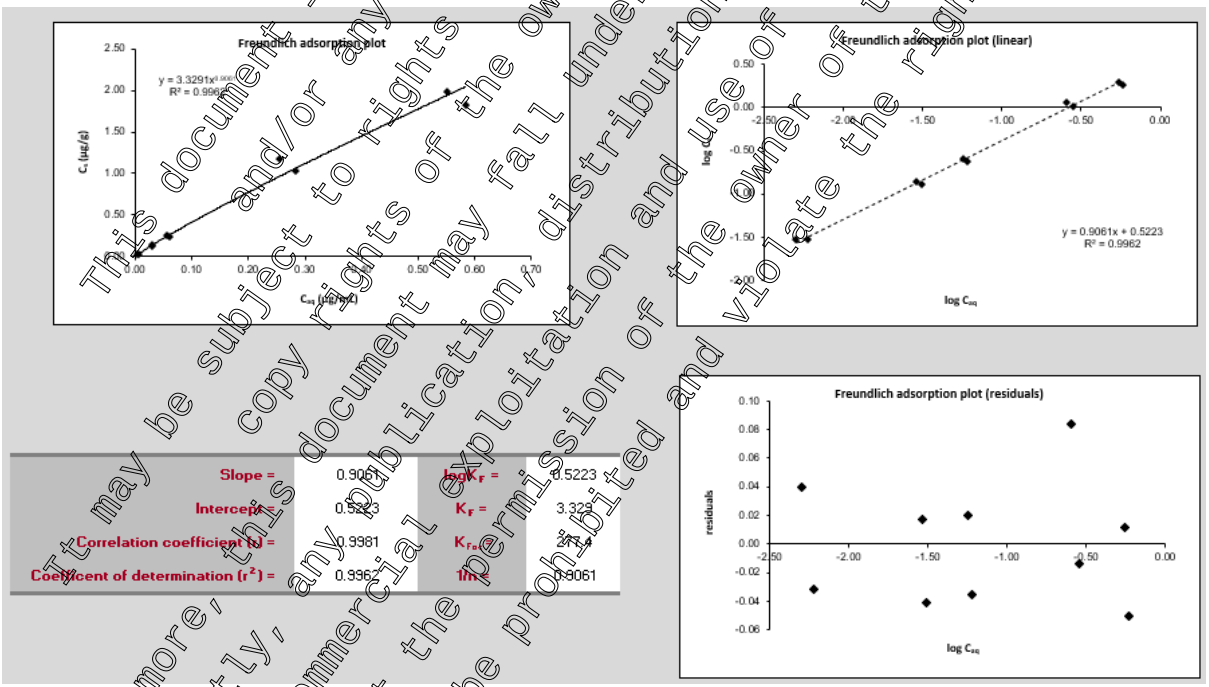
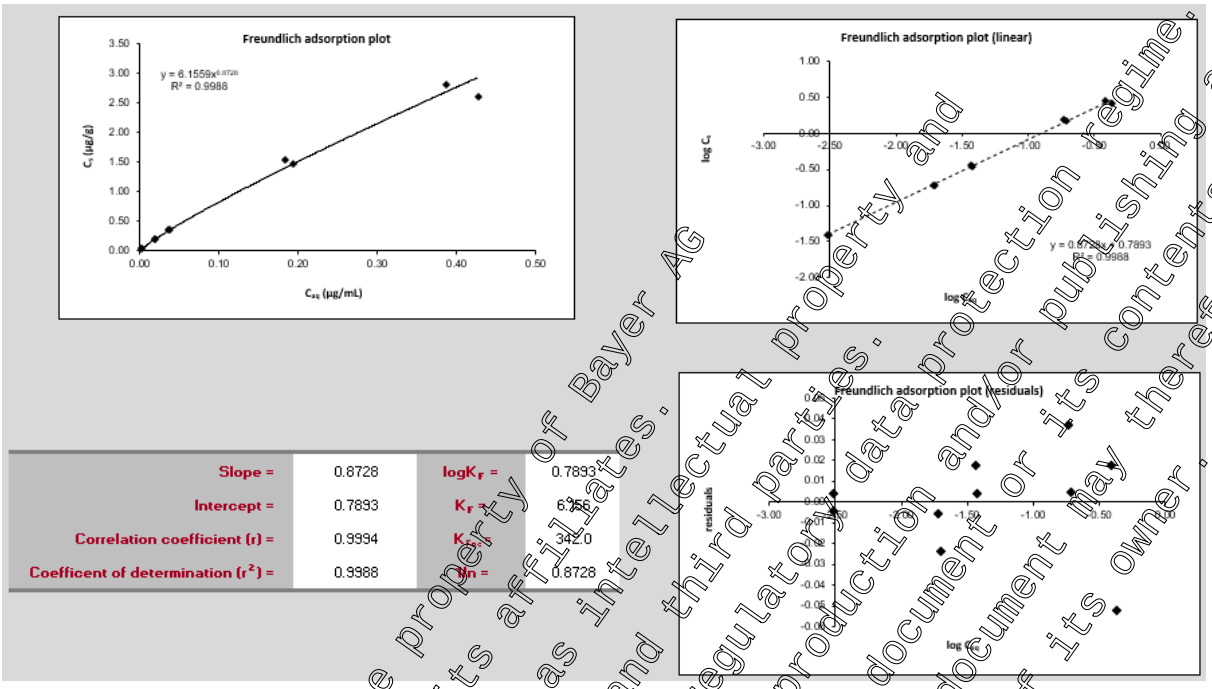
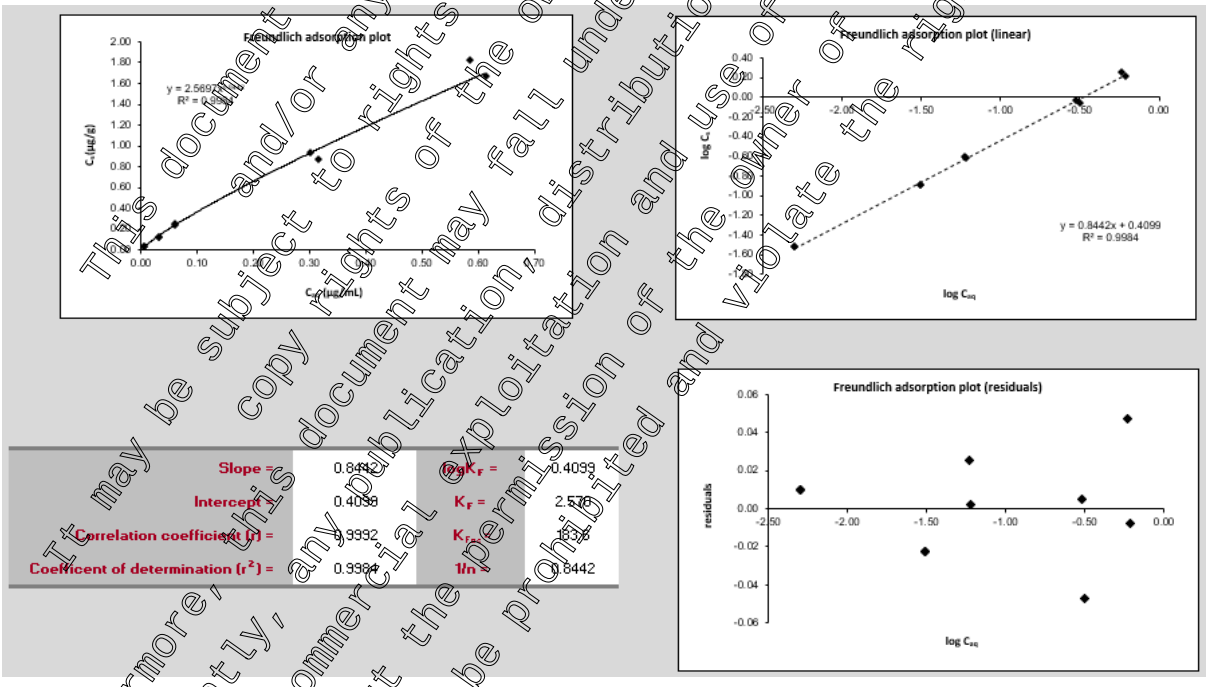


Figure 7.1.3.1.1- 26 Freundlich Isotherms of fluopicolide in Pikeville at 20°C



Slope =	0.8728	$\log K_F$ =	0.7893
Intercept =	0.7893	K_F =	6.156
Correlation coefficient (r) =	0.9994	K_{oc} =	342.0
Coefficent of determination (r^2) =	0.9988	1/n =	0.8728

Figure 7.1.3.1.1- 27 Freundlich Isotherms of fluopicolide in Sarotti at 20°C



Slope =	0.8442	$\log K_F$ =	0.4099
Intercept =	0.4099	K_F =	2.570
Correlation coefficient (r) =	0.9992	K_{oc} =	183.8
Coefficent of determination (r^2) =	0.9984	1/n =	0.8442

A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.1- 37. The impact on reported endpoints is summarised in Table 7.1.3.1.1- 38.

Table 7.1.3.1.1- 37: Summary of Quality Criteria and Regulatory Interpretation

Fluopicolide			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Abington	Sandy loam	AB	9	0	0
Lamberton	Loam	LB	8	1	0
Lignieres	Sandy loam	LN	9	0	0
Muenster	Loamy sand	MS	9	0	0
Pikeville	Loamy sand	PV	9	0	0
Sarotti	Silty clay loam	SR	9	0	0

Table 7.1.3.1.1- 38: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Abington	Sandy loam	AB	214.7	206.7	0.868	0.848
Lamberton	Loam	LB	331.9	334.1	0.844	0.848
Lignieres	Sandy loam	LN	363.1	355.5	0.888	0.873
Muenster	Loamy sand	MS	282.6	277.4	0.916	0.906
Pikeville	Loamy sand	PV	342.6	342	0.873	0.873
Sarotti	Silty clay loam	SR	185.6	183.6	0.851	0.844

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

These results indicate that fluopicolide is moderately absorbed to soil. The adsorption constant $K_{F(ads)}$ of fluopicolide ranged from 2.6 to 8.6 mL/g in the tested soils; the normalised adsorption constant $K_{OC(ads)}$ ranged from 185.6 to 363.1 mL/g. The Freundlich exponent 1/n was between 0.844 and 0.916, indicating that the concentration of the test item effects its adsorption behaviour in the concentration range examined.

There was no correlation between pH and adsorption for the investigated soils.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the soils were acceptable according to the quality criteria and therefore suitable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of fluopicolide in soil.

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

The adsorption and desorption of metabolites from fluopicolide have been investigated in fifteen studies (KCA 7.1.3.1.2/01 to KCA 7.1.3.1.2/15). Studies KCA 7.1.3.1.2/01 to KCA 7.1.3.1.2/08 were evaluated during the previous EU review. Studies KCA 7.1.3.1.2/09 to KCA 7.1.3.1.2/15 are new studies for Annex 1 renewal submission.

Metabolite	Report reference	Author, Year	Comment
M-01	KCA 7.1.3.1.2/01 M-235837-01-1	[redacted] 2001	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-03	KCA 7.1.3.1.2/02 M-241272-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered as supporting data.
M-03	KCA 7.1.3.1.2/03 M-221107-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-02	KCA 7.1.3.1.2/04 M-219828-01-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-05	KCA 7.1.3.1.2/05 M-241403-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-10	KCA 7.1.3.1.2/06 M-241404-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-11, M-12, M-13, M-14	KCA 7.1.3.1.2/07 M-223531-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered as supporting data for M-14.
M-01	KCA 7.1.3.1.2/08 M-224926-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-15	KCA 7.1.3.1.2/09 M-583208-01-1	[redacted] 2017	Submitted as additional information regarding fluopicolide confirmatory data on metabolite M-15, 2018. Reviewed and accepted by RMS Austria.
M-01	KCA 7.1.3.1.2/10 M-686388-01-1	[redacted] 2020	New data not yet reviewed.
M-05	KCA 7.1.3.1.2/11 M-587789-01-1	[redacted] 2017	New data not yet reviewed.
M-14	KCA 7.1.3.1.2/12 M-572869-01-1	[redacted] 2016	New data not yet reviewed.
M-14	KCA 7.1.3.1.2/13 M-686386-01-1	[redacted] 2020	New data not yet reviewed.
M-20	KCA 7.1.3.1.2/14 M-578760-01-1	[redacted] 2016	New data not yet reviewed.
M-02	KCA 7.1.3.1.2/15 M-686387-01-1	[redacted] 2020	New data not yet reviewed.

An overview of the sorption parameters measured for M-01, M-02, M-03, M-05, M-10, M-14 and M-15 in soil is provided below. No reliable sorption parameters have been derived for M-11, M-12, M-13 and M-20. Worst-case K_{OC}/K_{OM} values of 0 mL/g were therefore used in the groundwater modelling, with a default 1/n value of 1.0.

Summary of sorption parameters derived for M-01 (AE C653711)

Report reference	Soil	Soil Code	Texture	pH	OC (%)	K_f (mL/g)	K_{OC} (mL/g)	1/n
M-01 KCA 7.1.3.1.2/01 M-235837-01-1	Connecticut	RL-51	Sandy loam	4.8	0.9	0.241*	26*	1.141*
	North Dakota	RL-81	Sandy loam	7.7	5.7	1.761	31	0.809
	Florida	RM-014	Sand	6.3	1.4	0.529	38	0.916
	Washington	RM-019	Sand	4.9	4.2	1.890	45	0.918
	California	RM-022	Sandy clay loam	6.6	0.4	0.208	51	0.972
M-01 KCA 7.1.3.1.2/08 Connecticut M-224926-01-2	Connecticut	RL-51	Sandy loam	4.8	0.9	0.359**	39.9**	0.970**
M-01 KCA 7.1.3.1.2/10 M-686388-01-1	LUFA 2.1	2.1	Sand	5.2	0.59	0.103	17.5	0.958
	LUFA 2.3	2.3	Sandy loam	6.2	0.61	0.056	9.2	0.859
	LUFA 5M	5M	Sandy loam	7.1	1.10	0.162	14.8	0.888
	LUFA 6S	6S	Clay loam	7.3	1.78	0.265	14.9	0.872
	Frankenforst	FF	Silt loam	6.9	2.4	0.418	17.4	0.980
Geometric mean							**24.1**	**-**
Arithmetic mean							**-**	**0.914**

* value excluded from mean values
**recalculated and used for calculations

Summary of sorption parameters derived for M-02 (AE C657188)

Report reference	Soil	Soil Code	Texture	pH ($CaCl_2$)	OC (%)	K_f (mL/g)	K_{OC} (mL/g)	1/n
M-02 KCA 7.1.3.1.2/04 M-219828-01-1	Abington	03/06	Sandy loam	7.2	2.6	0.029	1.1	0.725
	Munster	03/07	Loamy sand	5.4	1.1	0.116	10.5	0.887
	Sarotti	03/10	Silt loam	7.5	1.3	0.082	6.3	0.709
M-02 KCA 7.1.3.1.2/15 M-686387-01-1	LUFA 2.1	2.1	Sand	5.2	0.59	0.047	8.0	1.031
	LUFA 2.3	2.3	Sandy loam	6.2	0.61	0.038	6.2	0.853
	LUFA 5M	5M	Sandy loam	7.1	1.1	0.154	14.0	0.989
	LUFA 6S	6S	Clay loam	7.3	1.78	0.145	8.2	1.105
	Frankenforst	FF	Silt loam	6.9	2.4	0.059	2.5	0.814
Geometric mean							**5.7**	**-**
Arithmetic mean							**-**	**0.889**

Summary of sorption parameters derived for M-03 (AE 0608000)

Report reference	Soil	Soil Code	Texture	pH ($CaCl_2$)	OC (%)	K_f (mL/g)	K_{OC} (mL/g)	1/n
M-03 KCA 7.1.3.1.2/02 M-241272-01-2	Munster	EFS-132	Loamy sand	4.8	0.9	-	73*	-
	Huntlosen	EFS-151	Loamy sand	7.7	5.7	-	112*	-
	Abington	EFS-128	Sandy loam	6.3	1.4	-	ND*	-
	Sarotti	EFS-133	Silt loam	4.9	4.2	-	ND*	-
M-03 KCA 7.1.3.1.2/03 M-221107-01-2	Ingleby	02/03	Sandy loam	4.1	3.5	2.86	82	0.961
	Huntlosen	03/04	Loamy sand	4.7	1.7	2.26	133	1.012
	Munster	03/07	Loamy sand	5.4	1.1	1.23	112	0.939
Geometric mean							**106.9**	-
Arithmetic mean								**0.971**

* value excluded from mean values

Summary of sorption parameters derived for M-05 (AE 1344122)

Report reference	Soil	Soil Code	Texture	pH ($CaCl_2$)	OC (%)	K_f (mL/g)	K_{OC} (mL/g)	1/n
M-05 KCA 7.1.3.1.2/05 M-241403-01-2	Abington	n/a	Sandy loam	7.2	2.6	0.294	11	0.883
	Munster	n/a	Loamy sand	5.4	1.1	0.544	49	0.954
	Sarotti	n/a	Silt loam	7.7	1.3	0.218	17	0.918
M-05 KCA 7.1.3.1.2/11 M-587780-01-1	[redacted]	331	Sandy loam	5.3	1.9	1.4793	77.9	0.974
	[redacted]	329	Silt loam	6.3	2	0.4915	24.6	0.985
	Dollendorf	330	Loam	7.3	4.5	0.6629	14.7	1.025
	[redacted]	327	Loamy sand	6.3	1.6	0.4671	29.2	0.984
Geometric mean (all soils)							**25.8**	-
Arithmetic mean (all soils)							-	**0.960**
Geometric mean (pH <7)							**40.7**	-
Arithmetic mean (pH <7)							-	**0.974**
Geometric mean (pH ≥7)							**14.0**	-
Arithmetic mean (pH ≥7)							-	**0.942**

Summary of sorption parameters derived for M-10 (AE 1344123)

Report reference	Soil	Soil Code	Texture	pH ($CaCl_2$)	OC (%)	K_d (mL/g)	K_{OC} (mL/g)	1/n
M-10 KCA 7.1.3.1.2/06 M-241404-01-2	Abington	n/a	Sandy loam	7.2	2.6	0.003[a]	0.07[a]	n/a
	Munster	n/a	Loamy sand	5.4	1.1	0.09[a]	8.2[a]	n/a
	Sarotti	n/a	Silt loam	7.5	1.3	0.14[a]	10.7[a]	n/a
Geometric mean							**1.8**	-

[a] Single point Kd and Koc values, mean values of 3 soil : solution ratios

Summary of sorption parameters derived for M-14 (AE 1388273)

Report reference	Soil	Soil Code	Texture	pH ($CaCl_2$)	OC [%]	K_f (mL/g)	K_{OC} (mL/g)	1/n
M-14 KCA 7.1.3.1.2/07 M-223531-01-2	n/a	n/a	n/a	6	n/a	n/a	19.2*	n/a
	n/a	n/a	n/a	2.5	n/a	n/a	133.4*	n/a
M-14 KCA 7.1.3.1.2/12 M-572869-01-1	[redacted]	-	Loam	5	1.8	0.1765	9.8	0.964
	[redacted]	-	Silt loam	6.1	1.9	0.2834	14.9	0.937
	Dollendorf II	-	Clay loam	7.3	4.8	0.5601	11.7	0.941
	L[redacted]	-	Sandy loam	6.5	1.5	0.1848	12.3	0.956
M-14 KCA 7.1.3.1.2/13 M-686386-01-1	LUFA 2.1	2.1	Sand	5.2	0.59	0.031	5.3	1.022
	LUFA 2.3	2.3	Sandy loam	6.2	0.61	0.028	4.6	0.908
	LUFA 5M	5M	Sandy loam	7.1	1.1	0.117	10.7	0.892
	LUFA 6S	6S	Clay loam	7.3	1.78	0.3	16.9	0.936
	Frankenforst	FF	Silt loam	6.9	2.4	0.238	9.9	0.923
Geometric mean							**9.9**	**-**
Arithmetic mean							**-**	**0.942**

* value excluded from mean value

Summary of sorption parameters derived for M-15 (AE 143903)

Report reference	Soil	Soil Code	Texture	pH ($CaCl_2$)	OC [%]	K_f (mL/g)	K_{OC} (mL/g)	1/n
M-15 KCA 7.1.3.1.2/09 M-585208-01-1	L[redacted]	[redacted]	Loamy sand	5.4	1.8	0.431	23.9	0.953
	Dollendorf II	DD	Clay loam	7.3	5.2	0.728	14.0	0.920
	H[redacted]	[redacted]	Silt loam	6.0	2.4	0.500	20.8	0.923
	[redacted]	[redacted]	Sandy loam	5.1	2.1	0.380	18.1	0.950
Geometric mean							**18.8**	**-**
Arithmetic mean							**-**	**0.937**

Data Point:	KCA 7.1.3.1.2/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Adsorption/desorption of (14C)2,6-dichlorobenzamide
Report No:	C034964
Document No:	M-235837-01-1
Guideline(s) followed in study:	USEPA (=EPA): 40 CFR 158.290, N 160-4, -5, 163-1
Deviations from current test guideline:	No
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Tests to investigate the adsorption/desorption characteristics of M-01 (referred to as 2,6-dichlorobenzamide and BAM in the report) were studied in five soils in batch equilibrium experiments in the laboratory in the dark at 25 ± 1 °C.

Soil	Soil Code	Texture (USDA)	pH	OC [%]
Connecticut	RL-51	Sandy loam	4.8	0.9
North Dakota	RL-81	Sandy loam	7.7	5.7
Florida	RM-014	Sand	6.3	1.4
Washington	RM-019	Sand	4.9	4.2
California	RM-022	Sandy clay loam	6.6	0.4

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratio of 1:5. Nominal test concentrations of 10, 5.0, 1.0, 0.5 and 0.2 µg/mL of [^{14}C]-M-01 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. 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.

For all soils, the recovery of radioactivity was quantitative with recoveries ranging from 95.1 % to 106.7 % of applied radioactivity. The stability of M-01 was confirmed in adsorption supernatants, desorption supernatants and where applicable in soil extracts.

The calculated Freundlich adsorption coefficients (K_f) ranged from 0.208 to 1.890 mL/g. In four of the soils investigated the correlation of data by linear regression was excellent but in one soil (RL-51) there was a clear outlier which resulted in a lower correlation. When the soil and solution concentrations for the nominal starting concentration of 0.5 µg/mL were excluded and the data re-evaluated by linear regression a better fit was obtained with the experimental data. This re-evaluation has been reported in a separate position paper (see KCA 7.1.3.1.2/08, M-224926-01-2). The Freundlich adsorption isotherm data reported for soils RL-81, RM-014, RM-019 and RM-022 and the re-evaluated data for soil RL-51 are summarized below. These values have been used in all subsequent risk assessments. In two soils, RL-51 and RM-22, the 1/n values were 0.97 with a linear relationship between the concentration in the soil and solution indicating the amount absorbed was independent of concentration. The relationship between the soil and solution concentration for the three remaining soils was non-linear with 1/n values ranging from 0.8085 to 0.9163. When corrected for organic carbon content of the soil, the K_{oc} values obtained ranged from 31 to 51 mL/g.

For all soils, a desorption cycle was obtained with an increase in the K_{oc} values. These results show that adsorption would be expected to be only partially reversible. The desorption coefficient (K_f) values obtained ranged from 2.577 to 6.397 mL/g. The values of 1/n for the desorption cycle were similar to those obtained for the adsorption cycle. The K_{oc} values for the desorption cycle ranged from 76 to 1560 mL/g.

Soil origin	Connecticut	North Dakota	Florida	Washington	California
Soil Code	RL-51	RL-81	RM-014	RM-019	RM-022
Soil type (USDA)	Sandy loam	Sandy loam	Sand	Sand	Sandy clay loam
pH	4.8	7.7	6.3	4.9	4.8
Organic carbon [%]	0.9	5.7	1.4	4.2	0.4
$K_F^{(ads)}$ [mL/g]	0.3588 [A]	1.761	0.529	1.89	0.208
1/n	0.970	0.8085	0.9163	0.9125	0.9718
$K_{F,OC}^{(ads)}$ [mL/g]	39.9 [A]	31	38	45	51
R^2	0.991	0.997	0.992	0.990	0.989
$K_F^{(des)}$ [mL/g]	4.652	4.35	2.577	4.178	6.397
1/n	1.1170	0.8877	1.0109	0.9663	1.0438
$K_{F,OC}^{(des)}$ [mL/g]	494	76	184	100	1560

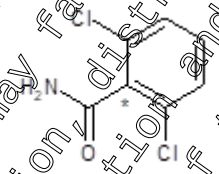
[A] Taken from report amendment M-224926-01-2

I. Materials and Methods

A. Materials

1. Test Item

[^{14}C-1-phenyl]-M-01 (referred to as 2,6-dichlorobenzamide in the report)



* = position of radiolabel.

Chemical name (IUPAC): 2,6-dichlorobenzamide

Batch number: DUPH016

Specific radioactivity: 6.86 mCi/mmol

Radiochemical purity: 97%

Stability of test compound: Stable during the batch equilibrium procedure.

2. Test Soil

Sorption tests were performed with five agricultural soils covering a wide range of pH, organic carbon content and texture. After collection, soils were air-dried at room temperature overnight and passed through a 2 mm sieve prior to use in the study. The characteristics of the soils summarised in Table 7.1.3.1.2- 1.

Table 7.1.3.1.2- 1: Physico-chemical properties of test soils

Parameter	Soil				
Soil Designation	**RL-51**	**Rl-81**	**RM-014**	**RM-019**	**RM-022**
Geographic Location					
State	Connecticut	North Dakota	Florida	Washington	California
Country	USA	USA	USA	USA	USA
Horizon (cm)	0 - 30	0 - 30	0 - 30	0 - 30	0 - 30
Textural Classification	Sandy loam	Sandy loam	Sand	Sand	Sandy clay loam
Sand (%)	74	62	99	97	49
Silt (%)	20	28	1	3	26
Clay (%)	6	10	0	0	25
pH	4.8	7.7	6.3	4.9	6.6
Organic Matter (%)	1.6	9.6	2.4	7.1	0.7
Organic Carbon (%)	0.9	5.7	1.4	4.2	0.4
Cation Exchange Capacity (meq/100 g)	7	23.1	6.3	8.5	16.3
Bulk density (g/cm^3)	1.32	0.84	1.3	0.92	1.26
Water Holding Capacity (%)					
at 1/3 bar	15.1	45.7	3.8	13.3	21.3

n.a.: not analysed

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of Teflon® centrifuge tubes (50 mL) with Teflon® screw caps.

In preliminary tests, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, and the appropriate adsorption and desorption equilibration times were determined.

The main test was performed in duplicate. M-01 was dissolved in 0.01M calcium chloride solution at nominal concentrations of 0.2, 0.8, 1, 5 and 10 mg/L. Soil samples were prepared at a soil to solution ratio of 1:5 and shaken at 25 °C. Following the preliminary tests, an equilibrium time of 24 hours was selected.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		5 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ 12-18 hours overnight
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 0.2, 0.5, 1.0, 5.0 and 10 µg/mL
	Analytically (LSC) measured concentrations	Concentrations in test solution: RL-51: 0.25, 0.54, 1.13, 5.58 and 10.94 µg/mL RL-81: 0.25, 0.58, 1.14, 5.52 and 11.04 µg/mL RM-014: 0.25, 0.60, 1.18, 5.85 and 11.60 µg/mL RM-019: 0.21, 0.57, 1.09, 5.27 and 10.62 µg/mL RM-022: 0.26, 0.56, 1.13, 5.47 and 10.94 µg/mL
Identity and concentration of co-solvent		Dosing stock made up in calcium chloride
Soil: Solution ratio		1:5 i.e. 5 g soil dry weight equivalent to 25 mL solution
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Equilibration conditions	Time	24 hrs
	Temperature	25±1°C
	Dark	In the dark
	Shaking method	Gyrotory shaker at 250 rpm
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	2000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 3 to 34% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		1
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 25 mL was used as equilibration solution.
Soil: Solution ratio		1:5 i.e. 5 g soil dry weight equivalent to 25 mL solution
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hours
	Temperature	25±1°C
	Dark	In the dark
	Shaking method	Gyrotory shaker at 250 rpm
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	2000 or 7000 rpm
	Duration	15 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

2. Analytical Procedures

After equilibration, soil and solution phases were separated by centrifugation and the concentration of M-01 in the water phase determined by LSC. M-01 remaining in the soil phase was then desorbed with 0.01M calcium chloride and the concentration determined by LSC. The radioactivity remaining in soil after the desorption phase was quantified by combustion.

Soil residues in two of the soils, RL-81 and RM-019, exceeded 10% of applied radioactivity after the desorption phase. Duplicate samples of the highest concentration (nominally 10 mg/L) were extracted at ambient temperature twice with methanol, followed by water and finally acetonitrile. Adsorption supernatants, desorption supernatants and where applicable soil extracts, from samples treated at the highest concentrations were analysed by HPLC. No significant adsorption to test vessels was detected over this time period.

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. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption. A soil-to-solution ratio of 1:5 was regarded adequate for all experiments as the K_d of all the soils was estimated to be less than 10. An equilibration time of 24 h was sufficient to reach adsorption equilibrium and was also used for desorption.

The average mass balance obtained during the range finding study was 96.07%.

B. Transformation of test substance: The stability of the test substance in contact with soil under the conditions of the definitive test was confirmed by HPLC analysis which demonstrated that M-01 was the predominant molecule observed in the solutions. M-01 represented at least 93% and 92% of the radioactivity in the adsorption and desorption solutions, respectively. Extraction of the desorbed soils and subsequent HPLC of the soil extracts also identified M-01 as the predominant molecule that was extractable from the soil representing at least 85% of the soil extractable residues.

C. Findings

Mean material balances were 101.1, 98.4, 99.7, 97.7 and 98.8% AR for soil Connecticut, North Dakota, Florida, Washington and California, respectively (summarised in Table 7.1.3.1.2- 2). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 2: Definitive test: Mass balance (% AR) of M-01

Test concentration (mg/L)	Connecticut	North Dakota	Florida	Washington	California
	RL-51	RL-81	RM-014	RM-019	RM-022
0.20	101.1	98.4	101.1	99.6	99.0
0.50	106.2	99.0	100.2	97.7	99.3
1.0	99.6	96.8	98.6	95.1	98.1
5.0	98.9	98.7	99.4	98.5	99.2
10.0	99.8	99.0	99.0	97.8	98.3
Mean	101.1	98.4	99.7	97.7	98.8
SD	3.0	0.9	1.0	1.7	0.5

Note: Mass balances were virtually quantitative. Values derived from mean values of duplicate samples in terms of percentages of AR. SD = standard deviation.

The results of adsorption tests of M-01 onto five soils are summarised in Table 7.1.3.1.2- 3 and Table 7.1.3.1.2- 4. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 1 to Figure 7.1.3.1.2- 5.

At the end of the adsorption phase 4.00 – 7.42% AR was adsorbed to soil Connecticut, 17.80 – 34.40% AR was adsorbed to soil North Dakota, 7.53 – 12.03% AR was adsorbed to soil Florida, 21.98 – 33.33% AR was adsorbed to soil Washington and 3.04 – 6.15% AR was adsorbed to soil California.

The calculated Freundlich adsorption coefficients (K_f) ranged from 0.208 to 1.890 mL/g. In four of the soils investigated the correlation of data by linear regression was excellent but in one soil (RL-51) there was a clear outlier which resulted in a lower correlation. When the soil and solution concentrations for the nominal starting concentration of 0.5 µg/mL were excluded and the data re-evaluated by linear regression a better fit was obtained with the experimental data. This re-evaluation has been reported in a separate position paper (KCA 7.1.3.1.2/08, M-224926-01-2). The Freundlich adsorption isotherm data reported for soils RL-81, RM-014, RM-019 and RM-022 and the re-evaluated data for soil RL-51 are summarized below. These values have been used in all subsequent risk assessments. In two soils, RL-51 and RM-22, the 1/n values were 0.97 with a linear relationship between the concentration in the soil and solution indicating the amount absorbed was independent of concentration. The relationship between the soil and solution concentration for the three remaining soils was non linear with 1/n values ranging from 0.8085 to 0.9163. When corrected for organic carbon content of the soil, the K_{oc} values obtained ranged from 31 to 51 mL/g.

Table 7.1.3.1.2- 3: Definitive test: Concentration of M-01 in aqueous and solid phase following 24 hours of adsorption.

Nominal Concentration (µg/mL)	Connecticut		North Dakota		Florida	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.20	0.240	0.070	0.164	0.430	0.220	0.126
0.20	0.235	0.098	0.172	0.386	0.223	0.110
0.50	0.529*	0.048*	0.405	0.831	0.533	0.320
0.50	0.533*	0.029*	0.407	0.820	0.540	0.293
1.0	1.052	0.406	0.792	1.688	1.038	0.701
1.0	1.048	0.419	0.831	1.478	1.055	0.622
5.0	5.166	2.045	4.319	5.776	5.328	2.544
5.0	5.217	1.779	4.289	6.092	5.375	2.297
10	10.233	3.446	8.929	10.300	10.689	4.468
10	10.357	2.844	9.075	9.620	10.726	4.268

* Excluded as outliers in the re-evaluation isotherm data (see KCA 7.1.3.1.2/08)

Nominal Concentration (µg/mL)	Washington		California	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.20	0.140	0.352	0.248	0.044
0.20	0.156	0.282	0.244	0.063
0.50	0.403	0.810	0.543	0.104
0.50	0.411	0.744	0.542	0.110
1.0	0.753	1.623	1.074	0.291
1.0	0.739	1.715	1.084	0.231
5.0	3.703	7.637	5.290	0.898
5.0	4.030	6.087	5.282	0.930
10	8.286	11.277	10.503	2.120
10	8.031	12.538	10.471	2.268

Table 7.1.3.1.2- 4: Summary of adsorption/desorption constants and correlation coefficients of M-01 in soil at 25 °C

Phase	Parameter	Units	Connecticut	North Dakota	Florida	Washington	California
			RL-51	RL-81	RM-014	RM-019	RM-022
Adsorption	$K_{F,ads}$	[mL/g]	0.3588	1.761	0.529	1.890	0.208
	1/n	-	0.970	0.8085	0.9163	0.9125	0.9718
	R^2	-	0.991	0.997	0.992	0.990	0.989
	$K_{OC,ads}$	[mL/g]	39.9	31	38	45	51
Desorption	$K_{F,des}$	[mL/g]	4.652	4.350	2.577	4.178	6.397
	1/n	-	1.1170	0.8877	1.0109	0.9663	1.0438
	R^2	-	0.998	0.991	0.995	0.992	0.969
	$K_{OC,des}$	[mL/g]	494	776	184	100	1560

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the report (Table 7.1.3.1.2- 3) were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet tool provided by EFSA (summarised in Table 7.1.3.1.2-5).

Table 7.1.3.1.2- 5: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Connecticut	North Dakota	Florida	Washington	California
Code	-	-	RL-51	RL-81	RM-014	RM-019	RM-022
Adsorption method	-	-	indirect	indirect	indirect	indirect	indirect
Soil solution ratio	g/mL		1:5	1:5	1:5	1:5	1:5
Mass balance of ^{14}C	%	>90%	98.9-106.2	96.8-99.0	98.6-101.1	95.1-99.6	98.1-99.3
f – due to loss processes (estimated)	%	-	5	5	5	5	5
Adsorbed percentage (δ)	%	>20%	4.00-7.42	17.80-34.40	7.53-12.03	21.98-33.33	3.04-6.15
K_D x soil:solution ratio		>0.3	0.05-0.08	0.21-0.52	0.08-0.14	0.27-0.50	0.03-0.05
$^{\#}K_{fE}$ / K_f	-	<1.2	14.42-16.19	1.40-1.45	2.75-2.97	1.29-1.26	-3.97 & -6.01
ads K_F	L/kg	-	0.359	1.761	0.529	1.890	0.208
95% confidence interval	-	*	0.308-0.417	1.665-1.863	0.480-0.584	1.691-2.112	0.184-0.235
ads 1/n	-	*	0.970	0.808	0.916	0.913	0.972
95% confidence interval	-	*	0.876-1.065	0.771-0.846	0.850-0.982	0.837-0.988	0.889-1.056
ads R^2	-	>0.975	0.9906	0.9967	0.9922	0.9897	0.9890
ads $K_{F\,oc}$	L/kg		39.8	309	37.8	45.0	52.0
Visual fit to Freundlich isotherm	-	-	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Residual plots randomly distributed	-	-	Good	Good	Good	Good	Good

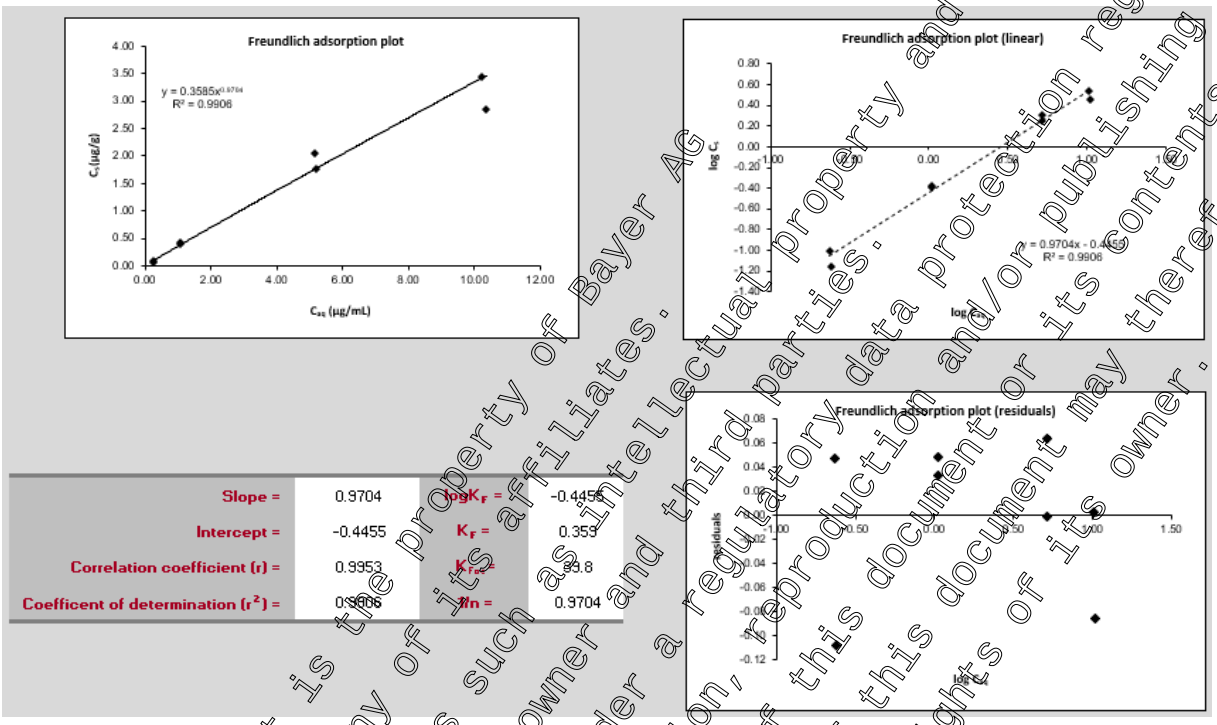
* Confidence intervals should be narrow

Relevant quality checks were performed to evaluate the acceptability of the study. Mass balances were good ranging from 95.1 -106.7% but the % adsorption of 3.0 - 34% was generally lower than the recommended 20%. The use of the indirect method was not appropriate based on a K_d * soil/solution ratio <0.3 in three of the soils. The graphical fits of the Freundlich equation based on the standard linear regression form using log-log transformed data alongside the associated residual plots was good. The R^2 of the standard linear regressions ranged from 0.989 to 0.997 and the visual fit of both the standard regression and the residual plots were acceptable.

Parental mass balance data is not presented in the report, however has been calculated to be *ca* 95% for soil RL-81. This value then assumed across all soils giving an "f" value of 5%. Considering the known

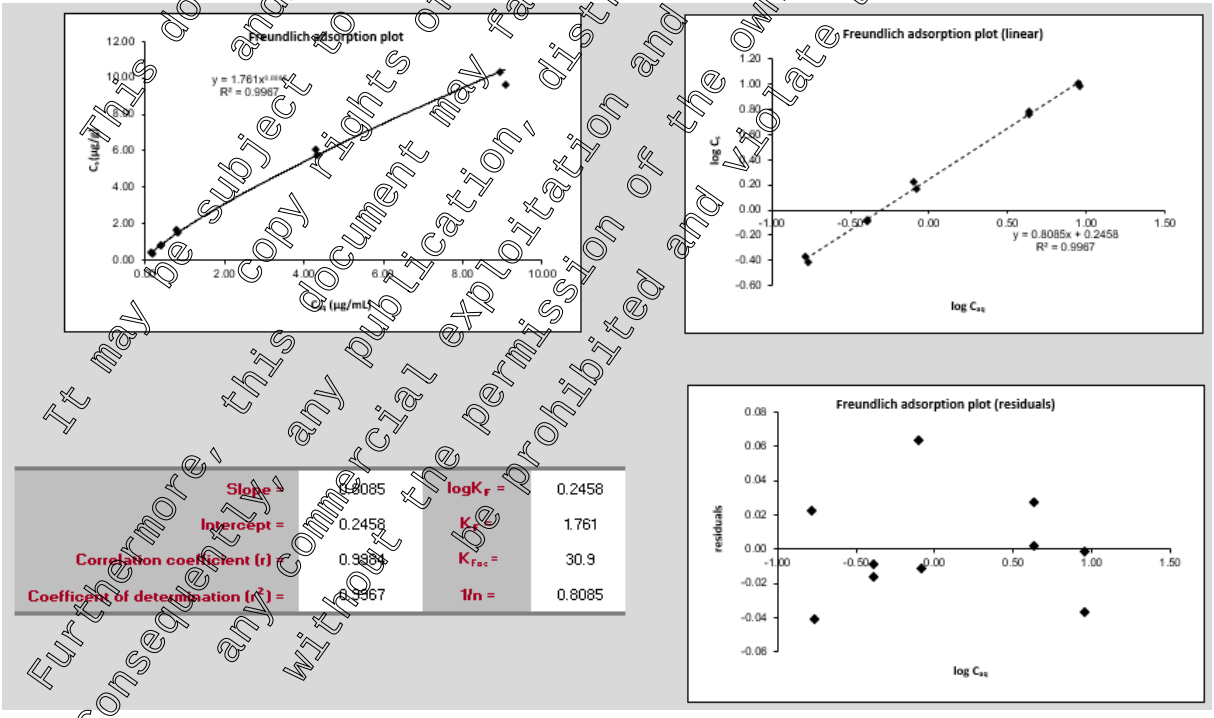
stability of M-01 in soil and water this value is considered to be conservative. Based upon this K_{fE} / K_f ratios were determined to be > 1.2 in all five soils.

Figure 7.1.3.1.2- 1: Freundlich Isotherms of M-01 in Soil Connecticut (RL-51) at 25°C



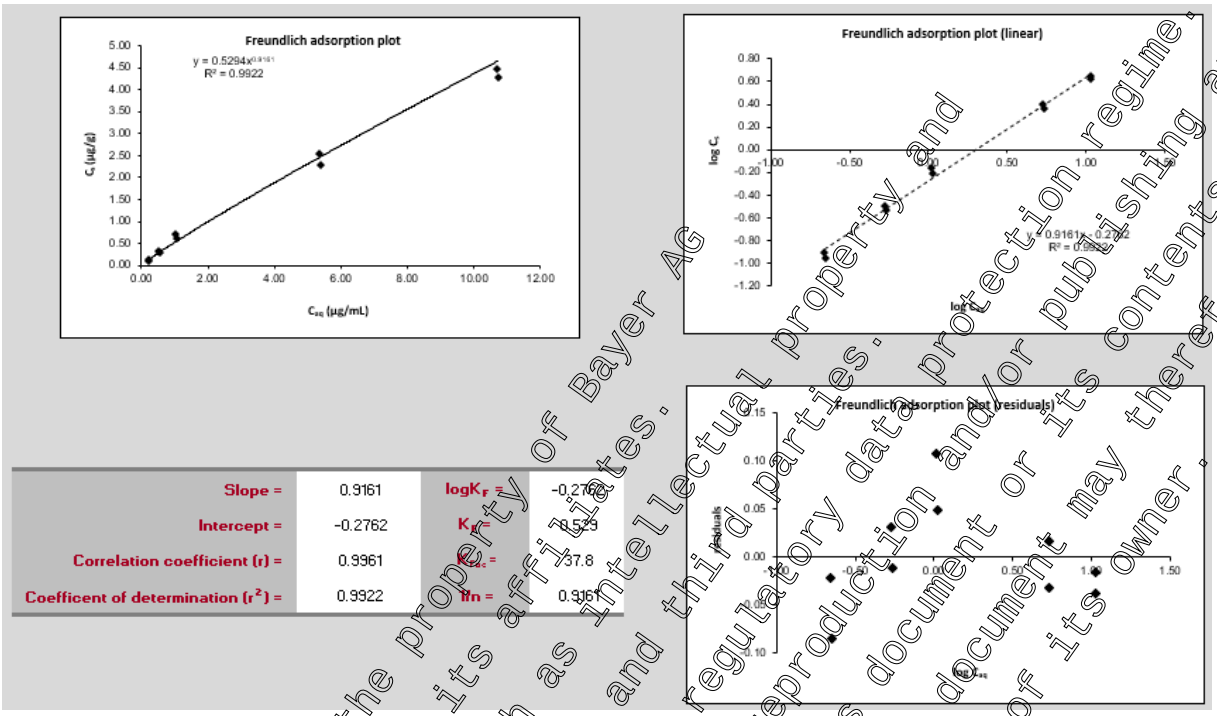
Slope =	0.9704	$\log K_F$ =	-0.4455
Intercept =	-0.4455	K_F =	0.359
Correlation coefficient (r) =	0.9953	K_{foc} =	33.8
Coefficent of determination (r^2) =	0.9906	1/n =	0.9704

Figure 7.1.3.1.2- 2: Freundlich Isotherms of M-01 in Soil North Dakota (RL-81) at 25°C



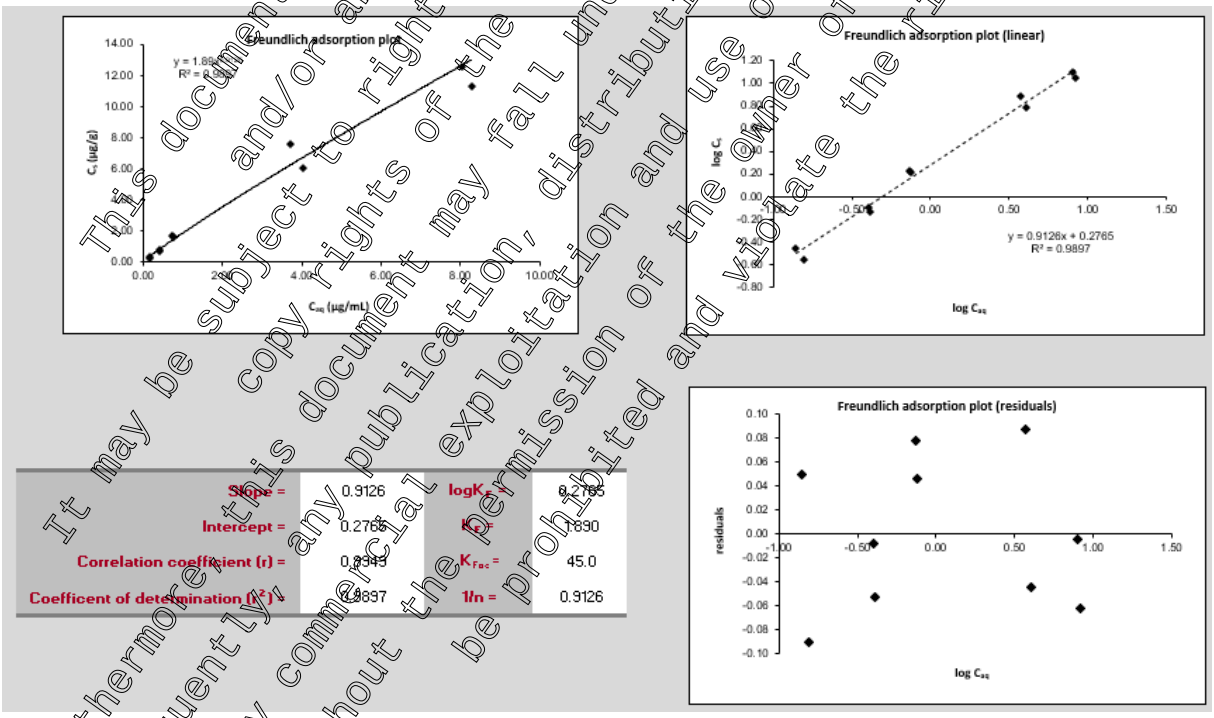
Slope =	0.8085	$\log K_F$ =	0.2458
Intercept =	0.2458	K_F =	1.761
Correlation coefficient (r) =	0.9984	K_{foc} =	30.9
Coefficent of determination (r^2) =	0.9967	1/n =	0.8085

Figure 7.1.3.1.2- 3: Freundlich Isotherms of M-01 in Soil Florida (RM-014) at 25°C



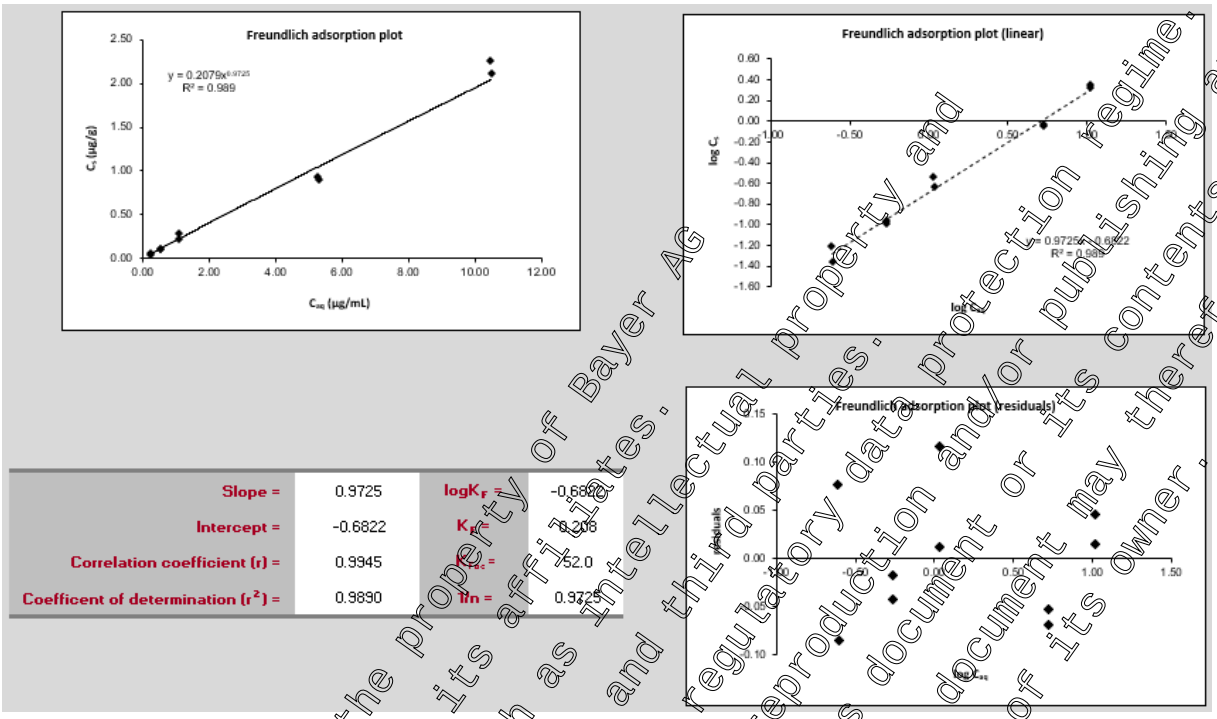
Slope =	0.9161	$\log K_F$ =	-0.2762
Intercept =	-0.2762	K_F =	0.529
Correlation coefficient (r) =	0.9961	K_{Foc} =	37.8
Coefficent of determination (r^2) =	0.9922	1/n =	0.9161

Figure 7.1.3.1.2- 4: Freundlich Isotherms of M-01 in Soil Washington (RM-019) at 25°C



Slope =	0.9126	$\log K_F$ =	0.2765
Intercept =	0.2765	K_F =	1.890
Correlation coefficient (r) =	0.9949	K_{Foc} =	45.0
Coefficent of determination (r^2) =	0.9897	1/n =	0.9126

Figure 7.1.3.1.2- 5: Freundlich Isotherms of M-01 in Soil California (RM-022) at 25°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- **6**. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 7.

Table 7.1.3.1.2- 6: Summary of Quality Criteria and Regulatory Interpretation

M-01			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Connecticut	Sandy loam	RL-51	6	0	3
North Dakota	Sandy loam	RL-81	6	2	1
Florida	Sand	RM-014	6	0	3
Washington	Sand	RM-019	7	1	1
California	Sandy clay loam	RM-022	6	0	3

Table 7.1.3.1.2- 7: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Connecticut	Sandy loam	RL-51	39.9	39.8	0.97	0.97
North Dakota	Sandy loam	RL-81	31	30.9	0.8085	0.808
Florida	Sand	RM-014	38	37.8	0.9163	0.916
Washington	Sand	RM-019	45	45	0.9125	0.913
California	Sandy clay loam	RM-022	51	52	0.9718	0.972

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.
Note: Outliers were excluded in re-evaluation isotherm data for 0.5 µg/mL Connecticut soil).

III. Conclusion

The adsorption constant $K_{F(ads)}$ of M-01 was between 0.208 and 1.89 mL/g for the tested soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 31 to 51 mL/g. The Freundlich exponent 1/n was between 0.8085 and 0.9718, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range in three out of five soils.

Adsorption was shown to be correlated with organic carbon content, there was no correlation with the pH of the soils.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that extent of adsorption of M-01 is too low to fully meet the proposed quality criteria for the indirect method but the compound is known to be stable and the study otherwise is well conducted.

A second soil adsorption study with M-01 using the OECD 106 direct method (see KCA 7.1.3.1.2/10) derived very similar endpoints, thus confirming the validity of this study.

Assessment and conclusion by applicant

Although the study has a number of deviations from the current version of OECD 106 (2002), it is considered valid to assess the adsorption and desorption characteristics of M-01 in soil.

Data Point:	KCA 7.1.3.1.2/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	The adsorption and desorption of AE0608000 in two soils
Report No:	M-241272-01-2
Document No:	M-241272-01-2
Guideline(s) followed in study:	OECD: 106; USEPA (=EPA): Section N, 163-1
Deviations from current test guideline:	Yes. M-03 (AE 0608000) was rapidly hydrolysed in 0.01 M calcium chloride solution and in mixtures of 0.01 M calcium chloride solution and neutral/alkaline soils. Single point Kd and Koc values were determined in two acidic soils.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

Tests to investigate the adsorption/desorption characteristics of M-03 (referred to as AE 0608000 in the report) were performed under conditions of batch equilibrium experiments in four soils.

Soil	Soil Code	Texture (USDA)	pH ($CaCl_2$)	OC (%)
Munster	EFS-132	Loamy sand	4.8	0.9
Huntlosen	EFS-151	Loamy sand	7.7	5.7
Abington	EFS-128	Sandy loam	8.3	1.4
Sarotti	EFS-133	Silt loam	4.9	4.2

The recovery of radioactivity was quantitative with recoveries ranging from 91.09 % to 101.83% of applied radioactivity.

M-03 was rapidly hydrolysed to form M-01 in 0.01 M calcium chloride solution and in mixtures of 0.01 M calcium chloride solution and neutral/alkaline soils, with half-lives of < 1 hour. Approximately 8% of applied radioactivity remained as M-03 in 0.01M calcium chloride solution after two hours. The amount of applied radioactivity remaining as M-03 after one hour in Abington and Sarotti soils was 15 and 12% respectively. M-03 was more stable in mixtures of 0.01 M calcium chloride solution and acidic soils. However some degradation was observed with approximately 87% and 95% of the radioactivity present in supernatants and soil extracts of acidic soil samples recovered as M-03 after one hour.

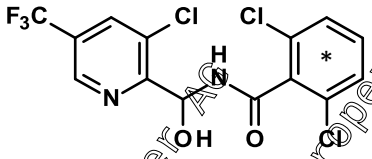
Single point K_d and K_{oc} values were determined in Munster and Huntlosen soils. K_d values ranged 1.09 to 2.19 mL/g. When corrected for organic carbon content of the soil, K_{oc} values obtained ranged from 60 to 135 mL/g with mean values of 73 and 112 mL/g for Munster and Huntlosen soils, respectively. Due to the instability of M-03 in Abington and Sarotti soils K_d and K_{oc} values were not determined in these neutral/ alkaline soils.

I. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-M-03 (referred to as AE 0608000 in the report)



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

Batch number:	SEL/1036
Specific Activity:	143.43 µCi/mg (318.415 dpm/µg)
Radiochemical Purity:	93%
Stability of test compound:	HPLC analysis showed M-03 was unstable in 0.01 M calcium chloride solution

2. Test Soil

Sorption tests were performed with four agricultural soils covering a wide range of pH, organic carbon content and texture. Soils were passed through a 2 mm sieve prior to use in the study. The characteristics of the soils summarised in Table 7.1.3.1.2- 8.

Table 7.1.3.1.2- 8: Physico-chemical properties of test soils

Characteristic / Code	Units	EFS-132	EFS-151	EFS-128	EFS-133
Origin	State, Country	Germany	Germany	England, UK	Germany
Location	City or Township	Munster	Huntlosen	Abington	Sarotti
Particle Size Analysis, USDA					
Total Sand	(0.05 - 2.0 mm)	82%	81.93%	73%	22%
Silt	(0.002 - 0.05 mm)	14%	12.07%	20%	62%
Clay	(<0.002 mm)	4%	6.00%	7%	16%
Textural Class	USDA	Loamy sand	Loamy sand	Sandy Loam	Silt Loam
Particle Size Analysis, ADAS:					
Total Sand	(0.063 - 2.00 mm)	80%	78.91%	71%	18%
Silt	(0.002 - 0.063 mm)	16%	15.09%	22%	66%
Clay	(< 0.002 mm)	4%	6.00%	7%	16%
Textural Class	ADAS	Loamy sand	Loamy sand	Sandy Loam	Silt Loam
Particle Size Analysis, BBA:					
Total Sand	(0.063 - 2.00 mm)	80%	NA	71%	18%
Silt	(0.002 - 0.063 mm)	16%	NA	22%	66%
Clay	(< 0.002 mm)	4%	NA	7%	16%
Textural Class	BBA	Silty Sand	NA	Loamy Sand	Clayey Silt
pH	Water (1:1)	5.7	6.3	7.7	7.5
	0.01 M CaCl2 (1:1)	4.9	5.2	7.2	7.1
	IM KCl	NA	5.3	NA	NA
Organic Carbon	%	1.8	1.6	3.2	2.0
Organic Matter	%	3.1	NA	5.4	3.4
Cation Exchange Capacity	meq/100g	6.1	NA	19.4	14.5
Water Holding Capacity	% at Saturation	40.6	39.6	65.4	62.4
	% at 1/10 bar	18.2	NA	23.2	34.0
	% at 1/3 bar	8.8	NA	17.9	20.9
	% at 15 bar	4.7	NA	14.3	11.5
Bulk Density (disturbed)	g/cm³	1.34	NA	1.20	1.16
Olsen Phosphorous	Ppm	92	774	65	86
Total Nitrogen	%	0.121	0.102	0.269	0.169
		% ppm	ppm	% ppm	% ppm
Cation	Calcium	23.4 285	489	83.9 3260	72.8 2110
	Magnesium	3.3 24	37.7	2.5 8	6.7 116
	Sodium	1.9 26	20.0	0.6 27	0.8 28
	Potassium	3.1 73	141	3.1 234	6.4 363
	Hydrogen	68.4 42	NA	9.9 19	13.3 19
	Manganese	NA	27.9	NA	NA
Soluble Salts	mmhos/cm	0.05	NA	0.33	0.22

NA : Not Analysed

B. Study Design

1. Experimental Conditions

The test system for the study was the soil and 0.01M calcium ion solution. The test system was dosed with M-03 and contained in capped Pyrex glass or polypropylene centrifuge tubes.

The stability of the test substance in calcium chloride solution was investigated. M-03 was dissolved in 0.01M calcium chloride solution at a concentration of 5 mg/L and incubated at 20 ºC. The solution was analysed by HPLC after 2 and 4 hours incubation.

The stability of M-03 in an acidic soil (Munster) and 0.01M calcium chloride solution was tested at a soil: solution ratio of 1:4. The samples were treated at an initial concentration of 5 mg/L and shaken at 20 ºC. After equilibration, soil and solution phases were separated by centrifugation and the percentage of M-03 remaining in the water phase determined by HPLC after 2 and 4 hours incubation.

The time to reach equilibrium was assessed in soils at timepoints up to 6 hours. Initially samples of Munster soil were shaken at 20 ºC with M-03 at a concentration of 5 mg/L in a 1:5 soil solution ratio for 2, 4 and 6 hours. In an attempt to improve the stability of M-03 lower soil : solution ratios were then used. Samples of each soil were shaken at 20 ºC with M-03 at a concentration of 3 mg/L in 1:2 soil solution ratios. Munster and Huntlosen soils were incubated for 1, 2 and 4 hours. Abington and Sarotti soils were incubated for 1 hour.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		5 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ overnight
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 3 or 5 10 µg/mL
	Analytically (LSC) measured concentrations	Concentrations in test solution: Munster soil, (1:4): 5.26 – 5.44 µg/g Munster, Huntlosen, Abington & Sarotti soil (1:2): 3.22 µg/g
Identity and concentration of co-solvent		Dosing stock made up in calcium chloride
Soil: Solution ratio		1:4 or 1:2 i.e. 5 g soil dry weight equivalent to 20 or 10 mL solution
Number of replicates	Control	Not reported
	Treatments	Munster soil (1:4): Single Munster soil (1:2): Duplicate Huntlosen soil (1:2): Duplicate Abington soil (1:2): Single Sarotti soil (1:2): Single
Equilibration conditions	Time	1 - 6 hrs
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	End-to-end
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	6000 rpm
	Duration	10 minutes
	Method of separating supernatant	Not reported

Desorption phase

Not applicable.

2. Analytical Procedures

After equilibration, soil and solution phases were separated by centrifugation and the radioactivity remaining in the water phase determined by LSC. The soils were extracted with acetonitrile and the radioactivity remaining in soil after extraction was quantified by combustion.

All supernatants and soil extracts were analysed by HPLC to determine the amount of M-03 present. The concentration of M-03 in soil and solution phases was determined based on the concentration of radioactivity in each phase corrected for the M-03 content measured by HPLC.

II. Results and Discussion

A. Results of preliminary tests

Both Pyrex glass and polypropylene centrifuge tubes were tested for adsorption of the test substance. M-03 was dissolved in 0.01M calcium chloride solution at a concentration of 0.01 mg/L and incubated for up to ca. 16 hours. The results showed no adsorption to either type of tube.

B. Transformation of test substance: M-03 was rapidly hydrolysed to form M-01 in 0.01 M calcium chloride solution and in mixtures of 0.01 M calcium chloride solution and neutral/alkaline soils, with half-lives of < 1 hour.

C. Findings

The mass balance was good for all samples, ranging from 91.09% to 101.83% (summarised in Table 7.1.3.1.2- 9). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 9: Definitive test: Mass balance (% AR) of M-03

Time (Hours)	Tube	Supernatants	Soil Extracts	Non-Extractable Residues	Total Recovery
Munster Soil (Soil-to-Solution Ratio 1:4; 5 ppm Tested)					
2	8	67.42	28.35	1.60	97.37
4	9	63.34	28.73	1.38	93.45
6	10	66.00	29.79	1.76	97.55
Munster Soil (Soil-to-Solution Ratio 1:2; 3 ppm Tested)					
1	14	44.74	43.79	2.56	91.09
1	15	46.60	46.57	1.98	95.15
2	16	48.80	47.66	2.47	98.93
2	17	49.22	47.45	2.52	99.19
4	18	43.35	48.39	2.82	94.56
4	19	44.58	49.60	2.87	97.05
Huntlosen Soil (Soil-to-Solution Ratio 1:2; 3 ppm Tested)					
1	20	43.61	52.53	3.09	99.23
1	21	42.04	54.34	3.62	100.00
2	22	43.16	50.37	4.20	97.73
2	23	43.87	49.94	4.96	98.77
4	26	37.26	58.48	3.97	99.71
4	27	37.44	56.31	3.79	97.54
Abington Soil (Soil-to-Solution Ratio 1:2; 3 ppm Tested)					
1	28	56.41	41.90	3.53	101.84
Sarotti Soil (Soil-to-Solution Ratio 1:2; 3 ppm Tested)					
1	29	59.60	39.07	3.16	101.83

The results of adsorption tests of M-03 onto four soils are summarised in Table 7.1.3.1.2- 10.

M-03 was rapidly hydrolysed to form M-01 in 0.01 M calcium chloride solution and in mixtures of 0.01 M calcium chloride solution and neutral/alkaline soils, with half-lives of < 1 hour. Approximately 8 % of applied radioactivity remained as M-03 in 0.01M calcium chloride solution after two hours. The amount of applied radioactivity remaining as M-03 after one hour in Abington and Sarotti soils was 15 and 12 % respectively. M-03 was more stable in mixtures of 0.01 M calcium chloride solution and acidic soils. However some degradation was observed with approximately 87 % and 95% of the radioactivity present in supernatants and soil extracts of acidic soil samples recovered as M-03 after one hour.

Single point K_d and K_{oc} values were determined in Munster and Huntlosen soils. K_d values ranged 1.09 to 2.19 mL/g. When corrected for organic carbon content of the soil, K_{oc} values obtained ranged from 60 to 135 mL/g, with mean values of 73 and 112 mL/g for Munster and Huntlosen soils, respectively. Due to the instability of M-03 in Abington and Sarotti soils K_d and K_{oc} values were not determined in these neutral / alkaline soils.

The K_d and K_{oc} values determined from HPLC corrected aqueous and soil extract concentrations were equivalent to the direct method. However, as these determinations were not performed at a full range of five concentrations no determination of K_f or K_{foc} values could be made.

Table 7.1.3.1.2- 10: Definitive test: Concentration of M-03 in aqueous and solid phase following adsorption period, K_d and K_{oc} values.

Time (hours)	Initial Conc	Solution phase			Soil Phase			K_d (mL/g)	K_{oc} (mL/g)	Mean K_{oc} (mL/g)
		Radioactivity	AE 0608000		Radioactivity	AE 0608000				
	(µg/mL)	(µg/g)	(%)	(µg/g)	(µg/g)	(%)	(µg/g)			
Munster soil, soil : solution ratio 1:4										
2	5.28	4.07	89.15	3.63	4.83	92.35	4.46	1.23	68	**73**
4	5.44	3.96	85.77	3.40	5.91	91.07	5.38	1.59	88	
6	5.26	3.94	84.21	3.32	5.27	90.68	4.77	1.44	80	
Munster soil, soil : solution ratio 1:2										
	3.22	2.12	88.72	1.88	2.20	93.04	2.05	1.09	60	
1	3.22	2.08	88.02	1.83	2.27	93.17	2.11	1.16	64	
2	3.22	1.97	86.80	1.71	2.51	92.80	2.33	1.36	76	
2	3.22	2.03	87.93	1.77	2.38	92.28	2.20	1.24	69	
4	3.22	1.94	84.64	1.64	2.56	91.77	2.35	1.43	79	
4	3.22	2.05	83.56	1.71	2.34	91.25	2.14	1.25	69	
Huntlosen soil, soil : solution ratio 1:2										
1	3.22	1.85	85.59	1.58	2.75	97.40	2.68	1.69	106	**112**
1	3.22	1.83	85.25	1.56	2.78	97.40	2.71	1.74	108	
2	3.22	1.93	84.49	1.63	2.58	94.44	2.44	1.49	93	
2	3.22	1.86	85.97	1.60	2.72	93.82	2.55	1.60	100	
4	3.22	1.71	78.83	1.35	3.01	94.78	2.85	2.12	132	
4	3.22	1.68	79.60	1.34	3.09	93.41	2.89	2.16	135	
Abington soil, soil : solution ratio 1:2										
1	3.22	2.46	5.55	0.14	1.53	29.27	0.45	ND	ND	ND
Sarotti soil, soil : solution ratio 1:2										
1	3.22	2.67	4.94	0.13	1.11	22.85	0.25	ND	ND	ND

D. Evaluation of the Data according to EFSA Evaluators Checklist

Freundlich isotherms were not generated in the study and therefore it was not possible to use the OECD 106 Checklist (v1) to evaluate the study.

III. Conclusion

M-03 was very rapidly hydrolysed in soil and calcium chloride solution. It was not possible to measure the soil adsorption coefficient of this metabolite in neutral and alkaline soils by the OECD soil batch equilibrium method due to its rapid hydrolysis. Greater stability of M-03 was observed under acidic conditions and single point K_d and K_{oc} values were determined in acidic soils.

The mean values for K_{oc} ranged from 73 to 112 mL/g, indicating M-03 was potentially mobile or of intermediate mobility in soil according to the Briggs classification.

Assessment and conclusion by applicant:

The study is considered supportive to assess the adsorption characteristics of M-03 in soil.

Data Point:	KCA 7.1.3.1.2/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE 0608000: Adsorption to and desorption from three acidic soils
Report No:	M-221107-01-2
Document No:	M-221107-01-2
Guideline(s) followed in study:	EU (=EEC): 95/36/EC
Deviations from current test guideline:	Yes. OECD 106 states soil selection should include a wide range of soil pH values. Freundlich isotherms were conducted in acidic soils only due to the instability of the metabolite in neutral and alkaline soils established in a separate study.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Tests to investigate the adsorption/desorption characteristics of M-03 (referred to as AE 0608000 in the report) were performed under conditions of batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C.

Soil	Soil Code	Texture (USDA)	pH ($CaCl_2$)	OC (%)
Ingleby	02/03	Sandy loam	4.1	3.5
Huntlosen	03/04	Loamy sand	4.7	1.7
Münster	03/07	Loamy sand	5.4	1.1

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1:2 all soils. Nominal test concentrations of 1.00, 0.30, 0.10, 0.03, and 0.01 mg/L of [^{14}C]-M-03 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption

was limited to 1 hour in the definitive test. Desorption phase of the study was carried out by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ solution for up to three desorption cycles of 1 hour.

The mean recoveries from each soil ranged from 95.8 to 99.3% of applied radioactivity. The mass balance for three individual tubes fell below 90% but in each case the mass balance for the corresponding duplicate tube was >90%. No significant degradation of M-03 was observed in adsorption or desorption supernatants and only M-03 was detected in soil extracts.

The calculated Freundlich adsorption coefficients (K_f) ranged from 1.23 to 2.86 mL/g. The value of 1/n ranged from 0.939 to 1.012, with a linear relationship between the concentration in the soil and solution for all the soils and the amount absorbed being largely independent of concentration. When corrected for organic carbon content of the soil, the K_{oc} values obtained ranged from 82 to 133 mL/g.

The K_{des} values ranged from 1.66 to 3.58 mL/g. The corresponding $K_{oc\ des}$ values ranged from 102 to 197 mL/g. For all soils, single point K_d values increased slightly with successive desorption cycles. This indicates that the absorbed radioactivity was increasingly difficult to desorb and thus the mobility of the compound may be less than suggested by the adsorption K_{oc} values.

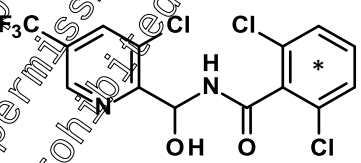
Soil	Ingleby	Huntlosen	Munster
Soil type (USDA)	Sandy loam	Loamy sand	Loamy sand
pH (0.01M $CaCl_2$)	4.1	4.7	5.4
Organic carbon [%]	3.5	1.7	1.1
$K_F^{(ads)}$ [mL/g]	2.86	2.26	1.23
1/n	0.961	1.012	0.939
$K_{F,OC}^{(ads)}$ [mL/g]	82	133	112
R^2	1.000	0.996	0.991
$K_F^{(des1)}$ [mL/g]	3.58	3.36	1.66
1/n	0.948	1.016	0.942
$K_{F,OC}^{(des1)}$ [mL/g]	102	197	151

II. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-M-03 (referred to as AE 0608000 in the report)



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

Batch number: SEL/1272

Specific Activity: 5.31 MBq/mg

Radiochemical Purity: 98.2% (TLC) to 97.1% (HPLC)

Stability of test compound: Stable in the application vehicle (acetonitrile acidified with 0.1% formic acid)

2. Test Soil

Sorption tests were performed with three agricultural soils. The soils were selected to cover a range of organic matter and clay content but all to have an acidic pH. The characteristics of the soils summarised in Table 7.1.3.1.2- 11.

Table 7.1.3.1.2- 11: Physico-chemical properties of test soils

Characteristic / Code	Units	Ingleby	Huntlosen	Munster
Soil ID	-	02/03	03/04	03/07
Origin	State, Country	UK	Germany	Germany
Location	City or Township	Ingleby	Huntlosen	Munster
Textural Class	USDA	Sandy Loam	Loamy Sand	Loamy Sand
Particle Size Analysis, ADAS:				
Total Sand	(0.063 - 2.00 mm)	73.38%	86%	75.45%
Silt	(0.002 - 0.063 mm)	16.48%	6%	20.78%
Clay	(< 0.002 mm)	10.13%	8%	3.77%
Textural Class	ADAS	Sandy Loam	Loamy Sand	Loamy Sand
pH	Water (1:5)	5.2	5.4	6.6
	1M KCl	3.9	4.7	5.5
	0.01 M $CaCl_2$	4.1	4.5	5.4
Organic Carbon	%	3.5	1.7	1.1
$Ca_{exchangeable}$	mEq/100g	2.4	NA	1.5
$Mg_{exchangeable}$	mEq/100g	0.1	NA	0.2
$Na_{exchangeable}$	mEq/100g	<0.05	NA	<0.05
$K_{exchangeable}$	mEq/100g	0.1	NA	0.4
$Mn_{exchangeable}$	mEq/100g	0.6	NA	<0.05
Cation Exchange Capacity	mEq/100g	NA	5.8	NA
$CaCO_3$ eq	g/kg	<0.05	NA	<0.05
Phosphorus total	mg/kg	565.9	NA	617.8
Nitrogen total	mg/kg	2534.0	NA	1077.9
Maximum Water Holding Capacity	g/100 g dry matter	53.8	29.9	46.5
Water Holding Capacity	% at 0.1 bar	22.7	NA	NA
	% at 0.33 bar	22.1	NA	NA

NA: Not Analysed

B. Study design

1. Experimental conditions:

The test system for the study was the soil and 0.01M calcium ion solution. The test system was dosed with M-03 and contained in thick walled borosilicate glass tubes with an external plastic coating. The tubes were approximately 125 mL capacity and sealed with a screw cap, which was lined with a Teflon seal.

Preliminary studies were carried out to check for adsorption to the tubes and to determine any background radioactivity in the soil. The appropriate soil: solution ratio, adsorption equilibrium time and desorption equilibrium time were derived from a previous study (KCA 7.1.3.1.2/02, M-241272-01-2).

The main test was performed in duplicate. M-03 was dissolved in acetonitrile acidified with 0.1% formic acid (60 µL per sample) which was used to prepare the nominal concentrations of 0.01, 0.03, 0.1, 0.3 and 1 mg/mL. Soil samples were prepared at a soil to solution ratio of 1:2 and shaken at 20 ºC. Based on the results of a previous study (KCA 7.1.3.1.2/02, M-241272-01-2), an equilibrium time of 1 hour was determined to be sufficient and limited breakdown of M-03.

For all soils one desorption cycle was conducted at all concentrations with the exception of the top rate, for which three desorption cycles were conducted. The volume of solution removed after the adsorption step was replaced by an equal volume of [^{14}C]-M-03 stock solution. Test vessels were then shaken for a one hour desorption phase.

In order to minimise any breakdown of M-03, calcium chloride supernatants were acidified with formic acid to pH 3 after decanting from soil.

Adsorption phase

Parameter		Description
Soil condition		Not reported
Soil sample weight		20 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ 24 hours minimum
Control		10 g soil: 50 mL 0.01M $CaCl_2$
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 0.01, 0.03, 0.10, 0.3 and 1 mg/L
	Analytically (LSC) measured concentrations	Concentrations in test solution: 0.0083, 0.0260, 0.0914, 0.2688 and 0.8674 mg/L
Identity and concentration of co-solvent		Dosing stock made up in acetonitrile with 0.1% formic acid
Soil: Solution ratio		1:2 i.e. 10 g soil dry weight equivalent to 20 mL solution
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Equilibration conditions	Time	1 hour
	Temperature	20±2°C
	Dark	In the dark
	Shaking method	End-over-end
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not reported
	Duration	10 minutes
	Method of separating supernatant	Not reported

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 39 to 67% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		1 for all concentrations apart from 1 mg/L where 3 desorption cycles were performed
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 40 mL was used as equilibration solution.
Soil: Solution ratio		1:4 i.e. 10 g soil dry weight equivalent to 40 mL solution
Number of replicates	Control	Duplicate
	Treatments	Duplicate
Desorption Equilibration conditions	Time	1 hour
	Temperature	20±2°C
	Dark	In the dark
	Shaking method	End-over-end
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not reported
	Duration	10 minutes
	Method of separating supernatant	Not reported

2. Analytical Procedures

After equilibration, soil and solution phases were separated by centrifugation and the concentration of M-03 in the water phase determined by HPLC. The adsorption supernatant of the highest concentration treatment for each soil was also analysed by TLC.

M-03 remaining in the soil phase was then desorbed with 0.01M calcium chloride and the concentration determined by LSC. An aliquot of the desorption supernatant of all the treatments for each soil were analysed by HPLC. The desorption supernatant of the highest concentration treatment for each soil was also analysed by TLC.

Following the final desorption cycle, each tube from each soil was solvent extracted using acetonitrile with 0.1% formic acid. Aliquots of each supernatant were analysed by LSC. An aliquot of the solvent extract of all the treatments for each soil were analysed by HPLC. The solvent extract of the highest concentration treatment for each soil was also analysed also by TLC.

The radioactivity remaining in soil after the desorption phase was quantified by combustion.

All supernatants, extracts and unextractable soil residues were assayed by LSC to determine levels of radioactivity. The mass balance was derived from the measured radioactivity using the known specific activity value of M-03 in the treatment solution.

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. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption.

B. Transformation of test substance

Selected supernatants and solvent extracts from the samples at the highest concentration were examined by HPLC.

In the Ingleby and Huntlosen soils the degradation was minimal with <3% of the applied radioactivity found to have degraded to M-01. The breakdown in the Münster soil was more significant and thus the samples from all concentrations were analysed. The total breakdown to M-01 at each concentration ranged from 7.0% to 10.0%. It was concluded that the degradation of M-03 that had occurred over the duration of the study was not sufficient to warrant an adjustment being made. All solvent extracts contained only M-03 thus the inference being that the minimal break down occurred in the calcium chloride solutions and not on the soils.

TLC showed similar results to those obtained by HPLC.

C. Findings

The mean recoveries from each soil ranged from 95.8 to 99.5 % of applied radioactivity. The mass balance for three individual tubes fell below 90% but in each case the mass balance for the corresponding duplicate tube was >90% (summarised in Table 7.1.3.1.2-12). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing. No significant degradation of M-03 was observed in adsorption or desorption supernatants and only M-02 was detected in soil extracts.

Table 7.1.3.1.2- 12: Definitive test: Mass balance of [phenyl-U-^{14}C]-M-03 (% AR)

Soil	Ingleby	Huntlosen	Münster
Min.	88.8	86.4	82.5
Max.	101.2	108.3	102.5
Mean	95.8	99.4	98.5
SD	4.2	7.0	6.0

SD = standard deviation

The results of adsorption tests of M-03 onto three soils are summarised in Table 7.1.3.1.2- 13, the Freundlich adsorption coefficients are summarised in Table 7.1.3.1.2- 14. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 6 to Figure 7.1.3.1.2- 8.

At the end of the adsorption phase 60.35 – 66.77% AR was adsorbed to soil Ingleby, 50.24 – 60.47% AR was adsorbed to soil Huntlosen and 39.38 – 56.03% AR was adsorbed to soil Münster.

The calculated Freundlich adsorption coefficients (K_f) ranged from 1.23 to 2.86 mL/g. The value of 1/n ranged from 0.939 to 1.012, with a linear relationship between the concentration in the soil and solution for all the soils and the amount absorbed being largely independent of concentration. When corrected for organic carbon content of the soil, the K_{oc} values obtained ranged from 82 to 133 mL/g.

The K_{des} values ranged from 1.66 to 3.58 mL/g. The corresponding $K_{oc\ des}$ values ranged from 102 to 197 mL/g, and for all soils, single point K_d values increased slightly with successive desorption cycles. This indicates that the absorbed radioactivity was increasingly difficult to desorb and thus the mobility of the compound may be less than suggested by the adsorption K_{oc} values.

Table 7.1.3.1.2- 13: Definitive test: Concentration of M-03 in aqueous and solid phase following 1 hour of adsorption.

Concentration of Test Item (µg/mL)	Ingleby		Huntlosen		Münster	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.008	0.0028	0.0105	0.0041	0.0078	0.0041	0.0075
0.008	0.0029	0.0103	0.0041	0.0076	0.0047	0.0063
0.026	0.0100	0.0299	0.0110	0.0281	0.0142	0.0211
0.026	0.0086	0.0329	0.0127	0.0249	0.0143	0.0205
0.091	0.0325	0.1110	0.0361	0.1034	0.0402	0.0938
0.091	0.0326	0.1110	0.0399	0.0967	0.0463	0.0803
0.269	0.1038	0.3122	0.1269	0.2595	0.1495	0.2052
0.269	0.1061	0.3079	0.1244	0.2677	0.1512	0.2113
0.867	0.3439	0.9936	0.4136	0.8468	0.5259	0.5846
0.867	0.3187	1.0427	0.4057	0.8715	0.5183	0.6005

Table 7.1.3.1.2- 14: Summary of adsorption/desorption constants and correlation coefficients of M-03 in soil at 20 °C

Phase	Soil	Units	02/03	03/04	03/07
			Ingleby	Huntlosen	Münster
Adsorption	$K_{F,ads}$	[mL/g]	2.86	2.26	1.23
	1/n	-	0.961	1.012	0.939
	R^2	-	1.000	0.996	0.991
	$K_{OC,ads}$	[mL/g]	82	133	112
Desorption	$K_{F,des}$	[mL/g]	3.58	3.36	1.66
	1/n	-	0.948	1.016	0.942
	R^2	-	0.999	0.990	0.978
	$K_{OC,des}$	[mL/g]	102	197	151

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the report were used as input data (Table 7.1.3.1.2- 13). Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 15**)**.

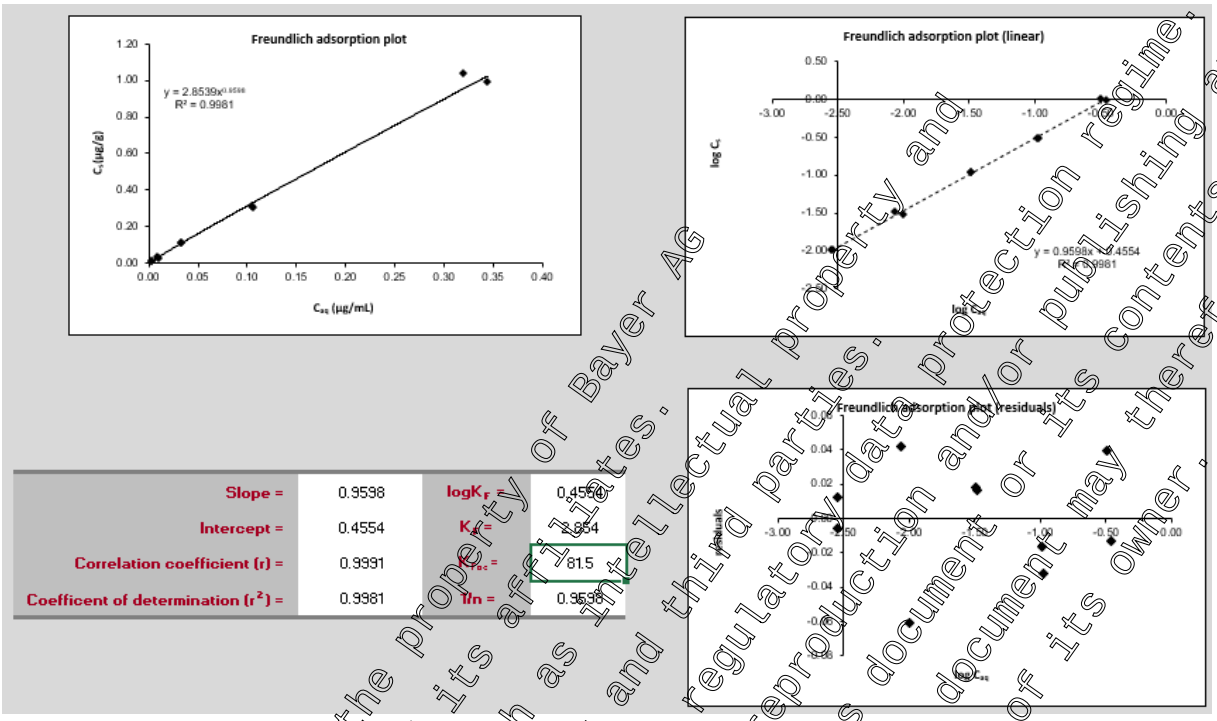
Table 7.1.3.1.2- 15: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Ingleby	Huntlosen	Münster
Code	-	-	02/03	03/04	03/07
Adsorption method	-	-	indirect	indirect	indirect
Soil solution ratio	g/mL		1:2	1:2	1:2
Mass balance of ^{14}C	%	>90%	88.8-101.2	86.4-108.3	82.5-102.6
f – due to loss processes	%	-	15.8	5.4	11.2
Adsorbed percentage (δ)	%	<20%	60.35-66.77	50.24-60.47	39.38-56.03
K_D x soil:solution ratio		>0.3	1.44-1.9	0.93-1.43	0.56-1.17
$^{\#}K_{fE}$ / K_f	-	<1.2	1.33-1.35	1.11-1.12	1.38-1.40
ads K_F	L/kg	-	2.854	2.256	1.227
95% confidence interval	-	*	2.505-3.251	1.782-2.856	0.902-1.668
ads 1/n	-		0.960	1.011	0.938
95% confidence interval			0.926-0.994	0.946-1.076	0.850-1.025
ads R^2	-	>0.975	0.998	0.994	0.987
ads $K_{F,OC}$	L/kg		81.6	132.7	111.5
Visual fit to Freundlich isotherm	-	-	Acceptable	Acceptable	Acceptable
Residual plots randomly distributed	-	-	Acceptable	Acceptable	Acceptable

Relevant quality checks were performed to evaluate the acceptability of the study. Generally the total mass balance was acceptable (i.e. >90%) with just three individual replicates (one per soil) falling below 90%. The acceptability of the analytical method was confirmed over the entire range of concentrations measured (reported LOQ of *ca* 0.0002 mg/L). The use of the indirect method may be deemed appropriate based on a K_D* soil:solution ratio > 0.3 in all soils however the K_{fE} / K_f ratio is > 1.2 in two out of the three soils. The R^2 of the standard linear regressions ranged from 0.987 to 0.998 and the visual fit of both the standard regression and the residual plots were acceptable.

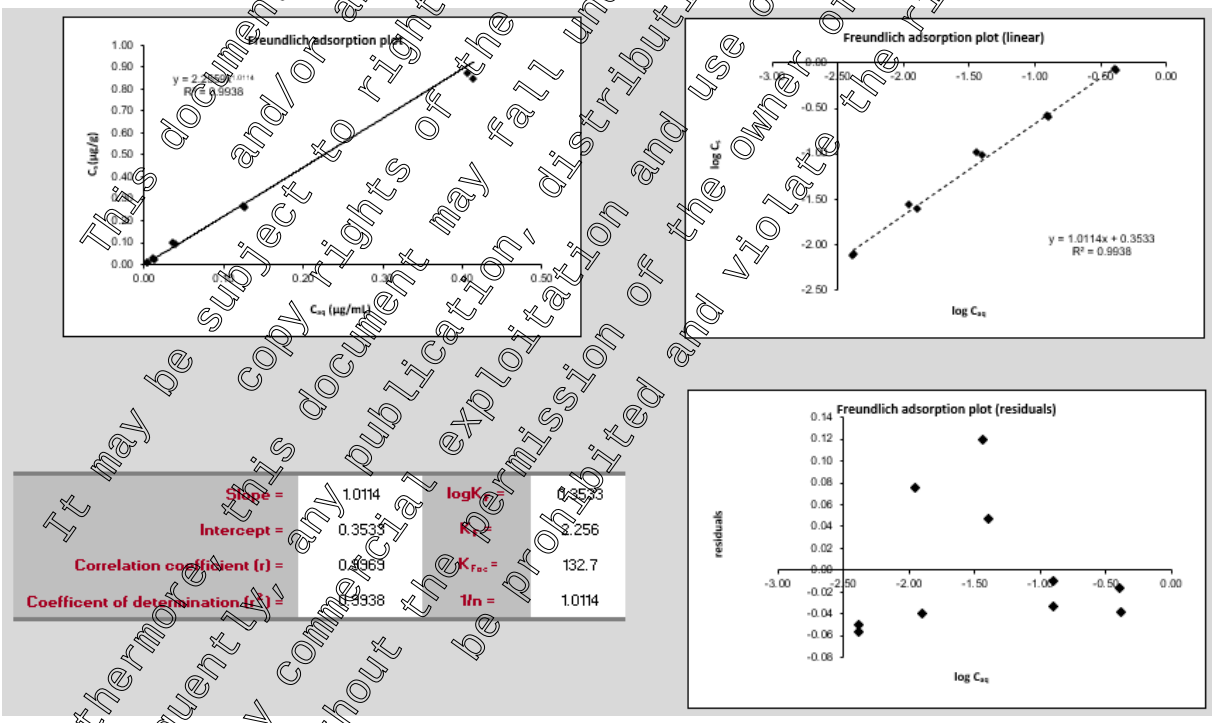
M-03 is unstable in solution and thus a short equilibration time (1 hour) was used to limit breakdown. There is no stated parental mass balance in the report but there is analytical data for the top concentration in each soil (also for the next three concentrations in the Münster soil where degradation was most significant). Based on the top concentration data parental mass balances of 84.2, 94.6 and 88.9% can be determined. Values determined at the lower concentrations in the Münster soil, where a single desorption cycle was performed, are in the same range (83.5 - 89.1%). It should be noted that degradation occurred in the aqueous phases but not in soil extracts.

Figure 7.1.3.1.2- 6: Freundlich Isotherms of M-03 in Soil Ingleby (02/03) at 20°C



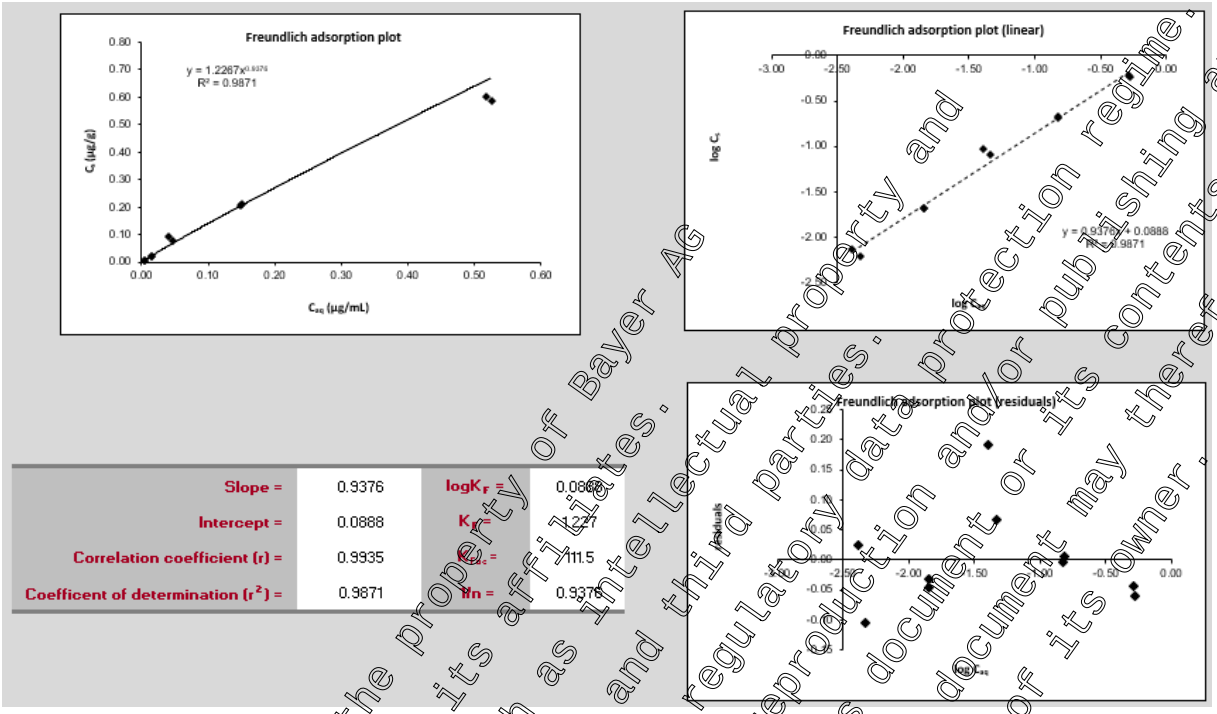
Slope =	0.9598	$\log K_F$ =	0.4554
Intercept =	0.4554	K_F =	2.854
Correlation coefficient (r) =	0.9991	K_{Foc} =	81.5
Coefficent of determination (r^2) =	0.9981	1/n =	0.9598

Figure 7.1.3.1.2- 7: Freundlich Isotherms of M-03 in Soil Huntlosen (03/04) at 20°C



Slope =	1.0114	$\log K_F$ =	0.3533
Intercept =	0.3533	K_F =	2.256
Correlation coefficient (r) =	0.9969	K_{Foc} =	132.7
Coefficent of determination (r^2) =	0.9938	1/n =	1.0114

Figure 7.1.3.1.2- 8: Freundlich Isotherms of M-03 in Soil Münster (03/07) at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 16. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 17.

Table 7.1.3.1.2- 16: Summary of Quality Criteria and Regulatory Interpretation

M-03			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Ingleby	Sandy loam	02/03	7	1	1
Huntlosen	Loamy sand	03/04	8	1	0
Münster	Loamy sand	03/07	7	1	1

Table 7.1.3.1.2- 17: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Ingleby	Sandy loam	02/03	82	81.5	0.961	0.960
Huntlosen	Loamy sand	03/04	133	132.7	1.012	1.011
Münster	Loamy sand	03/07	112	111.5	0.939	0.938

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

The adsorption constant $K_{F(ads)}$ of M-03 was between 1.23 and 2.86 mL/g for the tested soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 82 to 133 mL/g. The Freundlich exponent 1/n was between 0.939 and 1.012, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Adsorption was shown to be correlated with organic carbon content.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the results were acceptable according to the quality criteria and therefore suitable for regulatory use.

Assessment and conclusion by applicant: The study is considered valid to assess the adsorption and desorption characteristics of M-03 in soil.

Data Point:	KCA 7.1.3.1.2/04
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE C657188: Adsorption to and desorption from three soils
Report No:	C036060
Document No:	M-219828-01-1
Guideline(s) followed in study:	EU (=EEC): 95/36/EC; USEPA (=EPA): N, 163-1
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted in the DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption of M-02 (referred to as AE C657188 in the report) was characterised in three soils using the batch equilibrium method in the laboratory in the dark at 20 ± 1 °C. The three soils were a sandy loam (Abington), a loamy sand (Munster) and a silt loam (Sarotti) according to USDA soil classification.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
Abington	03/06	Cambridgeshire, UK	Sandy loam	7.2	2.6
Munster	03/07	Munster, Germany	Loamy sand	5.4	1.1
Sarotti	03/10	Hattersheim, Germany	Silt loam	7.5	1.3

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 1:1. Nominal test concentrations of 1.00, 0.30, 0.10, 0.03, and 0.01 mg/L of [^{14}C]-M-02 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for

48 hours in the definitive test. Desorption phase of the study was carried out by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ solution for up to three desorption cycles of 24 hours.

After the desorption phase of the study, all soils at all concentrations were extracted with acetonitrile/water, then air dried and combusted. No significant degradation was observed in any of the supernatants or extracts from the soil analysed.

The mass balance for the test soils ranged from 97.3% to 112.7% of applied radioactivity, but only one sample for one soil at the lowest treatment rate was above 110%. The average mass balance for all soils was 101.5 ± 1.5%.

The K_f and K_{des} values were determined using the Freundlich equation.

Soil origin	Abington	Munster	Sarotti
Soil type (USDA)	Sandy loam	Loamy sand	Silt loam
pH (0.01M $CaCl_2$)	7.2	5.4	7.5
Organic carbon [%]	2.6	1.1	1.3
$K_F^{(ads)}$ [mL/g]	0.029	0.116	0.082
1/n	0.725	0.887	0.709
$K_{F,OC}^{(ads)}$ [mL/g]	1.1	10.5	6.2
$K_F^{(des)}$ [mL/g]	0.046	0.137	0.132
1/n	0.711	0.857	0.599
$K_{F,OC}^{(des)}$ [mL/g]	1.8	12.5	10.2

K_{oc} values for the soils indicate that M-02 is mobile to very mobile in the test soils according to the Briggs classification. For all soils the first desorption K_{des} was similar to the corresponding adsorption values. At the highest treatment concentration for all soils, the ratio of the concentrations on the soil and in the supernatant increased for three successive desorptions, indicating that once adsorbed, M-02 was less readily desorbed.

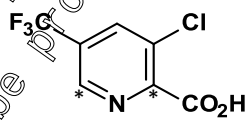
A supplemental experiment was conducted in order to investigate the effect of ageing upon the adsorption of M-02 to each of the soils. There was no trend for increased adsorption for up to three days ageing.

I. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-M-02 (referred to as AE C657188 in the report)



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

Specific Activity:	6.27 MBq/mg
Radiochemical Purity:	99.2% (TLC) 97.6% (HPLC)
Sample/Batch ID:	SEL/1189
Stability of test compound:	Stable during the batch equilibrium procedure.

2. Test Soil

Sorption tests were performed with three agricultural soils from UK and Germany selected to be the same as those used for the aerobic soil rate of degradation study. The soils were received partly air-dried, sieved to 2 mm and fully characterised, with respect to particle size distribution, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study.

Table 7.1.3.1.2- 18: Physico-chemical properties of test soils

Characteristic / Code		Munster	Abington	Sarotti
Soil ID		03/07	03/06	03/10
Origin		Germany	England, UK	Germany
Location		Munster	Great Abington, Cambridgeshire	Hattersheim
Textural Class (USDA)		Loamy sand	Sandy Loam	Silt Loam
Particle Size Analysis, ADAS:				
Total Sand (0.063 – 2.00 mm)	(%)	77	68	15
Silt (0.002 – 0.063 mm)	(%)	20	18	62
Clay (< 0.002 mm)	(%)	4	14	23
Textural Class (ADAS)		Loamy sand	Sandy Loam	Silty Clay Loam
pH				
Water		6.6	8.1	8.3
KCl		5.5	7.7	7.7
$CaCl_2$		5.4	7.2	7.5
Organic Carbon (%)		1.1	2.6	1.3
Organic Matter (%)		1.9	4.5	2.2
Maximum Water Holding Capacity (g/100 g dry matter)		46.5	57.1	52.1
$Ca_{exchangeable}$ (meq/100g)		1.5	19.9	34.3
$Mg_{exchangeable}$ (meq/100g)		0.2	1.6	1.6
$Na_{exchangeable}$ (meq/100g)		<0.05	0.1	0.1
$K_{exchangeable}$ (meq/100g)		0.4	1.3	0.9
$Mn_{exchangeable}$ (meq/100g)		<0.05	<0.05	<0.05
$CaCO_3$eq. (g/kg)		<0.05	73.5	13.4
Phosphorus total (mg/kg)		617.8	1586.3	728.8
Nitrogen total (mg/kg)		1077.9	2380.1	1470.2

B. Study Design

1. Experimental Conditions

The test system for the study was the soil and 0.01M calcium ion solution. The test system was dosed with M-02 and contained in thick-walled borosilicate glass tubes with external plastic coating. Tubes were approximately 125 mL capacity and capped with Teflon seals.

In preliminary tests, the adsorption of the test item to glassware, to determine any background radioactivity in the soil, the optimal soil-to-solution ratio and the appropriate adsorption and desorption equilibration times were determined.

The main test was performed in duplicate. M-02 was dissolved in 0.01 M calcium chloride solution at nominal concentrations of 0.01, 0.03, 0.1, 0.3 and 1 mg/L. Soil samples were prepared at a soil to solution ratio of 1:1 and shaken at in the dark at 20±1°C. Following the preliminary tests, an equilibrium time of 48 hours was selected.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		40 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ shaken overnight
Control (preliminary experiment)		No soil, test item in 0.01M $CaCl_2$ only
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 0.01, 0.03, 0.1, 0.3 and 1.0 µg/mL
	Initial concentrations of M-02 by LSC	Initial concentrations in test system (definitive study): 0.009, 0.028, 0.098, 0.291 and 0.946 µg/mL
Identity and concentration of co-solvent		Dosing stock made up in 10:90 acetonitrile:water
Soil: Solution ratio		1:1 i.e. 40 g soil dry weight equivalent to 40 mL solution
Number of replicates / Treatments		Duplicate
Equilibration conditions	Time	48 h
	Temperature	20 ± 1°C
	Dark	In the dark
	Shaking method	End-over-end shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not recorded
	Duration	10 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 2 to 26% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		3 for highest test treatment (1.0 µg/mL) 1 for lower test treatments (0.01, 0.03, 0.1 and 0.3 1.0 µg/mL)
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. Approximately 40 mL was used as equilibration solution.
Soil: Solution ratio		1:1 i.e. 40 g soil dry weight equivalent to 40 mL solution
Number of replicates	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hours
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	End-over-end shaker
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	Not reported
	Duration	16 minutes
	Method of separating supernatant	Supernatant was carefully decanted

2. Analytical Procedures

After equilibration, soil and solution phases were separated by centrifugation and the concentration of M-02 in the water phase determined by LSC. Following final desorption cycle M-02 remaining in the soil phase was then extracted with solvent (approximately 40 mL 50:50, acetonitrile:water) and the concentration determined by LSC. The radioactivity remaining in soil after the desorption phase was quantified by combustion.

The amount of radioactivity adsorbed to the soil was quite small compared to that in solution. Significant errors could arise if the amount on the soil is determined by subtracting the radioactivity in the solution from that applied. Accordingly, the results were normalised so that the radioactivity applied was set equal to the total radioactivity recovered.

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

Selected samples were analysed by high performance liquid chromatography (HPLC).

II. Results and Discussion

A. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption (mean recovery was 98.7%).

Levels of background radioactivity detected was negligible. No correction for background radioactivity was required.

At each soil:solution ratio tested all soils were below acceptable range (20-80% adsorbed). A 1:1 ratio was therefore selected for all subsequent experiments.

All three soils reached equilibrium after 48 hours and no significant breakdown of M-02 was observed following HPLC analysis of all soil adsorption supernatants. An adsorption equilibrium time of 48 hours was selected for the definitive study.

B. Transformation of test substance:

Selected supernatants and extracts were analysed by HPLC within 3 days of generation. Test substance accounted for greater than 97.8% radioactivity for all supernatants and soil extracts with the exception of one Munster soil extract (92.2%). No significant degradation of test substance occurred over the duration of the study, therefore, no adjustment for adsorption and desorption coefficient was required.

C. Findings

Material balances were 90-110% of applied with the exception of one sample from the lowest concentration in the loamy sand (Munster) soil which fell outside this acceptable range.

The mass balances for the three soils is summarised in the table below. The mean values for all concentrations in each soil ranged from 100.1 to 103.5%. The complete material balances for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 19: Definitive test: Mass balance of [2,6-pyridyl-^{14}C]-M-02 (% AR)

Soil	Abington (03/06)	Munster (03/07)	Sarotti (03/10)
Range	98.8 – 106.7	99.3 – 112.7	97.3 – 104.7
Mean	101.8	103.5	100.1
SD	3.01	4.50	2.36

SD = standard deviation.

For all soils the fit of log C_{sl} vs. log C_{wl} to a linear equation, was good with correlation coefficients between 0.995 and 0.997. The K_f values ranged from 0.029 mL/g in the sandy loam (Abington) to 0.12 mL/g in the loamy sand (Munster) indicating weak adsorption to soil. The relationship between the soil and solution concentrations for all soils was found to be non-linear with 1/n values less than 1. This was especially the case for the sandy loam (Abington) and the silt loam (Sarotti) with 1/n values of 0.73 and 0.71 respectively. This greater adsorption at lower concentration may indicate saturation of binding sites in these soils. The lower 1/n values were found for soils with higher pH.

The values for K_{oc} ranged from 1.1 to 10.5 mL/g, with a mean value of 6.0 mL/g, indicating that M-02 is potentially mobile to very mobile according to the Briggs classification. The values obtained did not appear to be directly related to any soil parameters measured.

The K_{des} values obtained for the first desorption cycle ranged from 0.046 to 0.14 mL/g. The values of 1/n for the first desorption cycle were similar to those obtained for the adsorption except for the silty clay loam/silt loam (Sarotti) which was lower at 0.60. The correlation coefficient of the linear fit was very good, ranging from 0.992 to 0.996 and so the determined K_{des} was reliable.

Table 7.1.3.1.2- 20: Summary of adsorption/desorption constants and correlation coefficients of M-02 in soil at 20°C

Phase	Soil	Units	Abington (03/06)	Münster (03/07)	Sarotti (03/10)
Adsorption	$K_{F,ads}$	[mL/g]	0.029	0.116	0.082
	1/n	-	0.725	0.887	0.709
	R^2	-	0.973	0.993	0.997
	$K_{OC,ads}$	[mL/g]	1.1	10.5	6.3
Desorption	$K_{F,des}$	[mL/g]	0.046	0.137	0.132
	1/n	-	0.711	0.857	0.599
	R^2	-	0.992	0.994	0.996
	$K_{OC,des}$	[mL/g]	1.75	12.5	10.2

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the report were used as input data for the checklist (Table 7.1.3.1.2- 21). Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 22).

Table 7.1.3.1.2- 21: Definitive test: Concentration of M-02 in aqueous and solid phase following 48 hours of adsorption

Nominal Concentration (µg/mL)	Abington		Münster		Sarotti	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.01	0.0015	0.0087	0.0021	0.0083	0.0026	0.0070
0.01	0.0010	0.0088	0.0016	0.0083	0.0026	0.0068
0.03	0.0018	0.0274	0.0036	0.0258	0.0054	0.0232
0.03	0.0020	0.0275	0.0042	0.0253	0.0054	0.0231
0.1	0.0054	0.0923	0.0132	0.0841	0.0132	0.0817
0.1	0.0049	0.0923	0.0126	0.0850	0.0133	0.0819
0.3	0.0158	0.2783	0.0332	0.2578	0.0298	0.2521
0.3	0.0084	0.2834	0.0359	0.2565	0.0302	0.2539
1.0	0.0234	0.9266	0.1004	0.8476	0.0806	0.8498
1.0	0.0367	0.9214	0.1077	0.8522	0.0772	0.8519

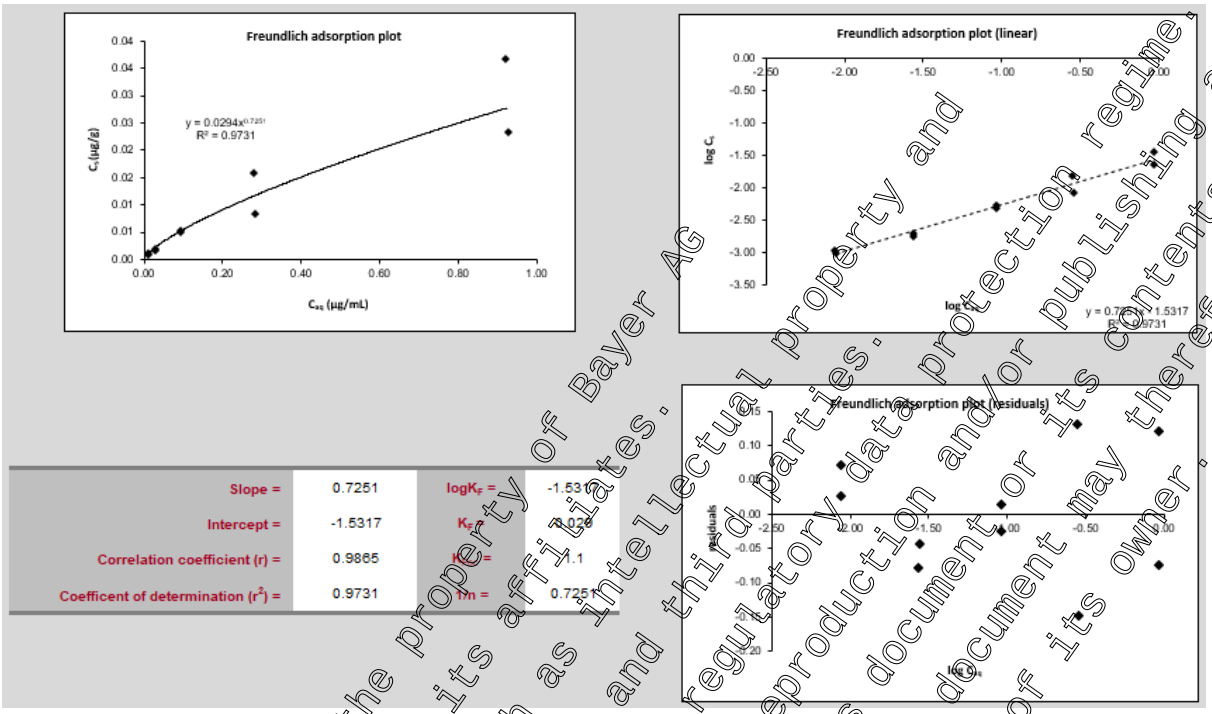
Table 7.1.3.1.2- 22: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Abington (03/06)	Münster (03/07)	Sarotti (03/16)
Adsorption method	-	-	Indirect	Indirect	Indirect
Soil solution ratio	g/mL	-	1:1	1:1	1:1
Mass balance of ^{14}C	%	>90%	101.6	103.0	99.9
f – due to loss processes (estimated)	%	-	5.9	3.0	9.8
Adsorbed percentage (δ)	%	>20%	2.05 – 5.67	8.37 – 14.00	9.95 – 26.13
K_D x soil:solution ratio		>0.3	0.03 – 0.13	0.12 – 0.25	0.09 – 0.38
K_{fE} / K_f	-	<1.2	-0.79 & - 0.53	1.43 & 1.41	27.55 & 65.74
ads K_F	L/kg		0.029	0.116	0.082
95% confidence interval	-	*	(0.022 – 0.039)	(0.099 – 0.135)	(0.075 – 0.090)
ads 1/n	-		0.725	0.887	0.709
95% confidence interval	-	*	(0.627 – 0.823)	(0.835 – 0.939)	(0.680 – 0.739)
ads R^2	-	>0.975	0.973	0.995	0.997
ads $K_{F,OC}$	L/kg	-	1.1	10.5	6.9
Visual fit to Freundlich isotherm	-	-	Acceptable	Acceptable	Acceptable
Residual plots randomly distributed	-	-	Acceptable	Acceptable	Acceptable

* Confidence intervals should be narrow

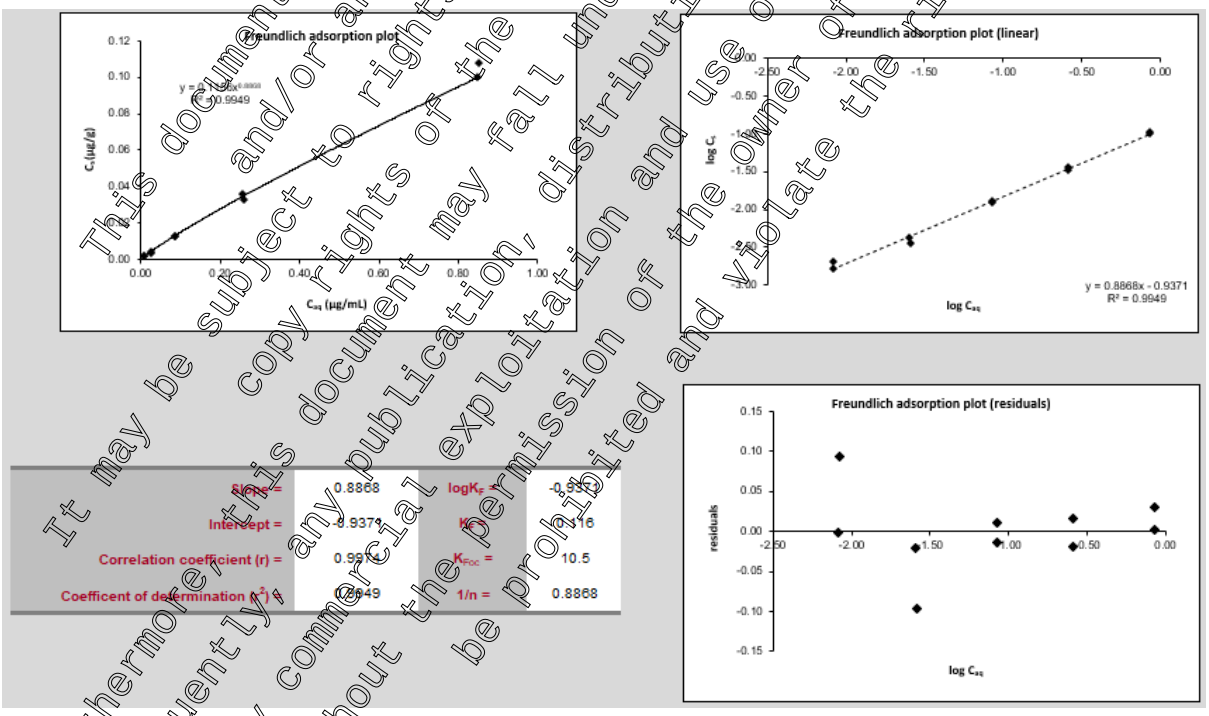
Relevant quality checks were performed to evaluate the acceptability of the study. The mean mass balances were acceptable (101.6, 103.0 and 99.9%) however at the lower concentrations some recoveries were high (up to 112.7%). The acceptability of the analytical method was confirmed over the entire range of concentrations measured (reported LOQ of 0.00016 mg/L for LSC; 0.02 – 1.6% AR). The use of the indirect method was however not appropriate based on a K_d* soil/solution ratio > 0.3 in all soils. The graphical fits of the Freundlich equation were acceptable with R^2 of the standard linear regressions ranging from 0.973 to 0.997. The visual fit of both the standard regression and the residual plots were also acceptable. Although no parental mass balance values were cited in the study report, there is sufficient data to estimate these at the top concentration.

Figure 7.1.3.1.2- 9: Freundlich Isotherms of M-02 in soil Abington (03/06) at 20°C



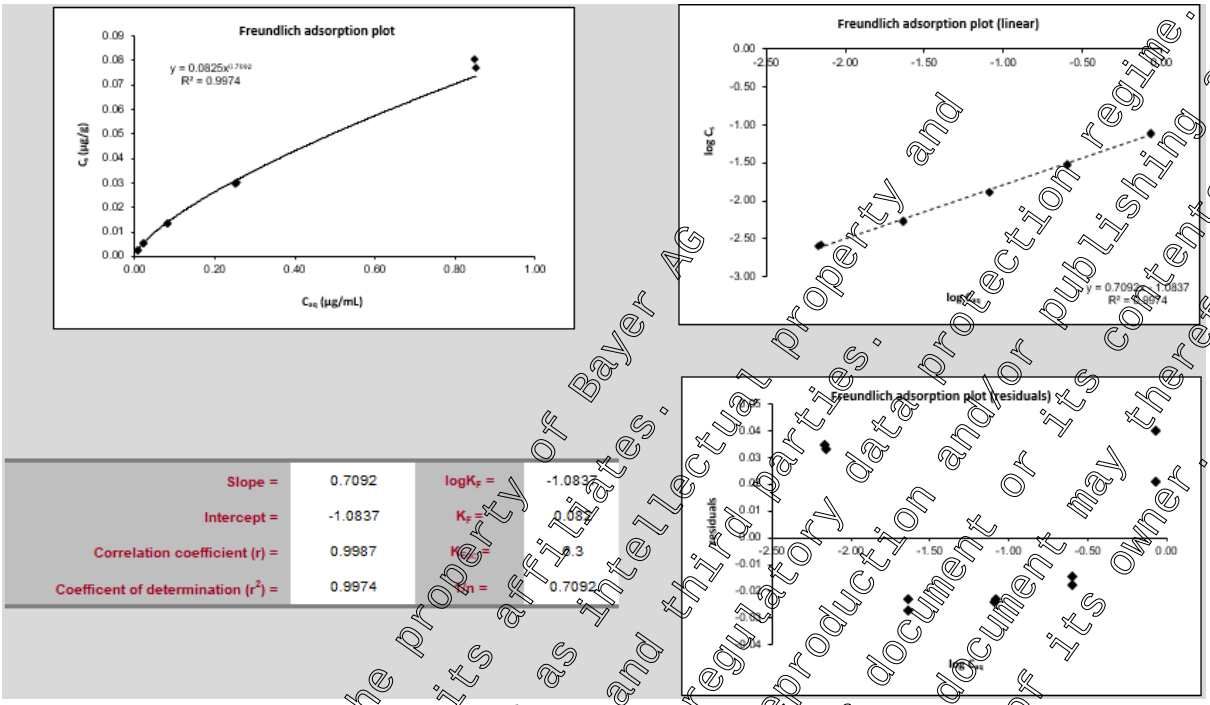
Slope =	0.7251	$logK_F$ =	-1.5317
Intercept =	-1.5317	K_F =	0.029
Correlation coefficient (r) =	0.9865	K_{OC} =	1.1
Coefficent of determination (r^2) =	0.9731	1/n =	0.7251

Figure 7.1.3.1.2- 10: Freundlich Isotherms of M-02 in soil Munster (03/07) at 20°C



Slope =	0.8868	$logK_F$ =	-0.9371
Intercept =	-0.9371	K_F =	0.116
Correlation coefficient (r) =	0.9974	K_{OC} =	10.5
Coefficent of determination (r^2) =	0.9949	1/n =	0.8868

Figure 7.1.3.1.2- 11: Freundlich Isotherms of M-02 in soil Sarotti (03/10) at 20°C



A summary of the quality criteria and regulatory interpretation is presented in **Table 7.1.3.1.2- 23**. . The impact on reported endpoints is summarised in Table 7.1.3.1.2- 24.

Table 7.1.3.1.2- 23: Summary of Quality Criteria and Regulatory Interpretation

M-02			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
Abington	Sandy loam	03/06	5	0	4
Münster	Loamy sand	03/07	6	0	3
Sarotti	Silty clay loam	03/10	6	2	1

Table 7.1.3.1.2- 24: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Abington	Sandy loam	03/06	1.13	1.1	0.725	0.725
Münster	Loamy sand	03/07	10.51	10.5	0.887	0.887
Sarotti	Silty clay loam	03/10	6.33	6.3	0.709	0.709

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

From the K_f values obtained (0.029 to 0.12 mL/g) it was concluded that M-02 is weakly adsorbed to soil. In all soils there was a non-linear to moderately linear relationship between the concentration in solution and the amount adsorbed to the soil, with values for 1/n ranging from 0.71 to 0.89. The K_{OC} values obtained ranged from 1.1 to 10.5 mL/g indicating that M-02 has potentially high mobility in soil.

The K_{des} values ranged from 1.8 to 12.5 mL/g and for all soils, was greater than the corresponding adsorption K_{oc} value, indicating that once adsorbed, M-02 was slightly less readily desorbed.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that extent of adsorption of M-02 is too low to fully meet the proposed quality criteria for the indirect method but study is otherwise well conducted.

A second OECD 106 study with M-02 using the direct method (see KCA 7.1.3.1.2/15) derived very similar endpoints, thus confirming the validity of this study.

Assessment and conclusion by applicant

The study is considered valid to assess the adsorption and desorption characteristics of M-02 in soil.

Data Point:	KCA 7.1.3.1.2/05
Report Author:	[redacted]
Report Year:	2003
Report Title:	Adsorption/desorption of [pyridine-2,6-14C] AE 1344122 degradate in three soil types
Report No:	M-241403-01-2
Document No:	M-241403-01-2
Guideline(s) followed in study:	EU (=EEC): 91/414/EEC; OECD 106
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially 120ecognized testing facilities:	Yes, conducted under GLP/Officially 120ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption characteristics of M-05 (referred to as AE 1344122 in the study report) was characterised in three soils using the batch equilibrium method in the laboratory in the dark at 20 ± 2 °C. The three soils were a sandy loam (Abington), a loamy sand (Munster) and a silt loam (Sarotti) according to USDA soil classification.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
Abington	03/06	Cambridgeshire, UK	Sandy loam	7.2	2.6
Munster	03/07	Munster, Germany	Loamy sand	5.4	1.1
Sarotti	03/10	Hattersheim, Germany	Silt loam	7.5	1.3

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 1:2. Nominal test concentrations of 1.0, 0.5, 0.1, 0.05, and 0.01 mg/L of [^{14}C]-M-05 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. 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 of 24 hours.

The aqueous supernatant after adsorption was separated by centrifugation, the soils were extracted and the amount of test item in the supernatants and soil extracts was analyzed by liquid scintillation counting (LSC) and HPLC. The sorption parameters were calculated using Freundlich isotherms.

The test item was reported to be stable throughout the study. The mass balance for the test soils ranged from 95 to 101%, 86 to 100%, and 98 to 102% AR for the Abington, Münster and Sarotti soils, respectively No degradation of M-05 was observed by HPLC analysis of the adsorption supernatants.

The K_f values for adsorption ranged from 0.218 to 0.544 mL/g. The K_f values for desorption ranged from 0.77 to 1.11 mL/g. A slight dependence on concentration was observed with 1/n values ranging from 0.88 to 0.95 for the adsorption and 0.85 to 0.95 for desorption. The $K_{oc\text{-}des}$ values were higher than the $K_{oc\text{-}ads}$, indicating that hysteresis occurs.

K_{oc} values indicate that M-05 is mobile in soil according to the Briggs classification.

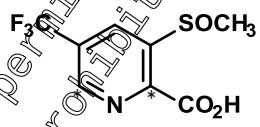
Soil origin	**Abington**	**Munster**	**Sarotti**
Soil type (USDA)	Sandy loam	Loamy sand	Silt loam
pH (0.01M $CaCl_2$)	7.2	6.4	7.5
Organic carbon [%]	2.6	1.1	1.3
$K_F^{(ads)}$ [mL/g]	0.294	0.544	0.218
1/n	0.8828	0.9538	0.9179
$K_{F,OC}^{(ads)}$ [mL/g]	11	49	17
$K_F^{(des)}$ [mL/g]	0.77	1.11	1.03
1/n	0.8499	0.9508	0.8886
$K_{F,OC}^{(des)}$ [mL/g]	30	101	79

I. Materials and Methods

A. Materials

1. Test Items

[2,6-Pyridyl-^{14}C]-M-05 (referred to as AE 1344122 in the report)



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

Specific Activity: 4973 Bq/ug (298,367 dpm/µg, 34.03 mCi/mmole)

Radiochemical Purity: 98.7%

Sample/Batch ID: SEL/1192 / C-937

Stability of test compound: Stable during the batch equilibrium procedure.

2. Test Soils

Sorption tests were performed with three agricultural soils from UK and Germany selected to be the same as those used for the aerobic soil rate of the degradation study. Surface soil was collected from top 30 cm and shipped to the test site, where it was maintained under alfalfa cover in a greenhouse.

The soils were fully characterised, with respect to texture, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study.

Table 7.1.3.1.2- 25: Physico-chemical properties of test soils

Characteristic / Code	Munster	Abington	Sarotti
Soil ID	03/07	03/06	03/10
Origin	Germany	England, UK	Germany
Location	Munster	Great Abington, Cambridgeshire	Hattersheim
Textural Class (USDA)	Loamy sand	Sandy Loam	Silt Loam
Particle Size Analysis, ADAS:			
Total Sand (0.063 – 2.00 mm) (%)	77	68	15
Silt (0.002 – 0.063 mm) (%)	20	18	62
Clay (< 0.002 mm) (%)	4	14	23
Textural Class (ADAS)	Loamy sand	Sandy Loam	Silty Clay Loam
pH			
Water	6.6	8.1	8.3
KCl	5.5	7.7	7.7
$CaCl_2$	5.4	7.2	7.5
Organic Carbon (%)	1.1	2.6	1.3
Organic Matter (%)	1.9	4.5	2.2
Maximum Water Holding Capacity (g/100 g dry matter)	46.5	57.1	52.1
$Ca_{exchangeable}$ (meq/100g)	15	19.9	34.3
$Mg_{exchangeable}$ (meq/100g)	0.2	1.6	1.6
$Na_{exchangeable}$ (meq/100g)	<0.05	0.1	0.1
$K_{exchangeable}$ (meq/100g)	0.4	1.3	0.9
$Mn_{exchangeable}$ (meq/100g)	<0.05	<0.05	<0.05
$CaCO_3$eq. (g/kg)	<0.05	73.5	13.4
Phosphorus total (mg/kg)	647.8	1586.3	728.8
Nitrogen total (mg/kg)	1077.9	2380.1	1470.2

B. Study Design

1. Experimental Conditions

The test system for the study was the soil and 0.01M calcium ion solution. The test system was dosed with M-05 and contained in 30 mL silanised borosilicate glass centrifuge tubes capped with Teflon-lined screw caps.

In preliminary tests, the adsorption of the test item to glassware, to determine any background radioactivity in the soil, the optimal soil-to-solution ratio and the appropriate adsorption and desorption equilibration times were determined.

The main test was performed in duplicate. M-05 was dissolved in 0.01M calcium chloride solution at nominal concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L. Soil samples were prepared at a soil to solution ratio of 1:2 and shaken at in the dark at 20 ± 2°C. Following the preliminary tests, an equilibrium time of 24 hours was selected.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		10 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ shaken overnight
Control (preliminary experiment)		No soil +test item in 0.01M $CaCl_2$ only
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 0.01, 0.05, 0.1, 0.5 and 1.0 µg/mL
	Initial concentrations of M-05 by LSC	0.01, 0.05, 0.10, 0.51 and 1.00 mg/L
Identity and concentration of co-solvent		Dosing stock made up in 1:1 acetonitrile:water
Soil: Solution ratio		1:2 i.e. 10 g soil dry weight equivalent to 20 mL solution
Number of replicates	Treatments	Duplicate
Equilibration conditions	Time	24 h
	Temperature	20 ± 1°C
	Dark	In the dark
	Shaking method	Benchtop shaker (low setting)
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	3100 G
	Duration	20 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 10 to 25% (definitive test).
Number of desorption cycles		1
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by an equal volume of fresh aqueous 0.01 M $CaCl_2$ solution
Soil: Solution ratio		1:2
Number of replicates	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hours
	Temperature	20±2°C
	Dark	In the dark
	Shaking method	Benchtop shaker (low setting)
Method of separation of supernatant		Centrifugation
Centrifugation	3100 G	Not reported
	20 minutes	10 minutes
	Supernatant was carefully decanted.	Supernatant was carefully decanted.

3. Analytical Procedures

After equilibration, soil and solution phases were separated by centrifugation and the concentration of M-05 in the water phase determined by LSC. Following final desorption cycle M-05 remaining in the soil phase was then extracted with acetone and the concentration determined by LSC. The radioactivity remaining in soil after the desorption phase was quantified by combustion.

Adsorption supernatants from the definitive test were analysed by HPLC coupled to a UV detector and flow-through radioactivity detector.

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. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption. The percentage of applied radioactivity in the $CaCl_2$ solution shaken for up to 72 hours was 98.4% AR (range 98.1 to 101.6%AR after 24 to 72 hours).

Levels of background radioactivity detected was negligible. No correction for background radioactivity was required.

The soil:solution ratio to be used for the definitive test was chosen to be 1:2, based on the percentage of M-05 adsorbed (23.3% for Abington, 29.7% for Munster, and 17.1% for silt loam).

The amount of radioactivity adsorbed after various equilibration time periods showed that equilibrium was obtained very rapid, with little change in the levels of radioactivity in solution between 2 and 72 hours. Therefore 24-hour equilibrium time was chosen for all three soils.

B. Transformation of test substance

The test item was reported to be stable throughout the study. Chromatographic analysis of adsorption supernatants showed that >99% of the recovered radioactivity was [^{14}C]-M-05.

C. Findings

Material balances for the definitive test ranged from 93.4 to 100.7% of the applied radioactivity for Abington, 95.2 to 100.2% for Munster, and 97.3 to 102.1% for Sarotti soils (mean of duplicate values). The mass balances for the three soils is summarised in the table below. The complete material balances for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 26: Definitive test: Mass balance (% AR) of M-05

Soil	Abington	Munster	Sarotti
1.0	98.1	95.2	98.7
0.50	100.7	100.2	99.0
0.10	93.4	95.9	97.3
0.05	97.6	97.4	98.0
0.01	99.8	99.3	102.1
Mean	97.9	97.6	99.0
SD	2.8	2.1	1.8

SD = standard deviation.

The results of adsorption tests of M-05 onto three soils are summarised in Table 7.1.3.1.2- 27 and Table 7.1.3.1.2- 28. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 12 to Figure 7.1.3.1.2- 14.

The equilibrium concentrations of [^{14}C]-M-05 in supernatants at the end of the adsorption phase were determined by calculating the difference between initial and equilibrium concentrations of aqueous solutions. The proportion of M-05 adsorbed on the soil ranged from 11.8 to 20.4% for Abington soil, 19.3 to 24.9% for Munster soil, and 9.8 to 13.5% for Sarotti soil.

The adsorption constant, K_f, for the Abington, Muenster, and Sarotti soils were 0.294, 0.544 and 0.218 mL/g, respectively. K_{oc} values were 19, 49 and 17 mL/g, respectively. The r^2 values for the regressions were 0.990 0.996, and 0.999 respectively.

The proportion of M-05 adsorbed to soil that was desorbed with calcium chloride solution ranged from 48.0 to 70.9% of adsorbed radioactivity for Abington, 56.7 to 68.9% for Munster, and 46.2 to 58.1% for Sarotti soils.

The K_{des} values ranged from 0.77 to 1.11 mL/g. The corresponding $K_{oc\ des}$ values ranged from 30 to 101 mL/g. The r^2 values for the regressions ranged from 0.965 to 0.999.

Table 7.1.3.1.2- 27: Definitive test: Concentration of M-05 in aqueous and solid phase following 24 hours of adsorption

Concentration of Test Item (µg/mL)	Abington		Münster		Sarotti	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.01	0.0041	0.0086	0.0053	0.0080	0.0029	0.0092
0.05	0.0166	0.0416	0.0249	0.0375	0.0122	0.0438
0.1	0.0410	0.0800	0.0483	0.0764	0.0254	0.0879
0.5	0.1196	0.4454	0.1948	0.4078	0.0987	0.4559
1.0	0.2729	0.8633	0.4718	0.7639	0.2020	0.8988

Table 7.1.3.1.2- 28: Summary of adsorption/desorption constants and correlation coefficients of M-05 in soil at 20°C

Phase	Soil	Units	Abington	Münster	Sarotti
Adsorption	$K_{F,ads}$	[mL/g]	0.294	0.544	0.218
	1/n	-	0.882	0.954	0.918
	R^2	-	0.990	0.996	0.999
	$K_{OC,ads}$	[mL/g]	14	49	17
Desorption	$K_{F,des}$	[mL/g]	0.77	1.11	1.03
	1/n	-	0.850	0.951	0.889
	R^2	-	0.965	0.993	0.999
	$K_{OC,des}$	[mL/g]	30	101	79

D. Evaluation of the Data according to EFSA Evaluators Checklist

The concentrations in the supernatant and the soil as given in the report were used as input data (Table 7.1.3.1.2- 27). Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation. The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 29).

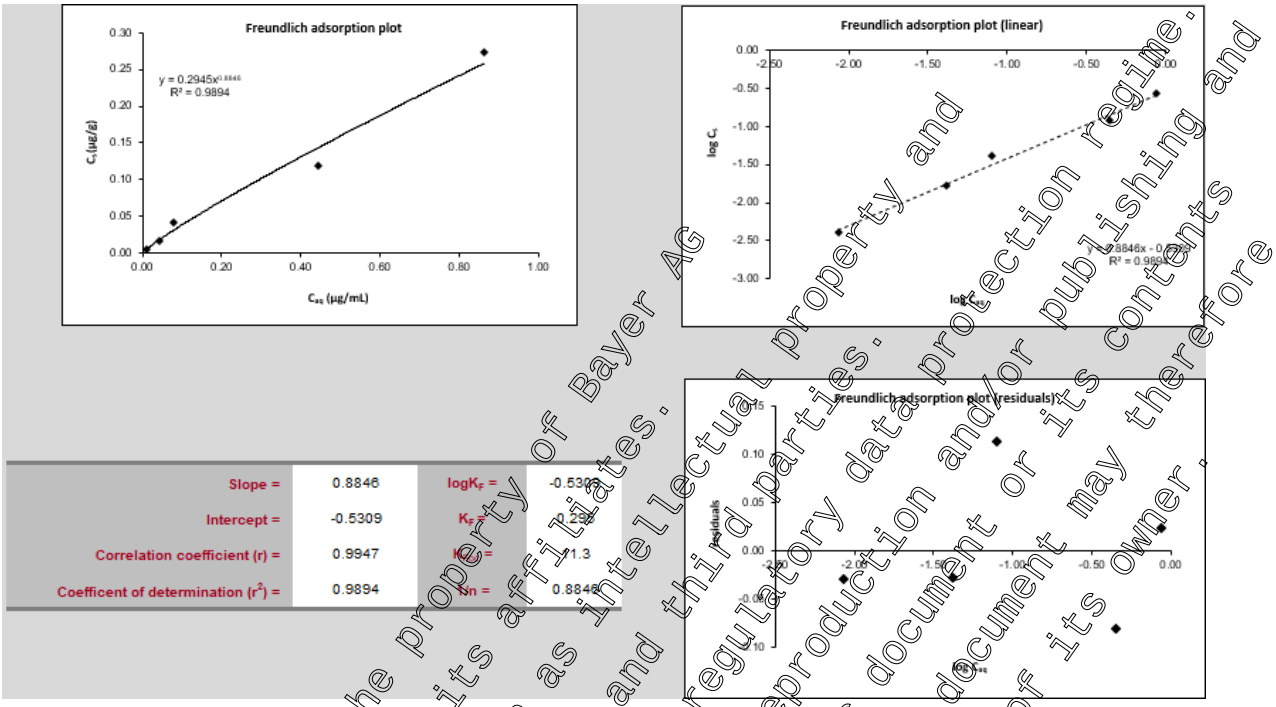
Table 7.1.3.1.2- 29: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	Abington	Münster	Sarotti
Adsorption method	-	-	Indirect	Indirect	Indirect
Soil solution ratio	g/mL	-	1:2	1:2	1:2
Mass balance of ^{14}C	%	>90%	95.0 – 100.7	86.2 – 100.1	97.5 – 102.1
f – due to loss processes (estimated)	%	-	9.9	13.3	9.5
Adsorbed percentage (δ)	%	>20%	11.8 – 21.8	19.3 – 27.3	9.7 – 16.4
K_D x soil:solution ratio		>0.3	0.13 – 0.26	0.24 – 0.33	0.11 – 0.16
$^{\#}K_{fE}$ / K_f	-	<1.2	3.63	2.29	16.32
ads K_F	L/kg		0.295	0.544	0.217
95% confidence interval	-	*	0.183 – 0.473	0.390 – 0.742	0.186 – 0.254
ads 1/n	-		0.885	0.954	0.916
95% confidence interval	-	*	0.717 – 1.053	0.846 – 1.062	0.860 – 0.973
ads R^2	-	>0.975	0.989	0.996	0.999
ads $K_{F,OC}$	L/kg	-	11.3	49.4	16.7
Visual fit to Freundlich isotherm	-	-	Acceptable	Acceptable	Acceptable
Residual plots randomly distributed	-	-	Acceptable	Acceptable	Acceptable

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance was generally acceptable (86.2 – 102.1%) but that the % adsorption of 9.7 – 27.3% was rather low. The LOQ of the analytical method (LSC) was determined to be <1% of applied at the lowest concentration and thus fully acceptable. Based on a Kd / soil/solution ratio <0.3 in two of the three soils, the indirect method of assessment was not most appropriate. The graphical fits of the Freundlich equation, based on the standard linear regression using log-log transformed data alongside the associated residual plots, were evaluated and found to be acceptable. The R^2 of the standard linear regressions performed on mean data ranged from 0.989 to 0.999 and the visual fit of both the standard regression and the residual plots were acceptable.

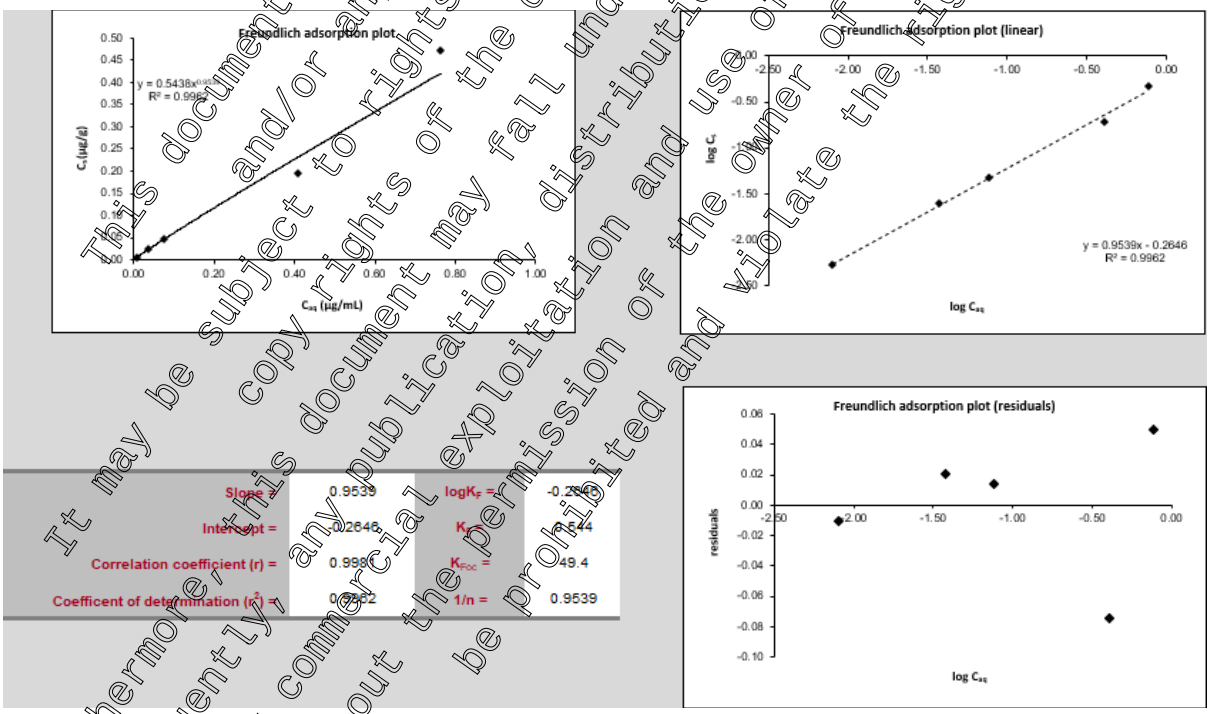
The study was conducted to an acceptable standard, however certain criteria that might be expected in a more modern study have not been investigated. In particular, there has been no specific attempt to confirm parental mass balance. The "f" values thus used in the evaluation of K_{fe} / K_f have been estimated as [(100% - mass balance) + total in combustion] with an assumption that no degradation seen in adsorption supernatant, desorption supernatant or solvent extract. HPLC data is only reported for adsorption supernatants. If a more rigorous extraction procedure had been attempted, it is possible that the "f" value might have been lower.

Figure 7.1.3.1.2- 12: Freundlich Isotherms of M-05 in soil Abington at 20°C



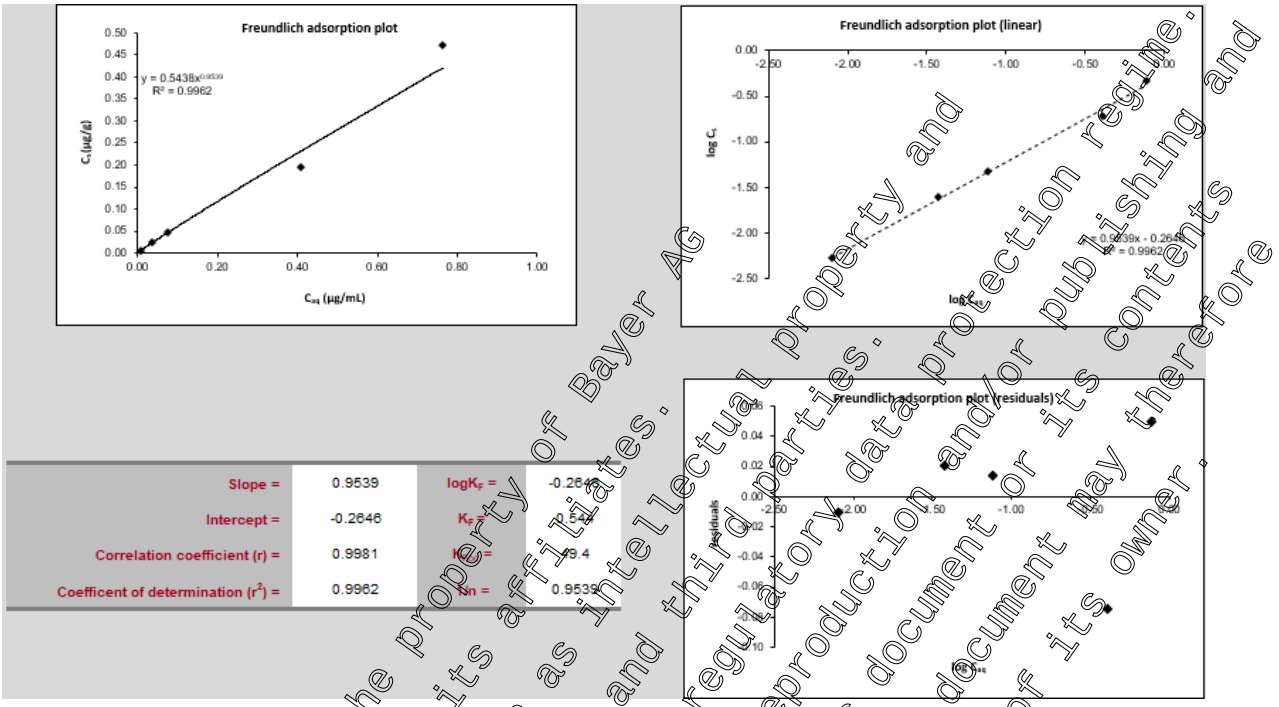
Slope =	0.8846	$\log K_F$ =	-0.5309
Intercept =	-0.5309	K_F =	0.293
Correlation coefficient (r) =	0.9947	K_{FOC} =	71.3
Coefficent of determination (r^2) =	0.9894	1/n =	0.8846

Figure 7.1.3.1.2- 13: Freundlich Isotherms of M-05 in soil Munster at 20°C



Slope =	0.9539	$\log K_F$ =	-0.2646
Intercept =	-0.2646	K_F =	0.544
Correlation coefficient (r) =	0.9981	K_{FOC} =	49.4
Coefficent of determination (r^2) =	0.9962	1/n =	0.9539

Figure 7.1.3.1.2- 14: Freundlich Isotherms of M-05 in soil Sarotti at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 30. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 31.

Table 7.1.3.1.2- 30: Summary of Quality Criteria and Regulatory Interpretation

M-05		Quality Criteria		
Soil Name	**Soil Type**	**Met**	**Partially Met**	**Not Met**
Abington	Sandy loam	4	0	5
Münster	Loamy sand	6	1	2
Sarotti	Silt loam	6	0	3

Table 7.1.3.1.2- 31 Impact on Endpoints

Soil Name	Soil Type	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
Abington	Sandy loam	11	11.3	0.883	0.885
Münster	Loamy sand	49	49.4	0.954	0.954
Sarotti	Silt loam	17	16.7	0.918	0.916

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. Note: The reported values have been used in modelling calculations.

III. Conclusion

The adsorption constant $K_{F(ads)}$ of M-05 was between 0.218 to 0.544 mL/g for the tested soils; the adsorption constant $K_{OC(ads)}$ normalised for organic carbon was in the range of 11 to 49 mL/g. The Freundlich exponent 1/n ranged from 0.883 to 0.954, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

According to the Briggs mobility classification, M-05 is considered to be mobile in the three soils.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that extent of adsorption of M-05 is rather low to fully meet all proposed quality criteria for the indirect method but study is otherwise well conducted.

A second soil adsorption study with M-05 has been conducted which largely meets the quality criteria for the indirect method (see KCA 7.1.3.1.1/11) and derived very similar endpoints, thus confirming the validity of this study.

Assessment and conclusion by applicant

The study is considered valid to assess the adsorption and desorption characteristics of M-05 in soil.

Data Point:	KCA 7.1.3.1.2/06
Report Author:	[redacted]
Report Year:	2003
Report Title:	Adsorption desorption of [Pyridine-2,6-14C] AE 1344123 degradate in three soil types
Report No:	M-241404-01-2
Document No:	M-241404-01-2
Guideline(s) followed in study:	EU (=EEC): 91/414/EEC; OECD 106
Deviations from current test guideline:	No. Preliminary test to determine the extent of adsorption indicated the compound was not adsorbed to soil to any significant extent. Consequently, Freundlich isotherms were not generated.
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognized testing facilities:	Yes, conducted under GLP/Officially 130ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption of M-10 (referred to as AE 1344123 in the report) was characterised in three soils using the batch equilibrium method. The three soils were a sandy loam (Abington), a loamy sand (Munster) and a silt loam (Sarotti) according to USDA soil classification. The study was carried out with air-dried soil in the dark at 20°C ± 2 °C.

Preliminary tests at a nominal concentration of 1 mg/L to determine the extent of adsorption indicated that M-10 was not adsorbed to soil to any significant extent at soil : solution ratios of 1:1, 1:2 and 1:5 with 91 to 100% of applied radioactivity remaining in the aqueous supernatant after 24 hours. It was concluded that Freundlich isotherms could not be reliably generated due to the low adsorption of the test substance to soil. M-10 was stable throughout the study.

Single point K_d and K_{oc} values are summarised below:

Soil	Abington		Münster		Sarotti	
	Sandy loam		Loamy sand		Silt loam	
% OC	2.6		1.1		1.3	
	K_d (mL/g)	K_{oc} (mL/g)	K_d (mL/g)	K_{oc} (mL/g)	K_d (mL/g)	K_{oc} (mL/g)
Preliminary Test 1						
Ratio 1:1	0.00	0.00	0.00	0.00	0.03	2.06
Ratio 1:2	0.00	0.00	0.20	18.1	0.004	3.29
Ratio 1:5	0.01	0.22	0.07	6.63	0.35	26.6
Mean	**0.003**	**0.07**	**0.09**	**8.2**	**0.14**	**10.7**

% OC = % organic carbon

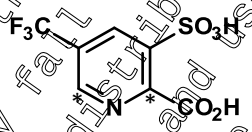
To provide additional information on the potential mobility of M-10, the extent of adsorption to soil after short ageing periods was investigated after application of the test item at an application rate of 1 mg/kg. The test systems were aged for 0, 3, and 8 days under aerobic conditions before each system was flooded with a 0.01 M calcium chloride solution to create a soil:solution ratio of 1:1. After desorption equilibration time of 24 hours, 0 to 6.8% of applied radioactivity was adsorbed to soil.

I. Materials and Methods

A. Materials

1. Test Items

[2,6-Pyridyl-^{14}C]-M-10 (referred to as AE 1344123 in the report)



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

Specific Activity: 31.3 mCi/mmole, 237,048 dpm/µg

Radiochemical Purity: 95.6% (HPLC)

Sample/Batch ID: SEL 1191/C-941

Stability of test compound: Stable during the equilibrium procedure.

2. Test Soils

Sorption tests were performed with three agricultural soils from UK and Germany selected to be the same as those used for the aerobic soil rate of the degradation study. Surface soil was collected from top 30 cm and shipped to the test site, where it was maintained under alfalfa cover in a greenhouse.

The soils were fully characterised, with respect to texture, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study.

Table 7.1.3.1.2- 32: Physico-chemical properties of test soils

Characteristic / Code	Munster	Abington	Sarotti
Soil ID	03/07	03/06	03/10
Origin	Germany	England, UK	Germany
Location	Munster	Great Abington, Cambridgeshire	Hattersheim
Textural Class (USDA)	Loamy sand	Sandy Loam	Silt Loam
Particle Size Analysis, ADAS:			
Total Sand (0.063 – 2.00 mm) (%)	77	68	15
Silt (0.002 – 0.063 mm) (%)	20	18	62
Clay (< 0.002 mm) (%)	4	14	23
Textural Class (ADAS)	Loamy sand	Sandy Loam	Silty Clay Loam
pH			
Water	6.6	8.1	8.3
KCl	5.5	7.7	7.7
$CaCl_2$	5.4	7.2	7.5
Organic Carbon (%)	1.1	2.6	1.3
Organic Matter (%)	1.9	4.5	2.2
Maximum Water Holding Capacity (g/100 g dry matter)	46.5	57.1	52.1
Soil moisture (%)	14.4	10.0	11.8
$Ca_{exchangeable}$ (meq/100g)	1.5	19.9	34.3
$Mg_{exchangeable}$ (meq/100g)	0.2	1.6	1.6
$Na_{exchangeable}$ (meq/100g)	<0.05	0.1	0.1
$K_{exchangeable}$ (meq/100g)	0.4	1.3	0.9
$Mn_{exchangeable}$ (meq/100g)	<0.05	<0.05	<0.05
$CaCO_3$eq. (g/kg)	<0.05	73.5	13.4
Phosphorus total (mg/kg)	617.8	1586.3	728.8
Nitrogen total (mg/kg)	1077.9	2380.1	1470.2

B. Study Design

1. Experimental Conditions

The test systems consisted of 30 mL silanised borosilicate glass centrifuge tubes capped with Teflon-lined screw caps. Preliminary tests to determine the adsorption of the test item to glassware (without soil) and to determine the optimal soil-to-solution ratio were conducted. The soils were pre-equilibrated by shaking with 0.01 M calcium chloride solution for at least 12 hours prior to the addition of test substance. The test was conducted at a nominal concentration of 1.0 µg/mL [2,6-Pyridyl-^{14}C]-M-10.

Soil Solution Preliminary Test

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		15, 10 and 5 g (dry weight) per replicate
Equilibration solution		0.01 M $CaCl_2$ shaken overnight
Control (preliminary experiment)		No soil (test item in 0.01 M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 1.0 µg/mL
	Initial concentrations of M-10 by LSC	Not stated
Identity and concentration of co-solvent		Dosing stock made up in 1:1 acetonitrile:water
Soil: Solution ratio		1:1, 1:2, and 1:5 i.e. 15, 10 or 5 g soil dry weight equivalent to 15 mL solution
Number of replicates		Duplicate
Equilibration conditions	Time	24 h
	Temperature	20 ± 2 °C
	Dark	In the dark
	Shaking method	Benchtop shaker (low setting)
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	3100 G
	Duration	20 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

As Freundlich isotherms could not be reliably generated due to the low adsorption of the test substance to soil, the aged sorption of M-10 was assessed.

The tests were performed at a concentration of 1 mg/kg dry weight of soil. The test item [2,6-Pyridyl-^{14}C]-M-10 dissolved in acetonitrile:water (1:1, v/v) (29 µL) was applied to soil. Soil samples were adjusted to a moisture content equivalent to 40% of maximum water holding capacity.

Duplicate samples of Abington, Münster and Sarotti soils were removed for analysis after 0, 2 and 8 days of incubation.

Aged Desorption Test

Parameter		Description
Soil condition		Soils moisture adjusted to 40% MWHC
Soil sample weight		15 g (dry weight) per replicate
Equilibration solution		0.01M $CaCl_2$ shaken overnight
Test item concentration	Nominal application rates	1.0 µg/g soil
	Initial concentrations of M-10	0.99 µg/g soil
Identity and concentration of co-solvent		Dosing stock made up in 1:1 acetonitrile:water, 29 µL
Soil: Solution ratio		1:1 i.e. 15 g soil (dry weight equivalent) to 15 mL solution
Number of replicates		Duplicate
Sampling		0, 3 and 8 days
Number of desorption cycles		1
Soil: Solution ratio		1:1
Number of replicates	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 hours
	Temperature	20±1°C
	Dark	In the dark
	Shaking method	Benchtop shaker (low setting)
Method of separation of supernatant		Centrifugation
Centrifugation	3100 G	Not reported
	20 minutes	10 minutes
	Supernatant was carefully decanted.	Supernatant was carefully decanted.

3. Analytical Procedures

Soil samples, as part of the soil solution preliminary test or after ageing, were shaken with 0.01M calcium chloride solution for 24 hours at 20 °C on a benchtop shaker (low setting). Aged soil samples were then extracted with methanol/water (1:1, v/v). An extraction step was not conducted for Day 8 samples.

Radioactivity in supernatants and soil extracts was determined by liquid scintillation counting (LSC). Following homogenisation, residues remaining in soil were determined by combustion.

Supernatants from the soil solution preliminary test were analysed by HPLC with radiodetection.

II. Results and Discussion

A. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption. The percentage of applied radioactivity in the $CaCl_2$ solution shaken for 24 hours was 89.0%.

Preliminary tests at a nominal concentration of 1 mg/L to determine the extent of adsorption indicated that M-10 was not adsorbed to soil to any significant extent at soil : solution ratios of 1:1, 1:2 and 1:5 with 91 to 100% of applied radioactivity remaining in the aqueous supernatant after 24 hours. It was concluded that Freundlich isotherms could not be reliably generated due to the low adsorption of the test substance to soil.

Table 7.1.3.1.2- 33: Preliminary test: Mass balance of M-10 (% AR, mean values)

Soil Solution Ratio	Abington		Münster		Sarotti	
	Supernatant	Adsorbed	Supernatant	Adsorbed	Supernatant	Adsorbed
1:1	100.6	-0.6	100.2	-0.2	97.4	2.6
1:2	100.0	-0.03	91.0	9.0	97.9	2.1
1:5	99.9	0.1	98.6	1.4	93.5	6.5

The amount of M-10 in supernatant from 1:2 soil solution ratio was 93.7%, 94.6% and 94.5% ROI for Abington, Munster and Sarotti soils, respectively, confirming that M-10 was stable throughout the study.

Single point K_d and K_{oc} values are summarised below:

Table 7.1.3.1.2- 34: Preliminary test: Single point K_d and K_{oc} values for M-10

Soil	Abington		Münster		Sarotti	
	Sandy loam		Loamy sand		Silt loam	
% OC	2.6		1.1		1.3	
	K_d (mL/g)	K_{oc} (mL/g)	K_d (mL/g)	K_{oc} (mL/g)	K_d (mL/g)	K_{oc} (mL/g)
Preliminary Test 1						
Ratio 1:1	0.00	0.00	0.00	0.00	0.03	2.06
Ratio 1:2	0.00	0.00	0.20	18.1	0.004	3.29
Ratio 1:5	0.01	0.25	0.07	6.63	0.35	26.6
Mean	**0.003**	**0.07**	**0.09**	**8.2**	**0.14**	**10.7**

% OC = % organic carbon

B. Results of aged sorption test

The aged sorption test showed that ageing did not increase the adsorption of the test item and there was no significant adsorption of M-10 to soil over 8 days. Initial Day 0 samples had low mass balance recoveries (< 90% AR) and were repeated. The final mass balance for the aged sorption samples ranged from 92.1 to 99.1%, 91.6 to 106.0%, and 91.9 to 100.7% AR for the Abington (sandy loam), Münster (loamy sand), and Sarotti (silt loam) soils, respectively.

Table 7.1.3.1.2- 35: Aged desorption test: Mass balance of M-10 (% AR)

DAT	Replicate	Supernatant	Extract	Remaining in soil	Mass balance
Abington (Sandy loam)					
Day 0	A1	55.3	-	30.9	86.2 [A]
	B1	55.3	-	30.8	86.2 [A]
	A2	61.1	23.4	14.5	99.0
	B2	60.1	23.6	14.7	98.4
Day 3	A	56.9	25.3	13.9	96.1
	B	58.0	23.6	15.6	97.1
Day 8	A	56.2	-	35.9	92.1
	B	62.5	-	36.6	99.1
Münster (Loamy sand)					
Day 0	A1	66.0	-	26.6	92.6 [A]
	B1	64.1	-	26.2	90.3 [A]
	A2	70.6	21.9	10.0	102.4
	B2	67.6	23.0	9.5	100.1
Day 3	A	72.7	23.5	9.7	106.0
	B	70.4	22.0	9.2	101.6
Day 8	A	64.0	-	30.4	94.4
	B	64.2	-	27.4	91.6
Sarotti (Silt loam)					
Day 0	A1	58.0	-	34.3	92.3 [A]
	B1	57.7	-	31.3	89.0 A
	A2	60.2	22.8	15.8	98.8
	B2	60.3	22.4	17.7	100.4
Day 3	A	59.1	23.6	17.9	100.7
	B	58.8	23.8	16.9	99.5
Day 8	A	56.2	-	34.6	91.9
	B	58.5	-	36.3	94.8

[A] Day 0 samples repeated
- Samples not extracted

After desorption equilibration time of 24 hours, 0 to 6.8% of applied radioactivity was adsorbed to soil.

Table 7.1.3.1.2- 36: Aged desorption test: Single point K_d and K_{oc} values for M-10

DAT	Initial (µg)	Solution concn. (µg/g)	Soil concn. (µg/g)	% adsorbed to soil	Mean K_d (mL/g)	Mean K_{OC} (mL/g)
Abington (Sandy loam)						
Day 0	0.99	1.05	-0.06	0 (-5.8) [A]	0.00	0.00
		1.03	-0.04	0 (-4.0)		
Day 3		0.98	0.02	1.8	0.01	0.49
		0.99	0.01	0.7		
Day 8		0.96	0.04	3.5	0.02	0.70
		1.07	-0.07	0 (-7.3)		
Münster (Loamy sand)						
Day 0	0.99	0.96	0.04	3.6	0.03	2.50
		0.93	0.07	6.8		
		1.03	-0.04	0 (-3.6)		
		1.00	-0.01	0 (-0.9)		
Day 3		1.06	-0.06	0 (-6.3)	0.00	0.00
		1.02	-0.03	0 (-2.7)		
Day 8		0.99	0.00	0.5	0.03	2.61
		0.94	0.05	5.0		
Sarotti (Silt loam)						
Day 0	0.99	0.97	0.02	2.1	0.01	0.54
		1.03	-0.03	0 (-3.1)		
		1.02	-0.03	0 (-3.0)		
Day 3		1.02	-0.03	0 (-2.6)	0.00	0.00
		1.02	-0.02	0 (-2.1)		
Day 8		0.97	0.02	2.0	0.01	0.80
		1.01	-0.02	0 (-2.0)		

[A] Negative numbers given in parenthesis where mass balance >100%. A value of zero used in determinations

II. Conclusion

Preliminary soil adsorption tests established Freundlich isotherms could not be reliably generated for M-10 (AE 1344123) due to its low adsorption to soil. Additional tests to assess the extent of adsorption after short ageing periods showed no significant change in the extent of adsorption.

Single point K_d values determined in preliminary tests ranged from 0.003 to 0.14 mL/g, with K_{oc} values ranging from 0.07 to 10.7 mL/g.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of M-10 in soil.

Data Point:	KCA 7.1.3.1.2/07
Report Author:	[redacted]
Report Year:	2003
Report Title:	Estimation of the adsorption coefficient (Koc) of AE C638206, metabolites AE 1388273 (P7), P2 (14C-labelled) and P3 (14C-labelled) on soil using high performance liquid chromatography (HPLC)
Report No:	M-223531-01-2
Document No:	M-223531-01-2
Guideline(s) followed in study:	OECD: Guideline 121, Jan. 2001
Deviations from current test guideline:	No
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The soil mobility of a number of fluopicolide metabolites was investigated using the OECD 121 HPLC based method. Soil adsorption coefficient (K_{OC}) values for the metabolites M-11/M-12 (called P2 in the report), M-13 (called P3 in the report) and M-14 (called AE 1388273 in the report) were calculated.

Sixteen reference standards, including four substances structurally related to the test items, of known K_{OC} values from batch equilibrium experiments were chromatographed using a CN-based HPLC method to determine an average capacity factor k'. Sodium nitrate was used to determine the HPLC system dead time (to). A regression line was plotted against the determined k' values and the known K_{OC} values (log k' vs. log K_{OC}) of the reference standards.

The test items were chromatographed during the same sample sequence as reference substances, and the average k' value was determined. The K_{OC} values for M-11/M-12, M-13 and M-14 were estimated by interpolation from the reference substance regression line. Reference substances structurally related to the test items were used to improve the accuracy of the method.

Linear regression of measured k' values against literature K_{OC} values and K_{OC} values for structurally related reference substances yielded a correlation coefficient of r = 0.8100 at pH 6 and r = 0.8520 at pH 2.5, respectively.

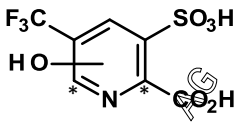
The estimated K_{OC} value for M-14 was 19.2 mL/g at pH 6.0 and 133.4 mL/g at pH 2.5. For M-13 an estimated K_{OC} value of 2.6 mL/g at pH 6 was obtained. At pH 2.5, no evaluation of the chromatograms was possible. No reliable results could be obtained for M-11/M-12 at either pH 6.0 or pH 2.5.

I. Materials and Methods

A. Materials

1. Test Items

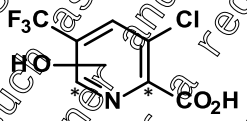
[2,6-Pyridyl-^{14}C]-M-11/M-12 (referred to as P2 (^{14}C labelled) in the report)



* Denotes position of [^{14}C]-radiolabel, 2 isomers

Radiochemical Purity:	Not stated
Sample ID:	HF24P2A
Source:	Isolated from leachate (KCA 7.1.4.2/02, M-218465-01-1) generated in a lysimeter study in which [2,6-Pyridyl-^{14}C]-fluopicolide was applied (KCA 7.1.4.2/01, M-218506-01-1)

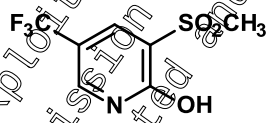
[2,6-Pyridyl-^{14}C]-M-13 (referred to as P3 (^{14}C labelled) in the report)



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

Radiochemical Purity:	Not stated
Sample ID:	HF24P302
Expiry Date:	Isolated from leachate (KCA 7.1.4.2/02, M-218465-01-1) generated in a lysimeter study in which [2,6-Pyridyl-^{14}C]-fluopicolide was applied (KCA 7.1.4.2/01, M-218506-01-1)

M-14 (referred to as AE 1388273 (P7) in the report)



Chemical Purity:	99.9%
Sample/Batch ID:	AZ 10592
Expiry Date:	25 March 2005

2. Reference Substances

Sixteen reference standards were used; Acetanilide, N,N-dimethyl-benzamide, Atrazine, Isoproturon, Triadimenol, Linuron, Methiocarb, Fenthion, Pyrazophos, Phenantrene, Cyfluthrin including four substances structurally related to the test items used to improve the accuracy of the method; fluopicolide, M-02 (AE C657188), M-05 (AE 1344122) and M-10 (AE 1344123). Sodium nitrate was used measure the dead time (t_0) of the HPLC system.

A. Study Design

1. Experimental Conditions

For M-11/M-12 and M-13 the entire amount of radiolabelled material was 8 and 1.1 µg respectively.

It was not possible to conduct standard batch equilibrium adsorption/desorption studies on these metabolites which were isolated from lysimeter leachate. Structural elucidation and accurate mass were determined but it was not possible to identify the position of hydroxyl groups on the pyridyl ring structure. Attempts to synthesis the most probable structures were unsuccessful and consequently the exact structure of metabolites M-11, M-12 and M-13 is not known. However small amounts of pure isolated radiolabelled material from the structural elucidation work were retained as analytical standards and were used to estimate the soil adsorption coefficients (K_{oc}).

Test items and reference standards were dissolved in methanol at a concentration of 1 mg/mL and then diluted to ca. 5 mg/L in methanol : 0.01M citrate buffer pH 6, 55:45 (v/v) prior to HPLC analysis.

HPLC was performed on analytical columns packed with a commercially available cyanopropyl solid phase containing both lipophilic and polar moieties. As required in OECD 121 for dissociable substances, two tests were performed with mobile phases at pH 6 and pH 2.5 so sorption behaviour of both ionized and non-ionized forms was assessed.

pH 6: Methanol : 0.01 M citrate-buffer adjusted to pH 6.0 with sodium hydroxide (55/45 v/v)

pH 2.5: Methanol : milli-Q-water adjusted to pH 2.5 with o-phosphoric acid (55/45 v/v).

Sodium nitrate was used to determine the HPLC system dead time (t_0).

2. Calculations

The capacity factors k' were calculated from the dead time (t_o) and retention times (t_R) of the test items and the selected reference standards. A regression line of the measured k' values of the reference standards against the known K_{oc} values (log k' vs. Log K_{oc}) was determined. The test items M-11/M-12, M-13 and M-14 were chromatographed during the same sample sequence as the reference substances and the average k' value was determined. K_{oc} values were estimated by interpolation from the reference substance regression line. The reference standards fluopicolide and three pyridyl ring metabolites M-02, M-05 and M-10, which are structurally closely related to the test items, were used to improve the accuracy of the method.

Calculations were performed using the computer software EXCEL (OFFICE 97®, Microsoft).

II. Results and Discussion

HPLC retention time data for the reference compounds are given below. The dead time (t_o) was determined to be 1.504 min at pH 6.0 and 1.362 min at pH 2.5 using sodium nitrate. Variability of the retention times from repetitive injections was low, confirming HPLC system stability throughout the analysis period.

The estimated K_{OC} value for M-14 is 19.2 mL/g at pH 6.0 and 133.4 mL/g at pH 2.5.

For M-13 an estimated K_{OC} value of 2.6 at pH 6 was obtained. At pH 2.5, no evaluation of the chromatograms was possible since the substance was eluted as a highly polar, broad area showing more than one unresolved peak.

No reliable results could be obtained for M-11/M-12 at pH 6 or pH 2.5. Due to the low amount of material available the detected peaks at different wavelengths were at or below the limit of detection.

Table 7.1.3.1.2- 37: HPLC retention time, K_{OC} and calculated K_{OC} at pH 6

Substance	Ret. Time (mins)	k'	Log k'	Mean log K'	K_{OC}	Log K_{OC}	Mean log K_{OC}
Reference standards							
Acetanilide	2.482 2.479	0.65 0.65	-0.187 -0.188	-0.19	17.8	1.25	1.25
N,N-dimethyl-benzamide	2.561 2.553	0.70 0.70	-0.153 -0.157	-0.15	33.1	1.52	1.52
Atrazine	2.702 2.690	0.80 0.79	-0.099 -0.103	-0.10	64.6	1.81	1.81
Isoproturon	2.855 2.852	0.90 0.90	-0.047 -0.048	-0.05	72.4	1.86	1.86
Aniline	2.482 2.483	0.65 0.65	-0.187 -0.187	-0.19	117	2.07	2.07
Traimenol	2.958 2.958	0.97 0.97	-0.015 -0.015	-0.01	251	2.40	2.40
Linuron	3.188 3.187	1.12 1.12	0.049 0.049	0.05	389	2.59	2.59
Methiocarb	2.924 2.924	0.94 0.94	-0.025 -0.025	-0.03	1259	3.10	3.10
Fenthion	3.876 3.870	1.58 1.57	0.0198 0.197	0.20	2042	3.31	3.31
Pyrazophos	3.819 3.818	1.54 1.54	0.187 0.187	0.19	4467	3.65	3.65
Phenanthrene	4.359 4.361	1.90 1.90	0.278 0.279	0.28	12303	4.09	4.09
Cyfluthrin	7.761 7.746	4.16 4.15	0.619 0.618	0.62	64300	4.81	4.81
M-02 (AE C657188)	1.608 1.604	0.07 0.07	-1.164 -1.178	-1.17	6.0	0.78	0.78
M-05 (AE 1344122)	1.629 1.627	0.08 0.08	-1.081 -1.088	-1.08	26	1.41	1.41
M-10 (AE 1344123)	1.436 1.429	-0.05 -0.05	Negative k', calculation not possible				
Fluopicolide	3.068 3.066	1.04 1.04	0.017 0.016	0.02	310	2.49	2.49
Test items							
M-14 (AE 1388273)	1.793 1.795	0.19 0.19	-0.711 -0.714	-0.712	19.3 19.2	1.29 1.28	1.28
M-11/M-12	No reliable peak obtained						
M-13	1.616 1.603	0.07 0.07	-1.129 -1.183	-1.156	2.9 2.3	0.46 0.35	0.41

Dead time was determined to be 1.504 mins

Table 7.1.3.1.2- 38: HPLC retention time, K_{OC} and calculated K_{OC} at pH 2.5

Substance	Ret. Time (mins)	k'	Log k'	Mean log K'	K_{OC}	Log K_{OC}	Mean log K_{OC}
Reference standards							
Acetanilide	2.477 2.471	0.82 0.81	-0.087 1.0.89	-0.09	17.8	1.25	1.25
N,N-dimethyl-benzamide	2.556 2.554	0.88 0.88	-0.057 -0.058	-0.06	33.1	1.52	1.52
Atrazine	2.734 2.736	1.01 1.01	0.003 0.004	0.00	64.6	1.81	1.81
Isoproturon	2.812 2.812	1.07 1.07	0.028 0.028	0.003	72.4	1.86	1.86
Traimenol	2.927 2.927	1.15 1.15	0.061 0.061	0.06	251	2.40	2.40
Linuron	3.119 3.117	1.29 1.29	0.111 0.110	0.11	389	2.59	2.59
Methiocarb	2.869 2.865	1.11 1.10	0.044 0.043	0.04	1259	3.10	3.10
Fenthion	3.694 3.681	1.71 1.70	0.234 0.231	0.23	2042	3.31	3.31
Pyrazopos	3.648 3.646	1.68 1.68	0.225 0.236	0.22	4467	3.65	3.65
Phenanthrene	4.088 4.076	2.00 1.99	0.302 0.300	0.30	12303	4.09	4.09
Cyfluthrin	6.849 6.811	4.03 4.00	0.605 0.602	0.60	64300	4.81	4.81
M-02 (AE C657188)	1.831 1.823	0.34 0.34	-0.462 -0.471	-0.47	6.0	0.78	0.78
M-05 (AE 1344122)	2.008 2.008	0.47 0.47	-0.326 -0.323	-0.32	26	1.41	1.41
M-10 (AE 1344123)	1.408 1.411	0.03 0.04	-1.467 -1.439	-1.45	1	0.00	0.00
Fluopicolide	2.982 2.979	1.19 1.19	0.076 0.075	0.08	310	2.49	2.49
Test items							
M-14 (AE 1388273)	2.355 2.359	0.73 0.73	-0.137 -0.135	-0.136	132.8 134.1	2.12 2.13	2.13
M-11/M-12	No reliable peak obtained						
M-13	No reliable peak obtained						

Dead time was determined to be 1.362 mins

III. Conclusion

It was not possible to estimate robust K_{oc} values for M-11/M-12 or M-13 using the OECD 121 method as the compounds were too polar to be retained. For these metabolites a K_{oc} value of 0 will be used in groundwater exposure assessments. For M-14 it was possible to estimate a K_{oc} value of 133.4 mL/g at pH 2.5 and 19.2 mL/g at pH 6. However two OECD 106 studies are available for this metabolite and are considered more reliable to establish the soil adsorption properties of M-14.

Assessment and conclusion by applicant:

The study is considered as supportive data to assess the soil adsorption characteristics of M-11, M-12 and M-13. For M-14 (AE 1388273) the results of this study have been superseded by two OECD 106 soil adsorption studies (see KCA 7.1.3.1.2/12 and KCA 7.1.3.1.2/13).

Data Point:	KCA 7.1.3.1.2/08
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE C653711: Re-evaluation of Freundlich adsorption isotherm of AE C653711 (BAM) (position paper)
Report No:	M-224926-01-2
Document No:	M-224926-01-2
Guideline(s) followed in study:	-
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially 143ecognized testing facilities:	No, not conducted under GLP/Officially 143ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption of M-01 (referred to as AE C653711 in the position paper) was characterised in five soils using the batch equilibrium method. In one of the soils (Connecticut RL-51) there was a clear outlier in the data at one concentration. Re-evaluation of the data, excluding the outlier, gave a significantly improved fit by linear regression (R^2 >0.99 vs 0.86). The K_d, K_{oc} and 1/n values were re-determined using the Freundlich equation for Soil RL-51 and these values are considered appropriate for use in risk assessments.

Phase	Soil	Units	Connecticut Sandy Loam RL-51
Adsorption	$K_{d,ads}$	[mL/g]	0.3588
	1/n	-	0.970
	R^2	-	0.991
	$K_{OC,ads}$	[mL/g]	39.9

I. Materials and Methods

The adsorption and desorption of M-01 (called AE C653711, 2,6-dichlorobenzamide in the statement) was investigated in five different soils (KCA 7.1.3.1.2/01, [redacted] 2001, M-235837-01-1). In four of the soils investigated the correlation of data by linear regression was excellent (R^2 >0.99) but in one soil there was a clear outlier which resulted in a lower correlation (R^2 0.86). This position paper was prepared to re-evaluate the Freundlich isotherm data for the Connecticut sandy loam soil RL-51 for use in risk assessments.

Table 7.1.3.1.2- 39: Soil and solution concentration of M-01 in Soil RL-51

Nominal Concentration (µg/mL)	Concentration achieved (µg/mL)	Replicate	Connecticut RL-51	
			Equilibrium Solution Concentration (LnCe)	Equilibrium Soil Concentration (Lnx/m)
0.20	0.25	A	-1.428	-2.655
		B	-1.449	-2.326
0.50	0.54	A	-0.636*	-3.038*
		B	-0.629*	-3.533*
1.0	1.13	A	0.05	-0.901
		B	0.047	-0.87
5.0	5.58	A	1.642	0.716
		B	1.652	0.576
10	10.94	A	2.326	1.237
		B	2.338	1.045

* Excluded as outlier in re-evaluation isotherm data

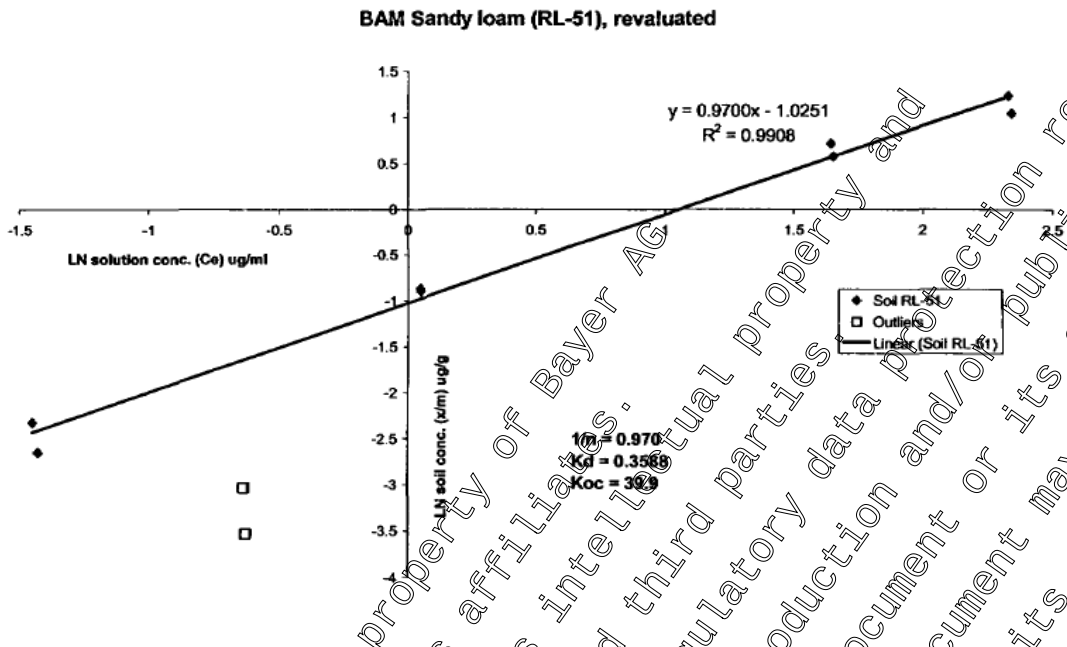
II. Results and Discussion

A significantly improved fit was obtained (R^2 0.99 vs 0.86) when the data for the nominal starting concentration of 0.5 µg/mL were excluded and the data re-evaluated by linear regression (Table 7.1.3.1.2- 40 and Figure 7.1.3.1.2- 15). The following K_d, K_{oc} and 1/n values were determined for Connecticut sandy loam soil RL-51.

Table 7.1.3.1.2- 40: Adsorption Freundlich Isotherm in Soil RL-51

	Re-evaluated Analysis
Correlation	0.9908
Slope (1/n)	0.970
Intercept	-1.0251
n	1.031
K_d	0.3588
K_{oc}	39.9
% organic carbon	0.9

Figure 7.1.3.1.2- 15: Re-evaluated Freundlich Adsorption Isotherm for Soil RL-51



III. Conclusion

K_d, K_{oc} and 1/n values were re-determined for Soil RL-51 and these values are considered appropriate for use in risk assessments.

Assessment and conclusion by applicant:

The statement is considered valid to assess the adsorption characteristics of M-01 in soil.

Data Poinut:	KCA 7.1.3.1.2/09
Report Author:	[redacted]
Report Year:	2017
Report Title:	[pyridyl-2,6-14C]AE 1413903: Adsorption / desorption on four soils
Report No:	S16-01250
Document No:	M-585208-01-1
Guideline(s) followed in study:	OECD Test Guideline No. 106 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009 US EPA OCSPP Test Guideline No. 835.1230
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted by RMS Austria 2018
GLP/Officially 146ecognized testing facilities:	Yes, conducted under GLP/Officially 146ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption / desorption behaviour of M-15 (referred to as AE 1413903 in the report) was studied in four soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
L[redacted]	[redacted]	Monheim, Germany	loamy sand	5.4	1.8
Dollendorf II	DD	Blankenheim, Germany	clay loam	7.3	5.2
H[redacted]	[redacted]	Burscheid, Germany	silt loam	6.0	2.4
[redacted]	[redacted]	Monheim, Germany	sandy loam	5.1	2.1

The adsorption phase of the study was investigated using samples of air-dried soil equilibrated in aqueous 0.01 M CaCl solution with soil-to-solution ratios of 1:2:1 (all soils). Test concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L of [^{14}C]-M-15 were applied in aqueous 0.01 M $CaCl_2$ solution. The desorption from pre-adsorbed soil samples was studied by addition of fresh aqueous 0.01 M $CaCl_2$ solution. Adsorption and desorption took place for 24 hours each. The test was performed in glass flasks with screw caps.

Following adsorption and desorption steps, the aqueous supernatants were separated by centrifugation and the amount of test item in the supernatant quantified by liquid scintillation counting (LSC). After the desorption step, radioactivity in air-dried soils was determined by combustion/LSC. The sorption parameters were calculated using Freundlich isotherms.

The test item was sufficiently stable throughout the study. Mean parental mass balances were 96.1% AR (soil AX), 95.9% (DD), 95.3% (HH) and 94.7% AR (soil WW).

Mean material balances were 95.7% AR for soil AX (range from 94.3 to 98.4% AR), 95.8% AR for soil DD (range from 91.8 to 101.5% AR), 94.6% AR for soil HH (range from 90.2 to 98.5% AR) and 94.2% AR for soil WW (range from 90.7 to 97.3% AR).

In the definitive adsorption test, radioactivity adsorbed was 34.6 – 39.9% AR (soil AX), 46.8 – 58.9% (DD), 38.8 – 47.1% (HH) and 31.5 – 35.7% (WW).

The calculated adsorption constants K_F of the Freundlich isotherms ranged from 0.380 to 0.728 for the tested soils. The Freundlich exponents 1/n ranged from 0.9196 to 0.9530, 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 were correlated with the organic carbon content of the soil to get a comparability of the adsorption behavior in different soils. For M-15 the K_{Foc} values ranged from 14.0 to 23.9 mL/g (mean: 19.2 mL/g).

At the end of the adsorption and desorption, 13.3 to 21.6%, -7.0 to 13.1%, 10.9 to 15.7% and 11.3 to 20.5% of the initially adsorbed amount were desorbed in soil [redacted], DD, [redacted] and [redacted], respectively. Negative values are observed due to the combination of a low desorption and variations in the measurement.

The desorption $K_{F(des)}$ and the normalized $K_{Foc(des)}$ values were not significantly higher than those obtained for adsorption.

There is no significant correlation between pH and adsorption for the investigated soils.

According to Briggs, M-15 can be classified as mobile for adsorption and desorption.

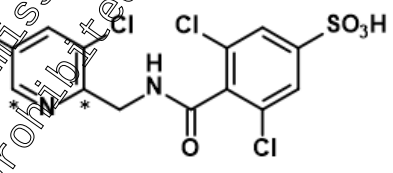
Soil	L[redacted]	Dollendorf II	H[redacted]	[redacted]
Soil type (USDA)	Loamy sand	Clay loam	Silt loam	Sandy loam
pH (0.01M $CaCl_2$)	5.4	7.3	6.0	5.1
Organic carbon [%]	1.8	5.2	2.4	2.1
$K_F^{(ads)}$ [mL/g]	0.431	0.728	0.500	0.380
1/n	0.953	0.920	0.923	0.950
$K_{F,OC}^{(ads)}$ [mL/g]	23.9	14.0	20.8	18.1
R^2	0.9999	0.9962	0.9997	0.9994
$K_F^{(des)}$ [mL/g]	0.475	0.825	0.598	0.412
1/n	0.945	0.916	0.932	0.940
$K_{F,OC}^{(des)}$ [mL/g]	26.4	15.9	24.9	19.6
R^2	0.9994	0.9975	0.9998	0.9987

1. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-M-15 (referred to as AE 1413903 in the report)



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

Specific Activity: 3.69 mCi/mmol

Radiochemical Purity: 98% (HPLC)

Sample/Batch ID: KML 10206

Stability of test compound: The test item was stable in aqueous 0.01 M $CaCl_2$ solution in the absence of soil. After incubation for 24 hours the test item was detected as 100 % of the injected radioactivity.

2. Test Soils

Sorption tests were performed with four agricultural soils from Germany selected to cover a representative range of soil physico-chemical properties. Soil samples were collected up to 20 cm depth, then transported to test facility where they were stored refrigerated (1-10°C). All soils were air-dried, sieved (≤2 mm) and sterilised prior to use. The soils were fully characterised with respect to texture, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study.

Table 7.1.3.1.2- 41: Physico-chemical properties of test soils

Characteristic / Code	L[redacted]	Dollendorf II	[redacted]	[redacted]
Soil ID	[redacted]	DD	[redacted]	[redacted]
Geographic Location	Monheim, Germany	Blankenheim, Germany	Burscheid, Germany	Monheim, Germany
Textural Classification (USDA)	Loamy sand	Clay loam	Silt loam	Sandy loam
Sand (2000 to ≥ 50 µm) (%)	79	25	55	
Silt (50 to ≥ 2 µm) (%)	18	62	32	
Clay (< 2 µm) (%)	3	13	13	
pH (0.01 M $CaCl_2$)	5.4	7.3	6.0	5.1
pH (water)	5.7	7.4	6.3	5.4
pH (saturated paste)	5.3	7.4	6.3	5.4
pH (1 N KCl)	5.2	7.0	5.1	4.8
Organic carbon (%)	1.8	5.2	2.4	2.1
Organic matter (%)	3.1	9.0	4.1	3.6
CEC (meq/100g soil)	7.9	20.7	11.1	10.0
Water Holding Capacity				
Maximum (g H_2O per 100 g DW)	49.9	89.5	61.1	55.7
at 1/10 bar, pF 2.0 (%)	15.0	43.0	35.3	23.0
Bulk Density (g/cm^3, disturbed)	1.19	0.96	1.02	1.08

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of glass flasks with screw caps.

In preliminary tests, the stability of the test item, the adsorption of the test item to glassware, the optimal soil-to-solution ratio and the appropriate adsorption and desorption equilibration times were determined.

Parental mass balance was established for all soils in preliminary tests. After equilibration for 48 hours, soil and solution phases were separated by centrifugation. Residues remaining on the soil after adsorption were exhaustively extracted three times with acetonitrile/water (4/1, *v/v*) for 30 minutes, followed microwave-assisted extraction with acetonitrile/water (4/1, *v/v*) at 60 °C for 15 minutes. After extraction radioactivity remaining in the soil was quantified by combustion.

Material balance was established for all soils in the definitive test. The main test was performed in duplicate. [^{14}C]-M-15 was dissolved in 0.01M calcium chloride solution at nominal concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L. Soil samples were prepared at a soil to solution ratio of 1.2:1 for all soils and shaken at in the dark at 20 ± 2°C. Following the preliminary tests, an equilibrium time of 24 hours was selected.

For all soils one desorption cycle was performed on all concentrations. The volume of solution removed after the adsorption step was replaced by an equal volume of [^{14}C]-M-15 stock solution. Test vessels were then shaken for a 24 hour desorption phase. After the desorption phase radioactivity remaining in the soil was quantified by combustion. 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.

Adsorption phase

Parameter		Description
Soil Condition		Soils were gently air-dried, sieved to ≤ 2 mm and equilibrated to study conditions for at least 16 hours with 25 mL aqueous 0.01 M $CaCl_2$ solution (corrected for soil moisture).
Have these soils been used for other laboratory studies?		The soils have been used in several degradation and sorption studies.
Soil sample weight		30 g dry weight equivalents per replicate.
Solution used for equilibration		aqueous 0.01 M $CaCl_2$ solution
Control used		$CaCl_2$ without soil
Test item concentrations	Nominal application rates	Nominal concentrations in test solution: 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, and 1.0 mg/L.
	Analytically measured concentrations	Concentrations in test solution: 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, and 1.0 mg/L.
Identity and concentration of solvent		methanol / water (1/1, v/v)
Soil-to-solution ratio		1.2:1 (i.e. 30 g soil dry weight to 25 mL solution (corrected for soil moisture).
pH of the equilibration solution (from preliminary test)	Initial	pH of aqueous 0.01 M $CaCl_2$ solution without soil: 6.53
	Final	pH with soil and test item after adsorption equilibrium: range 5.75 – 7.37
Number of replicats	Controls	Duplicate
	Treatments	Duplicate
Equilibration	Time	24 hours
	Temperature	21 °C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 rpm
	Shaking time	24 hours
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	1295 x *g*
	Duration	4 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

Desorption phase

Parameter		Description
Were the soil residues from the adsorption phase used?		Yes
Amount of test item present in the adsorbed state / adsorbed amount		The amounts of test item adsorbed to soil after asorption ranged from 31.4 to 58.9% AR
Number of desorption cycles		One desorption cycle was performed for each concentration.
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by fresh aqueous 0.01 M $CaCl_2$ solution. A total volume of 25 mL was used as equilibration solution.
Soil-to-solution ratio		1:5 l, i.e. 30 g soil dry weight to 25 mL solution (corrected for soil moisture).
Number of replications	Controls	Duplicate
	Treatments	Duplicate
Desorption equilibration	Time	24 hours
	Mean Temperature	21.1°C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 ± 2 rpm
	Shaking time	24 hours
Method of separation of supernatant		Centrifugation
Centrifugation	Speed	1295 x *g*
	Duration	4 min
	Method of separation of soil and solution	Supernatant was carefully decanted.

2. Analytical Procedures

After equilibration, soil and solution phases were separated by centrifugation and the concentration of M-15 in the water phase determined by LSC. M-15 remaining in the soil phase was then desorbed with 0.01M calcium chloride and the concentration determined by LSC. The radioactivity remaining in soil after the desorption phase was quantified by combustion.

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

Samples of supernatants, soil extracts and controls without soil from preliminary tests were analysed by HPLC. HPLC recovery was 101.3% AR and the LOQ was 5% AR. Supernatants and controls were characterized without any further processing whereas soil extracts were combined and concentrated (mean recovery on concentration 100 to 109%).

II. Results and Discussion

A. Results of preliminary tests

The test item was stable in aqueous 0.01 M $CaCl_2$ solution in the absence of soil. After incubation for 24 hours M-15 represented 100% AR. No adsorption of the test item to the test vessels was observed after shaking for 24 hours. A soil-to-solution ratio of 1.2:1 was used for all soils as the radioactivity adsorbed to soil represented 31.1 to 48.1% AR after 24 hours. An equilibration time of 24 hours was chosen for both the adsorption and desorption phase.

B. Transformation of test substance

For all soils at a soil/solution ratio of 1.2:1, mean parental mass balances of the test item were 96.1% AR (soil AX), 95.9% (DD), 95.3% (HH) and 94.7% AR (soil WW) over 48 hours equilibration time.

The stability was adequate to determine the test item distribution based on LSC measurements of the supernatant only in the adsorption and desorption experiments of the definitive test.

C. Findings

The radioactive material balance was calculated as sum of radioactivity detected in decanted supernatant solutions after the adsorption or desorption phase plus the radioactivity remaining in soil residues. Mean material balances were 95.6, 95.7, 94.5 and 94.1 AR for soils AX, DD, HH and WW, respectively (summarised in Table 7.1.3.1.2- 42). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table 7.1.3.1.2- 42: Definitive test: Mass balance of [2,6-Pyridyl-^{14}C]-M-15 (% AR)

Test concentration (mg/L)	[redacted]	Dollendorf II	[redacted]	[redacted]
1.0	94.3	94.0	98.5	95.4
0.50	95.7	96.8	96.0	94.8
0.10	94.6	94.7	95.3	92.2
0.05	94.8	91.8	90.3	90.8
0.01	98.5	101.5	92.4	97.3
Mean	95.6	95.7	94.5	94.1
SD	± 1.5	± 3.3	± 2.9	± 2.4

Mean values of duplicate samples
SD = standard deviation

The results of adsorption and desorption tests of [2,6-pyridyl-^{14}C]-M-15 onto four soils are summarised in Table 7.1.3.1.2- 43 to **Error! Reference source not found.**. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 16 to Figure 7.1.3.1.2- 19.

At the end of the adsorption phase 34.6 – 39.9% AR was adsorbed to soil AX, 46.8 – 58.9% AR was adsorbed to soil DD, 38.8 – 47.1% AR was adsorbed to soil HH, and 31.5 – 35.7% AR was adsorbed to soil WW.

The calculated Freundlich adsorption coefficients (K_f) ranged from 0.380 to 0.728 mL/g. The correlation coefficients of the individual isotherms ranged from 0.9962 to 0.9999. The Freundlich exponents 1/n ranged from 0.9196 to 0.9530, 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 were correlated with the organic carbon content of the soil to get a comparability of the adsorption behavior in different soils. For M-15 the K_{Foc} values ranged from 14.0 to 23.9 mL/g. There is no significant correlation between pH and adsorption.

Table 7.1.3.1.2- 43: Definitive test: Concentration of M-15 in aqueous and solid phase following 24 hours of adsorption.

Concentration (µg/mL)	L [redacted]		Dollendorf II		[redacted]		[redacted]	
	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.011	0.003	0.006	0.005	0.004	0.004	0.006	0.003	0.007
0.052	0.016	0.032	0.024	0.023	0.019	0.029	0.016	0.033
0.102	0.031	0.065	0.040	0.051	0.036	0.059	0.028	0.068
0.520	0.154	0.334	0.208	0.270	0.169	0.316	0.138	0.324
1.032	0.297	0.675	0.437	0.508	0.333	0.628	0.270	0.706

Table 7.1.3.1.2- 44: Summary of Freundlich adsorption/desorption constants K_f and K_{oc} values

Phase	Soil	Units	L [redacted]	Dollendorf II	[redacted]	[redacted]
Adsorption	$K_{F,ads}$	[mL/g]	0.431	0.728	0.500	0.380
	1/n	-	0.953	0.920	0.923	0.950
	R^2	-	0.9999	0.9962	0.9997	0.9994
	$K_{OC,ads}$	[mL/g]	23.9	14.0	20.8	18.1
Desorption	$K_{F,des}$	[mL/g]	0.475	0.825	0.598	0.412
	1/n	-	0.945	0.916	0.932	0.940
	R^2	-	0.9994	0.9975	0.9998	0.9987
	$K_{OC,des}$	[mL/g]	26.4	15.9	24.9	19.6

D. Evaluation of the Data according to EFSA Evaluators Checklist

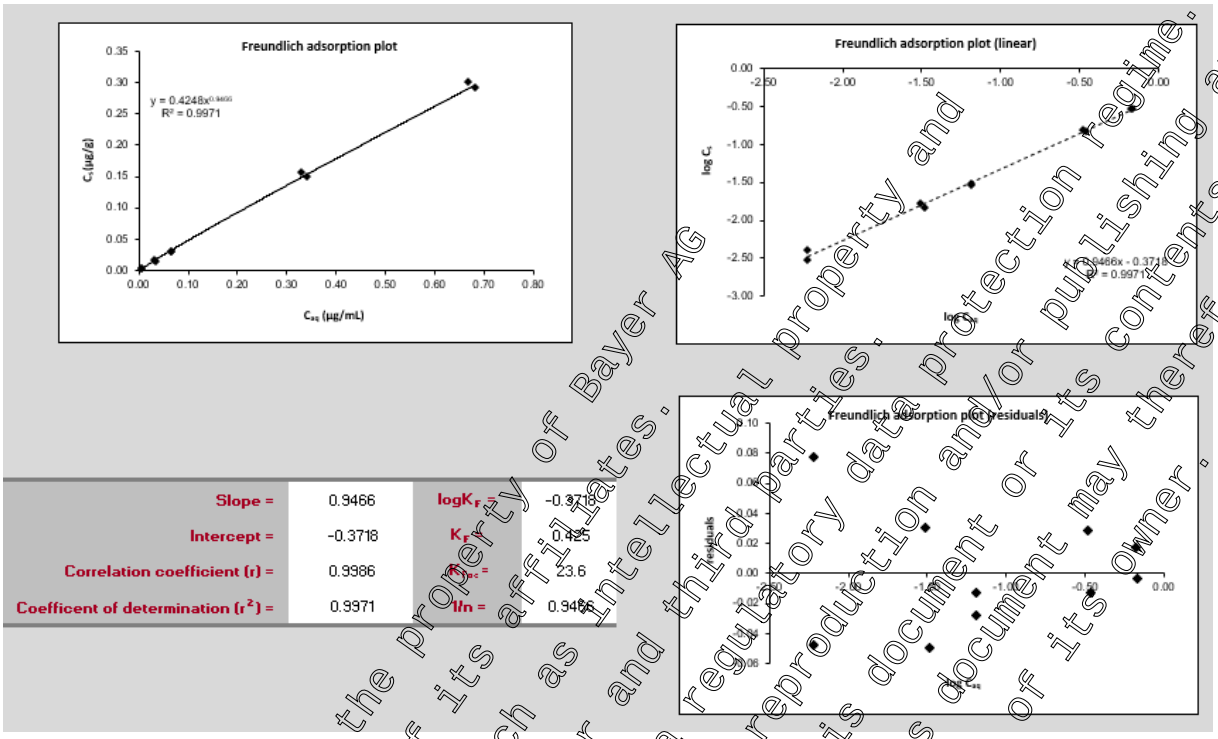
The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 45). The concentrations in the supernatant and the soil as given in the report were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.2- 45: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	L[redacted]	Dollendorf II	H[redacted]	[redacted]
Adsorption method	-	-	indirect	indirect	indirect	indirect
Soil solution ratio	g/mL		1.2:1	1.2:1	1.2:1	1.2:1
Mass balance of ^{14}C	%	>90%	94.0 – 100.9	89.1 – 102.2	88.5 – 102.3	90.5 – 95.3
f – due to loss processes (estimated)	%	-	3.9	4.1	4.7	5.3
Adsorbed percentage (δ)	%	>20%	34 – 45	43 – 64	39 – 46	32 – 45
K_D x soil:solution ratio		>0.3	0.52 – 0.80	0.76 – 1.50	0.62 – 0.86	0.46 – 0.60
$^{\#}K_{fE}$ / K_f	-	<1.2	1.12 & 1.13	1.09 & 1.09	1.14 & 1.13	1.20 & 1.20
ads K_F	L/kg	-	0.425	0.656	0.518	0.386
95% confidence interval	-	*	(0.374 – 0.482)	(0.511 – 0.842)	(0.482 – 0.557)	(0.358 – 0.417)
ads 1/n	-		0.947	0.894	0.943	0.957
95% confidence interval	-	*	(0.905 – 0.988)	(0.819 – 0.969)	(0.920 – 0.966)	(0.932 – 0.983)
ads R^2	-	>0.975	0.997	0.990	0.999	0.999
ads $K_{F,OC}$	L/kg		23.6	12.6	21.6	18.4
Visual fit to Freundlich isotherm	-	-	Good	Acceptable	Good	Good
Residual plots randomly distributed	-	-	Good	Good	Good	Good

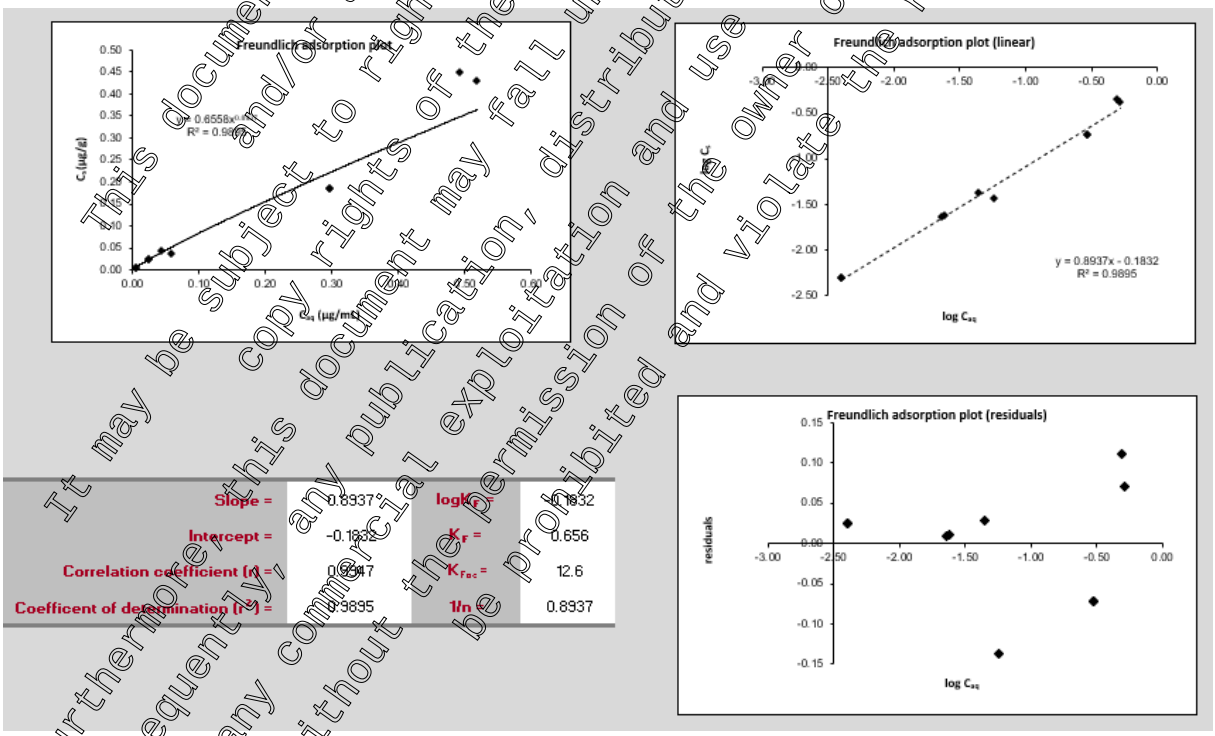
Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance of 88.5-102.3% (mass balances generally >90% with only two individual samples marginally < 90%) and % adsorption of 32-64% were all acceptable. The acceptability of the analytical method was confirmed over the entire range of concentrations measured (reported LOQ of LSC of 0.9 Bq stated to be 7x lowest measured value). The use of the indirect method was appropriate based on a Kd * soil/solution ratio > 0.3 in all soils. The graphical fits of the Freundlich equation based on the standard linear regression form using log-log transformed data alongside the associated residual plots were evaluated and found to be either acceptable or good. The R^2 of the standard linear regressions ranged from 0.990 to 0.999 and the visual fit of both the standard regression and the residual plots were acceptable.

Figure 7.1.3.1.2- 16: Freundlich Isotherms of M-15 in Soil L[redacted] ([redacted])



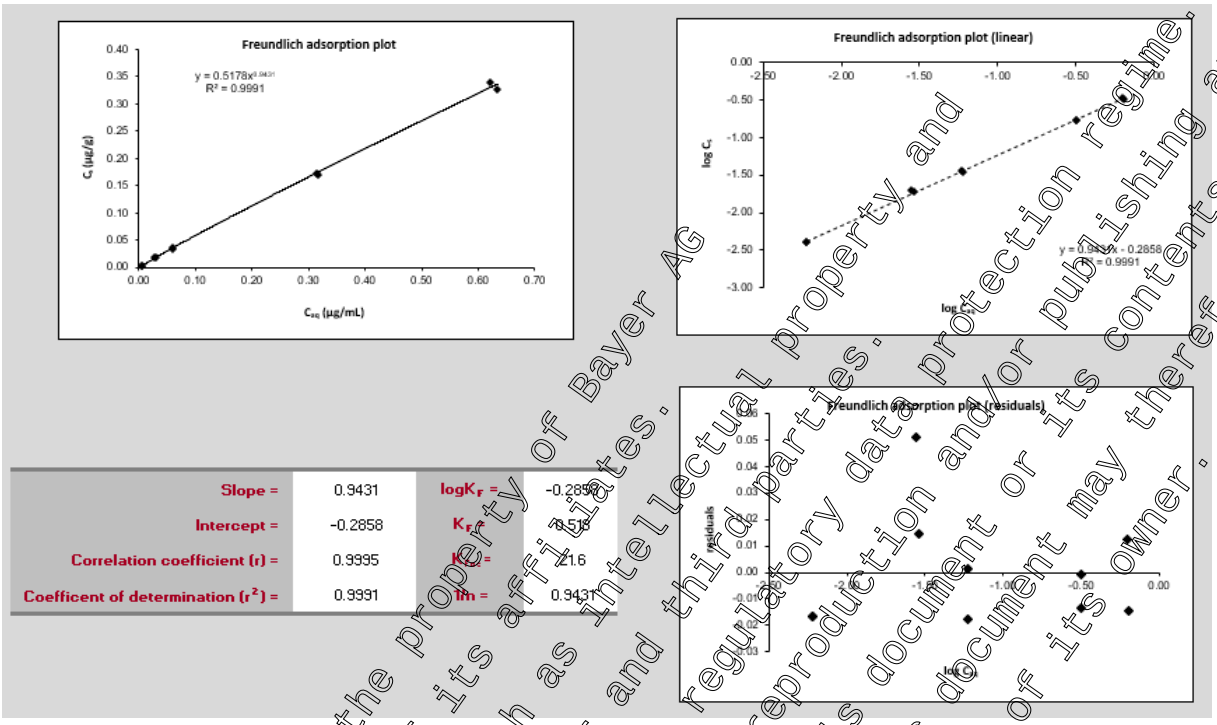
Slope =	0.9466	$\log K_F$ =	-0.3718
Intercept =	-0.3718	K_F =	0.425
Correlation coefficient (r) =	0.9986	K_{Foc} =	23.6
Coefficent of determination (r^2) =	0.9971	1/n =	0.9466

Figure 7.1.3.1.2- 17: Freundlich Isotherms of M-15 in Soil Dollendorf II (DD)



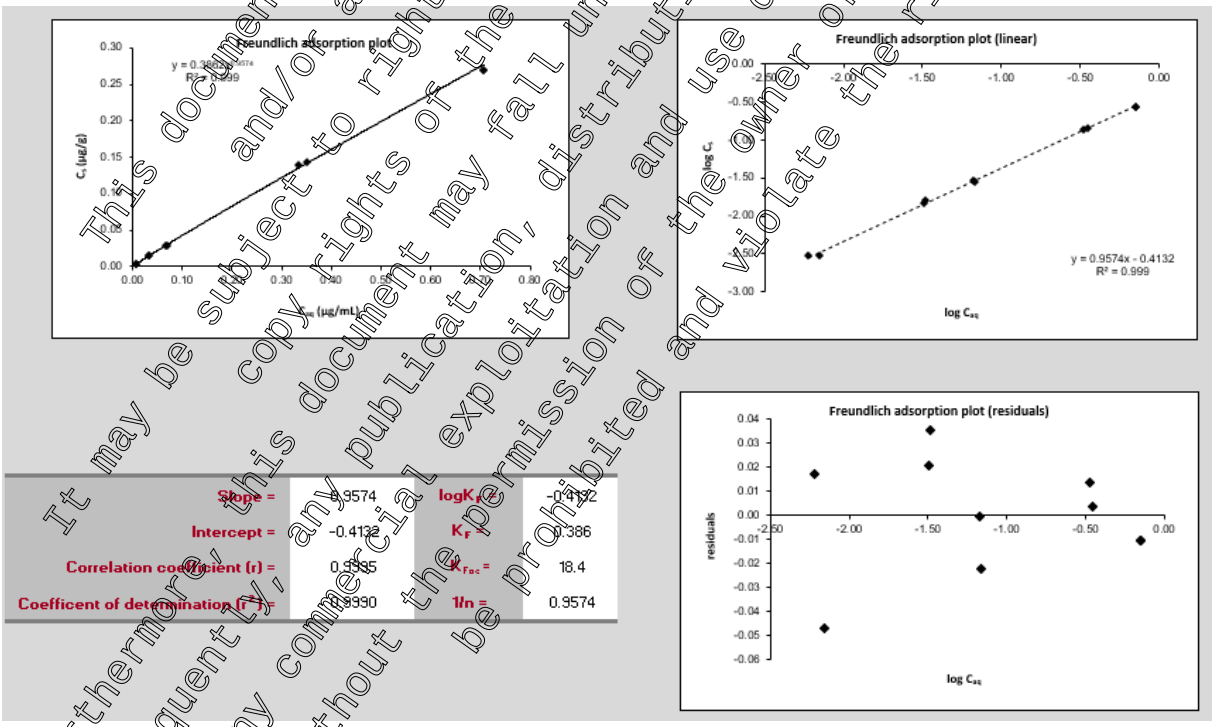
Slope =	0.8937	$\log K_F$ =	-0.1832
Intercept =	-0.1832	K_F =	0.656
Correlation coefficient (r) =	0.9947	K_{Foc} =	12.6
Coefficent of determination (r^2) =	0.9895	1/n =	0.8937

Figure 7.1.3.1.2- 18: Freundlich Isotherms of M-15 in Soil H[redacted] (HH)



Slope =	0.9431	$\log K_F$ =	-0.2858
Intercept =	-0.2858	K_F =	0.518
Correlation coefficient (r) =	0.9995	K_{Foc} =	21.6
Coefficent of determination (r^2) =	0.9991	1/n =	0.9431

Figure 7.1.3.1.2- 19: Freundlich Isotherms of M-15 in Soil [redacted] (WW)



Slope =	0.9574	$\log K_F$ =	-0.4132
Intercept =	-0.4132	K_F =	0.386
Correlation coefficient (r) =	0.9995	K_{Foc} =	18.4
Coefficent of determination (r^2) =	0.9990	1/n =	0.9574

A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 46. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 47.

Table 7.1.3.1.2- 46: Summary of Quality Criteria and Regulatory Interpretation

M-15			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
L[redacted]	loamy sand	[redacted]	9	0	0
Dollendorf II	clay loam	DD	8	1	0
[redacted]	silt loam	[redacted]	8	1	0
[redacted]	sandy loam	[redacted]	8	1	0

Table 7.1.3.1.2- 47: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
L[redacted]	loamy sand	[redacted]	23.9	23.6	0.953	0.947
Dollendorf II	clay loam	DD	14.0	12.6	0.920	0.894
H[redacted]	silt loam	[redacted]	20.8	21.6	0.923	0.943
[redacted]	sandy loam	[redacted]	18.0	18.4	0.950	0.957

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations.

III. Conclusion

The adsorption constants K_F of M-15 for the tested soils calculated based on the Freundlich isotherms ranged from 0.380 to 0.728 mL/g. The corresponding K_{Foc} values ranged from 14.0 to 23.9 mL/g. The Freundlich exponents 1/n were in the range of 0.920 to 0.953.

The desorption constants $K_{F(des)}$ of M-15 were not significantly higher than the respective adsorption constants, not indicating a strengthened binding of M-15 once adsorbed to soils representing conditions relevant for the environment.

Adsorption was shown to be correlated with organic carbon content. There was no significant correlation between pH and adsorption for the investigated soils.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed the results largely met the quality criteria and were therefore acceptable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption characteristics of M-15 (AE 1413903) in soil.

Data Point:	KCA 7.1.3.1.2/10
Report Author:	[redacted]
Report Year:	2020
Report Title:	Adsorption/desorption of [14C]-AE C653711 (BCS-AA65784) (BAM) M-01 in five soils
Report No:	S18-08481
Document No:	M-686388-01-1
Guideline(s) followed in study:	OECD Guideline No. 106, 2000 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009, 2013
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially 157ecognized testing facilities:	Yes, conducted under GLP/Officially 157ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption behaviour of M-01 (referred to as AE C653711 in the report) was studied in five soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C using the OECD 106 direct method with residues remaining on the soil after adsorption quantified directly.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
LUFA 2.1	2.1	Dudenhofen, Germany	Sand	5.2	0.59
LUFA 2.3	2.3	Offenbach, Germany	Sandy loam	6.2	0.61
LUFA 5M	5M	Mechtersheim, Germany	Sandy loam	7.1	1.10
LUFA 6S	6S	Siebeldingen, Germany	Clay loam	7.3	1.78
Frankenforst	FF	Königswinter, Germany	Silt loam	6.9	2.4

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1/1 (all soils). Test concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L of M-01 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. Due to the low adsorption, no desorption test was performed.

The aqueous supernatant after adsorption was separated by centrifugation, the soils were extracted and the amount of test item in the supernatants and soil extracts was analyzed by liquid scintillation counting (LSC) and HPLC. The sorption parameters were calculated using Freundlich isotherms.

The test item was stable throughout the study. Mean parental mass balances (without NER) were 97.4, 98.5, 96.8, 92.5 and 95.7 % AR in the preliminary and 96.7, 98.4, 95.3, 97.0 and 94.2 % AR in the definitive test, after 24 hours of adsorption for soil 2.1, 2.3, 5M, 6S and FF, respectively.

The calculated adsorption constants KF of the Freundlich isotherms ranged from 0.056 to 0.418 mL/g for the tested soils. The Freundlich exponents 1/n ranged from 0.859 to 0.980, 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_{Fads} were correlated with the organic carbon content of the soil to get a comparability of the adsorption behaviour in different soils. For M-01 the K_{foc} values ranged from 9.2 to 17.5 mL/g.

K_{oc} values for the soils indicate that M-01 is mobile in the test soils according to the Briggs classification.

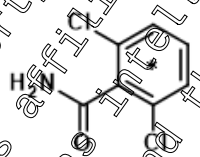
Soil origin	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
Soil type (USDA)	Sand	Sandy loam	Sandy loam	Clay loam	Silt loam
pH (0.01M $CaCl_2$)	5.16	6.24	7.13	7.31	6.9
Organic carbon [%]	0.59	0.61	1.1	1.78	2.4
$K_F^{(ads)}$ [mL/g]	0.103	0.056	0.162	0.265	0.418
1/n	0.958	0.859	0.888	0.872	0.980
$K_{F,OC}^{(ads)}$ [mL/g]	17.5	9.2	14.8	14.9	17.4

I. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-M-01 (referred to as AE C653711 in the report)



* = position of radiolabel.

Chemical name (IUPAC): 2,6-dichlorobenzamide

Batch number: KML10560

Specific radioactivity: 3.91 MBq/mg (0.106 µCi/mg)

Radiochemical purity: 97.5%

Stability of test compound: Stable during the batch equilibrium procedure with 100% AR remaining as M-02 after 48 hours.

2. Test Soils

The adsorption/desorption behaviour of M-01 was characterised in five soils using the batch equilibrium method. The five soils, taken from agricultural use areas, were categorised under the USDA classifications as a sand (Dudenhofen, Germany; LUFA 2.1), a sandy loam (Offenbach, Germany; LUFA 2.3), a sandy loam (Mechtersheim, Germany; LUFA 5M), a clay loam (Siebeldingen, Germany; LUFA 6S) and a silt loam (Königswinter, Germany; Frankenforst). After collection, soils were delivered sieved to a particle size of ≤ 2 mm and gently air-dried at ambient conditions. They were stored at room temperature at the receiving facility. The soil characteristics are given below in Table 7.1.3.1.2- 48.

Table 7.1.3.1.2- 48: Physico-chemical characteristics of test soils

Characteristic / Code	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S*	LUFA 6S*	Franken forst
Soil ID	2.1	2.3	5M	6S	6S	FF
Geographic Location	Dudenhofen Germany	Offenbach Germany	Mechter-sheim, Germany	Siebeld ingen Germany	Siebeld ingen Germany	Königs-winter Germany
GPS coordinates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Batch	1418	1318	3418	4518	1418	20170206
Textural Class (USDA)	Sand	Sandy Loam	Sandy Loam	Clay Loam	Clay Loam	Silt Loam
Sand (2000 to ≥ 50 µm)(%)	88.82	63.44	59.9	23.4	32.34	27
Silt (50 to ≥ 2 µm) (%)	8.75	30.08	31.1	40.9	34.03	54
Clay (< 2 µm) (%)	2.43	6.48	8.9	35.7	33.62	19
pH ($CaCl_2$)	5.16	6.24	7.13	7.31	7.24	6.9
Organic Carbon (%)	0.59	0.61	1.10	1.78	1.85	2.4
Cation exchange capacity (meq/100g)	5.2	11.8	20.0	16.4	24.0	18.5
Maximum Water Holding Capacity (g/100 g dry matter)	30.65	38.56	39.17	48.76	51.49	n.a.

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of either 50 mL glass flasks (conventional system) or commercially available 20 mL syringes (Braun,polypropylene, polyethylene) as incubation vessels and Falcon tubes™ (polypropylene) as recipient vessels for the aqueous phase removed later on (syringe method).

In preliminary tests, as well as a comparison of the conventional and syringe method being undertaken, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption equilibration times and the stability of the test item were determined.

The soil was weighed into the test vessels and pre-equilibrated over night with 5 mL 10 mM $CaCl_2$ solution with a soil-to-solution ratio of 1/1. The definitive test was performed at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L in 0.01M $CaCl_2$ solution using the syringe method. After application of M-01, the test systems were shaken for 24 hours in the dark at 20 ± 1 °C and centrifuged. The supernatants were decanted, the volumes determined by weighing and the radioactivity contents determined by LSC and characterized by radio-HPLC.

Five replicates per soil and concentration were used. The equilibration time was 24 hours for adsorption. An aliquot of the control flasks was taken at 0 h for LSC measurement in order to determine the initial mass of the test item. The definitive test was repeated for all soils with five replicates each at nominal concentration levels of 0.01 to 0.1 mg/L. The repetition was necessary as the actual applied concentrations deviated from the nominal concentrations.

After equilibration, soil and solution phases were separated by centrifugation. Residues remaining on the soil after adsorption were extracted and quantified directly.

In the definitive test, soil samples were exhaustively extracted with acetonitrile/water (4/1, *v/v*) at ambient temperature for 30 minutes, followed initially by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 60 °C for 20 minutes, and then extracted with acetonitrile/water (4/1, *v/v*) at ambient temperature for 30 minutes for a second time. Finally, the soil was extracted with acetone at ambient temperature for 30 minutes to aid drying. After each extraction step, extract and soil were separated by centrifugation.

For detailed information on experimental design see below. Desorption was not examined due to the low adsorption of M-01 on soil.

Adsorption

Parameter		Description
Soil Condition		Soils were pre-equilibrated to study conditions for at least 12 hours with 5 mL aqueous 0.01 M $CaCl_2$ solution
Soil sample weight		5 g per replicate.
Solution used for equilibration		aqueous 0.01 M $CaCl_2$ solution
Control used		$CaCl_2$ solution without soil
Test item concentrations	Nominal application rates	Nominal concentrations in test solution: 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L
	Analytically measured concentrations	Concentrations in test solution: 1.0 mg/L, 0.49 mg/L, 0.10 mg/L, 0.047 mg/L and 0.0092 mg/L.
Identity and concentration of extraction solvent		acetonitrile / water (80:20, *v/v*), acetone
Soil-to-solution ratio		1/1, i.e. 5 g soil dry weight to 5 mL solution (corrected for soil moisture).
pH of the equilibration solution (from definitive test)	Initial	pH of aqueous 0.01 M $CaCl_2$ solution without soil: 5.79
	Final	pH with soil and test item after adsorption equilibrium:range 6.70 – 8.31
Number of replicats	Controls	Two
	Treatments	Five
Incubation	Time	48 hours
	Temperature	20.9°C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 rpm
Centrifugation	Speed	2800 rpm
	Duration	35 to 140 min for $CaCl_2$ and 35 min for extracts
	Method of separation of soil and solution	Centrifugation (no filtration)

2. Analytical Procedures

Radioactivity in supernatants and soil extracts was determined by LSC. Radioactivity in the extracted soil was determined by combustion.

Samples of supernatants, soil extracts and controls without soil from the preliminary test and the definitive test were analysed by HPLC. For the definitive test, the highest and lowest test concentrations were analysed by HPLC. As M-01 was >95% ROI for all samples of highest and lowest concentration, the test item was deemed to be stable at the intermediate concentrations. ROI of the intermediate concentrations was formed taking the ROI mean value of highest and lowest test concentration. In cases where ROI was 100% (eg 2.3, 5M and 6S), K_d determination is based on LSC measurements of aqueous supernatant and soil extracts. HPLC recovery was quantitative (103.2%). The LOD of the analytical methods was 1279 dpm by HPLC and 45 dpm by TLC.

Samples of lowest concentration (K5) were characterized with thin layer chromatography (TLC).

Adsorption isotherms were calculated by linear regression analysis of the data according to the Freundlich equation. The amount of the test substance adsorbed was directly determined by extraction and analysis of soil. Desorption was not examined due to the low adsorption.

II. Results and Discussion

A. Results of preliminary tests

Adsorption percentage between conventional and syringe method were largely comparable. Using the syringe method, the residual pore water is minimized and therefore a more accurate determination of K_d and K_{oc} is possible. Therefore, the syringe method was chosen for the definitive test.

The test item was stable in aqueous 0.01 M $CaCl_2$ solution in the absence of soil. After incubation for 48 hours M-01 represented 100% AR. No significant adsorption of the test item to the test vessels was observed after shaking for 48 hours (< 3% AR). The test item has very low adsorption characteristics to soil and so a soil-to-solution ratio of 1:1 was used. An equilibration time of 24 hours was chosen for the adsorption phase. Plateau concentrations were established for all soils within 6 to 24 hours.

B. Transformation of test substance

The parental mass balances were determined at a soil-to-solution ratio of 1/1 for all soils. M-01 was stable throughout the study. Mean parental mass balances (excluding NER) were 97.4, 98.5, 96.8, 92.5 and 95.7% AR in the preliminary test for LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst soils, respectively.

The stability was adequate to determine the test item distribution based on LSC measurements of the adsorption phase extracts in the definitive test. No major degradation product was observed by HPLC analysis.

Mean parental mass balances of the test item in the definitive test were 96.7, 98.4, 95.3, 97.0 and 94.2 % AR for LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst soils, respectively.

C. Findings

Mean material balances were 97.0, 98.7, 95.2, 98.6 and 95.2% AR for soils LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst, respectively (summarised in Table 7.1.3.1.2- 49). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 49: Definitive test: Mass balance of [Phenyl-U-^{14}C]-M-01 (% AR)

Soil	Aquatic phase		Soil extract		Non extractable radioactivity		Total	
	% AR	SD	% AR	SD	% AR	SD	% AR	SD
LUFA 2.1	87.2	3.5	8.1	3.0	0.3	0.6	97.0	1.8
LUFA 2.3	91.3	3.4	7.1	2.3	0.3	0.3	98.7	3.3
LUFA 5M	78.5	2.7	16.8	3.4	0.5	0.3	95.2	2.5
LUFA 6S	71.9	6.7	25.1	6.4	2.0	2.0	98.6	4.0
Frankenforst	66.5	3.4	27.7	2.7	0.9	0.9	95.2	2.8

Note: Mass balances were quantitative. Overall mean values derived from five replicates at each of five concentrations provided in the report. SD = standard deviation.

The results of adsorption tests of [phenyl-U-^{14}C]-M-01 onto five soils are summarised in Table 7.1.3.1.2-50 and Table 7.1.3.1.2- 51. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 20 to Figure 7.1.3.1.2- 24.

At the end of the adsorption phase 11 to 15 % AR, 5 to 13 % AR, 20 to 23 %, 18 to 34 % and 29 to 37 % AR were absorbed in soils LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst, respectively.

The adsorption constant $K_{F(ads)}$ of M-01 was between 0.056 to 0.418 mL/g for the tested soils; the normalised adsorption constant $K_{OC(ads)}$ was in the range of 9.2 to 17.5 mL/g. The Freundlich exponent 1/n was between 0.814 and 1.105, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table 7.1.3.1.2- 50: Definitive test: Concentration of M-01 in aqueous and solid phase following 24 hours of adsorption.

Concn (µg/mL)	Rep	LUFA 2.1		LUFA 2.3		LUFA 5M		LUFA 6S*		Frankenforst	
		Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)
1.0	1	0.1023	0.8627	0.0536	0.9185	0.0415*	0.6009*	0.0340*	0.0705*	0.2549	0.6719
	2	0.0940	0.8897	0.0443	0.9268	0.0696*	0.8000*	0.2309	0.7091	0.2808	0.6746
	3	0.0645	0.9237	0.0598	0.9461	0.1106	0.8086	0.2352	0.6714	0.2852	0.6358
	4	0.1022	0.8914	0.0501	0.9051	0.1356*	0.7212*	0.2225	0.7034	0.3203	0.6560
	5	0.0690	0.8665	0.0646	0.8863	0.1239	0.7879	0.1676	0.7359	0.2949	0.6994
	Mean	**0.0864**	**0.8868**	**0.0545**	**0.9166**	**0.1110**	**0.7982**	**0.2140**	**0.7050**	**0.2872**	**0.6676**
0.49	1	0.0551	0.4155	0.0150	0.4396	0.0688	0.3853	0.1022	0.3431	0.1215	0.3409
	2	0.0478	0.4193	0.0204	0.4330	0.0803	0.3830	0.1169	0.3420	0.1360	0.3093
	3	0.0465	0.4302	0.0306	0.4380	0.0756	0.3902	0.0877*	0.3325*	0.1355*	0.3225
	4	0.0537	0.4301	0.0315	0.4390	0.0826	0.3913	0.1319	0.3503	0.1182	0.3332
	5	0.0425	0.4304	0.0231	0.4563	0.0743	0.3876	0.1213	0.3516	0.1452	0.3060
	Mean	**0.0491**	**0.4251**	**0.0241**	**0.4412**	**0.0762**	**0.3875**	**0.1181**	**0.3467**	**0.1315**	**0.3224**
0.10	1	0.0095	0.0824	0.0087	0.0882	0.0178	0.0732	0.0239	0.0814	0.0284	0.0659
	2	0.0097	0.0834	0.0097	0.0856	0.0127	0.0802	0.0236	0.0776	0.0337	0.0588
	3	0.0092	0.0716	0.0095	0.0843	0.0175*	0.0709*	0.0245	0.0798	0.0292	0.0615
	4	0.0112	0.0811	0.0045	0.0850	0.0186	0.0758	0.0200	0.0824	0.0274*	0.0567*
	5	0.0102	0.0919	0.0097	0.0883	0.0167	0.0787	0.0123	0.0810	0.0273	0.0644
	Mean	**0.0100**	**0.0821**	**0.0084**	**0.0863**	**0.0165**	**0.0769**	**0.0209**	**0.0807**	**0.0297**	**0.0626**
0.047	1	0.0053	0.0413	0.0051	0.0441	0.0076	0.0472	0.0162	0.0303	0.0136	0.0318
	2	0.0051	0.0408	0.0053	0.0419	0.0094	0.0378	0.0124	0.0335	0.0146	0.0306
	3	0.0052	0.0408	0.0038	0.0431	0.0081	0.0387	0.0152	0.0286	0.0154	0.0309
	4	0.0050	0.0427	0.0023	0.0445	0.0087	0.0369	0.0179	0.0273	0.0131	0.0333
	5	0.0042	0.0424	0.0045	0.0413	0.0095	0.0352	0.0160	0.0312	0.0158	0.0284
	Mean	**0.0050**	**0.0416**	**0.0042**	**0.0430**	**0.0087**	**0.0390**	**0.0156**	**0.0302**	**0.0145**	**0.0310**
0.0092	1	0.0007*	0.0050*	0.0008	0.0084	0.0022	0.0068	0.0028	0.0061	0.0024*	0.0043*
	2	0.0010	0.0074	0.0008	0.0086	0.002	0.0071	0.0026	0.0059	0.0031	0.0059
	3	0.0012	0.0083	0.0009	0.0088	0.0022	0.0068	0.0031	0.0061	0.0024	0.0062
	4	0.0007	0.0075	0.0010	0.0085	0.0017	0.0067	0.0036	0.0054	0.0030	0.0066
	5	0.0010	0.0078	0.0007	0.0084	0.0019	0.0072	0.0031	0.0061	0.0027	0.0061
	Mean	**0.0010**	**0.0078**	**0.0008**	**0.0085**	**0.0020**	**0.0069**	**0.0030**	**0.0059**	**0.0028**	**0.0062**

* Values excluded from mean value (mass balance < 90 % or >110 %)

Table 7.1.3.1.2- 51: Summary of Freundlich adsorption constants K_f and K_{oc} values

Soil	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Franken-forst
	2.1	2.3	5M	6S	FF
Textural class	Sand	Sandy Loam	Sandy Loam	Clay Loam	Silt Loam
K_f	0.103	0.056	0.162	0.265	0.418
1/n	0.955	0.859	0.888	0.872	0.977
K_{oc}	17.497	9.182	14.751	14.895	17.429
Correlation	0.990	0.966	0.991	0.968	0.995

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in

Table 7.1.3.1.2- **53**). The mean concentrations in the supernatant and the soil as given in Table 7.1.3.1.2-52 were used a input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.2- 52: Definitive test: Concentration of M-01 in aqueous and solid phase following 24 hours of adsorption used in checklist (mean values)

Soil ID	LUFA 2.1		LUFA 2.3		LUFA 5M		LUFA 6S		Frankenforst	
Concentration (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)
0.011	0.0010	0.0078	0.0008	0.0085	0.0020	0.0059	0.0030	0.0059	0.0028	0.0062
0.065	0.0050	0.0416	0.0042	0.0430	0.0087	0.0302	0.0156	0.0302	0.0145	0.0310
0.104	0.0100	0.0821	0.0084	0.0863	0.0165	0.0807	0.0209	0.0807	0.0297	0.0626
0.495	0.0491	0.4251	0.0241	0.4412	0.0762	0.3468	0.1181	0.3468	0.1313	0.3224
0.963	0.0864	0.8868	0.0545	0.9166	0.1200	0.7050	0.2140	0.7050	0.2872	0.6676

Note: µg/g in soil corrected for residual water entrained in the soil pellet

Table 7.1.3.1.2- 53: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
Code	-	-	**2.1**	**2.3**	**5M**	**6S**	**FF**
Adsorption method	-	-	direct	direct	direct	direct	direct
Soil solution ratio	g/mL		1:1	1:1	1:1	1:1	1:1
Mass balance of ^{14}C	%	>90%	97.4 (highest)	97.9 (highest)	91.6 (highest)	93.2 (highest)	95.0 (highest)
f – due to loss processes[#]	%	-	2.6	2.1	8.4	6.8	5.0
Adsorbed percentage (δ) [#]	%	>20%	11.55 – 18.88	7.10 – 14.75	17.15 – 24.82	20.30 – 35.[illegible]	12.53 – 27.10
K_D x soil : solution ratio [#]		>0.3	0.09 – 0.12	0.05-0.09	0.14-0.18	0.23-0.[illegible]	0.38-0.44
K_{fE} / K_f [#]	-	<1.2	1.29	1.32	0.70	1.30	1.08
ads K_F	L/kg	-	0.104	0.058	0.157	0.276	0.422
95% confidence interval	-	*	(0.089 – 0.122)	(0.039 – 0.086)	(0.129 – 0.192)	(0.147 – 0.518)	(0.364 – 0.498)
ads 1/n	-	-	0.956	0.860	0.880	0.881	0.979
95% confidence interval	-	*	(0.902 – 1.010)	(0.716 – 1.004)	(0.812 – 0.947)	(0.674 – 1.088)	(0.931 – 1.027)
ads R^2	-	>0.975	0.999	0.992	0.998	0.984	0.999
ads K_{foc}	L/kg	-	17.7	9.4	14.3	15.5	17.6
Visual fit to Freundlich isotherm	-	-	Good	Good	Good	Good	Good
Residual plots randomly distributed	-	-	Good	Good	Good	Good	Good

[#]**Note:** 3 checks (in grey) are excluded as not relevant for 'direct' studies, therefore number of checks reduced from 9 to 6.

Relevant quality checks were performed to evaluate the acceptability of the study. Three checks have been excluded from the overall assessment (i.e. adsorbed percentage, K_D x soil:solution ratio and K_{fE} / K_f) as they not relevant for 'direct' studies, the number of quality checks is reduced from 9 to 6.

These checks confirmed that the mass balance was acceptable (91.6 – 97.9%). The acceptability of the analytical method (LSC) was confirmed over the entire range of concentrations measured (reported LOQ of 60 dpm represents <1 % of the lowest test concentration). The R^2 of the standard linear regressions ranged from 0.984 to 0.999 for soils 2.1, 2.3, 5M, 6S and FF and the visual fit of both the standard regression and the residual plots were good.

The study has been conducted to a good standard. The test substance was stable (purity 100% in dosing stocks, adsorption supernatant and soil extracts).

The evaluation confirmed that all soils were acceptable according to the quality checks and acceptable for use in regulatory modelling. The results of the evaluation are summarised in the tables below.

Figure 7.1.3.1.2- 20: Freundlich Isotherms of M-01 in LUFA 2.1 soil at 20°C

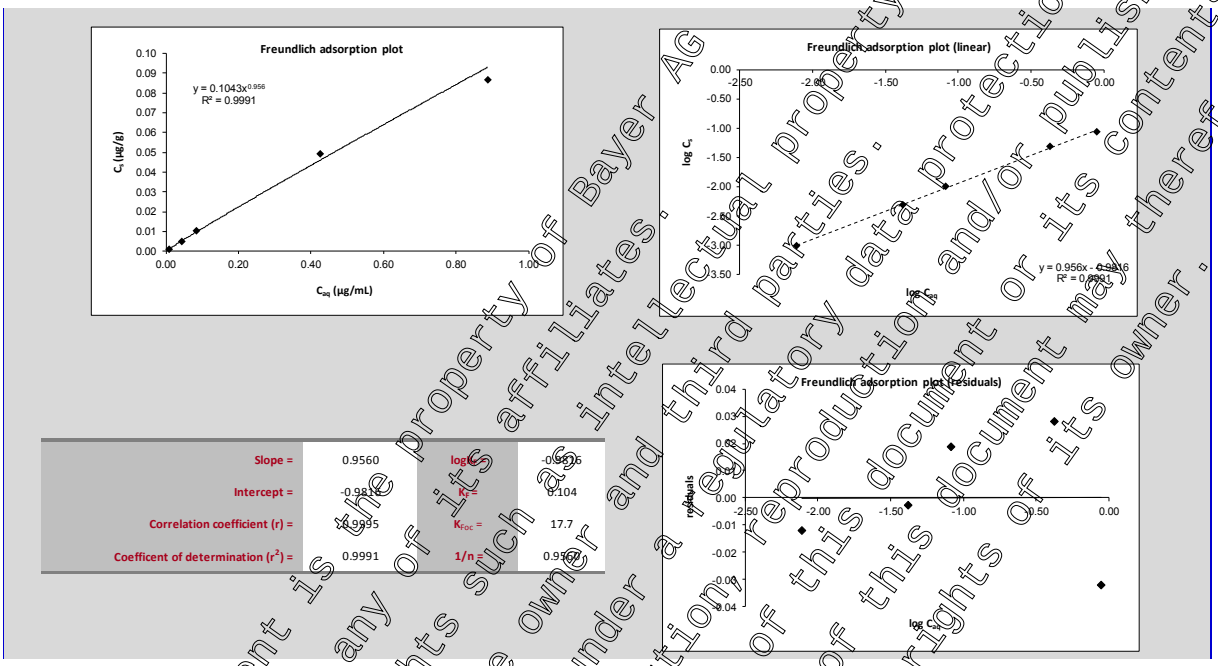


Figure 7.1.3.1.2- 21: Freundlich Isotherms of M-01 in LUFA 2.3 soil at 20°C

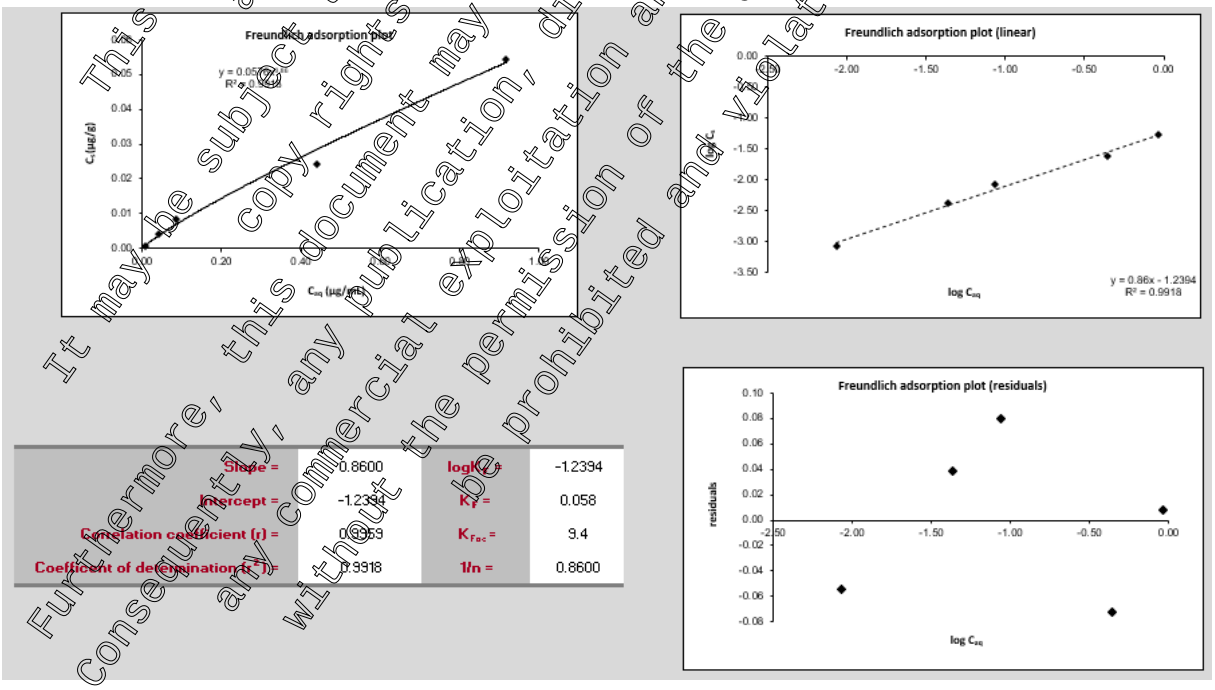
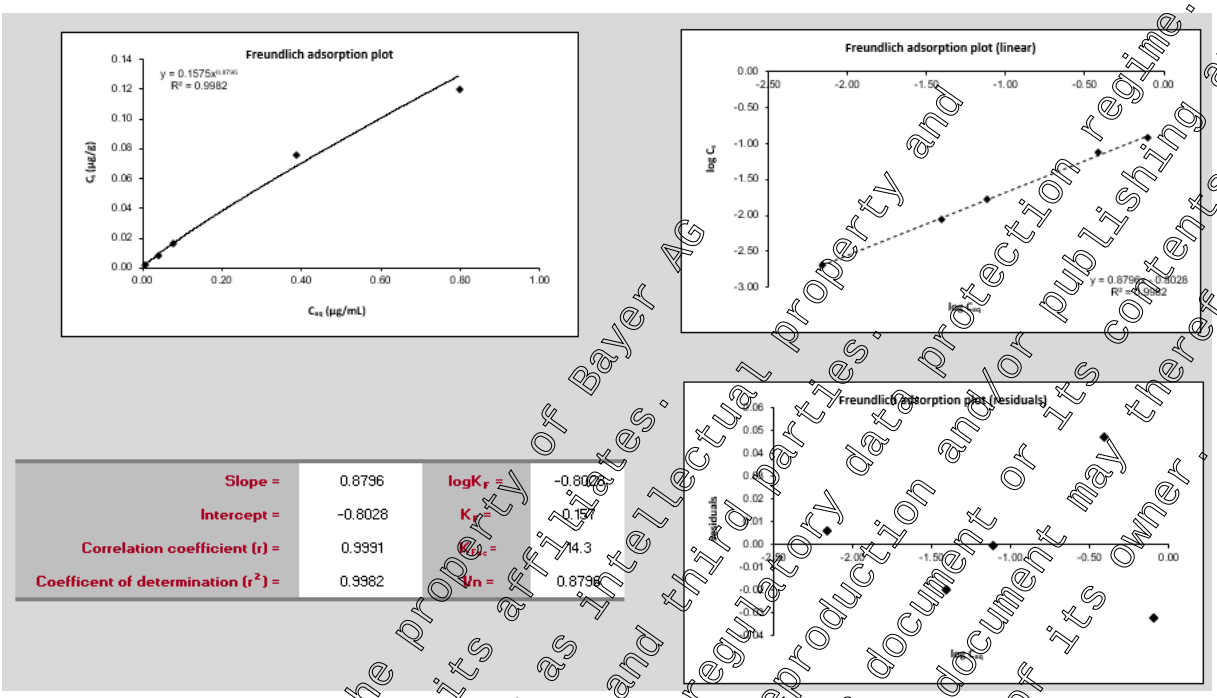
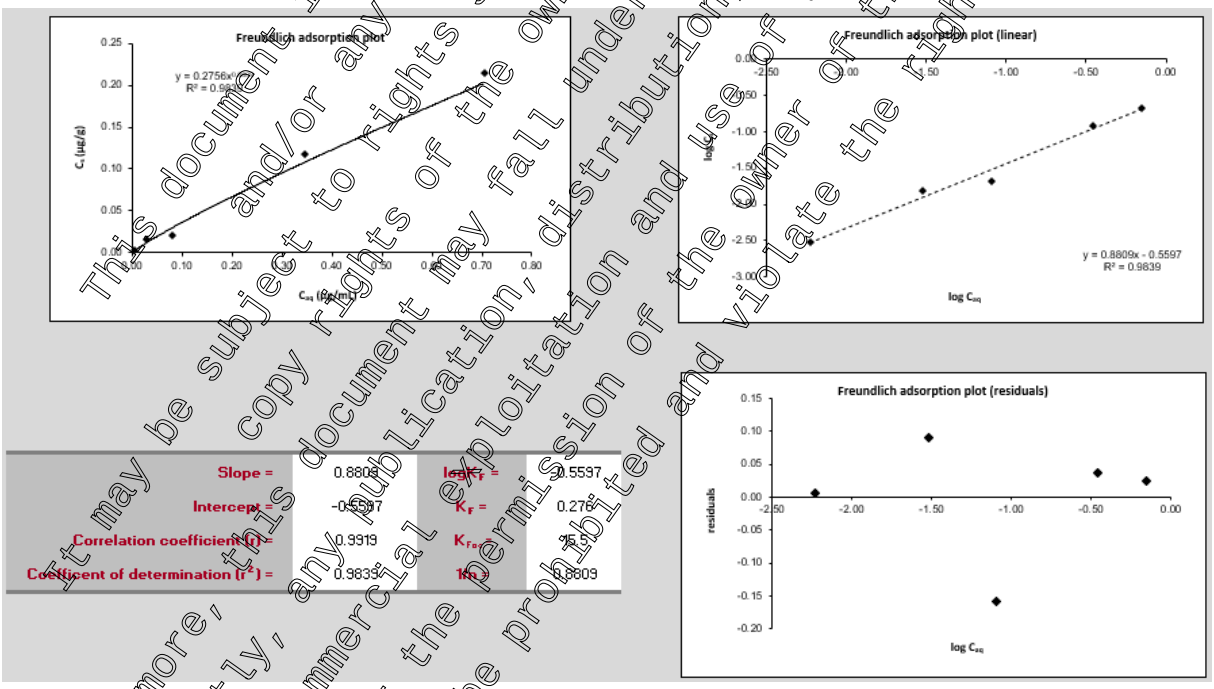


Figure 7.1.3.1.2- 22: Freundlich Isotherms of M-01 in LUFA 5M soil at 20°C



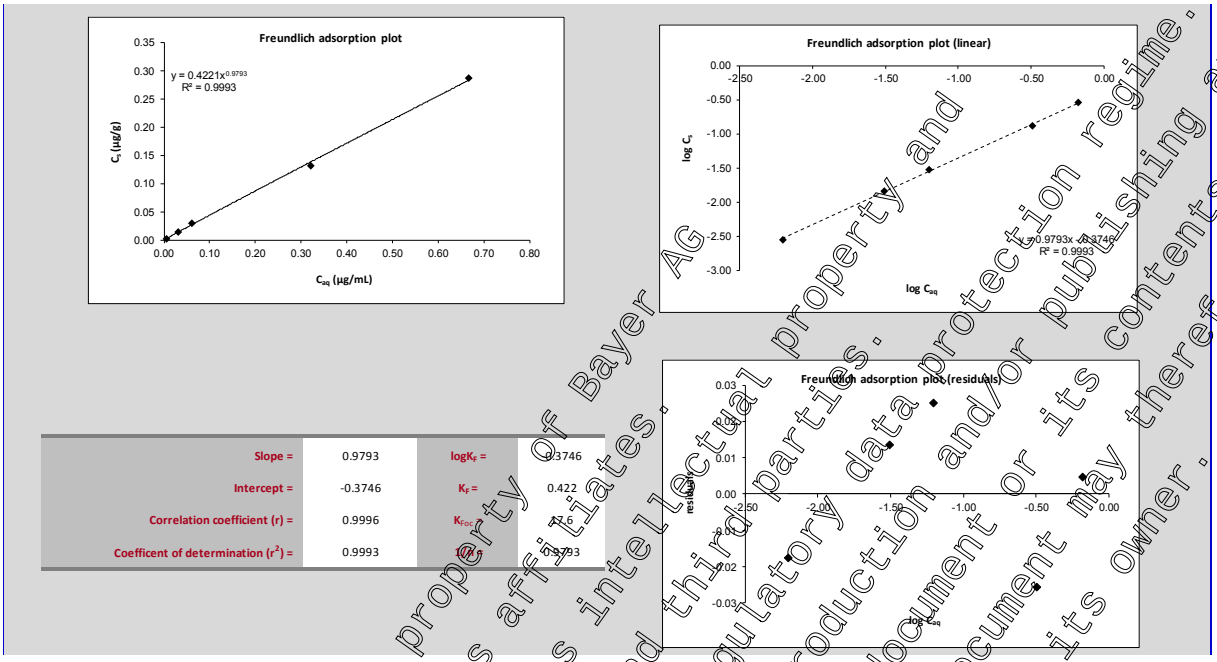
Slope =	0.8796	$logK_F$ =	-0.8028
Intercept =	-0.8028	K_F =	0.157
Correlation coefficient (r) =	0.9991	K_{oc} =	14.3
Coefficent of determination (r^2) =	0.9982	1/n =	0.8796

Figure 7.1.3.1.2- 23: Freundlich Isotherms of M-01 in LUFA 6S soil at 20°C



Slope =	0.8809	$logK_F$ =	-0.5597
Intercept =	-0.5597	K_F =	0.276
Correlation coefficient (r) =	0.9919	K_{oc} =	15.5
Coefficent of determination (r^2) =	0.9839	1/n =	0.8809

Figure 7.1.3.1.2- 24: Freundlich Isotherms of M-01 in Frankenforst soil at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 54. All four soils can be considered 'met' with regard to the quality criteria and therefore acceptable for regulatory use. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 55.

Table 7.1.3.1.2- 54: Summary of Quality Criteria and Regulatory Interpretation

M-01			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
LUFA 2.1	Sand	2.1	6	0	0
LUFA 2.3	Sandy Loam	2.3	6	0	0
LUFA 5M	Sandy Loam	5M	6	0	0
LUFA 6S	Clay Loam	6S	6	0	0
Frankenforst	Silt Loam	FF	6	0	0

Table 7.1.3.1.2- 55: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
LUFA 2.1	Sand	2.1	17.497	17.7	0.955	0.956
LUFA 2.3	Sandy Loam	2.3	9.182	9.4	0.859	0.860
LUFA 5M	Sandy Loam	5M	14.751	14.3	0.888	0.880
LUFA 6S	Clay Loam	6S	14.895	15.5	0.872	0.881
Frankenforst	Silt Loam	FF	17.429	17.6	0.977	0.979

The small differences between the reported values and the OECD calculation tool (v2) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

The adsorption constant $K_{F(ads)}$ of M-01 was between 0.056 to 0.418 mL/g for the tested soils, the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 9.2 to 17.5 mL/g. The Freundlich exponent 1/n ranged from 0.859 to 0.980, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the results were acceptable according to the quality criteria and therefore acceptable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption characteristics of M-01 (AE C653711) in soil.

Data Point:	KCA 7.1.3.1.2/11
Report Author:	[redacted]
Report Year:	2017
Report Title:	[Pyridyl-2,6-14C] AE 1344122: Adsorption / desorption in four different soils – Final report
Report No:	A84496
Document No:	M-587780-01-1
Guideline(s) followed in study:	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 (Adsorption/Desorption (Batch Equilibrium), October 2008
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially 169ecognized testing facilities:	Yes, conducted under GLP/Officially 169ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption characteristics of M-05 (referred to as AE 1344122 in the report) were studied in four soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 2 °C.

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
[redacted]	Monheim, Germany	Sandy loam	5.3	1.9
H[redacted]	Burscheid, Germany	Silt loam	6.3	2.0
Dollendorf II	Blankenheim, Germany	Loam	7.3	4.5
L[redacted]	Monheim, Germany	Loamy sand	6.3	1.6

The adsorption phase of the study was carried out using samples of air-dried soil equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1:1 for H[redacted] and L[redacted] soils and 1:2 for [redacted] and Dollendorf II soils. Test concentrations of 1.0, 0.3, 0.1, 0.03 and 0.01 mg/L of [^{14}C]-M-05 were applied in aqueous 0.01 M $CaCl_2$ solution. The desorption from pre-

adsorbed soil samples was studied by addition of fresh aqueous 0.01 M $CaCl_2$ solution. Adsorption and desorption took place for 24 hours each.

The adsorption parameters were calculated using the Freundlich adsorption isotherm. The mass balance of the soils in the definitive test was determined by LSC of the supernatants after adsorption, desorption and by combustion of the remaining soils. The total radioactivity recovery with respect to the individual vessel ranged from 94.4 to 106.6% of the applied radioactivity. Mean recovery rates were all within the range of 90 – 110% of the applied radioactivity.

The adsorption coefficients $K_{F\ ads}$ of [^{14}C] M-05 in the four test soils ranged from 0.467 to 1.479 mL/g based on the Freundlich equation. The corresponding adsorption coefficients $K_{F,OC,ads}$ normalised for organic carbon ranged from 14.7 to 77.9 mL/g. The Freundlich exponents 1/n were in the range of 0.974 to 1.025.

The measured $K_{F(des)}$ values ranged between 0.5490 and 1.7791 mL/g and $K_{F,OC\ (des)}$ values between 21.3 and 93.6 mL/g.

K_{oc} values indicate M-05 is mobile to intermediate mobility in soil according to the Briggs classification.

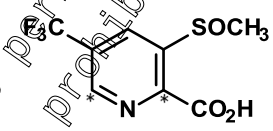
Soil	[redacted]	[redacted]	Dollendorf II	[redacted]
Soil type (USDA)	Sandy Loam	Silt Loam	Loam	Loamy Sand
pH (0.01M $CaCl_2$)	5.3	6.3	7.3	6.3
Organic carbon [%]	1.9	2.0	4.5	1.6
$K_F^{(ads)}$ [mL/g]	1.4793	0.4915	0.6629	0.4671
1/n	0.9736	0.9847	1.0248	0.9838
$K_{F,OC}^{(ads)}$ [mL/g]	77.9	24.6	14.7	29.2
R^2	1.0000	0.9999	0.9939	0.9997
$K_F^{(des)}$ [mL/g]	1.7791	0.6103	0.9564	0.5490
1/n	0.9771	0.9946	1.0267	0.9876
$K_{F,OC}^{(des)}$ [mL/g]	93.6	30.5	21.3	34.3
R^2	0.9998	0.9999	0.9790	0.9996

I. Materials and Methods

A. Materials

1. Test Items

[2,6-Pyridyl-^{14}C]-M-05 (referred to as AE 1344122 in the report)



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

Specific Activity:	4.95 MBq/mg
Radiochemical Purity:	>99% (HPLC)
Sample/Batch ID:	KML 10220
Stability of test compound:	Stable during the batch equilibrium procedure with >90% AR remaining as M-05 after 72 hours

2. Test Soils

Sorption tests were performed with four agricultural soils from Germany selected to cover a representative range of soil physico-chemical properties. Soil samples were collected up to 20 cm depth, then transported to test facility where they were stored refrigerated (1-10°C). All soils were air dried, sieved (≤2 mm) and sterilised prior to use. The soils were fully characterised with respect to texture, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study.

Table 7.1.3.1.2- 56: Physico-chemical properties of test soils

Characteristic / Code	[redacted]	[redacted]	Dollendorf II	[redacted]
Soil ID	[redacted]	[redacted]	DoII	[redacted]
Geographic Location	Monheim, Germany	Burscheid, Germany	Blankenheim, Germany	Monheim, Germany
Textural Classification (USDA)	Sandy loam	Silt loam	Loam	Loamy sand
Sand (2000 to ≥ 50 µm) (%)	55	21	39	79
Silt (50 to ≥ 2 µm) (%)	29	65	35	13
Clay (< 2 µm) (%)	16	14	26	8
pH (water)	5.6	6.6	7.5	6.6
pH ($CaCl_2$)	5.3	6.3	7.3	6.3
Organic carbon (%)	1.9	2.0	4.5	1.6
Organic matter (%)*	3.27	3.44	7.74	2.75
CEC (meq/100g soil)	9.9	11.0	19.8	8.5

*calculated using conversion factor % organic matter = % organic carbon x 1.72

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of borosilicate glass centrifuge tubes (42 mL or 83 mL) with Teflon® lined screw caps.

In preliminary tests, the adsorption of the test item to glassware, the optimal soil-to-solution ratio and the appropriate adsorption and desorption equilibration times were determined.

Parental mass balance was established for all soils in preliminary tests. After equilibration, soil and solution phases were separated by centrifugation. In [redacted], H[redacted] and L[redacted] soil, residues remaining on the soil after adsorption were exhaustively extracted with 0.01M $CaCl_2$/0.1M HCl (9:1, v/v), then methanol/water (1:1, v/v), followed by 0.1M EDTA (1-2 extractions) and pH 5 citrate buffer (1 – 2 extractions) for 10 to 30 minutes in an ultrasonic bath followed by a horizontal shaker for 30 minutes. For Dollendorf soil residues remaining on the soil after adsorption were exhaustively extracted with 0.1M EDTA, then pH 5 citrate buffer followed by a second extraction with 0.1M EDTA for 10 minutes in an ultrasonic bath followed by a horizontal shaker for 30 minutes.

Material balance was established for all soils in the definitive test. The main test was performed in duplicate. [^{14}C]-05 was dissolved in 0.01M calcium chloride solution at nominal concentrations of 0.01, 0.03, 0.1, 0.3 and 1.0 mg/L. Soil samples were prepared at a soil to solution ratio of 1:1 for H[redacted] and L[redacted] soils and 1:2 for [redacted] and Dollendorf soil and shaken at in the dark at 20 ± 2°C. Following the preliminary tests, an equilibrium time of 24 hours was selected.

For all soils one desorption cycle was performed on all concentrations. The volume of solution removed after the adsorption step was replaced by an equal volume of [^{14}C]-M-05 stock solution. Test vessels were then shaken for a 24 hour desorption phase. After the desorption phase radioactivity remaining in the soil was quantified by combustion. 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.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		10 g (dry weight) per replicate for [redacted] and Dollendorf 20g (dry weight) per replicate for H[redacted] and L[redacted]
Equilibration solution		0.01M $CaCl_2$, shaken ~16 hours
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentration in test solution: 1 mg/L, 0.3 mg/L, 0.1 mg/L, 0.03 mg/L and 0.01 mg/L
	Initial concentrations of [^{14}C] M-05 LSC	0.99 mg/L, 0.29 mg/L, 0.10 mg/L, 0.03 mg/L and 0.01 mg/L
Identity and concentration of co-solvent		Not specified
Soil: Solution ratio		1:2 i.e. 10 g soil dry weight equivalent to 20 mL solution [redacted] and Dollendorf) 1:1 i.e. 20 g soil dry weight equivalent to 20 mL solution (H[redacted] and L[redacted])
Number of replicates	Treatments	Duplicate
Equilibration conditions	Time	24 h
	Temperature	20 ± 2°C
	Dark	In the dark
	Shaking method	Mechanical overhead shaker (~20rpm)
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	~5000 rpm ~4200 G
	Duration	~10 minutes
	Method of separating supernatant	Supernatant was carefully decanted. Volumes measured gravimetrically

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 20.7 to 46.2% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		1 for all soils
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by an equal volume of fresh aqueous 0.01 M $CaCl_2$ solution
Soil: Solution ratio		1:2 i.e. 10 g soil dry weight equivalent to 20 mL solution ([redacted] and Dollendorf) 1:5 20 g soil dry weight equivalent to 20 mL solution (H[redacted] and [redacted])
Number of replicates	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 h
	Temperature	20 ± 2°C
	Dark	In the dark
	Shaking method	Mechanical overhead shaker (~20rpm)
Method of separation of supernatant		Centrifugation
Centrifugation	3100 G	~5000 rpm ~4200 G
	20 minutes	~10 minutes
	Supernatant was carefully decanted.	Supernatant was carefully decanted. Volumes measured gravimetrically

2. Analytical Procedures

Radioactivity in supernatants and soil extracts was determined by LSC. Radioactivity in the extracted soil was determined by combustion.

Samples of supernatants, soil extracts and controls without soil from the preliminary test only were analysed by HPLC.

The amount of test item adsorbed to the soil was calculated by subtracting the plateau (adsorption equilibrium) concentration in the supernatant solution from the initial concentration (applied concentration). By establishing the material balances it was verified that, beside the adsorption to the soils, no further processes had significantly contributed to the decline of the test item measured in the supernatants.

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. Results of preliminary tests

The test item was stable in aqueous 0.01 M $CaCl_2$ solution in the absence of soil. After incubation for 24 hours M-05 represented 100% AR. No adsorption of the test item to the test vessels was observed after shaking for 24 hours. A soil-to-solution ratio of 1:1 for H[redacted] and L[redacted] soils and 1:2 for [redacted] and Dollendorf II soils was used for the definitive tests. An equilibration time of 24 hours was chosen for both the adsorption and desorption phase.

B. Transformation of test substance

For the soils [redacted], H[redacted] and L[redacted] all parental mass balances were above 90% of the applied radioactivity over the 48 hour equilibration time cycle, with mass balances of between 91.2 and 92.9%. For Dollendorf II soil the parental mass balances was below 90% of the applied radioactivity at all sampling points. The preliminary test for soil Dollendorf II was repeated using a lower soil ratio of 1:2. Using this soil:solution ratio the parental mass balance was above 90% % AR over the entire test duration.

The stability was adequate to determine the test item distribution based on LSC measurements of the supernatant only in the adsorption and desorption experiments of the definitive test.

C. Findings

The radioactive material balance was calculated as sum of radioactivity detected in decanted supernatant solutions after the adsorption or desorption phase plus the radioactivity remaining in soil residues. Mean material balances were 97.4, 99.6, 99.6 and 102% AR for [redacted], H[redacted], Dollendorf II and L[redacted] soils, respectively (summarised in Table 7.1.3.1.2- 42). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity from the test systems or during sample processing.

Table 7.1.3.1.2- 57: Definitive test: Mass balance of [2,6-Pyridyl-^{14}C]-M-05 (% AR)

Test concentration (mg/L)	[redacted]	H[redacted]	Dollendorf II	L[redacted]
1.0	97.5	96.3	96.4	97.6
0.30	96.6	98.7	99.3	101
0.10	98.6	98.3	98.7	100
0.03	98.4	99.7	101	104
0.01	95.8	105	102	106
Mean	97.4	99.6	99.6	102
SD	1.6	3.1	2.5	3.1

Mean values of duplicate samples
SD = standard deviation

The results of adsorption and desorption tests of [2,6-pyridyl-^{14}C]-M-05 onto four soils are summarised in Table 7.1.3.1.2- 58 and

Table 7.1.3.1.2- **59**. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 25 to Figure 7.1.3.1.2- 28.

At the end of the adsorption phase 42.4-46.2%, 32.5-34.7%, 20.7-33.8% and 31.4-33.8% of the applied test material was adsorbed in [redacted], H[redacted], Dollendorf II and L[redacted], respectively.

The calculated Freundlich adsorption coefficients (K_f) ranged from 0.4671 to 1.4793 mL/g. The correlation coefficients of the individual isotherms ranged from 0.9939 to 1.000. The Freundlich exponents 1/n ranged from 0.9736 to 1.0248, 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 were correlated with the organic carbon content of the soil to get a comparability of the adsorption behavior in different soils. For M-05 the K_{Foc} values ranged from 14.7 to 77.9 mL/g.

Table 7.1.3.1.2- 58: Definitive test: Concentration of M-05 in aqueous and solid phase following 24 hours of adsorption

Nominal concn (µg/mL)	[redacted]		[redacted]		Dollendorf II		L[redacted]	
	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)
0.01	0.005	0.009	0.006	0.003	0.007	0.005	0.006	0.003
0.03	0.016	0.026	0.019	0.010	0.022	0.013	0.019	0.010
0.1	0054	0.087	0.063	0.033	0.075	0.043	0.064	0.033
0.3	0.163	0.253	0.192	0.096	0.226	0.122	0.198	0.091
1.0	0.562	0.847	0.656	0.325	0.704	0.554	0.671	0.316

Table 7.1.3.1.2- 59 Summary of Freundlich adsorption/desorption constants K_f and K_{oc} values

Phase	Soil	Units	[redacted]	[redacted]	Dollendorf II	L[redacted]
Adsorption	$K_{F,ads}$	[mL/g]	1.4793	0.4815	0.6629	0.4671
	1/n	-	0.9736	0.9847	1.0248	0.9838
	R^2	-	1.000	1.000	0.994	0.998
	$K_{oc,ads}$	[mL/g]	77.9	24.6	14.7	29.2
Desorption	$K_{F,des}$	[mL/g]	1.7791	0.6103	0.9564	0.5490
	1/n	-	0.9771	0.9946	1.0267	0.9876
	R^2	-	0.999	1.000	0.979	0.999

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 60). The concentrations in the supernatant and the soil as given in the report were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.2- 60: Results of the EFSA 106 data evaluation

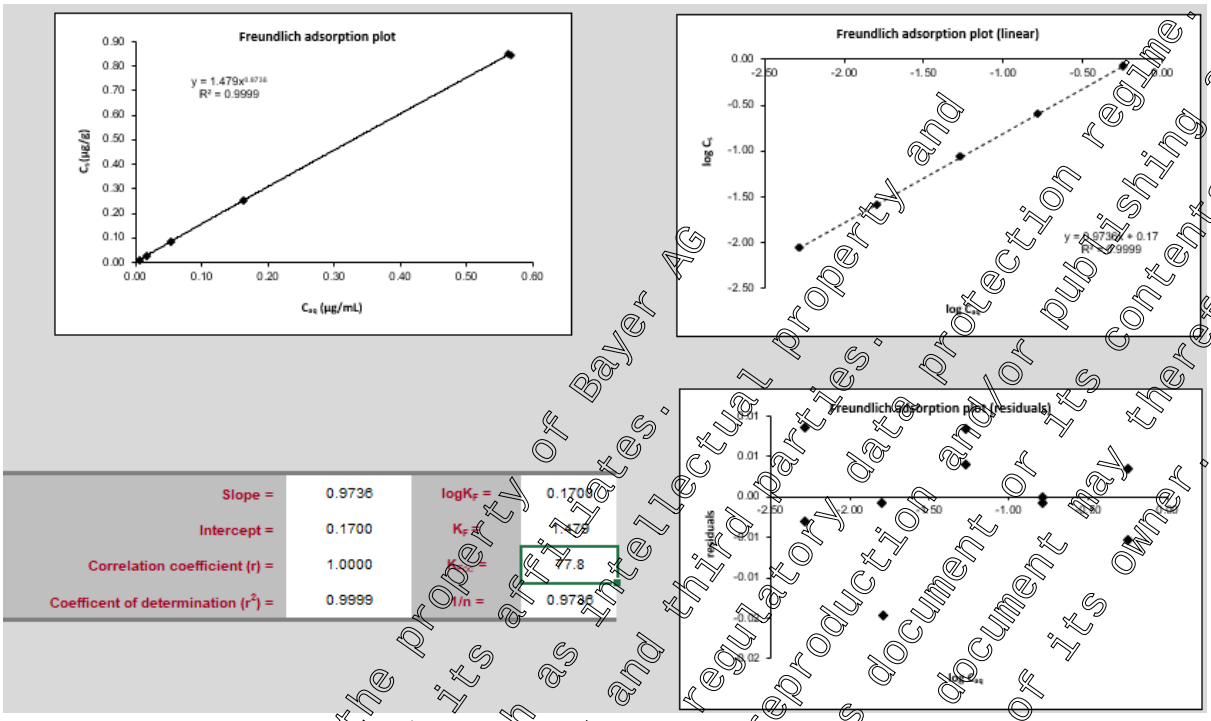
Soil	Units	Quality criteria	[redacted]	[redacted]	Dollendorf II	L[redacted]
Adsorption method	-	-	indirect	indirect	indirect	indirect
Soil solution ratio	g/mL	-	1:2	1:1	1:2	1:1
Mass balance of ^{14}C	%	>90%	94.4 – 99.5	96.0 – 105.3	96.0 – 103.9	97.2 – 106.6
f – due to loss processes (estimated)	%	-	7.9	8.8	6.3	7.1
Adsorbed percentage (δ)	%	>20%	42.38 – 48.14	32.46 – 37.65	20.70 – 29.73	31.34 – 35.94
K_D x soil:solution ratio		>0.3	0.74 – 0.87	0.49 – 0.54	0.27 – 0.52	0.46 – 0.51
$^{\#}K_{fE}$ / K_f	-	<1.2	1.23	1.37	1.23 & 1.40	1.29
ads K_F	L/kg	-	1.479	0.492	0.651	0.467
95% confidence interval	-	*	1.443 – 1.516	0.475 – 0.508	0.486 – 0.873	0.445 – 0.491
ads 1/n	-		0.974	0.985	1.020**	0.984
95% confidence interval	-	*	0.966 – 0.981	0.974 – 0.995	0.925 – 1.114**	0.968 – 0.999
ads R^2	-	>0.975	1.000	1.000	0.987**	1.000
ads $K_{F,OC}$	L/kg	-	77.8	24.6	14.5	29.2
Visual fit to Freundlich isotherm	-	-	Good	Good	Good	Good
Residual plots randomly distributed	-	-	Good	Good	Acceptable	Acceptable

* Confidence intervals should be narrow.
** Potential outlier value for Dollemdorf II; results in large range for 95% confidence intervals and lower R^2

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance of 94.4-106.6% and % adsorption of 20.7-48.1% were all acceptable. Although no LOQ value was reported the acceptability of the analytical method was confirmed with the lowest measured dpm values (LSC) significantly above reported background values over the entire range of concentrations measured. The use of the indirect method was considered largely appropriate based on a Kd * soil/solution ratio >0.3 in all except one soil (Dollendorf II) and was only marginally below 0.3 in this soil. However, the calculated K_{fE} / K_f ratio was slightly greater than 1.2 in all four soils due to the relatively low sorption and relatively high "f" values (6.3 – 8.8%, with all "parental mass balances" being >90%). The graphical fits of the Freundlich equation based on the standard linear regression formusing log-log transformed data alongside the associated residual plots was good with R^2 of the standard linear regressions ranging from 0.987 to 1.000 and the visual fit of both the standard regression and the residual plots being acceptable.

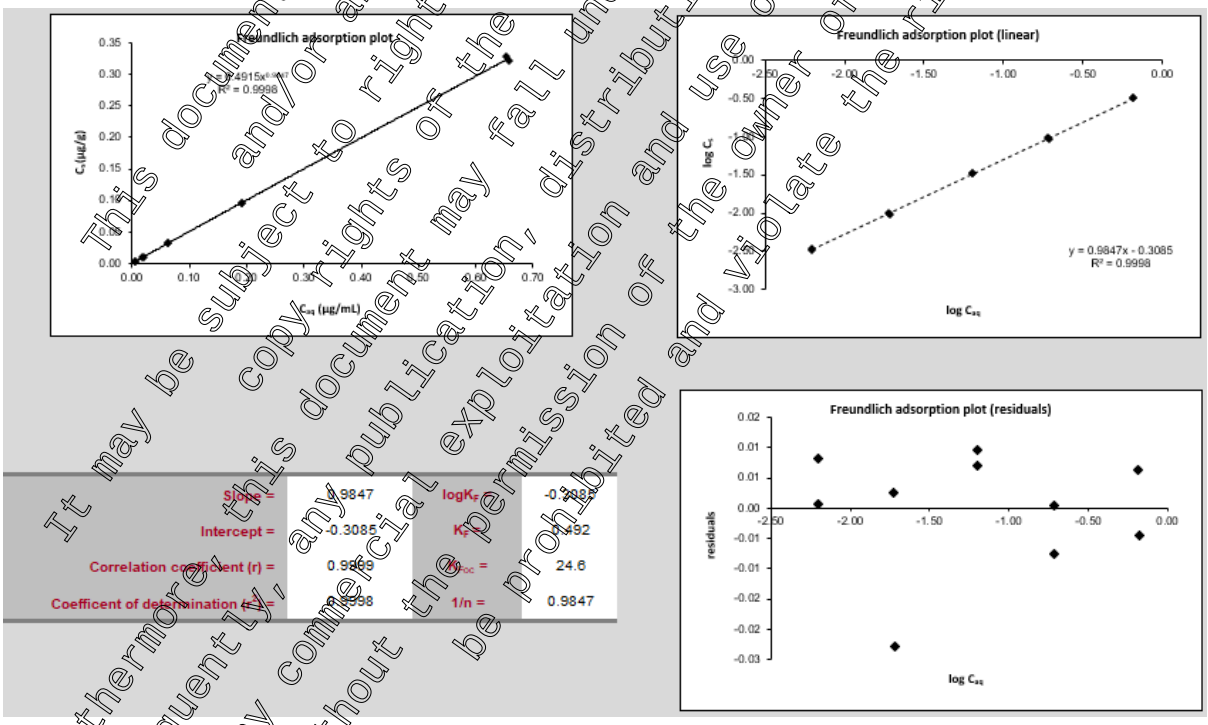
Overall, the study was conducted to a good standard and the overall assessment of the data confirms the study is acceptable.

Figure 7.1.3.1.2- 25: Freundlich Isotherms of M-05 in soil [redacted] at 20°C



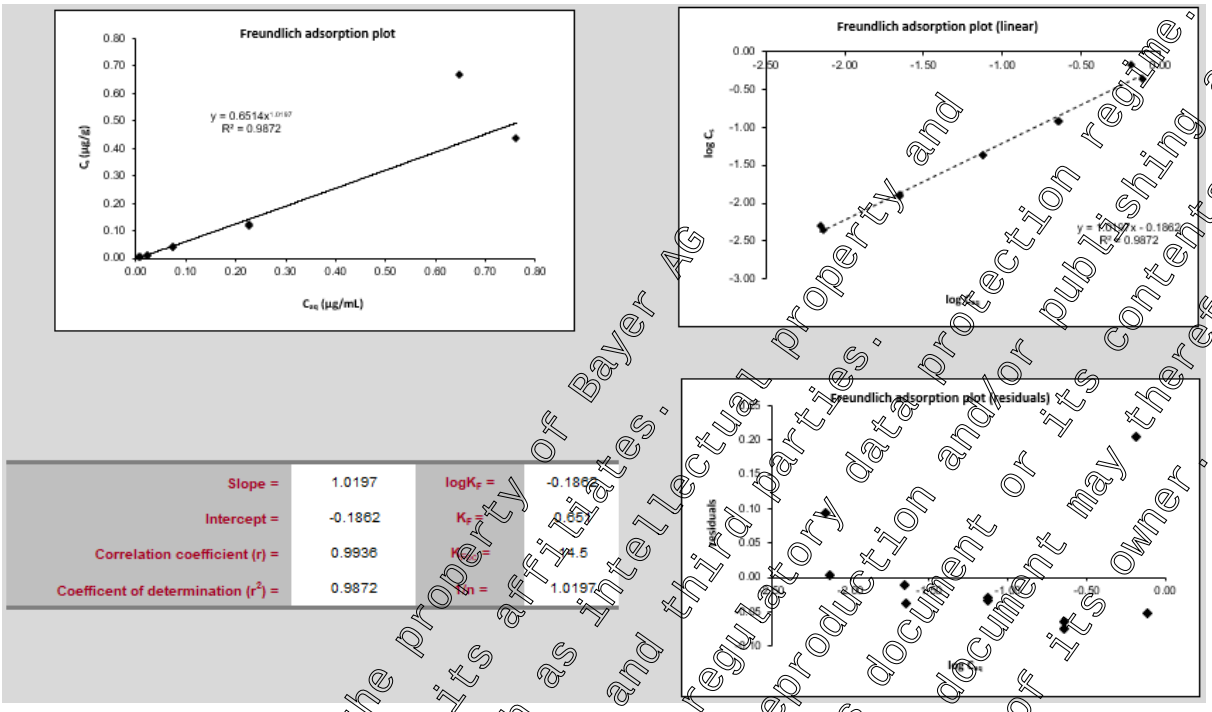
Slope =	0.9736	$logK_F$ =	0.1709
Intercept =	0.1700	K_F =	1.479
Correlation coefficient (r) =	1.0000	K_{foc} =	77.8
Coefficent of determination (r^2) =	0.9999	1/n =	0.9736

Figure 7.1.3.1.2- 26: Freundlich Isotherms of M-05 in soil [redacted] at 20°C



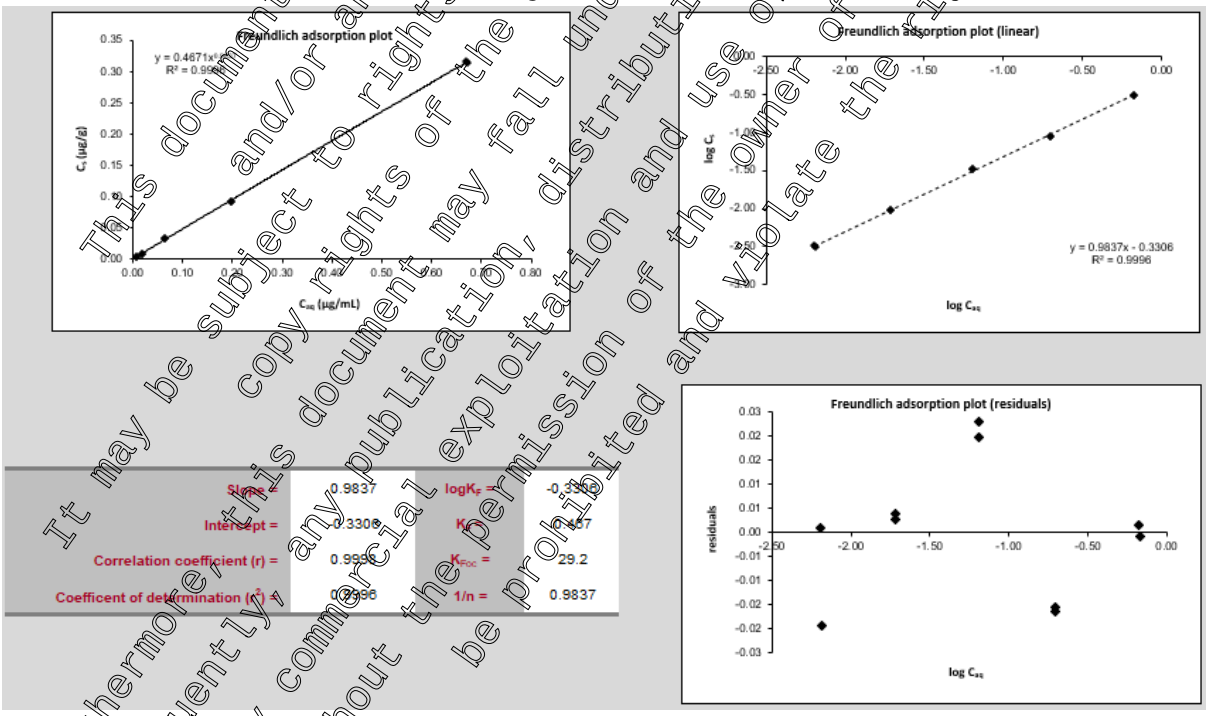
Slope =	0.9847	$logK_F$ =	-0.3085
Intercept =	-0.3085	K_F =	0.492
Correlation coefficient (r) =	0.9999	K_{foc} =	24.6
Coefficent of determination (r^2) =	0.9998	1/n =	0.9847

Figure 7.1.3.1.2- 27: Freundlich Isotherms of M-05 in soil Dollendorf II at 20°C



Slope =	1.0197	$\log K_F$ =	-0.1862
Intercept =	-0.1862	K_F =	0.651
Correlation coefficient (r) =	0.9936	K_{FOC} =	74.5
Coefficent of determination (r^2) =	0.9872	1/n =	1.0197

Figure 7.1.3.1.2- 28: Freundlich Isotherms of M-05 in soil L[redacted] at 20°C



Slope =	0.9837	$\log K_F$ =	-0.3306
Intercept =	-0.3306	K_F =	0.467
Correlation coefficient (r) =	0.9998	K_{FOC} =	29.2
Coefficent of determination (r^2) =	0.9996	1/n =	0.9837

A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 61. The impact on reported endpoints is summarised in

Table 7.1.3.1.2- **62**.

Table 7.1.3.1.2- 61: Summary of Quality Criteria and Regulatory Interpretation

M-05		Quality Criteria		
Soil Name	**Soil Type**	**Met**	**Partially Met**	**Not Met**
[redacted]	Sandy loam	8	0	1
H[redacted]	Silt loam	8	0	1
Dollendorf	Loam	7	1	1
L[redacted]	Loamy sand	8	0	1

Table 7.1.3.1.2- 62: Impact on Endpoints

Soil Name	Soil Type	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
[redacted]	Sandy loam	77.9	77.8	0.974	0.974
H[redacted]	Silt loam	24.6	24.6	0.985	0.985
Dollendorf	Loam	14.7	14.5	1.025	1.020
L[redacted]	Loamy sand	29.2	29.2	0.984	0.984

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

The Freundlich adsorption coefficients $K_{F,ads}$ of M-05 in the four test soils ranged from 0.467 to 1.479 mL/g. The corresponding adsorption coefficients $K_{F,OC,ads}$ normalised for organic carbon content ranged from 14.7 to 77.9 mL/g. The Freundlich exponents 1/n were in the range of 0.974 to 1.025.

According to the Briggs mobility classification, M-05 is considered mobile to intermediate mobility in the tested soils.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed the results largely met the quality criteria and were therefore acceptable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of M-05 in soil.

Data Point:	KCA 7.1.3.1.2/12
Report Author:	[redacted]
Report Year:	2016
Report Title:	[pyridine-2,6-14C] AE 1388273: Adsorption/desorption in four different soils
Report No:	AS493
Document No:	M-572869-01-1
Guideline(s) followed in study:	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 Adsorption/Desorption (Batch Equilibrium), October 2008
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially 180ecognized testing facilities:	Yes, conducted under GLP/Officially 180ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption/desorption characteristics of M-14 (referred to as AE 1388273 in the report) were studied in four soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 1 °C.

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
[redacted]	Monheim, Germany	Loam	5.0	1.8
H[redacted]	Burscheid, Germany	Silt loam	6.1	1.9
Dollendorf II	Blankenheim, Germany	Clay loam	7.3	4.8
L[redacted]	Monheim, Germany	Sandy loam	6.5	1.5

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1:1 for [redacted], H[redacted] and L[redacted] soils and 1:2 for Dollendorf II soil. Nominal test concentrations of 1.00, 0.30, 0.10, 0.03, and 0.01 mg/L of [^{14}C]-M-14 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. 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 adsorption parameters were calculated using the Freundlich adsorption isotherm. The mass balance of the soils in the definitive test was determined by LSC of the supernatants after adsorption, desorption and by combustion of the remaining soils. The total radioactivity recovery with respect to the individual vessel ranged from 89.4 to 100.5% of the applied radioactivity. Mean recovery rates were all within the range of 90 – 110% of the applied radioactivity.

The calculated adsorption constants K_F of the Freundlich isotherms ranged from 0.1765 mL/g to 0.5601 mL/g for the tested soils. The Freundlich exponents 1/n ranged from 0.9369 to 0.9639, 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_{Fads} were correlated with the organic carbon content of the soil to get a comparability of the adsorption behaviour in different soils. For M-14 the K_{Foc} values ranged from 9.8 to 14.9 mL/g.

The measured $K_{F(des)}$ values ranged between 0.2214 and 0.7523 mL/g and $K_{F,OC\ (des)}$ values between 12.3 and 19.9 mL/g.

K_{oc} values indicate that M-14 is mobile in the test soils according to the Briggs classification.

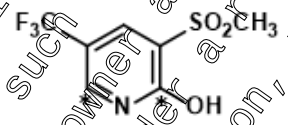
Soil	[redacted]	H[redacted]	Dollendorf II	L[redacted]
Soil type (USDA)	Loam	Silt loam	Clay loam	Sandy loam
pH (0.01M $CaCl_2$)	5.0	6.1	7.3	6.5
Organic carbon [%]	1.8	1.9	4.8	1.5
$K_F^{(ads)}$ [mL/g]	0.1765	0.2834	0.5601	0.1848
1/n	0.9639	0.9369	0.9411	0.9562
$K_{F,OC}^{(ads)}$ [mL/g]	9.8	14.9	11.7	12.3
R^2	0.9991	0.9999	0.9998	0.9992
$K_F^{(des)}$ [mL/g]	0.2214	0.3784	0.7523	0.2791
1/n	0.9558	0.9470	0.9190	0.9573
$K_{F,OC}^{(des)}$ [mL/g]	12.3	19.9	15.7	18.6

I. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-M-14 (referred to as AE 1388273 in the report)



*Denotes position of ^{14}C-radiolabel

Specific Activity: 3.54 MBq/mg

Radiochemical Purity: > 99% (HPLC)

Sample/Batch ID: KML 10158

Stability of test compound: Stable during the batch equilibrium procedure with >90% AR remaining as M-14 after 48 hours.

2. Test Soils

Sorption tests were performed with four agricultural soils from Germany selected to cover a representative range of soil physico-chemical properties. Soil samples were collected up to 20 cm depth, then transported to test facility where they were stored refrigerated (1-10°C). All soils were air dried, sieved (≤2 mm) and sterilised prior to use. The soils were fully characterised with respect to texture, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study.

Table 7.1.3.1.2- 63: Physico-chemical properties of test soils

Characteristic / Code	[redacted]	[redacted]	Dollendorf II	[redacted]
Soil ID	[redacted]	[redacted]	DoII	[redacted]
Geographic Location	Monheim, Germany	Burscheid, Germany	Blankenheim, Germany	Monheim, Germany
Textural Classification (USDA)	Loam	Silt loam	Clay loam	Sandy loam
Sand (2000 to ≥ 50 µm) (%)	40	15	25	75
Silt (50 to ≥ 2 µm) (%)	52	68	44	16
Clay (< 2 µm) (%)	19	17	31	9
pH (water)	5.3	6.4	7.6	6.8
pH ($CaCl_2$)	5.0	6.1	7.3	6.5
Organic carbon (%)	1.8	1.9	4.8	1.5
Organic matter (%)*	3.10	3.27	8.26	2.58
CEC (meq/100g soil)	9.7	10.5	18.8	8.2

*calculated using conversion factor % organic matter = % organic carbon x 1.72

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of borosilicate glass centrifuge tubes (42 mL or 83 mL) with Teflon® lined screw caps.

In preliminary tests, the adsorption of the test item to glassware, to determine any background radioactivity in the soil, the optimal soil-to-solution ratio and the appropriate adsorption and desorption equilibration times were determined.

A mass balance and parental mass balance was established for all soils in preliminary tests. After equilibration, soil and solution phases were separated by centrifugation. Residues remaining on the soil after adsorption were exhaustively extracted up to six times with acetonitrile/water (4/1, *v/v*) for 15 minutes in an ultrasonic bath, followed by a horizontal shaker for 30 minutes. After extraction radioactivity remaining in the soil was quantified by combustion.

The main test was performed in duplicate. [^{14}C]-M-14 was dissolved in 0.01M calcium chloride solution at nominal concentrations of 0.01, 0.03, 0.1, 0.3 and 1.0 mg/L. Soil samples were prepared at a soil to solution ratio of 1:1 for [redacted], H[redacted] and L[redacted] and 1:2 for Dollendorf and shaken at in the dark at 20 ± 1°C. Following the preliminary tests, an equilibrium time of 24 hours was selected.

For all soils one desorption cycle was performed on all concentrations. The volume of solution removed after the adsorption step was replaced by an equal volume of [^{14}C]-M-14 stock solution. Test vessels were then shaken for a 24 hour desorption phase.

Adsorption phase

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		20 g (dry weight) per replicate for [redacted] H[redacted] and L[redacted] 10g (dry weight) per replicate for Dollendorf
Equilibration solution		0.01M $CaCl_2$ shaken ≥16 hours
Control (preliminary experiment)		No
Test item concentration	Nominal application rates	Nominal concentration in test solution: 1.0, 0.3, 0.10, 0.03 and 0.01 mg/L
	Initial concentration	0.990 mg/L, 0.297 mg/L, 0.098 mg/L, 0.030 mg/L and 0.001 mg/L
Identity and concentration of co-solvent		Not specified
Soil:Solution ratio		1:2 i.e. 10 g soil dry weight equivalent to 20 mL solution (Dollendorf) 1:1 i.e. 20 g soil dry weight equivalent to 20 mL solution ([redacted] H[redacted] and L[redacted])
Number of replicates	Treatments	Duplicate
Equilibration conditions	Time	24 h
	Temperature	20 ± 2°C
	Dark	In the dark
	Shaking method	Mechanical overhead shaker (~20rpm)
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	~5000 rpm
	Duration	~10 minutes
	Method of separating supernatant	Supernatant was carefully decanted. Volumes measured gravimetrically

Desorption phase

Parameter		Description
Soil samples from adsorption phase used		Yes
Amount of test item present in the adsorbed state/adsorbed amount (mg a.i./kg soil)		The amounts of test item adsorbed to soil after adsorption ranged from 14.6 to 28.5% (reported for definitive test). See OECD 106 Checklist below for further details for each soil.
Number of desorption cycles		1 for all soils
Equilibrium solution and quantity used per treatment for desorption		The decanted solution was replaced by an equal volume of fresh aqueous 0.01 M $CaCl_2$ solution
Soil:Solution ratio		1:1 for soils [redacted], H[redacted] and L[redacted] i.e. 20 g soil to 20 mL solution 1:2 for soil Dollendorf II i.e. 10 g soil to 20 mL solution
Number of replicates	Treatments	Duplicate
Desorption Equilibration conditions	Time	24 h
	Temperature	20 ± 2°C
	Dark	In the dark
	Shaking method	Mechanical overhead shaker (~20rpm)
Method of separation of supernatant		Centrifugation
Centrifugation	3100 G	~5000 rpm
	20 minutes	~10 minutes
	Supernatant was carefully decanted.	Supernatant was carefully decanted. Volumes measured gravimetrically

2. Analytical Procedures

Radioactivity in supernatants and soil extracts was determined by LSC. Radioactivity in the extracted soil was determined by combustion.

Samples of supernatants, soil extracts and controls without soil from the preliminary test only were analysed by HPLC.

The amount of test item adsorbed to the soil was calculated by subtracting the plateau (adsorption equilibrium) concentration in the supernatant solution from the initial concentration (applied concentration). By establishing the material balances it was verified that, beside the adsorption to the soils, no further processes had significantly contributed to the decline of the test item measured in the supernatants.

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. Results of preliminary tests

Based on preliminary tests a soil-to-solution-ratio of 1:1 was selected for soils [redacted], H[redacted] and L[redacted] and a ratio of 1:2 for soil Dollendorf II in the definitive test. An equilibration time of 24 hours was chosen for both the adsorption and desorption phase.

B. Transformation of test substance

For the soils [redacted], H[redacted] and L[redacted] at a soil/solution ratio of 1:1, all parental mass balances were > 90% AR over 48 hours equilibration time, with mass balances ranging from 90.6 to 93.3% AR. For Dollendorf II soil the parental mass balance was < 90% AR at a soil/solution ratio of 1:1 at 24 and 48 hours. The preliminary test for Dollendorf II soil was repeated using a lower soil/solution ratio of 1:2 and the parental mass balance was established as > 90% AR at 48 hours (92.9% AR).

The stability was adequate to determine the test item distribution based on LSC measurements of the adsorption phase extracts in the definitive test.

C. Findings

The radioactive material balance was calculated as sum of radioactivity detected in decanted supernatant solutions after the adsorption or desorption phase plus the radioactivity remaining in soil residues. The total mass balance ranged from 89.4% to 100.5% AR in individual samples. Mean mass balances for duplicate samples were within the range of 90 – 110% AR (see Table 7.1.3.1.2- 64).

Table 7.1.3.1.2- 64: Definitive test: Mass balance of [2,6-pyridyl-^{14}C]-M-14 (% AR)

Test concentration (mg/L)	[redacted]	H[redacted]	Dollendorf II	L[redacted]
1.0	94.4	90.3	92.9	92.0
0.50	94.0	92.3	92.2	93.7
0.10	95.3	93.5	92.7	95.5
0.05	94.5	92.9	91.1	93.9
0.01	94.5	95.6	96.8	94.3
Mean	94.5	92.9	93.1	93.8
SD	1.4	2.0	2.7	1.2

Mean values of duplicate samples
SD = standard deviation

The results of adsorption tests of [2,6-pyridyl-^{14}C]-M-14 onto four soils are summarised in Table 7.1.3.1.2- 65 and

Table 7.1.3.1.2- 66. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 29 to Figure 7.1.3.1.2- 32.

At the end of the adsorption phase 14.6-18.0%, 21.7-28.5%, 21.3-27.4% and 14.7-19.8% of the applied test material was adsorbed in [redacted] H[redacted], Dollendorf II and L[redacted] soils, respectively.

The adsorption constant $K_{F\ ads}$ of M-14 was between 0.1765 to 0.5601 mL/g for the tested soils; the normalised adsorption constant $K_{OC\ ads}$ was in the range of 9.8 to 14.9 mL/g. The Freundlich exponent

1/n was between 0.9369 to 0.9639, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

The Freundlich desorption coefficients $K_{F\ des}$ ranged from 0.2214 to 0.7523 mL/g with Freundlich exponents (1/n) ranging from 0.9190 to 0.9573. Normalisation to the soil organic carbon contents led to $K_{F,OC\ des}$ values of 12.3 to 19.9 mL/g.

Table 7.1.3.1.2- 65: Definitive test: Concentration of M-14 in aqueous and solid phase following 24 hours of adsorption

Nominal concn (µg/mL)	[redacted]		[redacted]		Dollendorf II		L[redacted]	
	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)
0.01	0.004	0.001	0.004	0.002	0.003	0.004	0.004	0.001
0.03	0.013	0.003	0.012	0.006	0.011	0.011	0.011	0.004
0.1	0.042	0.010	0.042	0.019	0.036	0.034	0.037	0.011
0.3	0.127	0.031	0.129	0.055	0.107	0.098	0.114	0.036
1.0	0.427	0.101	0.429	0.168	0.359	0.294	0.396	0.114

Table 7.1.3.1.2- 66 Summary of Freundlich adsorption/desorption constants K_f and K_{oc} values

Phase	Soil	Units	[redacted]	H[redacted]	Dollendorf II	L[redacted]
Adsorption	$K_{F,ads}$	[mL/g]	0.1765	0.2834	0.5601	0.1848
	1/n	-	0.9639	0.9369	0.9411	0.9562
	R^2	-	0.9991	0.9999	0.9998	0.9992
	$K_{OC,ads}$	[mL/g]	9.8	14.9	11.7	12.3
Desorption	$K_{F,des}$	[mL/g]	0.2214	0.3784	0.7523	0.2791
	1/n	-	0.9558	0.9470	0.9190	0.9573
	R^2		0.9994	1.0000	0.9997	0.9994
	$K_{OC,des}$	[mL/g]	12.3	19.9	15.7	18.6

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in

Table 7.1.3.1.2- **68**). The concentrations in the supernatant and the soil as given in the report were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.2- 67: Definitive test: Concentration of M-14 in aqueous and solid phase following 24 hours of adsorption used in checklist.

Concn (µg/mL)	[redacted]		[redacted]		Dollendorf II		L[redacted]	
	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)	Solution (µg/mL)	Soil (µg/g)
0.010	0.0081	0.0017	0.0071	0.0027	0.0073	0.0051	0.0079	0.0020
	0.0080	0.0018	0.0070	0.0028	0.0071	0.0054	0.0083	0.0016
0.0029	0.0243	0.0051	0.0217	0.0077	0.0214	0.0160	0.0239	0.0057
	0.0245	0.0049	0.0219	0.0076	0.0218	0.0152	0.0241	0.0054
0.097 [A]	0.0826	0.0146	0.0729	0.0246	0.0734	0.0481	0.0810	0.0168
	0.0826	0.0146	0.0732	0.0243	0.0731	0.0485	0.0819	0.0159
0.293 [B]	0.2456	0.0483	0.2235	0.0709	0.2257	0.1369	0.2435	0.0521
	0.2497	0.0441	0.2237	0.0707	0.2230	0.1426	0.2467	0.0489
0.978 [C]	0.8346	0.1450	0.7594	0.2223	0.7696	0.4225	0.8402	0.1451
	0.8192	0.1605	0.7664	0.2152	0.7658	0.4301	0.8302	0.1552

[A] 0.098 for [redacted]; [B] 0.294 for HaH and 0.295 for [redacted]; [C] 0.979 for [redacted] and 0.985 for [redacted]

Table 7.1.3.1.2- 68: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	[redacted]	[redacted]	Dollendorf II	L[redacted]
Adsorption method	-	-	indirect	indirect	indirect	indirect
Soil solution ratio	g/mL	-	1:1	1:1	1:2	1:1
Mass balance of ^{14}C	%	>90%	91.7-97.3	89.4-96.7	90.6-100.5	91.8-96.0
f – due to loss processes (estimated)	%	-	6.1	9.4	7.1	7.9
Adsorbed percentage (δ)	%	>20%	14.66-19.89	21.72-30.05	21.31-29.04	14.70-21.17
K_D x soil:solution ratio	-	0.3	0.17-0.22	0.28-0.40	0.27-0.38	0.17-0.25
#K_{fE} / K_f	-	<1.2	1.84 & 1.70	1.72 & 1.76	1.50 & 1.49	2.17 & 2.02
ads K_F	L/kg	-	0.176	0.283	0.560	0.185
95% confidence interval	-	*	0.161-0.193	0.273-0.294	0.529-0.592	0.163-0.209
ads 1/n	-	-	0.964	0.937	0.941	0.957
95% confidence interval	-	*	0.934-0.994	0.926-0.948	0.923-0.959	0.915-0.998
ads R^2	-	>0.975	0.999	1.000	0.999	0.997
ads $K_{F,OC}$	L/kg	-	9.8	14.9	11.7	12.3
Visual fit to Freundlich isotherm	-	-	Good	Good	Good	Good
Residual plots randomly distributed	-	-	Good	Good	Good	Good

* Confidence intervals should be narrow

Relevant quality checks were performed to evaluate the acceptability of the study. These checks confirmed that the mass balance of 89.4-100.5% was acceptable. At the soil:solution ratio of 1:1 the % adsorption was below the set quality criteria of 20% in two of the soils ([redacted] and L[redacted]). Although no LOQ value is reported [either as % applied or as mg/kg (mg/L)] the acceptability of the analytical method was confirmed with the lowest measured dpm values (LSC) significantly above reported background values over the entire range of concentrations measured. The $K_{d\,x}$ soil/solution ratio was <0.3 in two of the four soils ([redacted] and L[redacted]). Additionally, the calculated K_{fE} / K_f ratio was >1.2 in all fours soils due to the low sorption and relatively high "f" values (6.7 – 9.4% despite all parental mass balances being >90%).

The graphical fits of the Freundlich equation based on the standard linear regression form using log-log transformed data alongside the associated residual plots were all good, with the R^2 of the standard linear regressions ranging from 0.997 to 1.000 and the visual fit of both the standard regression and the residual plots were good.

An overall assessment of the data confirms the study is acceptable.

Figure 7.1.3.1.2- 29: Freundlich Isotherms of M-14 in soil [redacted] at 20°C

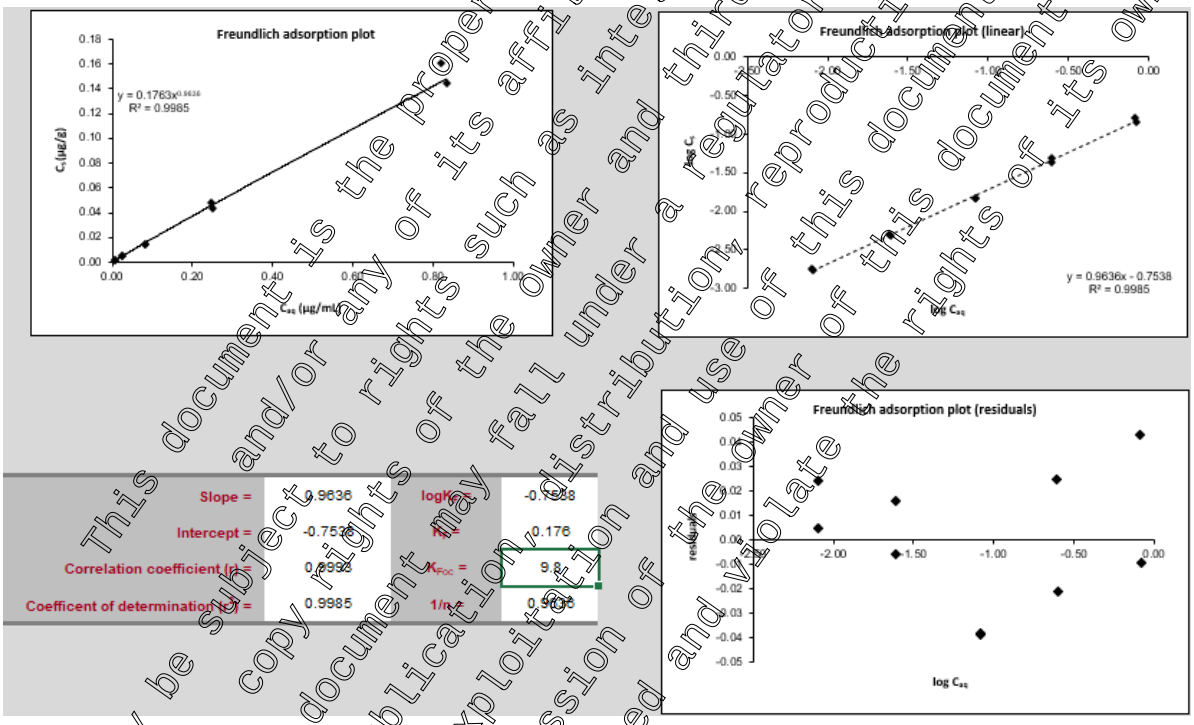
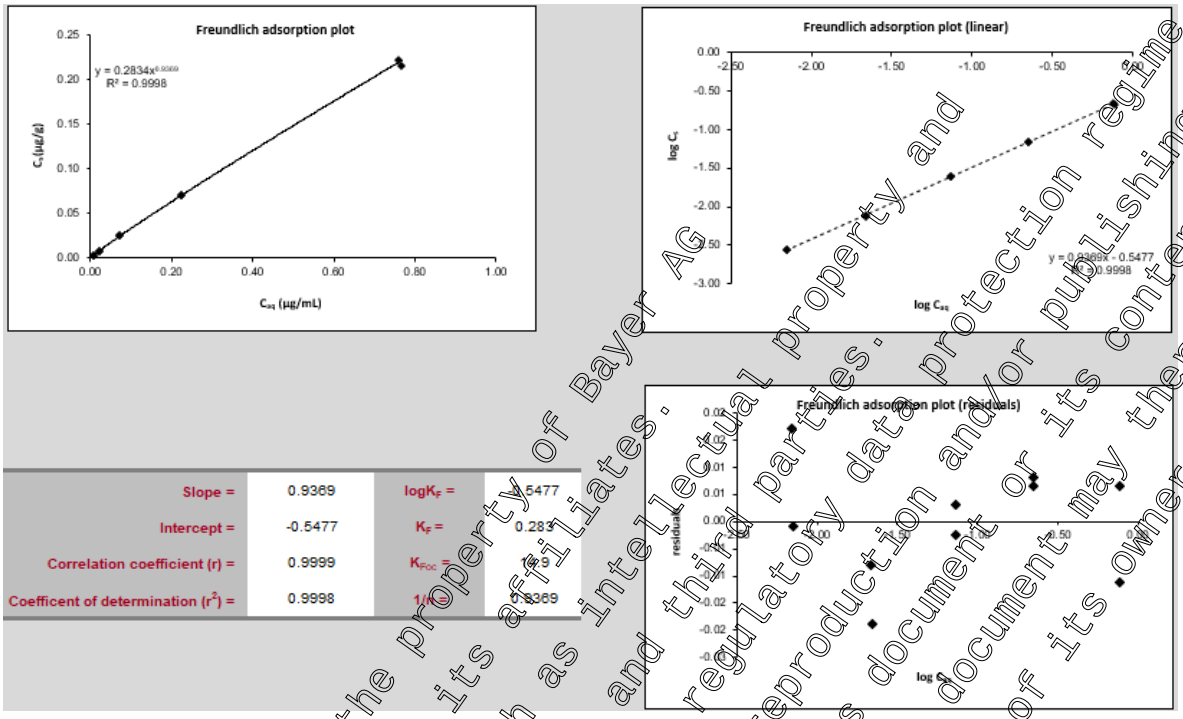
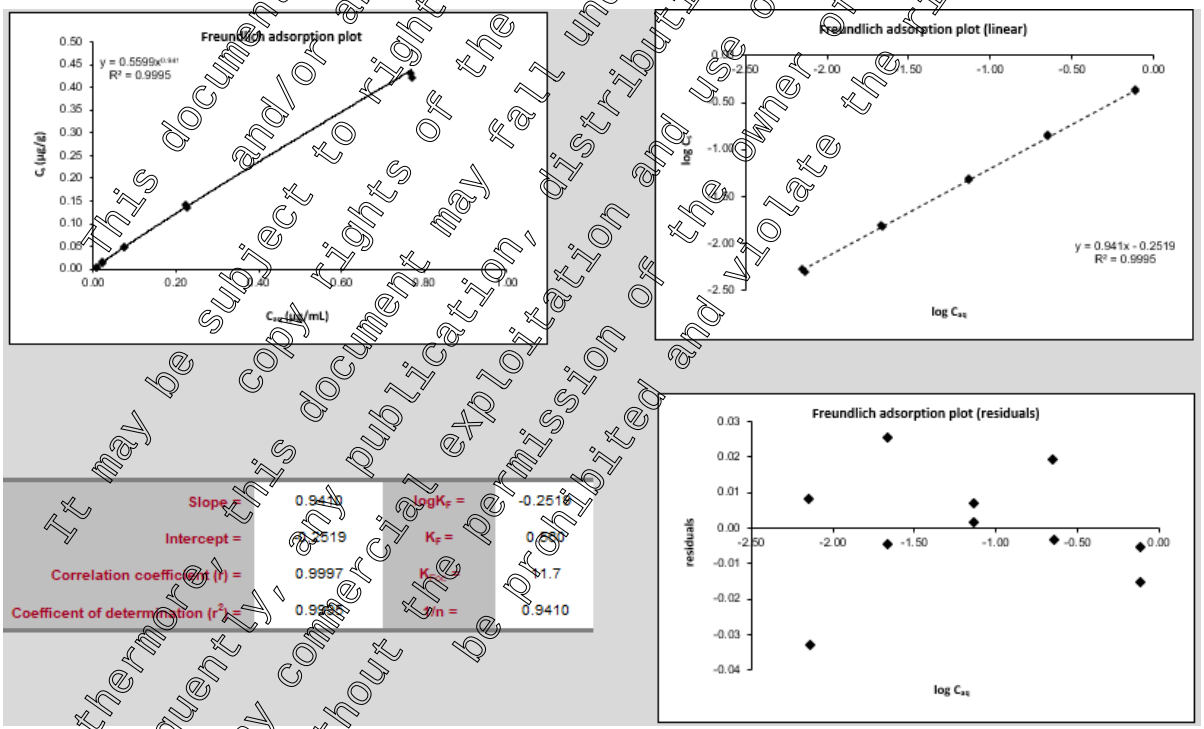


Figure 7.1.3.1.2- 30: Freundlich Isotherms of M-14 in soil H[redacted] at 20°C



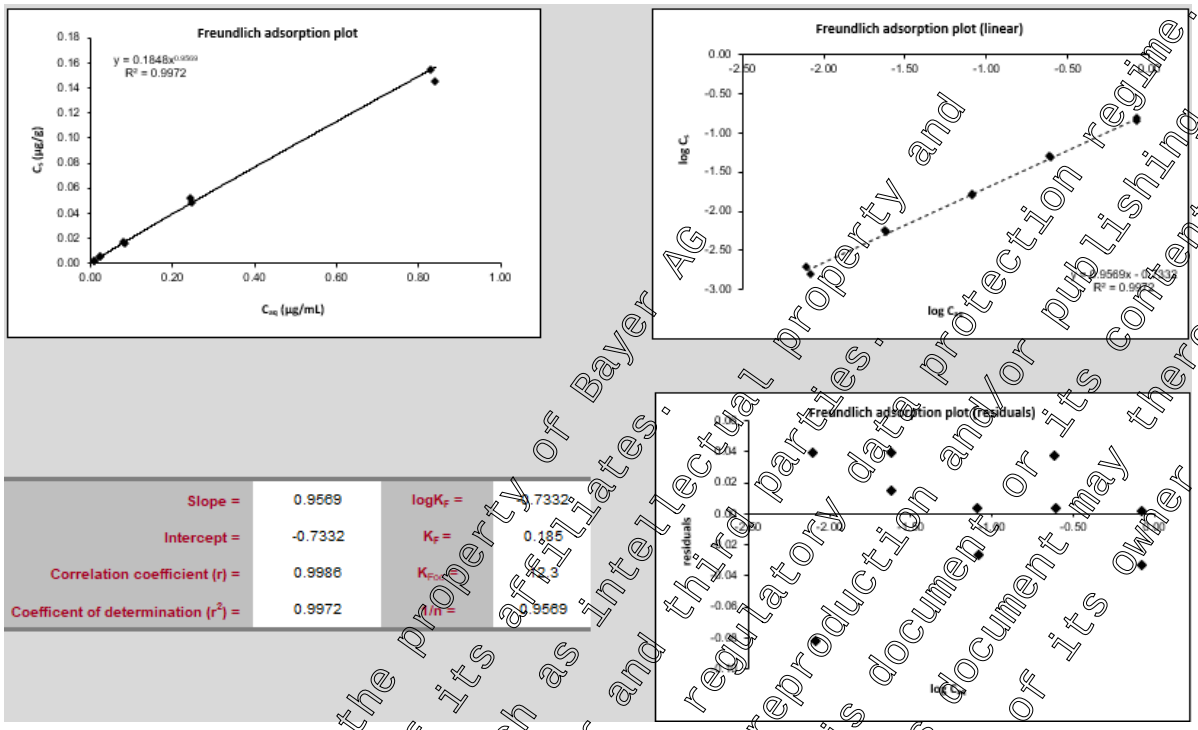
Slope =	0.9369	$\log K_F$ =	-0.5477
Intercept =	-0.5477	K_F =	0.283
Correlation coefficient (r) =	0.9999	K_{FOC} =	14.9
Coefficent of determination (r^2) =	0.9998	1/n =	0.9369

Figure 7.1.3.1.2- 31: Freundlich Isotherms of M-14 in soil Dollendorf II at 20°C



Slope =	0.9410	$\log K_F$ =	-0.2519
Intercept =	-0.2519	K_F =	0.560
Correlation coefficient (r) =	0.9997	K_{FOC} =	11.7
Coefficent of determination (r^2) =	0.9995	1/n =	0.9410

Figure 7.1.3.1.2- 32: Freundlich Isotherms of M-14 in soil L[redacted] at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 69. The impact on reported endpoints is summarised in

Table 7.1.3.1.2- **70**.

Table 7.1.3.1.2- 69: Summary of Quality Criteria and Regulatory Interpretation

M-14		Quality Criteria		
Soil Name	**Soil Type**	**Met**	**Partially Met**	**Not Met**
[redacted]	Loam	6	0	3
H[redacted]	Silt loam	7	1	1
Dollendorf	Clay loam	7	1	1
L[redacted]	Sandy loam	6	1	2

Table 7.1.3.1.2- 70: Impact on Endpoints

Soil Name	Soil Type	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
[redacted]	Loam	9.8	9.8	0.9639	0.964
H[redacted]	Silt loam	14.9	14.9	0.9369	0.937
Dollendorf	Clay loam	11.7	11.7	0.9411	0.941
L[redacted]	Sandy loam	12.3	12.3	0.9562	0.957

The small differences between the reported values and the OECD calculation tool (v1) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

The adsorption coefficients $K_{F\ ads}$ of M-14 in the four test soils ranged from 0.1765 to 0.5601 mL/g based on Freundlich equation. The corresponding organic carbon normalised adsorption coefficients $K_{F,OC\ ads}$ ranged from 9.8 to 14.9 mL/g. The Freundlich exponents 1/n were in the range of 0.9369 to 0.9639.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that extent of adsorption of M-14 is too low to meet all the proposed quality criteria for the indirect method but the compound was shown to be stable and the study otherwise is well conducted.

A second soil adsorption study with M-14 using the OECD 106 direct method (see KCA 7.1.3.1.2/13) derived very similar endpoints, thus confirming the validity of this study.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption characteristics of M-14 (AE 1388273) in soil.

Data Point:	KCA 7.1.3.1.2/13
Report Author:	[redacted]
Report Year:	2020
Report Title:	Adsorption/desorption [14C]-AE 1388273 (BCS-BA63474) M-14 in five soils
Report No:	S18-08483
Document No:	M-686386-02-1
Guideline(s) followed in study:	OECD Guideline No. 106, 2000 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009, 2013
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognized testing facilities:	Yes, conducted under GLP/Officially recognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption behaviour of M-14 (referred to as AE 1388273 in the report) was studied in five soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 1 °C using the OECD 106 direct method with residues remaining on the soil after adsorption quantified directly.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
LUFA 2.1	2.1	Dudenhofen, Germany	Sand	5.2	0.59
LUFA 2.3	2.3	Offenbach, Germany,	Sandy loam	6.2	0.61
LUFA 5M	5M	Mechtersheim, Germany	Sandy loam	7.1	1.10
LUFA 6S	6S	Siebeldingen, Germany	Clay loam	7.3	1.78
Frankenforst	FF	Königswinter, Germany	Silt loam	6.9	2.4

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1/1 (all soils). Test concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L of [^{14}C]-M-14 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. Due to the low adsorption, no desorption test was performed.

The aqueous supernatant after adsorption was separated by centrifugation, the soils were extracted and the amount of test item in the supernatants and soil extracts was analyzed by liquid scintillation counting (LSC) and HPLC. The sorption parameters were calculated using Freundlich isotherms.

The test item was stable throughout the study. Mean parental mass balances after 24 hours of adsorption were 100.5, 95.5, 92.4, 92.5 and 90.4 % in the preliminary test 2 and 100.2, 100.9, 99.7, 96.8 and 98.0 % in the definitive test, for soil 2.1, 2.3, 5M, 6S and FF, respectively.

The calculated adsorption constants K_F of the Freundlich isotherms ranged from 0.028 to 0.300 mL/g for the tested soils. The Freundlich exponents 1/n ranged from 0.892 to 1.022, 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_{Fads} were correlated with the organic carbon content of the soil to get a comparability of the adsorption behaviour in different soils. For M-14 the K_{Foc} values ranged from 4.6 to 16.9 mL/g.

K_{oc} values indicate that M-14 is mobile to very mobile in soil according to the Briggs classification.

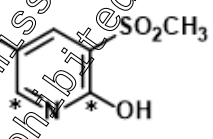
Soil origin	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
Soil type (USDA)	Sand	Sandy loam	Sandy loam	Clay loam	Silt loam
pH (0.01M $CaCl_2$)	5.16	6.24	7.13	7.31	6.9
Organic carbon [%]	0.59	0.61	1.1	1.78	2.4
$K_F^{(ads)}$ [mL/g]	0.031	0.028	0.117	0.300	0.238
1/n	1.022	0.908	0.892	0.936	0.936
$K_{F,OC}^{(ads)}$ [mL/g]	5.3	4.6	10.7	16.9	9.9

I. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-M-14 (referred to as AE 1388273, BCS-BA63474 in the report)



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

Specific Activity:	3.54 MBq/mg (0.096 µCi/mg)
Radiochemical Purity:	> 99%
Sample/Batch ID:	KML 10563
Stability of test compound:	Stable during the batch equilibrium procedure with 100% AR remaining as M-14 after 48 hours.

2. Test Soils

The adsorption/desorption behaviour of M-14 was characterised in five soils using the batch equilibrium method. The five soils, taken from agricultural use areas, were categorised under the USDA classifications as a sand (Dudenhofen, Germany; LUFA 2.1), a sandy loam (Offenbach, Germany; LUFA 2.3), a sandy loam (Mechtersheim, Germany; LUFA 5M), a clay loam (Siebeldingen, Germany; LUFA 6S) and a silt loam (Königswinter, Germany; Frankenforst). After collection, soils were delivered sieved to a particle size of ≤ 2 mm and gently air-dried at ambient conditions. They were stored at room temperature at the receiving facility. The soil characteristics are given below in Table 7.1.3.1.2- 71.

Table 7.1.3.1.2- 71: Physico-chemical characteristics of test soils

Characteristic / Code	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S*	LUFA 6S*	Franken forst
Soil ID	2.1	2.3	5M	6S	6S	FF
Geographic Location	Dudenhofen Germany	Offenbach Germany	Mechter-sheim, Germany	Siebeld ingen Germany	Siebeld ingen Germany	Königs-winter Germany
GPS coordinates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Batch	1418	1318	3418	4518	1418	20170206
Textural Class (USDA)	Sand	Sandy Loam	Sandy Loam	Clay Loam	Clay Loam	Silt Loam
Sand (2000 to ≥ 50 µm)(%)	88.82	63.44	59.9	23.4	32.34	27
Silt (50 to ≥ 2 µm) (%)	8.75	30.08	31.1	40.9	34.03	54
Clay (< 2 µm) (%)	2.43	6.48	8.9	35.7	33.62	19
pH ($CaCl_2$)	5.16	6.24	7.13	7.31	7.24	6.9
Organic Carbon (%)	0.59	0.61	1.10	1.78	1.85	2.4
Cation exchange capacity (meq/100g)	5.2	11.8	20.0	16.4	24.0	18.5
Maximum Water Holding Capacity (g/100 g dry matter)	30.65	38.56	39.17	48.76	51.49	n.a.

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of either 50 mL glass flasks (conventional system) or commercially available 20 mL syringes (Braun,polypropylene, polyethylene) as incubation vessels and Falcon tubes™ (polypropylene) as recipient vessels for the aqueous phase removed later on (syringe method).

In preliminary tests, as well as a comparison of the conventional and syringe method being undertaken, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption equilibration times and the stability of the test item were determined.

The soil was weighed into the test vessels and pre-equilibrated over night with 0.01M $CaCl_2$ solution at a soil-to-solution ratio of 1/1. The definitive test was performed at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L in 0.01M $CaCl_2$ solution using the syringe method. After application of M-14, the test systems were shaken for 24 hours in the dark at 20 ± 1 °C and centrifuged. The supernatants were decanted, the volumes determined by weighing and the radioactivity contents determined by LSC and characterized by radio-HPLC.

Five replicates per soil and concentration were used. The equilibration time was 24 hours for adsorption. An aliquot of the control flasks was taken at 0 h for LSC measurement in order to determine the initial mass of the test item. The definitive test was repeated for all soils at nominal concentration levels of 0.01 and 0.1 mg/L. The repetition was necessary as the actual applied concentrations deviated from the nominal concentrations.

After equilibration, soil and solution phases were separated by centrifugation. Residues remaining on the soil after adsorption were extracted and quantified directly.

In the definitive test, soil samples were exhaustively extracted three times with acetonitrile/water (4:1, *v/v*) at ambient temperature for 30 minutes. Finally, the soil was extracted with acetone at ambient temperature for 30 minutes to aid drying. After each extraction step, extract and soil were separated by centrifugation.

For detailed information on experimental design see below. Desorption was not examined due to the low adsorption of M-14 on soil.

Adsorption

Parameter		Description
Soil Condition		Soils were equilibrated to study conditions for at least 12 hours with 5 mL aqueous 0.01 M $CaCl_2$ solution.
Soil sample weight		5 g per replicate.
Solution used for equilibration		aqueous 0.01 M $CaCl_2$ solution.
Control used		$CaCl_2$ solution without soil
Test item concentrations	Nominal application rates	Nominal concentrations in test solution: 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L
	Analytically measured concentrations	Concentrations in test solution: 0.96, 0.50, 0.10, 0.065 and 0.011 mg/L
Identity and concentration of extraction solvent		acetonitrile / water (80/20, *v/v*), acetone
Soil-to-solution ratio		1:1, i.e. 5 g soil dry weight to 5 mL solution (corrected for soil moisture)
pH of the equilibration solution (from definitive test)	Initial	pH of aqueous 0.01 M $CaCl_2$ solution without soil: 4.89
	Final	pH with soil and test item after adsorption equilibrium: range 6.41 – 8.26
Number of replicats	Controls	Two
	Treatments	Five
Incubation	Time	24 hours
	Temperature	20 ± 1 °C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 rpm
Centrifugation	Speed	2800 rpm
	Duration	35 to 140 min for $CaCl_2$ and 35 min for extracts
	Method of separation of soil and solution	Centrifugation (no filtration)

2. Analytical Procedures

Radioactivity in supernatants and soil extracts was determined by LSC. Radioactivity in the extracted soil was determined by combustion.

Samples of supernatants, soil extracts and controls without soil from the preliminary test and the definitive test were analysed by HPLC. For the definitive test, the highest and lowest test concentrations were analysed by HPLC. In cases where ROI was 100%, K_d determination is based on LSC measurements of aqueous supernatant and soil extracts. HPLC recovery was quantitative (96.6%). The LOD of analytical method was 1271 dpm by HPLC and 13 dpm by TLC.

Samples of lowest concentration (K5) were characterized with thin layer chromatography (TLC).

Adsorption isotherms were calculated by linear regression analysis of the data according to the Freundlich equation. The amount of the test substance adsorbed was directly determined by extraction and analysis of soil. Desorption was not examined due to the low adsorption.

II. Results and Discussion

A. Results of preliminary tests

Adsorption percentage between conventional and syringe method were comparable. Using the syringe method, the residual pore water is minimized and therefore a more accurate determination of K_d and K_{oc} is possible. Therefore, the syringe method was chosen for the definitive test.

The test item was stable in aqueous 0.01 M $CaCl_2$ solution in the absence of soil. After incubation for 48 hours M-14 represented 100% AR. No adsorption of the test item to the test vessels was observed after shaking for 48 hours. The test item has very low adsorption characteristics to soil and so a soil-to-solution-ratio of 1:1 was used. An equilibration time of 24 hours was chosen for the adsorption phase.

B. Transformation of test substance

The parental mass balances were determined at a soil-to-solution ratio of 1/1 for all soils. M-14 was stable throughout the study. Mean parental mass balances (excluding NER) were 100.5, 95.5, 92.4, 92.5 and 90.4% AR in the preliminary test for LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst soils, respectively.

The stability was adequate to determine the test item distribution based on LSC measurements of the adsorption phase extracts in the definitive test. No major degradation product was observed by HPLC analysis.

Mean parental mass balances of the test item in the definitive test were 100.2, 100.9, 99.7, 96.8 and 98.0% AR for LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst soils, respectively.

C. Findings

Mean material balances were 100.1, 101.4, 100.9, 99.5 and 100.6% AR for soils LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst, respectively (summarised in Table 7.1.3.1.2- 72). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 72: Definitive test: Mass balance of [2,6-Pyridyl-^{14}C]-M-14 (% AR)

Soil	Aquatic phase		Soil extract		Non extractable radioactivity		Total	
	% AR	SD	% AR	SD	% AR	SD	% AR	SD
LUFA 2.1	97.0	3.1	3.2	1.4	0.2	0.2	100.1	2.7
LUFA 2.3	97.3	2.9	3.5	1.1	0.5	0.4	101.4	2.1
LUFA 5M	86.5	4.0	13.2	3.8	1.2	0.7	100.9	2.3
LUFA 6S	71.7	4.2	25.2	5.4	2.8	1.6	99.5	2.9
Frankenforst	76.4	4.2	21.6	4.5	2.1	1.2	100.6	3.1

Note: Mass balances were quantitative. Overall mean values derived from five replicates at each of five concentrations provided in the report. SD = standard deviation.

The results of adsorption tests of [2,6-pyridyl-^{14}C]-M-14 onto five soils are summarised in Table 7.1.3.1.2- 73 and Table 7.1.3.1.2- 74. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 33 to Figure 7.1.3.1.2- 37.

At the end of the adsorption phase 1 to 5% AR, 1 to 5% AR, 10 to 17%, 25 to 32% AR and 21 to 26% of the applied test material was adsorbed in LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst, respectively.

The adsorption constant $K_{F(ads)}$ of M-14 was between 0.028 to 0.300 mL/g for the tested soils; the normalised adsorption constant $K_{OC(ads)}$ was in the range of 4.6 to 16.9 mL/g. The Freundlich exponent 1/n was between 0.892 to 1.022, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table 7.1.3.1.2- 73: Definitive test: Concentration of M-14 in aqueous and solid phase following 24 hours of adsorption.

Concn (µg/mL)	Rep	LUFA 2.1		LUFA 2.3		LUFA 5M		LUFA 6S*		Frankenforst	
		Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)
0.963	1	0.0366	0.9522	0.0337	0.9051	0.0579	0.8500	0.1607	0.7302	0.2572	0.7013
	2	0.0238	0.9390	0.0120	0.8671	0.0778	0.8570	0.2065	0.6601	0.1708	0.7881
	3	0.0312	0.8777	0.0206	0.9361	0.0934	0.8731	0.2138	0.6833	0.1709	0.7060
	4	0.0280	0.9056	0.0240	0.9789	0.0854	0.8456	0.1781	0.7099	0.1805	0.7469
	5	0.0381	0.9057	0.0276	1.4844	0.1073	0.8149	0.1588	0.7380	0.1380	0.7988
	Mean	**0.0315**	**0.9160**	**0.0236**	**1.0343**	**0.0844**	**0.8481**	**0.1896**	**0.7043**	**0.1835**	**0.7482**
0.495	1	0.0179	0.4779	0.0120	0.4855	0.0628	0.4347	0.1209	0.3330	0.1001	0.3898
	2	0.0186	0.4805	0.0180	0.4793	0.0491	0.4171	0.1390	0.3262	0.0941	0.3853
	3	0.0178	0.4724	0.0199	0.4862	0.07	0.4131	0.0608*	0.3259*	0.0948	0.4128
	4	0.0134	0.5097	0.0245	0.4774	0.0564	0.4171	0.1254	0.3350	0.0969	0.3805
	5	0.0145	0.4804	0.0199	0.4880	0.0605	0.4120	0.1115	0.3304	0.0976	0.3756
	Mean	**0.0164**	**0.4842**	**0.0188**	**0.4833**	**0.0598**	**0.4188**	**0.1264**	**0.3311**	**0.0966**	**0.3888**
0.104	1	0.0056	0.0998	0.0048	0.0969	0.0142	0.0890	0.0253	0.0767	0.0220	0.0805
	2	0.0015	0.0973	0.0036	0.0977	0.0142	0.0887	0.0273	0.0724	0.0230	0.0780
	3	0.0057	0.1050	0.0048	0.0956	0.0132	0.0888	0.0268	0.0755	0.0257	0.0764
	4	0.0029	0.0993	0.0032	0.0986	0.0133	0.0905	0.0340	0.0757	0.0144	0.0786
	5	0.0026	0.1021	0.0022	0.1008	0.0127	0.0905	0.0239	0.0760	0.0221	0.0785
	Mean	**0.0037**	**0.1007**	**0.0037**	**0.0979**	**0.0135**	**0.0895**	**0.0275**	**0.0753**	**0.0214**	**0.0784**
0.065	1	0.0007	0.0562	0.0041*	0.0739*	0.0103	0.0553	0.0133	0.0433	0.0166	0.0508
	2	0.0006*	0.0580*	0.0025	0.0610*	0.0119	0.0530	0.0221	0.0419	0.0165*	0.0527*
	3	0.0005	0.0560	0.0033	0.0634	0.0098	0.0581	0.0148	0.0488	0.0205	0.0458
	4	0.0003*	0.0546*	0.0043*	0.0686*	0.0146	0.0511	0.0138	0.0453	0.0212	0.0442
	5	0.0005	0.0497	0.0017	0.0645	0.0132	0.0514	0.0225	0.0411	0.0159*	0.0505*
	Mean	**0.0006**	**0.0539**	**0.0025**	**0.0630**	**0.0120**	**0.0538**	**0.0173**	**0.0441**	**0.0194**	**0.0488**
0.011	1	0.0005	0.0105	0.0004	0.0107	0.0017	0.0087	0.0029	0.0074	0.0025	0.0077
	2	0.0003	0.0101	0.0002	0.0104	0.0017	0.0092	0.0027	0.0078	0.0025	0.0081
	3	0.0003	0.0108	0.0006	0.0105	0.0017	0.0092	0.0032	0.0080	0.0022	0.0076
	4	0.0005	0.0107	0.0004	0.0105	0.0015	0.0093	0.0029	0.0078	0.0027	0.0077
	5	0.0006	0.0105	0.0006	0.0106	0.0007	0.0071	0.0034	0.0081	0.0025	0.0077
	Mean	**0.0004**	**0.0105**	**0.0004**	**0.0105**	**0.0015**	**0.0087**	**0.0030**	**0.0078**	**0.0025**	**0.0077**

* Values included in mean value (mass balance < 90 % or >110 %)

Table 7.1.3.1.2- 74: Summary of Freundlich adsorption constants K_f and K_{oc} values

Soil	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
	2.1	2.3	5M	6S	FF
Textural class	Sand	Sandy Loam	Sandy Loam	Clay Loam	Silt Loam
K_f	0.031	0.028	0.117	0.300	0.238
1/n	1.022	0.908	0.892	0.936	0.923
K_{oc}	5.25	4.588	10.667	16.851	9.899
Correlation	0.916	0.951	0.968	0.985	0.983

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 76) The mean concentrations in the supernatant and the soil as given in Table 7.1.3.1.2- 75 were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.2- 75: Definitive test: Concentration of M-14 in aqueous and solid phase following 24 hours of adsorption used in checklist.

Concn (µg/mL)	LUFA 2.1		LUFA 2.3		LUFA 5M		LUFA 6S*		Frankenforst	
	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)
0.011	0.0004	0.0105	0.0004	0.0105	0.0015	0.0087	0.0030	0.0078	0.0025	0.0077
0.065	0.0006	0.0539	0.0025	0.0630	0.0120	0.0538	0.0173	0.0441	0.0194	0.0469
0.104	0.0037	0.1007	0.0057	0.0979	0.0135	0.0895	0.0275	0.0753	0.0214	0.0784
0.495	0.0164	0.4842	0.0188	0.4833	0.0597	0.4188	0.1264	0.3311	0.0966	0.3888
0.963	0.0315	0.9160	0.0236	1.0343	0.0844	0.8481	0.1896	0.7043	0.1835	0.7482

Table 7.1.3.1.2- 76: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
Code	-	-	**2.1**	**2.3**	**5M**	**6S**	**FF**
Adsorption method	-	-	direct	direct	direct	direct	direct
Soil solution ratio	g/mL		1:1	1:1	1:1	1:1	1:1
Mass balance of ^{14}C	%	>90%	98.6 (highest)	99.3 (highest)	98.2 (highest)	96.4 (highest)	97.8 (highest)
f – due to loss processes[#]	%	-	1.4	0.7	1.8	3.6	2.2
Adsorbed percentage (δ)[#]	%	>20%	0.05 – 16.42	2.44 – 6.14	11.90-13.15	20.59-33.11	21.47-27.27
K_D x soil:solution ratio[#]		>0.3	0.01-0.04	0.02-0.04	0.10-0.14	0.26-0.49	0.24-0.41
K_{fE} / K_f[#]	-	<1.2	1.40	0.91	1.17	1.15	1.07
ads K_F	L/kg	-	0.031	0.029	0.118	0.309	0.244
95% confidence interval	-	*	(0.006 – 0.166)	(0.018 – 0.046)	(0.066 – 0.210)	(0.217 – 0.435)	(0.152 – 0.391)
ads 1/n	-	-	1.038	0.904	0.886	0.939	0.920
95% confidence interval	-	*	(0.516 – 1.741)	(0.726 – 1.081)	(0.675 – 1.098)	(0.819 – 1.060)	(0.754 – 1.086)
ads R^2	-	<0.975	0.917	0.989	0.983	0.995	0.990
ads $K_{F,OC}$	L/kg	-	5.2	4.7	10.7	17.3	10.2
Visual fit to Freundlich isotherm	-	-	Acceptable	Acceptable	Acceptable	Acceptable	Good
Residual plots randomly distributed	-	-	Acceptable	Acceptable	Good	Good	Acceptable

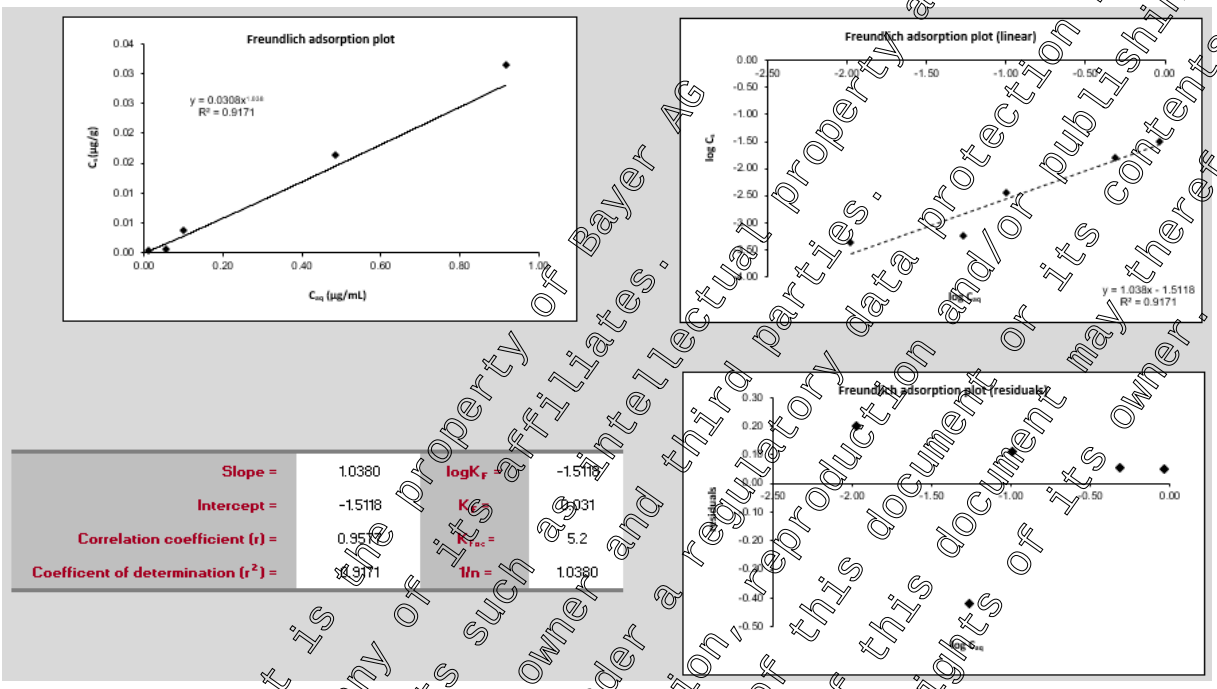
[#]**Note:** 3 checks (in grey) are excluded as not relevant for 'direct' studies, therefore number of checks reduced from 9 to 6.

Relevant quality checks were performed to evaluate the acceptability of the study. Three checks have been excluded from the overall assessment (i.e. adsorbed percentage, K_D x soil:solution ratio and K_{fE} / K_f) as they not relevant for 'direct' studies, the number of quality checks is reduced from 9 to 6.

These checks confirmed that the mass balance was acceptable (>90%). The acceptability of the analytical method (LSC) was confirmed over the entire range of concentrations measured (reported LOQ of 60 dpm represents <1 % of the lowest test concentration). The R^2 of the standard linear regressions ranged from 0.983 to 0.995 for soils 2.3, 5M, 6S and FF meeting the quality criteria but were <0.975 in soil 2.1. The visual fit of both the standard regression and the residual plots were good or acceptable.

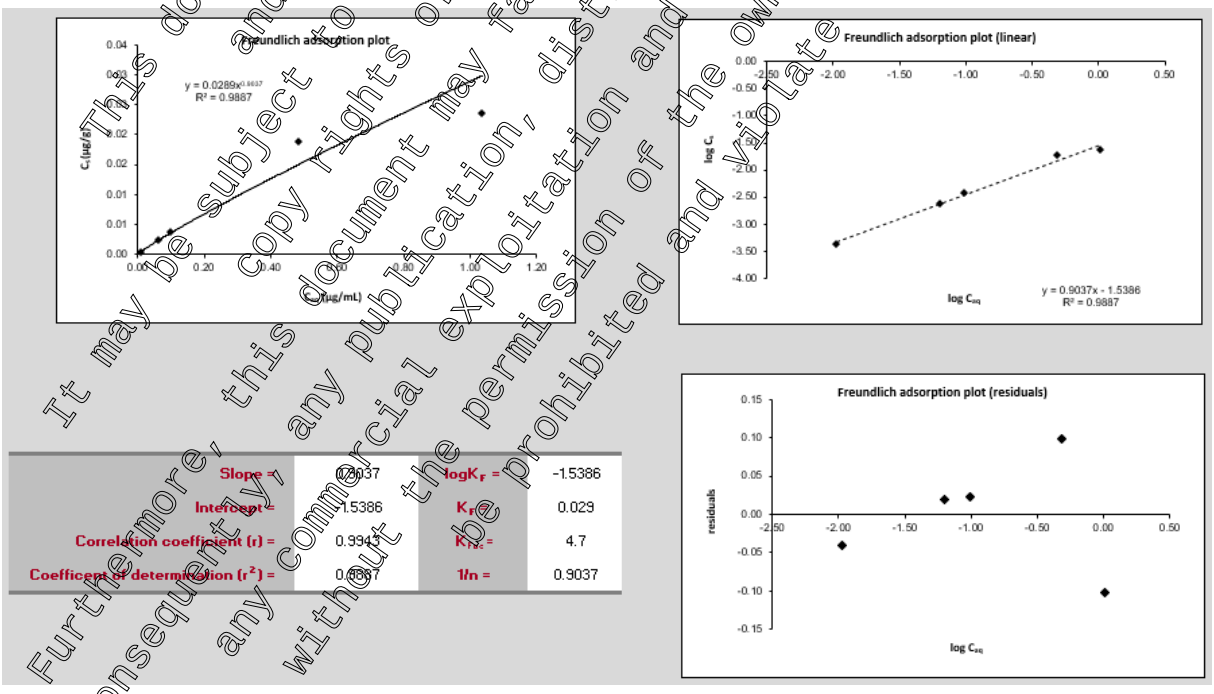
The study has been conducted to a good standard. The test substance was stable (purity 100% in dosing stocks, adsorption supernatant and soil extracts). The evaluation confirmed all soils were acceptable according to the quality checks. The results of the evaluation are summarised in the tables below.

Figure 7.1.3.1.2- 33: Freundlich Isotherms of M-14 in LUFA 2.1 soil at 20°C



Slope =	1.0380	$\log K_F$ =	-1.5118
Intercept =	-1.5118	K_F =	0.031
Correlation coefficient (r) =	0.9574	K_{foc} =	5.2
Coefficent of determination (r^2) =	0.9171	1/n =	1.0380

Figure 7.1.3.1.2- 34: Freundlich Isotherms of M-14 in LUFA 2.3 soil at 20°C



Slope =	0.9037	$\log K_F$ =	-1.5386
Intercept =	-1.5386	K_F =	0.029
Correlation coefficient (r) =	0.9943	K_{foc} =	4.7
Coefficent of determination (r^2) =	0.9887	1/n =	0.9037

Figure 7.1.3.1.2- 35: Freundlich Isotherms of M-14 in LUFA 5M soil at 20°C

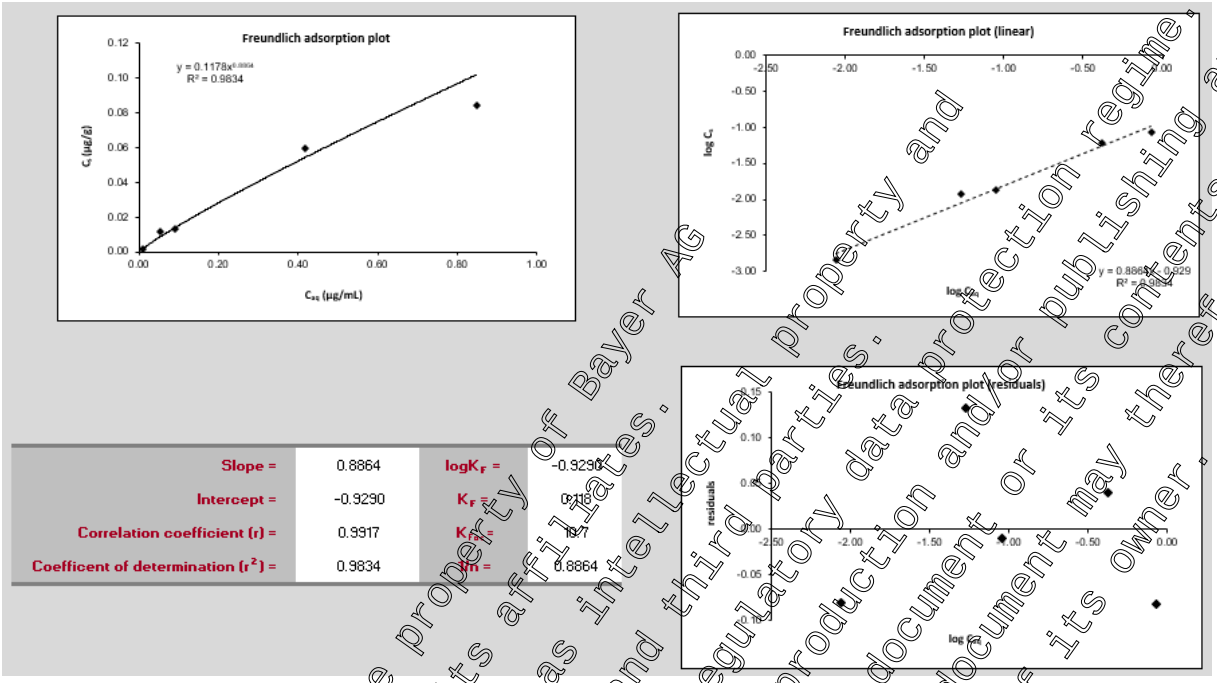


Figure 7.1.3.1.2- 36: Freundlich Isotherms of M-14 in LUFA 6S soil at 20°C

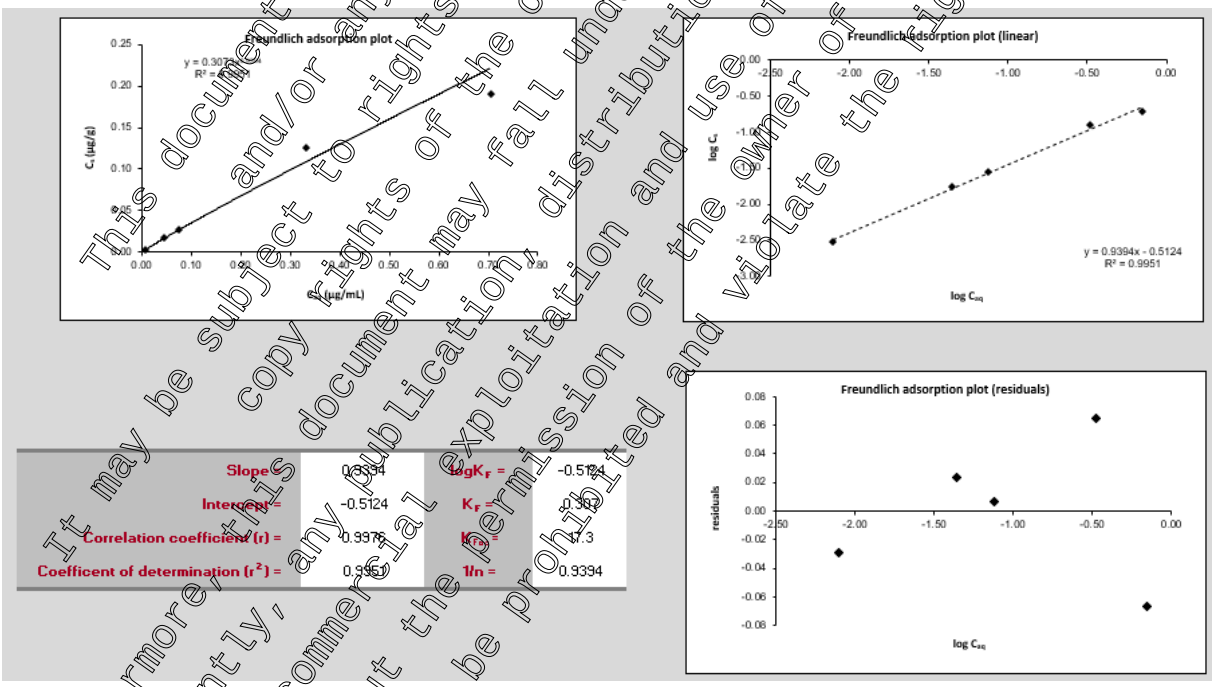
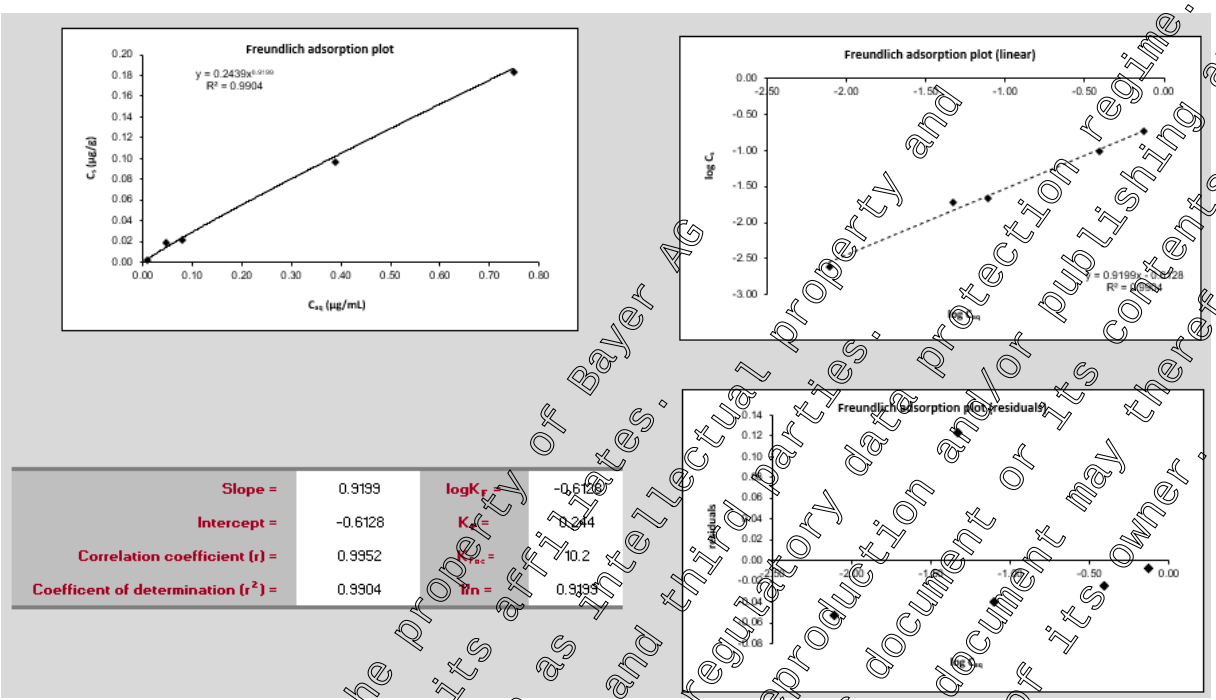


Figure 7.1.3.1.2- 37: Freundlich Isotherms of M-14 in Frankenforst soil at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 77. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 78.

Table 7.1.3.1.2- 77: Summary of Quality Criteria and Regulatory Interpretation

M-14			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
LUFA 2.1	Sand	2.1	4	0	2
LUFA 2.3	Sandy Loam	2.3	6	0	0
LUFA 5M	Sandy Loam	5M	6	0	0
LUFA 6S	Clay Loam	6S	6	0	0
Frankenforst	Silt Loam	FF	6	0	0

Note: 3 checks excluded as not relevant for 'direct' studies, therefore number of checks reduced from 9 to 6.

Table 7.1.3.1.2- 78: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
LUFA 2.1	Sand	2.1	5.3	5.2	1.022	1.038
LUFA 2.3	Sandy Loam	2.3	4.6	4.7	0.908	0.904
LUFA 5M	Sandy Loam	5M	10.7	10.7	0.892	0.886
LUFA 6S	Clay Loam	6S	16.9	17.3	0.936	0.939
Frankenforst	Silt Loam	FF	9.9	10.2	0.923	0.920

The small differences between the reported values and the OECD calculation tool (v2) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

The adsorption constant $K_{F(ads)}$ of M-14 was between 0.028 to 0.300 mL/g for the tested soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 4.6 to 16.9 mL/g. The Freundlich exponent 1/n ranged from 0.892 to 1.022, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the results were acceptable according to the quality criteria and therefore acceptable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption characteristics of M-14 (AE 1388273) in soil.

Data Point:	KCA 7.1.3.1.2/14
Report Author:	[redacted]
Report Year:	2016
Report Title:	[pyridine-2,6-14C] BCS-BX16566-ammonium-salt (BCS-DC10532): Adsorption/desorption in four different soils
Report No:	AS494
Document No:	M-578760-01-1
Guideline(s) followed in study:	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 Adsorption/Desorption (Batch Equilibrium), October 2008
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognized testing facilities:	Yes, conducted under GLP/Officially recognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption characteristics of M-20 (referred to as BCS-BX16566-Ammonium salt in the report) were studied in four soils in batch equilibrium experiments. The study was carried out with air-dried soil in the dark at 20°C ± 2 °C.

Soil	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
[redacted]	Monheim, Germany	Sandy loam	5.3	1.9
H[redacted]	Burscheid, Germany	Silt loam	6.3	2.0
Dollendorf II	Blankenheim, Germany	Loam	7.3	4.5
L[redacted]	Monheim, Germany	Loamy sand	6.3	1.6

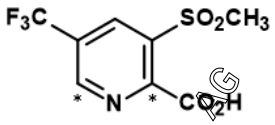
Preliminary tests at a nominal concentration of 1 mg/L to determine the extent of adsorption indicated that M-20 was not adsorbed to soil to any significant extent at soil : solution ratios of 1:1, 1:2 and 1:10 with 98.0 to 100.4% of applied radioactivity remaining in the aqueous supernatant after 24 hours. It was concluded that Freundlich isotherms could not be reliably generated due to the low adsorption of the test substance to soil. M-20 was stable throughout the study.

I. Materials and Methods

A. Materials

1. Test Items

[2,6-Pyridyl-^{14}C]-M-20 (referred to as BCS-BX16566-Ammonium salt in the report)



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

Specific Activity:	3.19 MBq/mg
Radiochemical Purity:	>99%
Sample/Batch ID:	KML 10051
Stability of test compound:	The test item was stable in aqueous 0.01 M $CaCl_2$ solution in absence of soil. After incubation for 24 hours M-20 was detected at 98.6 % AR.

2. Test Soils

Sorption tests were performed with four agricultural soils from Germany selected to cover a representative range of soil physico-chemical properties. Soil samples were collected up to 20 cm depth, then transported to test facility where they were stored refrigerated (1-10°C). All soils were air-dried, sieved (≤2 mm) and sterilised prior to use. The soils were fully characterised, with respect to texture, pH, CEC and organic carbon content. The moisture content of each soil was determined prior to use in the study. The characteristics of the soils summarised in Table 7.1.3.1.2- 79.

Table 7.1.3.1.2- 79: Physico-chemical properties of test soils

Characteristic / Code	[redacted]	[redacted]	Dollendorf II	L[redacted]
Soil ID	[redacted]	[redacted]	DoII	[redacted]
Geographic Location	Monheim, Germany	Burscheid, Germany	Blankenheim, Germany	Monheim, Germany
Textural Classification (USDA)	Sandy loam	Silt loam	Loam	Loamy sand
Sand (2000 to ≥ 50 µm) (%)	55	21	39	79
Silt (50 to ≥ 2 µm) (%)	29	65	35	13
Clay (< 2 µm) (%)	16	14	26	8
pH (water)	5.6	6.6	7.5	6.6
pH ($CaCl_2$)	5.3	6.3	7.3	6.3
Organic carbon (%)	1.9	2.0	4.5	1.6
Organic matter (%)	3.27	3.44	7.74	2.75
CEC (meq/100g soil)	9.9	11.0	19.8	8.5

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of borosilicate glass centrifuge tubes (42 mL or 83 mL) with Teflon® lined screw caps.

In preliminary tests, the adsorption of the test item to glassware and optimal soil-to-solution ratio were determined. The test was conducted at a nominal concentration of 1.0 mg/L [2,6-Pyridyl-^{14}C]-M-20.

Soil Solution Preliminary Test

Parameter		Description
Soil condition		Soils were air-dried and sieved to 2 mm
Soil sample weight		20, 10 and 2 g (dry weight)
Equilibration solution		0.01M $CaCl_2$ shaken overnight
Control (preliminary experiment)		No soil (test item in 0.01M $CaCl_2$ only)
Test item concentration	Nominal application rates	Nominal concentrations in test solution: 1.0 mg/L
	Initial concentrations of M-20 by LSC	Not stated
Identity and concentration of co-solvent		Dosing stock made up in 1:1 acetonitrile:water
Soil: Solution ratio		1:1, 1:2, and 1:10 i.e. 20, 10 or 2 g soil dry weight equivalent to 20 mL solution
Number of replicates		Single replicate
Equilibration conditions	Time	24 h
	Temperature	20 ± 2 °C
	Dark	In the dark
	Shaking method	Benchtop shaker (low setting)
Method of separation of supernatant		Centrifugation
Centrifugation	Speed (rpm)	4200 G
	Duration	10 minutes
	Method of separating supernatant	Supernatant was carefully decanted.

3. Analytical Procedures

Soil samples were shaken with 0.01M calcium chloride solution for 24 hours at 20 °C on a benchtop shaker (low setting). Radioactivity in supernatants was determined by liquid scintillation counting (LSC).

Supernatants from the stability in 0.01M $CaCl_2$ preliminary test were analysed by HPLC.

II. Results and Discussion

A. Results of preliminary tests

Pre-tests on adsorption to the walls of test vessels by shaking an aqueous solution of the test substance in the absence of soil showed no adsorption. The percentage of applied radioactivity in the $CaCl_2$ solution shaken for 24 hours was 98.6%.

Preliminary tests at a nominal concentration of 1 mg/L to determine the extent of adsorption indicated that M-20 was not adsorbed to soil to any significant extent at soil : solution ratios of 1:1, 1:2 and 1:10 with 98.0 to 100.4% of applied radioactivity remaining in the aqueous supernatant after 24 hours. It was concluded that Freundlich isotherms could not be reliably generated due to the low adsorption of the test substance to soil and the study was terminated.

Table 7.1.3.1.2- 80: Preliminary test: Mass balance of M-20 (% AR, mean values)

Soil Solution Ratio	[redacted]		H[redacted]		Dollendorf II		L[redacted]	
	Supernatant	Adsorbed	Supernatant	Adsorbed	Supernatant	Adsorbed	Supernatant	Adsorbed
1:1	98.2	1.8	99.1	0.9	98.0	2.0	99.2	0.8
1:2	100.4	-0.4	100.2	-0.2	99.2	0.8	100.0	0.0
1:10	100.0	0.0	101.1	-1.1	102.7	-2.7	98.1	1.9

III. Conclusion

Preliminary soil adsorption tests established Freundlich isotherms could not be reliably generated for M-20 (BCS-BX16566) due to its low adsorption to soil.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption and desorption characteristics of M-20 in soil.

Data Point:	KCA 7.1.3.1.2/15
Report Author:	[redacted]
Report Year:	2020
Report Title:	Adsorption/Desorption of [14C]-AE C657188 (BCS-AB43478) (PCA) M-02 in five soils
Report No:	S18-08482
Document No:	M-686387-01-1
Guideline(s) followed in study:	OECD Guideline No. 106, 2000 Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009, 2013
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially 207ecognized testing facilities:	Yes, conducted under GLP/Officially 207ecognized testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The adsorption behaviour of M-02 (referred to as AE C657188 in the report) was studied in five soils in batch equilibrium experiments in the laboratory in the dark at 20 ± 1 °C using the OECD 106 direct method with residues remaining on the soil after adsorption quantified directly.

Soil	Soil ID	Source	Texture (USDA)	pH ($CaCl_2$)	OC (%)
LUFA 2.1	2.1	Dudenhofen, Germany	Sand	5.2	0.59
LUFA 2.3	2.3	Offenbach, Germany,	Sandy loam	6.2	0.61
LUFA 5M	5M	Mechtersheim, Germany	Sandy loam	7.1	1.10
LUFA 6S	6S	Siebeldingen, Germany	Clay loam	7.3	1.78
Frankenforst	FF	Königswinter, Germany	Silt loam	6.9	2.4

The adsorption phase of the study was carried out using air-dried soils equilibrated in aqueous 0.01 M $CaCl_2$ solution with soil-to-solution ratios of 1:1 (all soils). Test concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L of M-02 were applied in aqueous 0.01 M $CaCl_2$ solution. Adsorption took place for 24 hours in the definitive test. Due to the low adsorption, no desorption test was performed.

The aqueous supernatant after adsorption was separated by centrifugation, the soils were extracted and the amount of test item in the supernatants and soil extracts was analyzed by liquid scintillation counting (LSC) and HPLC. The sorption parameters were calculated using Freundlich isotherms.

The test item was stable throughout the study. Mean parental mass balances were 102.7, 105.8, 100.0, 97.4 and 104.1% AR in the preliminary and 93.4, 95.2, 95.5, 96.1 and 93.9% AR in the definitive test after 24 hours of adsorption for soils 2.1, 2.3, 5M, 6S and FF, respectively.

The calculated adsorption constants K_F of the Freundlich isotherms ranged from 0.038 to 0.154 mL/g for the tested soils. The Freundlich exponents 1/n ranged from 0.853 to 1.105, 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_{Fads} were correlated with the organic carbon content of the soil to get a comparability of the adsorption behaviour in different soils. For M-02 the K_{Foc} values ranged from 2.5 to 14.0 mL/g.

K_{oc} values for the soils indicate that M-02 is mobile in the test soils according to the Briggs classification.

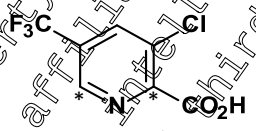
Soil origin	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
Soil type (USDA)	Sand	Sandy loam	Sandy loam	Clay loam	Silt loam
pH (0.01M $CaCl_2$)	5.16	6.24	7.13	7.31	6.9
Organic carbon [%]	0.59	0.61	1.1	1.78	2.4
$K_F^{(ads)}$ [mL/g]	0.047	0.038	0.154	0.145	0.059
1/n	1.031	0.853	0.989	1.105	0.814
$K_{F,OC}^{(ads)}$ [mL/g]	8.0	6.2	14.0	8.2	2.5

I. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-M-02 (referred to as AE C657188, PCA BCS-AB43478 in the report)



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

Specific Activity: 6.5 MBq/mg (0.176 µCi/mg)

Radiochemical Purity: 98%

Sample/Batch ID: KML 10684

Stability of test compound: Stable during the batch equilibrium procedure with 100% AR remaining as M-02 after 48 hours.

2. Test Soils

The adsorption/desorption behaviour of M-02 was characterised in five soils using the batch equilibrium method. The five soils, taken from agricultural use areas, were categorised under the USDA classifications as a sand (Dudenhofen, Germany; LUFA 2.1), a sandy loam (Offenbach, Germany; LUFA 2.3), a sandy loam (Mechtersheim, Germany; LUFA 5M), a clay loam (Siebeldingen, Germany; LUFA 6S) and a silt loam (Königswinter, Germany; Frankenforst). After collection, soils were delivered sieved to a particle size of ≤ 2 mm and gently air-dried at ambient conditions. They were stored at room temperature at the receiving facility. The soil characteristics are given below in Table 7.1.3.1.2- 81.

Table 7.1.3.1.2- 81: Physico-chemical characteristics of test soils

Characteristic / Code	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S*	LUFA 6S*	Franken forst
Soil ID	2.1	2.3	5M	6S	6S	FF
Geographic Location	Dudenhofen Germany	Offenbach Germany	Mechter-sheim, Germany	Siebeld ingen Germany	Siebeld ingen Germany	Königs-winter Germany
GPS coordinates	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Batch	1418	1318	3418	4518	1418	20170206
Textural Class (USDA)	Sand	Sandy Loam	Sandy Loam	Clay Loam	Clay Loam	Silt Loam
Sand (2000 to ≥ 50 µm)(%)	88.82	63.44	59.9	23.4	32.34	27
Silt (50 to ≥ 2 µm) (%)	8.75	30.08	31.1	40.9	34.03	54
Clay (< 2 µm) (%)	2.43	6.48	8.9	35.7	33.62	19
pH ($CaCl_2$)	5.16	6.24	7.13	7.31	7.24	6.9
Organic Carbon (%)	0.59	0.61	1.10	1.78	1.85	2.4
Cation exchange capacity (meq/100g)	5.2	11.8	20.0	16.4	24.0	18.5
Maximum Water Holding Capacity (g/100 g dry matter)	30.65	38.56	39.17	48.76	51.49	n.a.

B. Study Design

1. Experimental Conditions

The test vessels in the experiments consisted of either 50 mL glass flasks (conventional system) or commercially available 20 mL syringes (Braun,polypropylene, polyethylene) as incubation vessels and Falcon tubes™ (polypropylene) as recipient vessels for the aqueous phase removed later on (syringe method).

In preliminary tests, as well as a comparison of the conventional and syringe method being undertaken, the adsorption of the test item to the test system surface, the optimal soil-to-solution ratio, the appropriate adsorption equilibration times and the stability of the test item were determined.

The soil was weighed into the test vessels and pre-equilibrated over night with 0.01M $CaCl_2$ solution at a soil-to-solution ratio of 1/1. The definitive test was performed at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L in 0.01M $CaCl_2$ solution using the syringe method. After application of M-02, the test systems were shaken for 24 hours in the dark at 20 ± 1 °C and centrifuged. The supernatants were decanted, the volumes determined by weighing and the radioactivity contents determined by LSC and characterized by radio-HPLC.

Five replicates per soil and concentration were used. The equilibration time was 24 hours for adsorption. An aliquot of the control flasks was taken at 0 h for LSC measurement in order to determine the initial mass of the test item. The definitive test was repeated for all soils at nominal concentration levels of 0.01 and 0.1 mg/L. The repetition was necessary as the actual applied concentrations deviated from the nominal concentrations.

After equilibration, soil and solution phases were separated by centrifugation. Residues remaining on the soil after adsorption were extracted and quantified directly.

In the definitive test, soil samples were exhaustively extracted with acetonitrile/water (4/1, *v/v*) at ambient temperature for 30 minutes, followed initially by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 60 °C for 20 minutes, and then extracted with acetonitrile/water (4/1, *v/v*) at ambient temperature for 30 minutes for a second time. Finally, the soil was extracted with acetone at ambient temperature for 30 minutes to aid drying. After each extraction step, extract and soil were separated by centrifugation.

For detailed information on experimental design see below. Desorption was not examined due to the low adsorption of M-02 on soil.

Adsorption

Parameter		Description
Soil Condition		Soils were equilibrated to study conditions for at least 12 hours with 5 mL aqueous 0.01 M $CaCl_2$ solution
Soil sample weight		5 g per replicate.
solution used for equilibration		aqueous 0.01 M $CaCl_2$ solution
Control used		$CaCl_2$ solution without soil
Test item concentrations	Nominal application rates	Nominal concentrations in test solution: 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L
	Analytically measured concentrations	Concentrations in test solution: 1.04, 0.52, 0.10, 0.047 and 0.011 mg/L
Identity and concentration of extraction solvent		acetonitrile / water (80:20, *v/v*), acetone
Soil-to-solution ratio		1/1, i.e. 5 g soil dry weight to 5 mL solution (corrected for soil moisture).
pH of the equilibration solution (from definitive test)	Initial	pH of aqueous 0.01 M $CaCl_2$ solution without soil: 5.68
	Final	pH with soil and test item after adsorption equilibrium: range 6.42 - 8.21
Number of replicats	Controls	Two
	Treatments	Five
Incubation	Time	24 hours
	Temperature	20 ± 1 °C
	Dark	Yes
	Shaking method	Mechanical shaker, 150 rpm
Centrifugation	Speed	2800 rpm
	Duration	35 to 140 min for $CaCl_2$ and 35 min for extracts
	Method of separation of soil and solution	Centrifugation (no filtration)

2. Analytical Procedures

Radioactivity in supernatants and soil extracts was determined by LSC. Radioactivity in the extracted soil was determined by combustion.

Samples of supernatants, soil extracts and controls without soil from the preliminary test and the definitive test were analysed by HPLC. For the definitive test, the highest and lowest test concentrations were analysed by HPLC. As M-02 >95% ROI for all samples of highest and lowest concentration, the test item was deemed to be stable at the intermediate concentrations. ROI of the intermediate concentrations was formed taking the ROI mean value of highest and lowest test concentration. In cases where ROI was 100%, K_d determination is based on LSC measurements of aqueous supernatant and soil extracts. HPLC recovery was quantitative (100.6%). The LOD of the HPLC method was 1229 dpm.

Samples of lowest concentration (K5) were characterized with thin layer chromatography (TLC).

Adsorption isotherms were calculated by linear regression analysis of the data according to the Freundlich equation. The amount of the test substance adsorbed was directly determined by extraction and analysis of soil. Desorption was not examined due to the low adsorption.

II. Results and Discussion

A. Results of preliminary tests

Adsorption percentage between conventional and syringe method were comparable. Using the syringe method, the residual pore water is minimized and therefore a more accurate determination of K_d and K_{oc} is possible. Therefore, the syringe method was chosen for the definitive test.

The test item was stable in aqueous 0.01 M $CaCl_2$ solution in the absence of soil. After incubation for 48 hours M-02 represented 100% AR. No adsorption of the test item to the test vessels was observed after shaking for 48 hours. The test item has very low adsorption characteristics to soil and so a soil-to-solution-ratio of 1:1 was used. An equilibration time of 24 hours was chosen for the adsorption phase.

B. Transformation of test substance

The parental mass balances were determined at a soil-to-solution ratio of 1/1 for all soils. M-02 was stable throughout the study. Mean parental mass balances (excluding NER) were 102.7, 105.8, 100.0, 97.4 and 104.1% AR in the preliminary test for LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst soils, respectively.

The stability was adequate to determine the test item distribution based on LSC measurements of the adsorption phase extracts in the definitive test. No major degradation product was observed by HPLC analysis.

Mean parental mass balances of the test item in the definitive test were 93.4, 95.2, 95.5, 96.1 and 93.9% AR for LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst soils, respectively.

C. Findings

Mean material balances were 94.6, 95.6, 97.8, 100.2 and 96.6% AR for soils LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst, respectively (summarised in Table 7.1.3.1.2- 82). The complete material balances found for all soils and concentrations demonstrated that there was no significant loss of radioactivity dissipated from the test systems or during sample processing.

Table 7.1.3.1.2- 82: Definitive test: Mass balance of [2,6-Pyridyl-^{14}C]-M-02 (% AR)

Soil	Aquatic phase		Soil extract		Non extractable radioactivity		Total	
	% AR	SD	% AR	SD	% AR	SD	% AR	SD
LUFA 2.1	89.1	3.5	4.0	1.1	1.5	1.5	94.6	2.0
LUFA 2.3	89.6	5.4	4.9	1.7	0.5	0.6	95.6	2.0
LUFA 5M	82.7	4.5	12.8	3.5	2.3	0.5	97.8	3.3
LUFA 6S	85.7	5.4	9.9	4.7	5.5	1.2	100.2	1.6
Frankenforst	85.3	6.9	6.9	7.2	3.4	3.9	96.6	2.1

Note: Mass balances were quantitative. Overall mean values derived from five replicates at each of five concentrations provided in the report. SD = standard deviation.

The results of adsorption tests of [2,6-pyridyl-^{14}C]-M-02 onto five soils are summarised in Table 7.1.3.1.2- 83 and Table 7.1.3.1.2- 84. The plots of the calculated Freundlich isotherms for all soils are presented in Figure 7.1.3.1.2- 38 to Figure 7.1.3.1.2- 42.

At the end of the adsorption phase 8 to 13% AR, 7 to 14% AR, 12 to 22% AR, 8 to 20% AR and 7 to 23% AR was adsorbed in LUFA 2.1, LUFA 2.3, LUFA 5M, LUFA 6S and Frankenforst, respectively.

The adsorption constant $K_{F(ads)}$ of M-02 was between 0.038 to 0.154 mL/g for the tested soils; the normalised adsorption constant $K_{OC(ads)}$ was in the range of 2.5 to 14.0 mL/g. The Freundlich exponent 1/n was between 0.814 and 1.105, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

Table 7.1.3.1.2- 83: Definitive test: Concentration of M-02 in aqueous and solid phase following 24 hours of adsorption.

Concn (µg/mL)	Rep	LUFA 2.1		LUFA 2.3		LUFA 5M		LUFA 6S		Frankenforst	
		Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)
1.04	1	0.0487	0.9343	0.0735	0.8886	0.1369	0.8541	0.1465	0.8270	0.0580	0.9333
	2	0.0231	0.9596	0.0175	0.9814	0.1207	0.8548	0.1764	0.8246	0.0597	0.9111
	3	0.0449	0.9241	0.0400	0.9663	0.1279	0.8417	0.1777	0.8843	0.0565	0.9314
	4	0.0500	0.9386	0.0559	0.9351	0.1075	0.8544	0.1677	0.8381		0.9059*
	5	0.0511	0.9104	0.0245	1.0106	0.1485	0.8246	0.1966	0.9018	0.0688	0.9314
	Mean	**0.0436**	**0.9334**	**0.0423**	**0.9564**	**0.1283**	**0.8459**	**0.1730**	**0.8551**	**0.0608**	**0.9268**
0.52	1	0.0228	0.4964	0.0164	0.4913	0.0663	0.4425	0.0343	0.4485	0.0392	0.4841
	2	0.0252	0.4802	0.0160	0.4895	0.0556	0.4170	0.0214	0.4376	0.0222	0.4771
	3	0.0220	0.4748	0.0197	0.4733	0.0725	0.4684	0.0484	0.4312	0.0409	0.4725
	4	0.0230	0.4581	0.0246	0.4558	0.0845	0.4562	0.0552	0.4523	0.0241	0.4741
	5	0.0290	0.4840	0.0151	0.5255	0.0650	0.4455	0.0476	0.4161	0.0268	0.4763
	Mean	**0.0244**	**0.4787**	**0.0184**	**0.4871**	**0.0688**	**0.4459**	**0.0414**	**0.4365**	**0.0306**	**0.4768**
0.10	1	0.0033	0.0956	0.0065	0.0926	0.0120	0.0886	0.0061	0.0909	0.0063	0.0863
	2	0.0041	0.0979	0.0083	0.0863	0.0111	0.0884	0.0062*	0.0812*	0.0076	0.0926
	3	0.0035	0.0942	0.0035	0.0962	0.0110	0.0881	0.0070	0.0894	0.0056	0.0883
	4	0.0036	0.0944	0.0057	0.0977	0.0112	0.0918	0.0072	0.0929	0.0089	0.0920
	5	0.0031	0.0957	0.0048	0.0956	0.0115	0.0963	0.0047	0.0966	0.0053	0.0901
	Mean	**0.0035**	**0.0956**	**0.0058**	**0.0937**	**0.0114**	**0.0906**	**0.0062**	**0.0924**	**0.0067**	**0.0899**
0.047	1	0.0020	0.0410	0.0028	0.0417	0.0091	0.0340	0.0066	0.0381	0.0063*	0.0335*
	2	0.0025*	0.0386*	0.0028	0.0415	0.0089	0.0376	0.0074	0.0377	0.0076	0.0348
	3	0.0033	0.0395	0.0035	0.0391	0.0089	0.0355	0.0086	0.0339	0.0071	0.0354
	4	0.0015	0.0419	0.0035*	0.0342*	0.0098	0.0381	0.0098	0.0359	0.0072	0.0353
	5	0.0021	0.0420	0.0026	0.0407	0.0107	0.0396	0.0060	0.0376	0.0055	0.0376
	Mean	**0.0022**	**0.0411**	**0.0029**	**0.0408**	**0.0095**	**0.0369**	**0.0077**	**0.0367**	**0.0069**	**0.0358**
0.011	1	0.0004	0.0091	0.0007	0.0094	0.0009	0.0086	0.0010	0.0087	0.0011	0.0083
	2	0.0006	0.0091	0.0005	0.0093	0.0010	0.0086	0.0009	0.0092	0.0006	0.0085
	3	0.0004	0.0091	0.0005	0.0094	0.0012	0.0085	0.0003	0.0092	0.0011	0.0083
	4	0.0003	0.0099	0.0006	0.0098	0.0012	0.0085	0.0005	0.0095	0.0015*	0.0072*
	5	0.0002	0.0099	0.0008	0.0102	0.0014	0.0087	0.0009	0.0096	0.0012	0.0086
	Mean	**0.0004**	**0.0094**	**0.0006**	**0.0096**	**0.0011**	**0.0086**	**0.0007**	**0.0093**	**0.0010**	**0.0084**

* Values not included in mean value (mass balance < 90 % or >110 %).

Table 7.1.3.1.2- 84: Summary of Freundlich adsorption constants K_f and K_{oc} values

Soil	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
	2.1	2.3	5M	6S	FF
Textural class	Sand	Sandy Loam	Sandy Loam	Clay Loam	Silt Loam
K_f	0.047	0.038	0.154	0.145	0.059
1/n	1.031	0.853	0.989	1.105	0.814
K_{oc}	7.960	6.186	13.974	8.171	2.477
Correlation	0.973	0.947	0.973	0.918	0.938

D. Evaluation of the Data according to EFSA Evaluators Checklist

The results of the determination of the Freundlich Isotherm were analysed using the Excel-sheet provided by EFSA (summarised in Table 7.1.3.1.2- 86). The mean concentrations in the supernatant and the soil as given in Table 7.1.3.1.2- 85 were used as input data. Derived data were generated from the accurate raw data values and rounded appropriately. As a consequence, recalculation of certain individual derived data from its tabular precursors will, in some instances, show minor rounding variation.

Table 7.1.3.1.2- 85: Definitive test: Concentration of M-02 in aqueous and solid phase following 24 hours of adsorption used in checklist (mean values)

Concn (µg/mL)	LUFA 2.1		LUFA 2.3		LUFA 5M		LUFA 6S		Frankenforst	
	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)	Soil (µg/g)	Soln (µg/mL)
0.011	0.0004	0.0094	0.0006	0.0096	0.0011	0.0086	0.0007	0.0093	0.0010	0.0084
0.047	0.0022	0.0411	0.0029	0.0408	0.0095	0.0369	0.0077	0.0367	0.0069	0.0358
0.10	0.0035	0.0956	0.0058	0.0937	0.0114	0.0906	0.0062	0.0924	0.0067	0.0899
0.52	0.0244	0.4787	0.0184	0.4871	0.0688	0.4459	0.0414	0.4365	0.0306	0.4768
1.04	0.0436	0.9334	0.0423	0.9564	0.1283	0.8459	0.1730	0.8551	0.0608	0.9268

Table 7.1.3.1.2- 86: Results of the EFSA 106 data evaluation

Soil	Units	Quality criteria	LUFA 2.1	LUFA 2.3	LUFA 5M	LUFA 6S	Frankenforst
Code	-	-	**2.1**	**2.3**	**5M**	**6S**	**FF**
Adsorption method	-	-	direct	direct	direct	direct	direct
Soil solution ratio	g/mL		1:1	1:1	1:1	1:1	1:1
Mass balance of ^{14}C	%	>90%	91.7 (highest)	93.6 (highest)	92.8 (highest)	102.3 (highest)	95.9 (highest)
f – due to loss processes#	%	-	8.3	6.4	7.2	-2.3	4.1
Adsorbed percentage (δ)#	%	>20%	8.10 – 15.33	7.07 – 14.02	12.89 – 22.06	11.10 – 22.65	9.03 – 24.52
K_D x soil:solution ratio#		>0.3	0.04 – 0.05	0.04 – 0.07	0.12 – 0.24	0.06 – 0.19	0.06 – 0.18
K_{fE} / K_f#	-	<1.2	5.96	3.61	1.64	0.88	4.59
ads K_F	L/kg	-	0.048	0.041	0.154	0.144	0.061
95% confidence interval	-	*	(0.032 – 0.073)	(0.028 – 0.061)	(0.070 – 0.340)	(0.035 – 0.595)	(0.026 – 0.144)
ads 1/n	-		1.024	0.873	0.989	1.094	0.813
95% confidence interval	-	*	(0.873 – 1.176)	(0.731 – 1.015)	(0.710 – 1.268)	(0.587 – 1.599)	(0.512 – 1.113)
ads R²	-	<0.975	0.994	0.992	0.977	0.940	0.962
ads $K_{F,OC}$	L/kg		8.1	6.8	14.0	8.1	2.6
Visual fit to Freundlich isotherm	-	-	Good	Acceptable	Good	Acceptable	Good
Residual plots randomly distributed	-	-	Good	Good	Good	Good	Good

#**Note:** 3 checks (in grey) are excluded as not relevant for 'direct' studies**,** therefore number of checks reduced from 9 to 6.

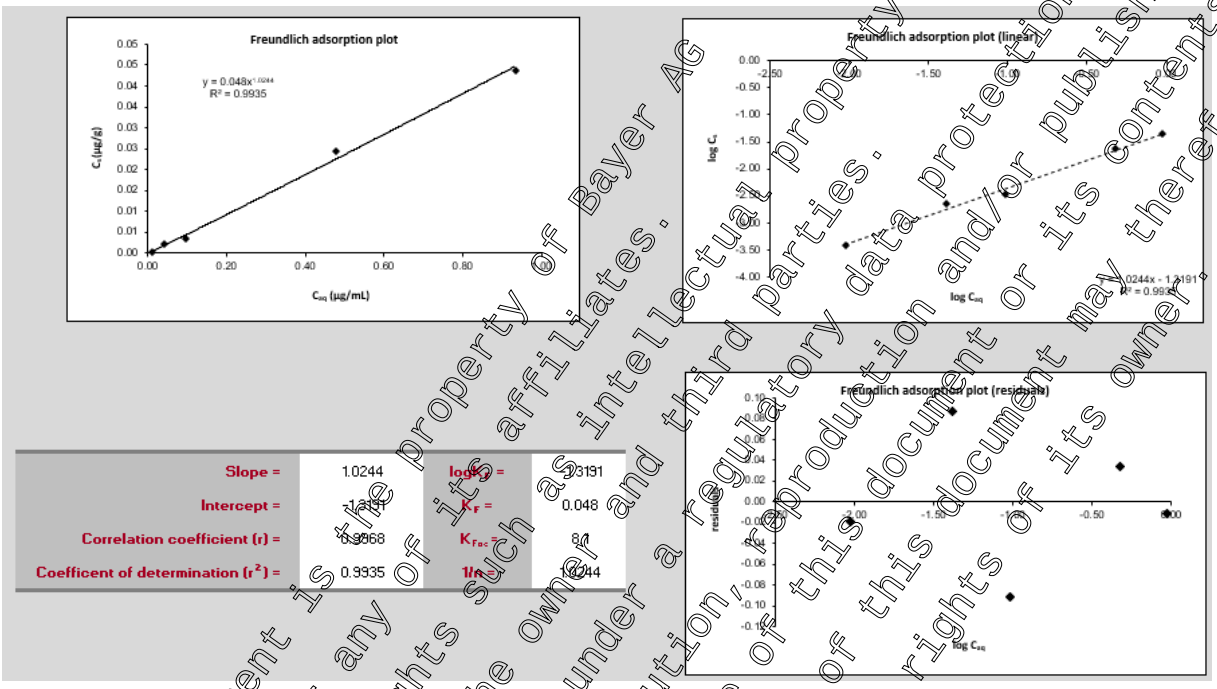
Relevant quality checks were performed to evaluate the acceptability of the study. Three checks have been excluded from the overall assessment (i.e. adsorbed percentage, K_D x soil:solution ratio and K_{fE} / K_f) as they not relevant for 'direct' studies, the number of quality checks is reduced from 9 to 6.

These checks confirmed that the mass balance was acceptable (91.7 – 102.3%). The acceptability of the analytical method (LSC) was confirmed over the entire range of concentrations measured (reported LOQ of 60 dpm represents <1 % of the lowest test concentration). The R^2 of the standard linear regressions ranged from 0.977 to 0.994 for soils 2.1, 2.3 and 5M, meeting the quality criteria but were <0.975 in soils 6S and FF. The visual fit of both the standard regression and the residual plots were good.

The study has been conducted to a good standard. The test substance was stable (purity 100% in dosing stocks, adsorption supernatant and soil extracts).

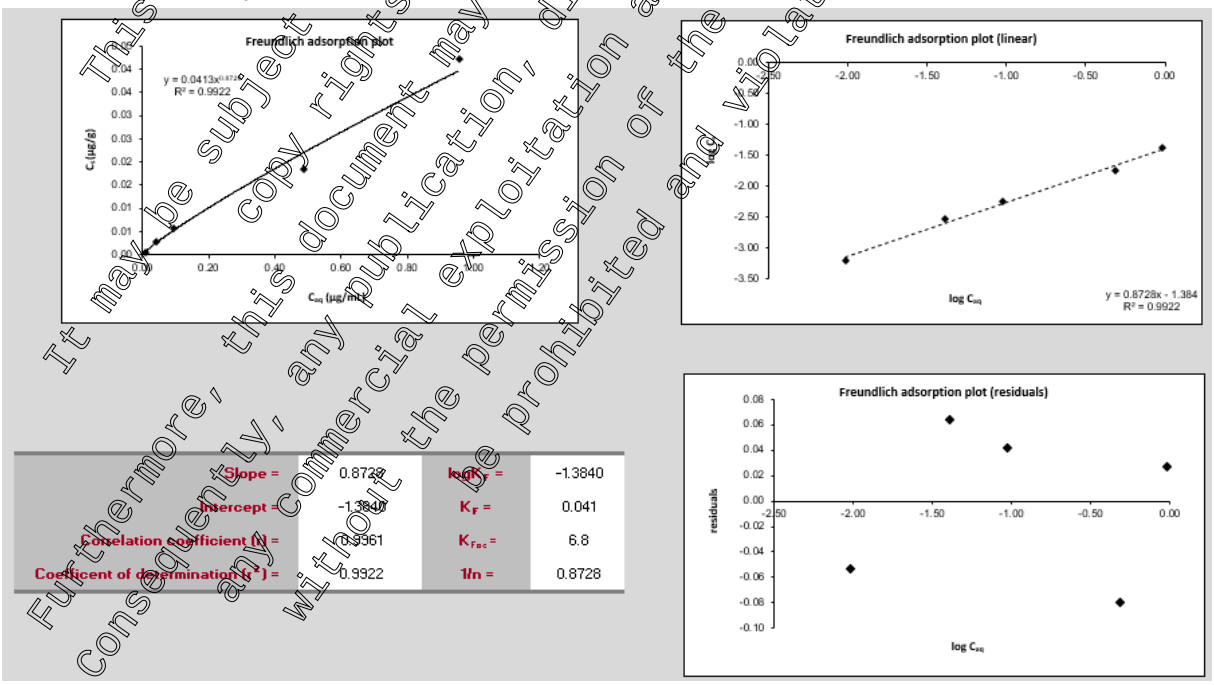
The evaluation confirmed that all soils were acceptable according to the quality checks and acceptable for use in regulatory modelling. The results of the evaluation are summarised in the tables below.

Figure 7.1.3.1.2- 38: Freundlich Isotherms of M-02 in LUFA 2.1 soil at 20°C



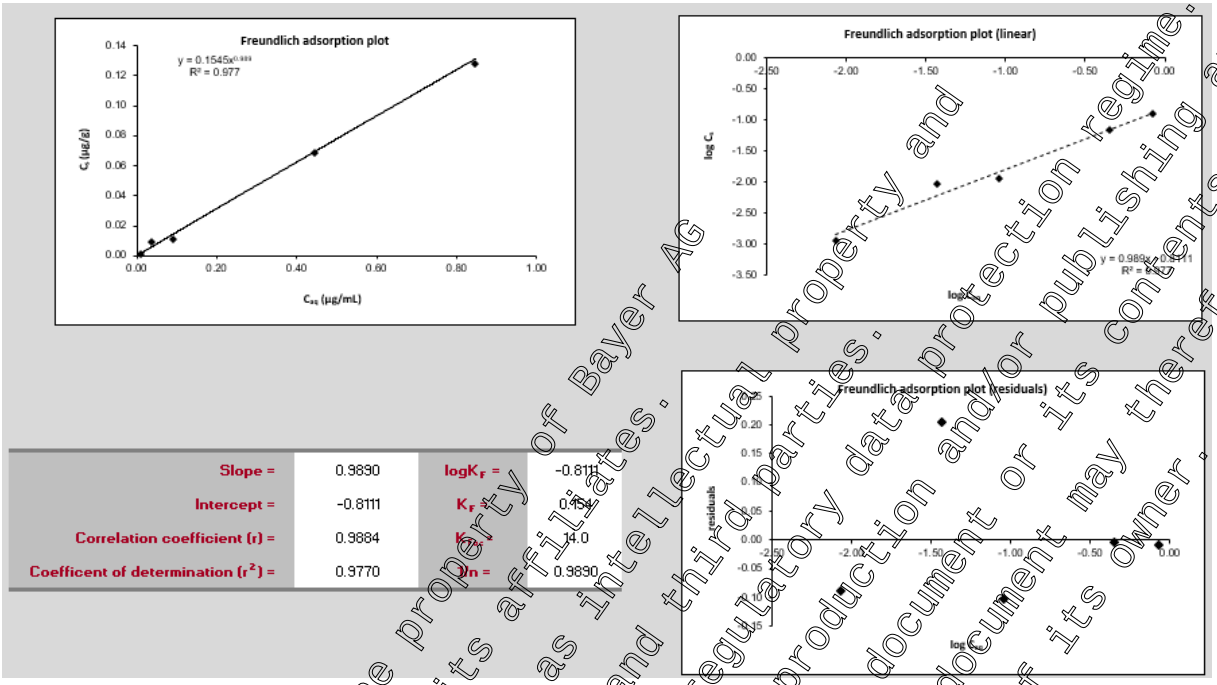
Slope =	1.0244	log K_F =	-1.3191
Intercept =	-1.3191	K_F =	0.048
Correlation coefficient (r) =	0.9968	K_{Foc} =	8.0
Coefficent of determination (r^2) =	0.9935	1/n =	1.0244

Figure 7.1.3.1.2- 39: Freundlich Isotherms of M-02 in LUFA 2.3 soil at 20°C



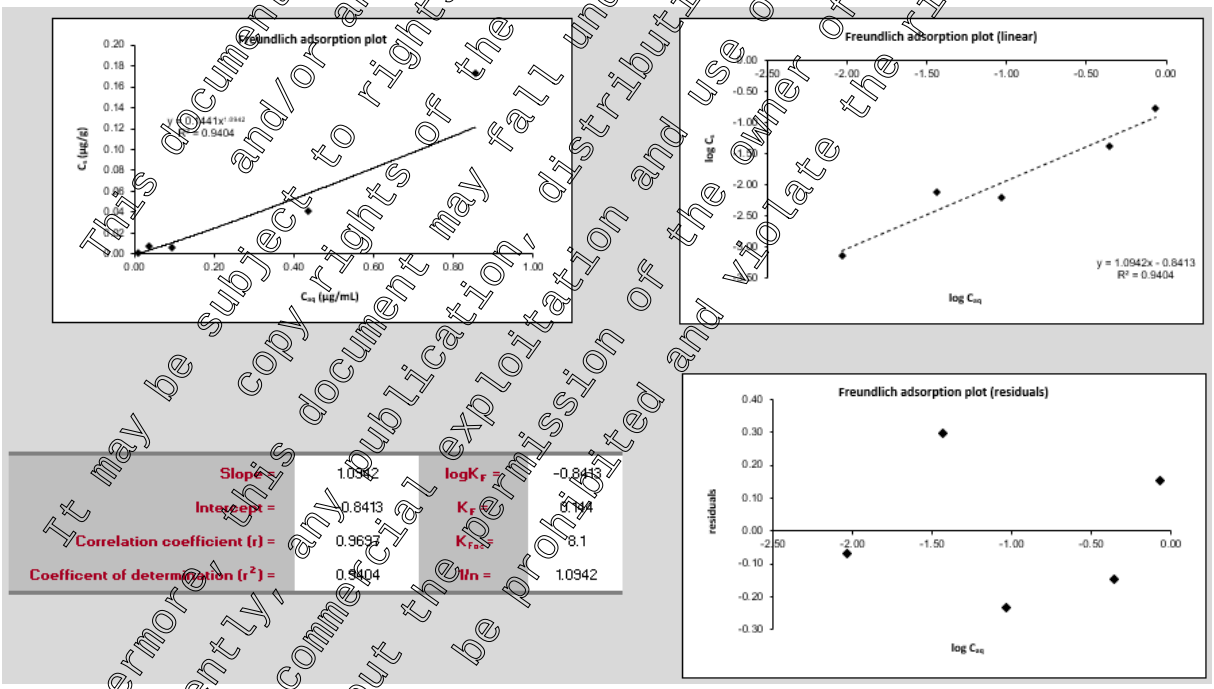
Slope =	0.8728	log K_F =	-1.3840
Intercept =	-1.3840	K_F =	0.041
Correlation coefficient (r) =	0.9961	K_{Foc} =	6.8
Coefficent of determination (r^2) =	0.9922	1/n =	0.8728

Figure 7.1.3.1.2- 40: Freundlich Isotherms of M-02 in LUFA 5M soil at 20°C



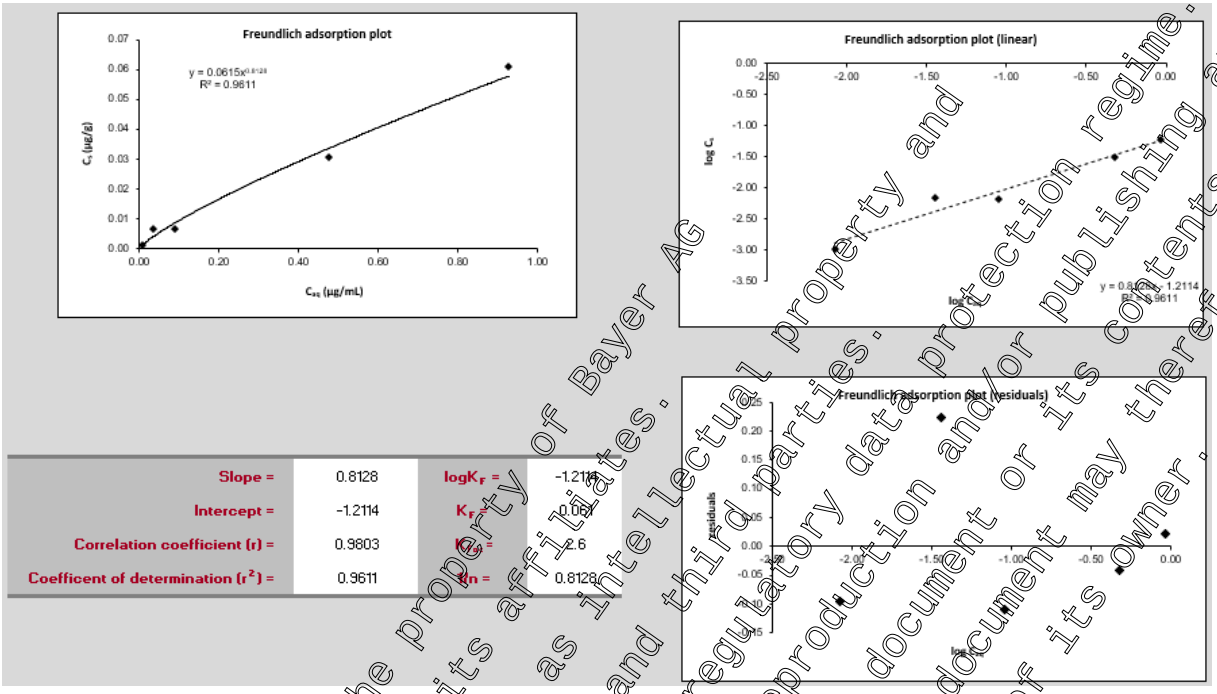
Slope =	0.9890	$\log K_F$ =	-0.8111
Intercept =	-0.8111	K_F =	0.154
Correlation coefficient (r) =	0.9884	K_{oc} =	14.0
Coefficent of determination (r^2) =	0.9770	1/n =	0.9890

Figure 7.1.3.1.2- 41: Freundlich Isotherms of M-02 in LUFA 6S soil at 20°C



Slope =	1.0942	$\log K_F$ =	-0.8413
Intercept =	-0.8413	K_F =	0.144
Correlation coefficient (r) =	0.9693	K_{oc} =	8.1
Coefficent of determination (r^2) =	0.9404	1/n =	1.0942

Figure 7.1.3.1.2- 42: Freundlich Isotherms of M-02 in Frankenforst soil at 20°C



A summary of the quality criteria and regulatory interpretation is presented in Table 7.1.3.1.2- 87. All four soils can be considered 'met' with regard to the quality criteria and therefore acceptable for regulatory use. The impact on reported endpoints is summarised in Table 7.1.3.1.2- 88.

Table 7.1.3.1.2- 87: Summary of Quality Criteria and Regulatory Interpretation

M-02			Quality Criteria		
Soil Name	**Soil Type**	**Code**	**Met**	**Partially Met**	**Not Met**
LUFA 2.1	Sand	2.1	6	0	0
LUFA 2.3	Sandy Loam	2.3	6	0	0
LUFA 5M	Sandy Loam	5M	6	0	0
LUFA 6S	Clay Loam	6S	5	0	1
Frankenforst	Silt Loam	FF	5	0	1

Note: 3 checks excluded as not relevant for direct studies, therefore number of checks reduced from 9 to 6.

Table 7.1.3.1.2- 88: Impact on Endpoints

Soil Name	Soil Type	Code	K_{foc} (Reported)	K_{foc} (OECD tool)	1/n (Reported)	1/n (OECD tool)
LUFA 2.1	Sand	2.1	8.0	8.1	1.03	1.02
LUFA 2.3	Sandy Loam	2.3	6.2	6.8	0.85	0.87
LUFA 5M	Sandy Loam	5M	14.0	14.0	0.99	0.99
LUFA 6S	Clay Loam	6S	8.2	8.1	1.11	1.09
Frankenforst	Silt Loam	FF	2.5	2.6	0.81	0.81

The small differences between the reported values and the OECD calculation tool (v2) are considered to be due to rounding in the calculations. The reported values have been used in modelling calculations.

III. Conclusion

The adsorption constant $K_{F(ads)}$ of M-02 was between 0.038 and 0.154 mL/g for the tested soils; the respective normalized adsorption constant $K_{OC(ads)}$ was in the range of 2.5 to 14.0 mL/g. The Freundlich exponent 1/n ranged from 0.853 to 1.105, indicating that the concentration of the test item affects its adsorption behaviour in the examined concentration range.

The OECD 106 Checklist (v1) was used to evaluate the study. The evaluation confirmed that the results were acceptable according to the quality criteria and therefore acceptable for regulatory use.

Assessment and conclusion by applicant:

The study is considered valid to assess the adsorption characteristics of M-02 (AE C657188) in soil.

CA 7.1.3.2 Aged sorption

The aged sorption of fluopicolide has been investigated in five studies. Study KCA 7.1.3.2/01 and KCA 7.1.3.2/02 were evaluated during the previous EU review and were accepted to assess the time dependent sorption of fluopicolide, but the results were not accepted for use in FOCUS groundwater assessments by EFSA. For reasons elaborated in the study summaries, both are now considered only as supporting data as the study design does not conform to current aged sorption guidelines.

Studies KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05 are provided as new studies not yet reviewed.

Report reference	Author, Year	Comment
KCA 7.1.3.2/01 M-224207-01-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered as supporting data.
KCA 7.1.3.2/02 M-241049-01-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered as supporting data.
KCA 7.1.3.2/03 M-555570-01-1	[redacted] 2016	New data not yet reviewed.
KCA 7.1.3.2/04 M-550687-01-1	[redacted] 2016	New data not yet reviewed.
KCA 7.1.3.2/05 M-655086-01-1	[redacted] 2019	New data not yet reviewed.
KCA 7.1.3.2/06 M-685678-02-1	[redacted] 2020	New data not yet reviewed.
KCA 7.1.3.2/07 M-687157-01-1	[redacted] 2020	New data not yet reviewed.

In addition, two kinetic evaluation reports (KCA 7.1.3.2/06 and KCA 7.1.3.2/07) are also not yet reviewed. KCA 7.1.3.2/06 evaluates the data from sixteen soils in the three new time-dependent sorption studies according to current guidance on aged sorption. The adsorption of fluopicolide to soil increased significantly with time under laboratory conditions, with mean aged-sorption parameters of F_{ne} 0.508 and k_{des} 0.0356.

Lower-tier degradation study data (laboratory and field) must be combined with the aged-sorption data prior to inclusion in PEC_{gw} exposure assessments. KCA 7.1.3.2/07 evaluates the data from four other aerobic soil degradation studies (5 soils, 6 datasets) and eight field studies (12 soils) to derive $DegT_{50eq}$ values, which when combined with the higher-tier aged-sorption values yield an overall geometric mean $DegT_{50eq}$ of 121 days for use in exposure modelling (in combination with the mean aged-sorption parameters: F_{ne} 0.508; k_{des} 0.0356).

Data Point:	KCA 7.1.3.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE C638206: Aged desorption from four European soils collected from field dissipation trial sites
Report No:	C038099
Document No:	M-224207-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Yes. The study design does not conform to current aged sorption guidelines as the study duration is too short and had insufficient timepoints
Previous evaluation:	yes, evaluated and accepted DAR (2005) and Addendum 1 to the DAR (2007)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aged desorption behaviour of fluopicolide was initially characterised in four soils using an adapted version of the Freundlich batch equilibrium method. The degradation and time-dependence of sorption of fluopicolide was studied under aerobic conditions in the laboratory in the dark at 20 °C and 45% of the maximum water holding capacity (Senas and Rödelsee soils) or pF2 (Philippsburg and Huntlosen soils) for 21 days.

Soil	Texture (USDA)	pH ($CaCl_2$)	% Organic Carbon
Philippsburg	sandy loam	6.3	0.6
Senas	clay loam	7.6	1.5
Huntlosen	loamy sand	5.3	1.6
Rödelsee	clay	7.0	1.5

[Phenyl-U-^{14}C]-labelled fluopicolide was applied to soil samples at application rates of 0.11, 0.45 and 1.79 mg/kg dry weight. The radiochemical purity and specific activity were > 99% and 5.5 MBq/mg, respectively.

Samples were taken for extraction and analysis immediately after treatment (day 0) and after 3, 10 and 21 days of incubation. Each soil sample was subject to three desorption phases at 20°C in the dark. Following preliminary studies an equilibrium time of 48 hours for the first desorption and two hours for the subsequent desorption cycles were selected for each soil. After equilibration, soil and solution phases were separated by centrifugation and the concentration of fluopicolide in the water phase determined by LSC. After the final desorption phase all soils were extracted with acetonitrile. Desorption supernatants and soil extracts, from all samples treated at the highest concentration and selected samples treated at the two lower concentrations, were analysed by HPLC.

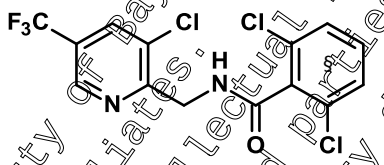
The study design does not conform to current aged sorption guidelines as study duration is too short and had insufficient timepoints. Nevertheless the aged sorption of fluopicolide to soil was measured and showed a significant increase with time. Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.18 to 2.12 (mean 1.78) over 21 days incubation.

I. Materials and Methods

A. Materials

1. Test Items

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



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

Specific Activity: 5.50 MBq/mg

Radiochemical Purity: >99%

2. Test Soil

The study was performed using four soils as characterized in Table 7.1.3.2- 1.

Table 7.1.3.2- 1: Physico-chemical properties of test soils

Parameter	Soil			
Soil Designation	Philippsburg	Senas	Huntlosen	Rodelsee
Geographic Location City Country	Mühlfeldhof, Philippsburg, Baden-Württemburg, Germany	Mas Bel Air, Senas France	Grossenkneten, Huntlosen, Germany	Kirchenplatz, Rodelsee, Germany
Batch Number	03/02	03/03	03/04	03/05
Textural Classification (USDA)	Sandy Loam	Clay Loam	Loamy Sand	Clay
Textural Classification (ADAS)	Sandy Loam	Clay Loam	Loamy Sand	Clay
Sand [63 - 2000 µm] (%)	79	19	78	37
Silt [2 – 63 µm] (%)	10	49	16	19
Clay [< 2 µm] (%)	11	31	6	44
pH				
in $CaCl_2$ (1:1)	6.3	7.6	5.3	7.0
in H_2O (1:5)	7.1	8.3	6.3	7.9
in KCl (1:1)	6.5	7.5	5.2	7.1
Organic Carbon (%)	0.6	1.5	1.6	1.5
$Ca_{exchangeable}$ (mg/kg)	638	6848	489	2556
$Mg_{exchangeable}$ (mg/kg)	18.5	95.3	37.7	462
$Na_{exchangeable}$ (mg/kg)	62.9	29.8	20.0	8.9
$K_{exchangeable}$ (mg/kg)	228	347	141	862
$Mn_{exchangeable}$ (mg/kg)	0.97	1.26	27.92	0.72
$CaCO_3$ (%)	0	41.0	0	0
Phosphorus total(mg/kg)	513	1084	774	1214
Nitrogen total (%w/w)	0.057	0.150	0.102	0.160
Water Holding Capacity				
Maximum (g H_2O per 100 g DW)	33.0	49.9	39.6	52.4
at saturation (%)	35.8	49.7	39.8	54.4
at 0.05 bar (%)	14.7	31.2	22.2	29.7
at 0.1 bar (%)	13.6	26.3	14.9	26.5
at 0.33 bar (%)	7.1	19.3	8.8	19.7
at 2 bar (%)	5.8	16.1	7.0	17.0
at 15 bar (%)	3.5	11.8	3.7	13.7
Moisture Content During Incubation (%)	11.6	22.5	14.9	23.6

B. Study Design

1. Experimental Conditions

Tests were performed in static systems consisting of a borosilicate glass tube containing 20 g soil (dry weight equivalents). The aged samples were loosely capped and incubated at a temperature of 20 ºC in darkness for the required period. As soil laboratory studies conducted with fluopicolide had not detected significant quantities of carbon dioxide or other volatile metabolites, volatile traps were not used.

The tests were performed at a concentration of approximately 0.11, 0.45 and 1.79 mg/kg dry weight of soil. The test item [phenyl-U-^{14}C]-fluopicolide dissolved in acetonitrile (100 µL) was applied drop wise onto the soil surface. Soil samples were adjusted to a moisture content equivalent to approximately 45% of the maximum moisture holding capacity (Senas and Rodelsee) or pF 2 (Philippsburg and Huntlosen). The samples were incubated at 20 °C under aerobic conditions in the dark for 21 days.

After the required ageing period (immediately after treatment for the zero ageing samples) 0.01M calcium chloride solution was added to the samples. Sufficient calcium chloride solution was added to give a soil to solution ratio of 1:3. Each soil sample was subject to three desorption phases at 20°C in the dark. Following preliminary studies an equilibrium time of 48 hours for the first desorption and two hours for the subsequent desorption cycles were selected for each soil. After equilibration, soil and solution phases were separated by centrifugation and the concentration of fluopicolide in the water phase determined by LSC. After the final desorption phase all soils were extracted with acetonitrile. The radioactivity remaining in soil after extraction was quantified by combustion. In a standard OECD adsorption/desorption study with fluopicolide carried out concurrently with this study using identical glassware and the same batches of soils, it was shown that no significant adsorption to the test vessels occurred.

Desorption supernatants and soil extracts, from all samples treated at the highest concentration and selected samples treated at the two lower concentrations, were analysed by HPLC. Fluopicolide was the major component detected in all the desorbate samples analysed. The metabolite AE C653711 was detected at a maximum of 2.4%, 6.3%, 12.1% and 17.4% of applied radioactivity following zero, three, ten and twenty one days ageing, respectively. No other degradation products were observed. The degree of degradation of fluopicolide observed in the two lower application rates was comparable to that observed in the highest application rate. Thus for each combination of soil and ageing period, the degree of degradation of fluopicolide in each desorbate at the highest application rate was applied as a correction factor to the two lower application rates. Fluopicolide alone was detected in soil extracts in both unaged and aged soil samples.

2. Sampling

Duplicate samples of each soil were removed for analysis at four timepoints only (0, 3, 10 and 21 days of incubation) at each concentration.

3. Analytical Procedures

After the required ageing period (immediately after treatment for the zero ageing samples) 0.01M calcium chloride solution was added to the samples. Sufficient calcium chloride solution was added to give a soil to solution ratio of 1:3. Each soil sample was subject to three desorption phases at 20°C in the dark on an end-over-end shaker. Following preliminary studies an equilibrium time of 48 hours for the first desorption and two hours for the subsequent desorption cycles were selected for each soil. After each extraction step, extract and soil were separated by centrifugation.

After the final desorption phase soil samples were extracted with acetonitrile at ambient temperature for 2 hours on an end-over-end shaker. The radioactivity remaining in soil after extraction was quantified by combustion. In a standard OECD adsorption/desorption study with fluopicolide carried out concurrently with this study using identical glassware and the same batches of soils, it was shown that no significant adsorption to the test vessels occurred.

Desorption supernatants and soil extracts, from all samples treated at the highest concentration and selected samples treated at the two lower concentrations, were analysed by HPLC. Fluopicolide was the major component detected in all the desorbate samples analysed. The metabolite M-01 (AE C653711) was detected at a maximum of 2.4%, 6.3%, 12.1% and 17.4% of applied radioactivity following zero, three, ten and twenty one days ageing, respectively. No other degradation products were observed. The degree of degradation of fluopicolide observed in the two lower application rates was comparable to that observed in the highest application rate. Thus for each combination of soil and ageing period, the degree of degradation of fluopicolide in each desorbate at the highest application rate was applied as a correction factor to the two lower application rates. Fluopicolide alone was detected in soil extracts in both unaged and aged soil samples.

Following homogenisation, non-extractable residues (NER) in extracted soils were determined by combustion.

4. Determination of degradation kinetics

No degradation kinetics were determined in the report.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the test soils incubated at 20 °C following application of [phenyl-U-^{14}C]- fluopicolide are summarized in Table 7.1.3.2- 2 to Table 7.1.3.2- 5.

Table 7.1.3.2- 2: Degradation of [phenyl-U-^{14}C]-fluopicolide in Philippsburg soil under aerobic conditions at 20 °C [% AR]

Compound	Mean SD	Incubation time (DAT)											
		1.79 mg/kg				0.45 mg/kg				0.11 mg/kg			
		0	3	10	21	0	3	10	21	0	3	10	21
Fluopicolide	Mean	90.5	85.6	80.5	73.6	[A]	89.9	-	-	[B]	91.8	-	-
	SD	0.2	0.3	1.5	0.6	-	-	-	-	-	-	-	-
M-01 (AE C653711)	Mean	2.4	5.9	8.4	16.0	[A]	0.0	-	-	[B]	0.0	-	-
	SD	0.1	0.4	1.5	0.7	-	-	-	-	-	-	-	-
$CaCl_2$ solution 1	Mean	52.5	49.4	45.5	41.6	49.0	42.6	39.3	36.1	46.3	38.2	36.6	35.4
	SD	0.3	0.0	0.1	0.4	0.1	1.4	0.1	1.0	0.6	1.8	0.3	0.4
$CaCl_2$ solution 2	Mean	18.2	17.3	16.0	15.3	17.7	14.5	15.2	14.0	17.7	15.7	14.1	13.1
	SD	0.1	0.2	0.1	0.1	0.2	2.4	0.3	0.0	0.0	-	0.0	0.0
$CaCl_2$ solution 3	Mean	7.4	7.2	6.8	7.0	7.8	9.7	6.9	6.8	8.1	7.7	6.7	6.1
	SD	0.0	0.0	0.2	0.1	0.1	-	0.1	0.1	0.0	-	0.0	0.2
Acetonitrile ext	Mean	14.5	17.7	20.4	25.7	18.1	24.1	26.9	32.6	22.0	28.4	32.6	35.1
	SD	0.1	0.0	0.3	0.5	0.2	-	0.1	0.7	0.3	-	0.2	0.4
Total Extractable Residues	Mean	92.8	91.5	88.8	89.6	92.6	89.9	88.3	89.5	94.2	91.8	90.0	89.7
	SD	0.1	0.1	0.0	0.1	0.1	-	0.1	0.3	0.3	-	0.4	0.6
Non-Extractable Residues	Mean	3.2	4.5	6.2	5.9	4.1	6.4	7.9	6.9	4.8	7.5	9.2	10.0
	SD	0.1	0.1	0.2	0.0	0.1	-	0.2	0.0	0.2	-	0.3	1.7
Total Recovery	Mean	96.0	96.1	95.0	95.5	96.8	96.3	96.2	96.4	99.0	99.2	99.3	99.7
	SD	0.0	0.3	0.2	0.1	0.2	-	0.1	0.4	0.0	-	0.1	2.3

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, SD: standard deviation
All values expressed as percentage of total applied radiolabel
[A] HPLC analysis of first $CaCl_2$ solution and acetonitrile extract detected only fluopicolide
[B] HPLC analysis of acetonitrile extract detected only fluopicolide

Table 7.1.3.2- 3: Degradation of [phenyl-U-^{14}C]-fluopicolide in Senas soil under aerobic conditions at 20 °C [% AR]

Compound	Mean SD	Incubation time (DAT)											
		1.79 mg/kg				0.45 mg/kg				0.11 mg/kg			
		0	3	10	21	0	3	10	21	0	3	10	21
Fluopicolide	Mean	90.4	85.6	74.9	71.2	[A]	-	77.1		[B]	-	80.7	-
	SD	0.1	0.1	3.0	0.7	-	-	-		-	-	-	-
M-01 (AE C653711)	Mean	0.0	5.5	11.0	17.0	[A]	-	7.7	-	[B]	-	6.7	
	SD	0.0	0.0	1.2	0.4	-	-		-	-	-	-	-
$CaCl_2$ solution 1	Mean	39.8	34.7	33.9	34.2	34.7	29.6	37.3	30.2	32.8	31.2	26.0	28.0
	SD	0.1	0.3	0.0	0.6	0.4	0.8	0.6	0.5	0.0	4.3	0.2	0.5
$CaCl_2$ solution 2	Mean	20.2	18.8	18.6	17.4	19.3	17.4	16.8	16.0	10.7	16.7	15.9	15.5
	SD	0.1	0.1	0.1	0.4	0.4	0.4	0.2	0.1	8.0	0.0	0.0	0.1
$CaCl_2$ solution 3	Mean	10.7	10.8	10.2	9.3	11.0	10.6	10.0	9.0	14.4	10.7	9.9	9.1
	SD	0.2	0.0	0.2	0.1	0.0	0.3	0.0	0.1	3.2	0.0	0.1	0.2
Acetonitrile ext	Mean	19.7	26.7	23.1	27.3	23.3	31.0	30.9	32.6	31.9	38.4	35.9	37.8
	SD	0.1	0.3	1.6	0.9	0.2	1.2	0.5	0.4	4.0	0.1	0.6	0.4
Total Extractable Residues	Mean	90.3	91.0	85.9	88.2	88.3	88.6	85.0	87.8	89.8	97.0	87.7	90.4
	SD	0.0	0.2	1.9	0.3	0.6	2.7	0.3	0.0	0.8	4.5	0.2	0.4
Non-Extractable Residues	Mean	5.7	5.8	8.1	7.2	6.8	6.7	10.7	8.3	8.8	8.2	11.7	9.0
	SD	0.2	0.1	0.2	0.1	0.0	0.3	0.5	0.0	0.9	0.2	0.4	0.0
Total Recovery	Mean	96.1	96.9	94.1	95.4	95.1	95.3	95.7	96.1	98.6	105.2	99.4	99.5
	SD	0.1	0.1	1.8	0.3	0.6	3.1	0.2	0.1	0.0	4.3	0.1	0.5

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, SD: standard deviation
All values expressed as percentage of total applied radiolabel
[A] HPLC analysis of first $CaCl_2$ solution and acetonitrile extract detected only fluopicolide
[B] HPLC analysis of acetonitrile extract detected only fluopicolide

Table 7.1.3.2- 4: Degradation of [phenyl-U-^{14}C]-fluopicolide in Huntlosen soil under aerobic conditions at 20 °C [% AR]

Compound	Mean SD	Incubation time (DAT)											
		1.79 mg/kg				0.45 mg/kg				0.11 mg/kg			
		0	3	10	21	0	3	10	21	0	3	10	21
Fluopicolide	Mean	86.0	84.2	82.5	86.1	[A]	-	85.3	87.8	[B]	-	86.5	90.7
	SD	-	0.1	0.0	0.4	-	-	-	-	-	-	-	-
M-01 (AE C653711)	Mean	0.0	0.0	1.6	1.1	[A]	-	0.0	0.0	[B]	-	0.0	0.0
	SD	-	-	0.3	0.0	-	-	-	-	-	-	-	-
$CaCl_2$ solution 1	Mean	15.6	13.9	15.2	13.2	13.5	12.2	12.0	11.7	15.0	11.9	11.6	11.2
	SD	0.3	0.1	2.7	0.1	0.9	0.4	0.0	-	0.0	0.1	0.0	0.5
$CaCl_2$ solution 2	Mean	11.7	10.1	9.8	9.9	10.6	9.1	9.3	8.8	11.0	9.2	9.0	8.6
	SD	-	0.4	0.6	0.2	0.7	0.0	0.2	-	0.0	0.0	0.1	0.2
$CaCl_2$ solution 3	Mean	8.5	7.9	7.0	7.4	7.8	7.0	6.9	6.9	8.5	7.1	6.6	6.5
	SD	-	0.2	0.0	0.3	0.4	0.2	0.1	-	0.3	-	0.0	0.0
Acetonitrile ext	Mean	49.9	52.3	52.0	56.7	48.9	55.8	56.8	60.1	54.2	59.1	59.2	64.5
	SD	-	0.3	3.6	0.5	3.2	0.2	0.5	-	0.6	-	0.1	0.8
Total Extractable Residues	Mean	86.0	84.1	84.0	87.2	80.8	84.2	84.9	87.5	88.7	87.3	86.4	90.8
	SD	-	0.0	0.3	0.3	5.3	0.1	0.3	-	0.3	-	0.0	0.1
Non-Extractable Residues	Mean	10.3	12.6	11.7	9.3	10.7	13.2	12.5	10.2	11.5	13.6	14.3	11.0
	SD	-	0.0	0.8	0.1	0.4	0.2	0.2	-	0.0	-	0.3	0.2
Total Recovery	Mean	96.2	96.7	95.8	96.6	92.0	97.4	97.4	97.7	100.2	100.9	100.7	101.8
	SD	-	0.1	1.1	0.5	5.7	0.0	0.1	-	0.3	-	0.3	0.2

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, SD: standard deviation
All values expressed as percentage of total applied radiolabel
[A] HPLC analysis of first $CaCl_2$ solution and acetonitrile extract detected only fluopicolide
[B] HPLC analysis of acetonitrile extract detected only fluopicolide

Table 7.1.3.2- 5: Degradation of [phenyl-U-^{14}C]-fluopicolide in Rödelsee soil under aerobic conditions at 20 °C [% AR]

Compound	Mean SD	Incubation time (DAT)											
		1.79 mg/kg				0.45 mg/kg				0.11 mg/kg			
		0	3	10	21	0	3	10	21	0	3	10	21
Fluopicolide	Mean	87.0	85.9	76.2	73.0	^{A}	-	-	80.6	^{B}	-	-	82.6
	SD	-	0.1	0.6	0.5	-	-	-	-	-	-	-	-
M-01 (AE C653711)	Mean	2.2	3.7	8.5	13.5	^{A}	-	-	7.7	^{B}	-	-	6.8
	SD	-	0.2	0.3	0.5	-	-	-	-	-	-	-	-
$CaCl_2$ solution 1	Mean	34.2	32.1	27.5	28.2	32.8	28.1	27.2	26.9	29.0	25.4	22.5	23.6
	SD	2.5	0.1	0.8	0.5	0.2	0.2	0.6	0.2	0.5	0.2	1.1	0.4
$CaCl_2$ solution 2	Mean	22.2	19.9	19.5	18.9	20.4	18.9	17.6	17.7	13.4	18.1	17.4	16.0
	SD	1.1	0.0	0.1	0.0	0.4	0.3	0.0	0.2	6.0	0.0	0.5	0.2
$CaCl_2$ solution 3	Mean	11.6	12.0	11.7	10.6	11.8	11.6	11.1	10.5	14.0	11.4	10.7	10.0
	SD	-	0.1	0.3	0.0	0.1	0.1	0.0	0.2	2.3	0.3	0.5	0.1
Acetonitrile ext	Mean	19.8	25.7	25.9	28.9	24.2	30.6	29.9	33.2	31.2	36.6	35.7	39.3
	SD	0.0	0.1	0.2	0.5	0.2	0.4	0.2	0.2	3.0	0.4	1.4	0.1
Total Extractable Residues	Mean	89.1	89.6	84.5	86.5	89.1	89.2	85.7	88.3	87.5	91.5	86.3	88.9
	SD	-	0.1	0.4	0.0	0.1	1.0	0.3	0.0	1.2	0.4	0.7	0.5
Non-Extractable Residues	Mean	7.7	7.3	11.3	9.5	9.4	8.8	12.6	10.5	12.6	10.9	14.8	12.0
	SD	-	0.0	0.3	0.1	0.1	0.0	0.3	0.0	1.0	0.6	0.2	0.1
Total Recovery	Mean	96.7	96.9	95.9	96.1	98.5	98.0	98.4	98.8	100.2	102.4	101.1	100.9
	SD	-	0.1	0.1	0.1	0.2	1.1	0.1	0.0	0.3	0.1	0.5	0.4

n.d.: not detected, n.a.: not analysed, DAT: days after treatment, SD: standard deviation
All values expressed as percentage of total applied radiolabel
^{A} HPLC analysis of first $CaCl_2$ solution and acetonitrile extract detected only fluopicolide
^{B} HPLC analysis of acetonitrile extract detected only fluopicolide

B. Material Balance

Mean mass balances were 97.0% AR for Philippsburg soil (range from 97.0 to 102.0% AR), 97.3% AR for Senas soil (range from 97.3 to 109.4% AR), 97.7% AR for Huntlosen soil (range from 86.3 to 102.0% AR) and 98.7% AR for Rödelsee soil (range from 95.8 to 102.5% AR).

The results confirm there were no significant losses of radioactivity during sample processing.

C. Extractable and Non-Extractable Residues

Desorbable residues in aqueous 0.01 M $CaCl_2$ solution ranged from 54.4 to 78.4% AR in Philippsburg soil over the course of the study (mean 66.0% AR), declining from 75.0% AR (mean of 3 concentration levels) at DAT-0 to 58.5% AR at DAT-21 (mean of 3 concentration levels). In Senas soil desorbable residues ranged from 51.4 to 70.7% AR over the course of the study (mean 59.3 % AR), declining from 64.5% AR (mean of 3 concentration levels) at DAT-0 to 56.3% AR at DAT-21 (mean of 3 concentration levels). In Huntlosen soil desorbable residues ranged from 25.6 to 36.1% AR over the course of the study (mean 30.2% AR), declining from 33.8% AR (mean of 3 concentration levels) at DAT-0 to 27.9% AR at DAT-21 (mean of 3 concentration levels). Desorbable residues in aqueous 0.01 M $CaCl_2$ solution ranged from 48.4 to 69.3% AR in Rödelsee soil over the course of the study (mean 57.4 % AR), declining from 62.4% AR (mean of 3 concentration levels) at DAT-0 to 54.1% AR at DAT-21 (mean of 3 concentration levels).

Total extractable residues (i.e. residues desorbed by aqueous 0.01 M $CaCl_2$ solution and residues in organic soil extracts) decreased slightly from DAT-0 to DAT-21 from 93.2 to 89.6% AR in Philippsburg soil, from 89.5 to 88.8% AR in Senas soil, and from 88.5 to 87.9% AR in Rödelsee soil (all values mean of 3 concentrations). In Huntlosen soil, total extractable residues increased slightly over the same period, from 85.0 to 89.2% AR (mean of 3 concentrations).

Non-extractable soil residues changed concurrently with the slight change in extractable radioactivity in all soils. Non-extractable residues (NER) increased slightly from DAT-0 to DAT-21 from 4.0 to 7.6% AR in Philippsburg soil, from 7.1 to 8.2% AR in Senas soil, and from 10.3 to 10.7% AR in Rödelsee soil and decreased slightly from 10.9 to 10.2% AR in Huntlosen soil (all values mean of 3 concentrations).

D. Volatile Radioactivity

Not applicable. Volatile traps were not used in the study.

E. Degradation of Parent Compound

Chromatographic analysis was conducted for all extracts in samples treated at the top concentration (1.79 mg/kg) only. The amount of fluopicolide in the total soil extracts (i.e. in three aqueous desorption solutions and organic soil extract) decreased from 90.5% at DAT-0 to 73.6% AR at DAT-21 in Philippsburg soil, from 90.4 to 71.2% AR in Senas soil and from 87.0 to 73.0% AR in Rödelsee soil. In Huntlosen soil, levels of fluopicolide ranged from 82.5% (DAT 10) to 86.1% AR (DAT 21) with no overall decline.

Degradation of fluopicolide was accompanied by the formation of one degradation product, M-01 (AE C653711) which was observed at a maximum of 17.0% AR at DAT-21 in Senas soil. M-01 was identified by co-chromatography with an analytical standard.

F. Degradation Kinetics

The study duration is too short to determine robust degradation kinetics for fluopicolide.

G: Time-Dependent Sorption

The sorption of fluopicolide in soil increased steadily with time. Apparent sorption coefficients ($K_{d,app}$) increased from DAT-0 to DAT-21 in Philippsburg, Senas and Rodelsee soils, with values for the first desorption cycle increasing by a factor of 2 after 21 days ageing. In Huntlosen soil $K_{d, app}$ values (for the first desorption) were in the range 13.88 to 21.44; significantly higher than the results obtained for the other three soils. The values are summarised in Table 7.1.3.2- 6.

Table 7.1.3.2- 6: Apparent sorption coefficients ($K_{d, app}$) (expressed as mL/g)

DAT	Philippsburg			Senas			Huntlosen			Rödelsee		
Cycle [A]	1	2	3	1	2	3	1	2	3	1	2	3
0	2.09	3.78	7.22	3.10	4.42	6.35	13.88	17.69	23.34	3.23	4.04	5.60
3	2.34	4.21	7.13	5.01	6.25	8.46	14.69	18.86	22.40	4.05	5.41	6.52
10	2.96	5.53	9.58	4.92	6.17	8.19	21.44	24.73	27.55	4.77	5.82	7.51
21	4.42	7.34	11.60	5.73	8.03	11.39	16.41	19.88	22.87	6.14	8.25	11.81
Factor [B]	2.12	1.94	1.61	1.85	1.82	1.79	1.18	1.12	0.98	1.91	2.04	2.11
Mean Factor [C]	1.76											

Apparent Sorption Coefficients ($K_{d, app}$) are called Desorption Coefficients in the report.
[A] Desorption cycle
[B] Calculated as $K_{d, app}$ DAT-21 divided by $K_{d, app}$ DAT-0
[C] Mean factor of desorption cycle 1 for all soils

III. Conclusion

The objective of the study was to investigate the sorption of fluopicolide, determined under equilibrium conditions, following its aging in soil under aerobic conditions in the dark under laboratory conditions. Apparent sorption coefficients ($K_{d,app}$) increased over the incubation period (21 days) by a factor of 1.18 to 2.12 (mean 1.78) in the four soils tested.

However the study design does not conform to current test guidelines and has been superseded by three new time dependent sorption studies conducted over longer incubation periods with more timepoints (see KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05). Consequently, the study is no longer considered acceptable to assess the time dependent sorption of fluopicolide.

Assessment and conclusion by applicant:

The study is considered as supporting information only to assess the aged sorption of fluopicolide in soil as it does not meet current requirements for conducting aged sorption studies for use in regulatory assessments (CRD, 2019).

Data Point:	KCA 7.1.3.2/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	Route and rate of degradation of [2,6-14C pyridinyl] and [U-14C-benzoyl]-AE C638206 in a European sandy loam under laboratory aerobic conditions at 20 deg. C and determination of aged in situ Kd values at 25 degrees C
Report No:	B004071
Document No:	M-241049-01-1
Guideline(s) followed in study:	EU (=EEC): 95/36/EC of July 1995
Deviations from current test guideline:	Yes. The study design does not conform to current aged sorption guidelines as the aged sorption phase of the study had insufficient timepoints
Previous evaluation:	yes, evaluated and accepted Tests on aerobic degradation evaluated and accepted in the DAR (2005). Tests on aged sorption evaluated in the DAR (2005) and Addendum 1 to the DAR (2007).
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aged desorption behaviour of fluopicolide was initially characterised as part of a study conducted to investigate the route and rate of degradation fluopicolide in a European sandy loam soil. The main phase of the study is described in full under Point CA 7.1.1.1 (KCA 7.1.1.1/01). Samples for the aged sorption phase were incubated concurrently with route and rate samples under aerobic conditions in the laboratory at 20°C in the dark at a moisture content equivalent to pF 2 for 120 days.

Soil	Texture (USDA)	pH (CaCl2)	% Organic Carbon
Abington	sandy loam	7.1	2.2

[Phenyl-U-^{14}C]-labelled fluopicolide or [2,6-pyridyl-^{14}C]-labelled fluopicolide was applied to soil samples at an application rate equivalent to 0.41 mg/kg dry weight. The radiochemical purity was > 99 % for both radiolabelled test items. The specific activities were 5.33 and 5.88 MBq/mg for [phenyl-U-^{14}C]- and [2,6-pyridyl-^{14}C]-labelled fluopicolide, respectively.

Samples were taken for extraction and analysis immediately after treatment (day 0) and after 28, 77 and 120 days of incubation. Soil samples were first desorbed with 0.01M calcium chloride solution for 24 hours at 25 °C at a soil : solution ratio of 1:5 (w/w) to determine the desorbable portion of the test item from aged soil. The soil residue was then exhaustively extracted with up to four successive extractions with acetonitrile / water (4 / 1, v/v) at ambient temperature followed by one Soxhlet extraction using acetonitrile. Desorption supernatants and soil extracts were analysed by reverse phase high performance liquid chromatography (HPLC).

Thus the design does not conform to current aged sorption guidelines as the aged sorption phase of the study had insufficient timepoints. Nevertheless the aged sorption of fluopicolide to soil was measured and showed a significant increase with time. Apparent sorption coefficients ($K_{d, app}$) increased with time by a mean factor of 2.87 for both radiolabelled experiments over 120 days incubation.

I. Materials and Methods

The full details of this study are described under Point CA 7.1.1.1 (KCA 7.1.1.1/01).

Aged sorption soil samples were first shaken with 0.01M calcium chloride solution for 24 hours in an incubation chamber at 24.4 ± 0.5 °C on an end-over-end shaker to determine the desorbable portion of the test item from aged soil. A soil-to-solution ratio of 1:5 was used for all soils. After the desorption step, supernatant and soil were separated by centrifugation. Soil samples were then extracted up to four times with acetonitrile/water (4/1, v/v) at ambient temperature followed by a Soxhlet extraction with acetonitrile.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for aged sorption soil samples incubated at 20 °C following application of [phenyl-U-^{14}C]- and [2,6-pyridyl-^{14}C]-fluopicolide are summarized below in Table 7.1.3.2- 7 and Table 7.1.3.2- 8.

Table 7.1.3.2- 7: Degradation of [phenyl-U-^{14}C]-fluopicolide in Abington soil under aerobic conditions [% AR]- Aged Sorption Phase

Compound	Incubation time (DAT)			
	0	28	77	120
Fluopicolide	95.5	85.5	80.1	76.0
M-01 (AE C653711)	0.0	12.9	12.7	20.6
$CaCl_2$ solution	39.8	32.8	32.0	36.3
Ambient extract	55.7	61.5	56.8	53.1
Soxhlet extract	n.a.	4.1	4.0	8.5
Total extractable radioactivity [A]	95.5	98.4	92.8	97.9
Non-extractable radioactivity	1.9	1.2	3.0	2.7
^{14}C-Carbon dioxide including other volatiles [B]	n.a.	0.3	0.7	0.0
Total radioactivity	97.4	99.9	96.5	100.6

n.a.: not analysed, n.d.: not detected, DAT: days after treatment
All values expressed as percentage of total applied radiolabel
[A] The total extractable radioactivity was calculated as sum of ambient and Soxhlet extracts.
[B] Other volatile radioactivity was < 0.05 % AR at any time point

Table 7.1.3.2- 8: Degradation of [2,6-pyridyl-^{14}C]-fluopicolide in Abington soil under aerobic conditions [% AR]- Aged Sorption Phase

Compound	Incubation time (DAT)			
	0	28	77	120
Fluopicolide	95.8	91.8	83.2	81.3
M-02 (AE C657188)	0.0	1.9	2.7	3.4
Unidentified (R_t 5 min)	0.0	0.8	1.9	2.0
$CaCl_2$ solution	39.9	30.4	29.8	30.2
Ambient extract	57.4	60.4	52.8	54.9
Soxhlet extract	n.a.	4.3	3.3	6.9
Total extractable radioactivity [A]	97.3	95.1	87.9	90.1
Non-extractable radioactivity	2.0	2.6	6.2	7.4
^{14}C-Carbon dioxide including other volatiles [B]	n.a.	0.7	1.9	0.5
Total radioactivity	99.3	98.4	96.0	98.0

n.a.: not analysed, n.d.: not detected, DAT: days after treatment
All values expressed as percentage of total applied radiolabel
[A] The total extractable radioactivity was calculated as sum of ambient and Soxhlet extracts.
[B] Other volatile radioactivity was < 0.05 % AR at any time point

B. Material Balance

For aged sorption samples of Abington soil, material balances ranged from 96.5 to 100.6% AR for [phenyl-U-^{14}C]-fluopicolide and from 96.0 to 99.3% AR for [2,6-pyridyl-^{14}C]-fluopicolide.

There were no signs for losses of radioactivity during work-up and processing.

C. Extractable and Non-Extractable Residues

Desorbable residues in aqueous 0.01 M $CaCl_2$ solution ranged from 32.0 to 39.8% AR and from 29.8 to 39.9% AR in samples treated with [phenyl-U-^{14}C]- and [2,6-pyridyl-^{14}C]-labelled fluopicolide, respectively.

Total extractable residues (i.e. residues desorbed by aqueous 0.01 M $CaCl_2$ solution and residues in organic soil extracts) at DAT 0 were 95.5% and 95.8% AR in samples treated with [phenyl-U-^{14}C]- and [2,6-pyridyl-^{14}C]-labelled fluopicolide, respectively.

For aged sorption samples incubated with [phenyl-U-^{14}C]-fluopicolide, total extractable radioactivity ranged from 92.8% AR (DAT 77) to 98.4% AR (DAT 28). The total of non-extractable residues (NER) ranged from 1.2% AR (DAT 28) to 3.0% AR (DAT 77).

For samples incubated with [2,6-pyridyl-^{14}C]-fluopicolide, total extractable radioactivity decreased from 97.3% AR on DAT 0 to 90.1% AR after 120 days of incubation (minimum 87.9% AR on DAT 77). NER was 2.0% AR on DAT 0 and increased to 7.4% AR by the end of the study (DAT 120).

D. Volatile Radioactivity

Levels of ^{14}C-carbon dioxide were minimal throughout the aged sorption phase, reaching maximum values of 0.7% AR in samples treated with [phenyl-U-^{14}C]-fluopicolide) and 1.9% AR in samples treated with [2,6-pyridyl-^{14}C]-fluopicolide. Other volatile radioactivity was < 0.05% AR at all timepoints.

E. Degradation of Parent Compound

Following application of [phenyl-U-^{14}C]-fluopicolide, the amount of parent in the total soil extracts of aged sorption samples (i.e. in aqueous desorption supernatant, ambient extracts and Soxhlet soil extract) decreased from 95.5% at DAT-0 to 76.0% AR by the end of the study at DAT 120. In the corresponding aged sorption samples treated with [2,6-pyridyl-^{14}C]-fluopicolide, the amount of parent in the total soil extracts decreased from 95.8% at DAT-0 to 81.3% AR by DAT 120.

Degradation of [phenyl-U-^{14}C]-fluopicolide was accompanied by the formation of the degradation product M-01 (AE C653711). M-01 was detected at a maximum of 20.6% AR at DAT 120 in aged sorption samples.

Degradation of [2,6-pyridyl-^{14}C]-fluopicolide aged sorption samples was accompanied by the formation of the degradation product M-02 (AE C657188) and a minor unidentified metabolite. M-02 was detected at a maximum of 3.4% AR at DAT 120. The unidentified polar metabolite amounted to a maximum of 2.0% AR at DAT 120.

M-01 and M-02 were identified by co-chromatography with an analytical standard.

F. Degradation Kinetics

No degradation kinetics for fluopicolide have been determined for the aged sorption samples.

G: Time-Dependent Sorption

The sorption of fluopicolide in soil increased steadily with time. Apparent sorption coefficients ($K_{d, app}$) increased from DAT-0 to DAT-120 in Abington soil by a mean factor of 2.87 for both radiolabelled experiments. The values are summarised in Table 7.1.3.2- 9.

Table 7.1.3.2- 9: Apparent sorption coefficients ($K_{d, app}$) (expressed as mL/g)

DAT	Abington		
	[phenyl-U-^{14}C]-fluopicolide	[2,6-pyridyl-^{14}C]-fluopicolide	Mean
0	6.2	7.9	7.1
28	16.4	20.4	18.4
77	18.0	21.6	19.8
120	17.0	23.7	20.4
Factor [A]	2.74	3.00	2.87

Apparent Sorption Coefficients ($K_{d, app}$) are called In Situ K_d in the report.
[B] Calculated as $K_{d, app}$ DAT-120 divided by $K_{d, app}$ DAT-0

III. Conclusion

The objective of this phase of the study was to investigate the sorption of fluopicolide, determined under equilibrium conditions, following its aging in soil under aerobic conditions in the dark under laboratory conditions. Apparent sorption coefficients ($K_{d, app}$) increased over the incubation period (120 days) by a factor of 2.74 in phenyl label treated soil and 3.00 in pyridyl label treated soil (mean factor 2.87).

However the study design does not conform to current test guidelines and has been superseded by three new time dependent sorption studies conducted with more timepoints (see KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05). Consequently, the study is no longer considered acceptable to assess the time dependent sorption of fluopicolide.

Assessment and conclusion by applicant:

The study is considered as supporting information only to assess the aged sorption of fluopicolide in soil as it does not meet current requirements for conducting aged sorption studies for use in regulatory assessments (CRD, 2019).

Data Point:	KCA 7.1.3.2/03
Report Author:	[redacted]
Report Year:	2016
Report Title:	[Phenyl-UL-14C]Fluopicolide: Degradation and time - Dependent sorption in soils
Report No:	EnSa-15-0475
Document No:	M-555570-01-1
Guideline(s) followed in study:	OECD Test Guideline No. 307 US EPA OCSPP Test Guideline No. 835.4100 / 835.4200 OECD Test Guideline No. 106 (only in parts) Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in the regulatory process; Food and Environmental Agency, York, UK, 2012
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation and time-dependence of sorption of fluopicolide was studied in four soils under aerobic conditions in the laboratory in the dark at 20 ± 1 °C and 54.3% of the maximum water holding capacity for 126 days. In addition, the rate of degradation of fluopicolide was determined in the study.

Full details of this study are described under Point CA 7.1.2.1.1 (KCA 7.1.2.1.1/06).

Soil	Texture (USDA)	pH ($CaCl_2$)	% Organic Carbon
L[redacted]	sandy loam	6.5	1.5
Dollendorf II	clay loam	7.3	4.8
L[redacted]	loam	5.0	1.8
H[redacted]	silt loam	6.1	1.9

[Phenyl-U-^{14}C]-labelled fluopicolide was applied to soil samples at an application rate of 0.44 mg/kg dry weight. The radiochemical purity and specific activity were > 98% and 5.50 MBq/mg, respectively.

Samples were taken for extraction and analysis immediately after treatment (day 0) and after 2, 7, 10, 14, 28, 57, 85 and 126 days of incubation. Soil samples were first desorbed with 0.01M calcium chloride solution for 24 hours at 20 °C at a soil : solution ratio of 1:3 (w/w) to determine the desorbable portion of the test item from aged soil. The soil residue was then exhaustively extracted with three further successive extractions with acetonitrile/water 4/1 (v/v) at ambient temperature, followed by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 70 °C. Desorption supernatants and concentrated soil extracts were analysed by reverse phase high performance liquid chromatography (HPLC).

The effect of aged sorption to soil was determined for fluopicolide and showed a significant increase with time. Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.96 to 2.98 (mean 2.60).

I. Materials and Methods

The full details of this study are described under Point CA 7.1.2.1.1 (KCA 7.1.1.1/06).

Standard OECD 106 batch equilibrium tests were performed on the same batches of soils to derive the equilibrium sorption parameters (see KCA 7.1.3.1.1/03, M-544194-02-1).

The extraction method used in the study was the same as that used in two other aged sorption studies (see KCA 7.1.3.2/04, M-550687-01-1 and KCA 7.1.3.2/05, M-655056-01-1). Soil samples were first shaken with 0.01M calcium chloride solution for 24 hours at 20 °C on an overhead shaker at 16 rpm to determine the desorbable portion of the test item from aged soil. A soil-to-solution ratio of 1:3 was used for all soils. Soil samples were then extracted three times with acetonitrile/water (4/1, v/v) at ambient temperature followed by a microwave extraction with acetonitrile/water (4/1, v/v) at 70 °C. After each extraction step, extract and soil were separated by centrifugation.

II. Results and Discussion

A. Data

The datasets used for the fluopicolide kinetic sorption evaluation are summarised below in Table 7.1.3.2- 10 to Table 7.1.3.2- 13.

Table 7.1.3.2- 10: Total mass and aqueous concentration of fluopicolide (C_{des}) in L[redacted] soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	14	28	57	85	126
Total mass (µg)	A	41.17	41.24	38.12	37.49	35.80	33.03	27.02	23.68	20.11
	B	41.81	40.74	38.50	36.60	35.09	32.97	27.52	23.66	21.12
	Mean	41.49	40.99	38.31	37.05	35.45	33.00	27.27	23.67	20.62
	SD	0.32	0.25	0.19	0.45	0.35	0.03	0.25	0.01	0.51
C_{des} (µg/mL)	A	0.0458	0.0421	0.0362	0.0325	0.0298	0.0248	0.0185	0.0151	0.0108
	B	0.0468	0.0416	0.0347	0.0325	0.0297	0.0241	0.0179	0.0135	0.0123
	Mean	0.0463	0.0418	0.0349	0.0325	0.0298	0.0245	0.0182	0.0143	0.0115
	SD	0.0005	0.0003	0.0002	0.0000	0.0000	0.0004	0.0003	0.0008	0.0007

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 11: Total mass and aqueous concentration of fluopicolide (C_{des}) in Dollendorf II soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	14	28	57	85	126
Total mass (µg)	A	37.74	40.23	37.31	35.83	35.11	31.81	25.12	23.49	20.13
	B	37.12	40.26	37.12	36.61	34.95	32.06	23.17	22.35	20.49
	Mean	37.43	40.25	37.22	36.22	35.03	31.94	24.15	22.92	20.31
	SD	0.31	0.02	0.10	0.39	0.08	0.13	0.98	0.57	0.18
C_{des} (µg/mL)	A	0.0192	0.0166	0.0154	0.0144	0.0150	0.0121	0.0088	0.0074	0.0061
	B	0.0188	0.0167	0.0154	0.0146	0.0135	0.0118	0.0095	0.0079	0.0055
	Mean	0.0190	0.0166	0.0154	0.0145	0.0143	0.0120	0.0091	0.0077	0.0058
	SD	0.0002	0.0001	0.0000	0.0001	0.0007	0.0001	0.0004	0.0003	0.0003

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 12: Total mass and aqueous concentration of fluopicolide (C_{des}) in L[redacted] soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	14	28	57	85	126
Total mass (µg)	A	41.85	40.19	34.03	32.73	29.78	25.79	20.14	16.86	13.84
	B	41.52	40.05	33.71	31.75	30.58	25.13	20.16	17.15	13.96
	Mean	41.69	40.12	33.87	32.24	30.18	25.46	20.15	17.01	13.90
	SD	0.16	0.07	0.16	0.49	0.40	0.33	0.01	0.15	0.06
C_{des} (µg/mL)	A	0.0405	0.0357	0.0266	0.0241	0.0210	0.0162	0.0109	0.0082	0.0057
	B	0.0397	0.0354	0.0267	0.0238	0.0226	0.0167	0.0117	0.0087	0.0060
	Mean	0.0401	0.0356	0.0267	0.0240	0.0218	0.0165	0.0113	0.0084	0.0059
	SD	0.0004	0.0001	0.0001	0.0002	0.0008	0.0003	0.0004	0.0002	0.0002

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 13: Total mass and aqueous concentration of fluopicolide (C_{des}) in H[redacted] soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	14	28	57	85	126
Total mass (µg)	A	40.97	39.69	35.83	35.09	35.32	30.81	26.29	22.36	19.28
	B	40.74	40.44	36.12	35.18	33.44	30.69	25.51	22.87	19.15
	Mean	40.86	40.07	35.98	35.14	33.38	30.75	25.90	22.62	19.22
	SD	0.11	0.38	0.15	0.04	0.06	0.06	0.39	0.26	0.07
C_{des} (µg/mL)	A	0.0342	0.0296	0.0245	0.0224	0.0195	0.0173	0.0119	0.0091	0.0067
	B	0.0334	0.0294	0.0235	0.0220	0.0197	0.0168	0.0120	0.0096	0.0064
	Mean	0.0338	0.0295	0.0240	0.0222	0.0196	0.0171	0.0119	0.0093	0.0066
	SD	0.0004	0.0001	0.0005	0.0002	0.0001	0.0002	0.0000	0.0003	0.0002

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

B: Extraction efficiency

Total extractable residues (i.e. residues desorbed by aqueous 0.01 M $CaCl_2$ solution and residues in organic soil extracts) at DAT 0 were 97.8 %, 87.8%, 96.8 % and 95.7% AR in L[redacted], Dollendorf II, L[redacted] and H[redacted] soils, respectively, which represents 98.5%, 90.5%, 97.9% and 97.6% of the recovered radioactivity. In Dollendorf soil although the extraction efficiency of the method is 95% at DAT 0 by DAT 2 the extraction efficiency was 96.4 % AR. It is concluded all residues of fluopicolide potentially available for leaching were extracted by this method.

C: Time-Dependent Sorption

The sorption of fluopicolide in soil increased steadily with time. Apparent sorption coefficients ($K_{d,app}$) increased from DAT-0 to DAT-126 in all four soils. The values are summarised in Table 7.1.3.2-14.

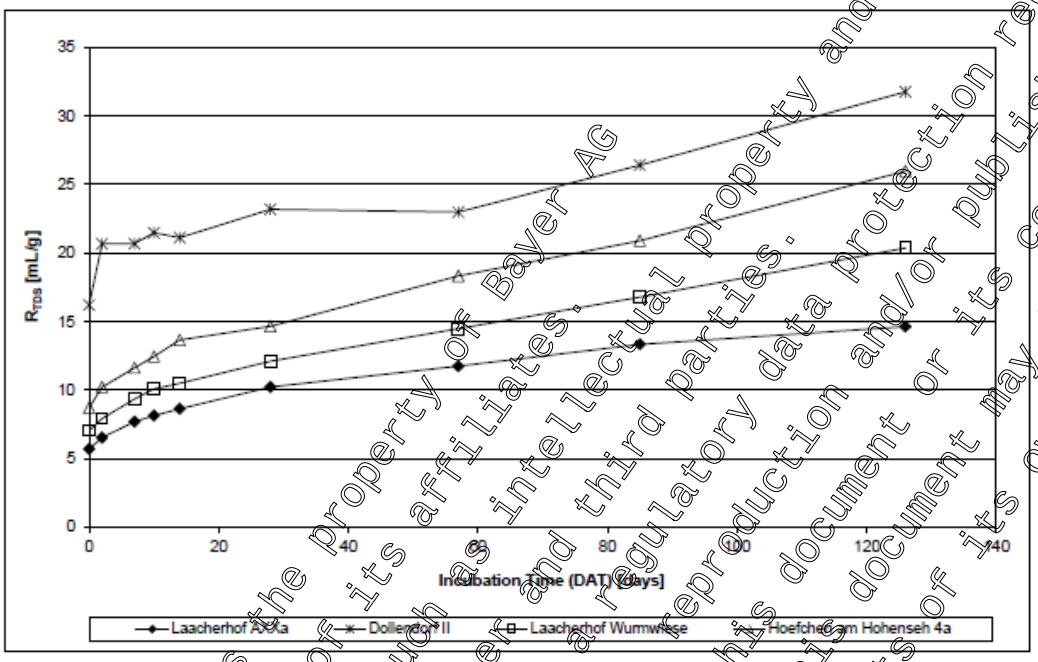
Table 7.1.3.2- 14: Apparent sorption coefficients (Kd, app) (expressed as mL/g)

DAT	Mean SD	L[redacted]	Dollendorf	L[redacted]	[redacted]
0	Mean	5.69	16.19	7.02	8.7
	SD	± 0.00	± 0.00	± 0.01	± 0.01
2	Mean	6.52	20.66	7.9	10.21
	SD	± 0.00	± 0.00	± 0.00	± 0.02
7	Mean	7.69	20.67	9.33	12.61
	SD	± 0.02	± 0.00	± 0.01	± 0.03
10	Mean	8.13	21.47	10.08	12.44
	SD	± 0.02	± 0.01	± 0.01	± 0.01
14	Mean	8.63	21.12	10.49	13.65
	SD	± 0.01	± 0.06	± 0.03	± 0.01
28	Mean	10.21	23.18	12.1	14.64
	SD	± 0.02	± 0.02	± 0.04	± 0.01
57	Mean	11.74	22.98	14.47	18.32
	SD	± 0.03	± 0.00	± 0.04	± 0.02
85	Mean	13.32	26.41	16.78	20.89
	SD	± 0.07	± 0.07	± 0.02	± 0.02
126	Mean	14.62	31.76	20.36	25.97
	SD	± 0.05	± 0.07	± 0.03	± 0.03
Factor[A]		2.57	1.96	2.90	2.98
Mean Factor		2.60			

Apparent Sorption Coefficients ($K_{d, app}$) are called Time-Dependent Sorption Ratios (R_{TDS}) in the report.
[A] Calculated as $K_{d, app}$ DAT-120 divided by $K_{d, app}$ DAT-0

A plot of the apparent sorption coefficients ($K_{d, app}$) with time in the four soils is shown in Figure 7.1.3.2-1.

Figure 7.1.3.2- 1: Apparent sorption coefficients ($K_{d, app}$) with time



Apparent Sorption Coefficients ($K_{d, app}$) are called Time-Dependent Sorption Ratios (R_{TDS}) in the report.

III. Conclusion

The adsorption of fluopicolide to soil increased significantly with time under laboratory conditions at 20 °C and a soil moisture content of 54.3% of the maximum water holding capacity. Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.96 to 2.98 (mean 2.60).

Assessment and conclusion by applicant:

The study is considered valid to assess the aged sorption of fluopicolide in soil and meets current requirements for conducting aged sorption studies for use in regulatory assessments (CRD, 2019).

Data Point:	KCA 7.1.3.2/04
Report Author:	[redacted]
Report Year:	2016
Report Title:	[Phenyl-UL-14C]Fluopicolide: Degradation and time- dependent sorption in 6 soils from field dissipation trials
Report No:	EnSa-15-0510
Document No:	M-550687-01-1
Guideline(s) followed in study:	OECD Test Guideline No. 307 US EPA OPPTS Test Guideline No. 835.4100 / 835.4200 OECD Test Guideline No. 106 (only in parts) Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in the regulatory process; Food and Environmental Agency, York, UK, 2012
Deviations from current test guideline:	Study: none; Analytical methods part: none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation and time-dependence of sorption of fluopicolide was studied in six soils under aerobic conditions in the laboratory in the dark at 20 ± 1 °C and 53.9% of the maximum water holding capacity for 120 days. In addition, the rate of degradation of fluopicolide was determined in the study.

Full details of this study are described under Point CA 7.1.2.1.1 (KCA 7.1.2.1.1/07).

Soil	Texture (USDA)	pH ($CaCl_2$)	% Organic Carbon
H[redacted]	Silt loam	6.1	0.7
Great Chishill	Clay	7.3	2.1
Parcey/Meslay	Loam	6.7	1.3
Mas du Coq	Clay loam	7.6	0.9
Albaro/Marcomcini	Silty clay	7.2	2.1
Vilobi d'Onyar	Sandy loam	6.3	0.8

[Phenyl-U-^{14}C]-labelled fluopicolide was applied to soil samples at an application rate of 0.44 mg/kg dry weight. The radiochemical purity and specific activity were > 99% and 5.73 MBq/mg, respectively.

Samples were taken for extraction and analysis immediately after treatment (day 0) and after 7, 10, 30, 44, 59, 91 and 120 days of incubation. Soil samples were first desorbed with 0.01M calcium chloride solution for 24 hours at 20 °C at a soil : solution ratio of 1:3 (w/w) to determine the desorbable portion of the test item from aged soil. The soil residue was then exhaustively extracted with three further successive extractions with acetonitrile/water 4/1 (v/v) at ambient temperature, followed by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 70 °C. Desorption supernatants and concentrated soil extracts were analysed by reverse phase high performance liquid chromatography (HPLC).

The effect of aged sorption to soil was determined for fluopicolide and showed a significant increase with time. Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.93 to 3.12 (mean 2.49).

I. Materials and Methods

Full details of this study are described under Point CA 7.1.2.1.1 (KCA 7.1.2.1.1/07).

Standard OECD 106 batch equilibrium tests were performed on the same batches of soils to derive the equilibrium sorption parameters (see KCA 7.1.3.1.1/04, M-550735-01-1). Five of the six soils used in this study were collected in November 2014 from sites used for fluopicolide terrestrial field dissipation conducted between May 2015 and 2017.

The extraction method used in the study was the same as that used in two other aged sorption studies (see KCA 7.1.3.2/03, M-555570-01-1 and KCA 7.1.3.2/05, M-655056-01-1). Soil samples were first shaken with 0.01M calcium chloride solution for 24 hours at 20 °C on an overhead shaker at 16 rpm to determine the desorbable portion of the test item from aged soil. A soil-to-solution ratio of 1:3 was used for all soils. Soil samples were then extracted three times with acetonitrile/water (4/1, *v/v*) at ambient temperature followed by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 70 °C. After each extraction step, extract and soil were separated by centrifugation.

II. Results and Discussion

The datasets used for the fluopicolide kinetic sorption evaluation are summarised below in Table 7.1.3.2-15 to Table 7.1.3.2- 20.

Table 7.1.3.2- 15: Total mass and aqueous concentration of fluopicolide (C_{des}) in H[redacted] soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	30	44	59	91	120
Total mass (µg)	A	42.54	40.75	38.56	36.42	30.15	27.61	26.35	22.08	20.19
	B	42.89	40.61	38.1	35.65	30.38	28.43	26.16	22.34	20.53
	Mean	42.72	40.68	38.33	36.04	30.27	28.02	26.26	22.21	20.36
	SD	0.18	0.07	0.23	0.39	0.12	0.41	0.10	0.13	0.17
C_{des} (µg/mL)	A	0.0694	0.0614	0.0518	0.0488	0.0361	0.0298	0.0271	0.0203	0.0174
	B	0.0691	0.0611	0.0506	0.0484	0.0351	0.0311	0.0278	0.0212	0.0179
	Mean	0.0693	0.0613	0.0512	0.0486	0.0356	0.0304	0.0275	0.0207	0.0176
	SD	0.0002	0.0002	0.0006	0.0002	0.0005	0.0007	0.0004	0.0004	0.0003

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 16: Total mass and aqueous concentration of fluopicolide (C_{des}) in Great Chishill soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	30	44	59	91	120
Total mass (µg)	A	42.45	40.86	38.14	37.05	35.64	34.12	33.6	30.39	29.09
	B	42.26	40.82	38.74	37.01	35.23	34.2	33.35	30.74	29.49
	Mean	42.36	40.84	38.44	37.03	35.44	34.16	33.48	30.57	29.29
	SD	0.10	0.02	0.30	0.02	0.21	0.04	0.13	0.17	0.20
C_{des} (µg/mL)	A	0.0427	0.0378	0.0330	0.0329	0.0258	0.0233	0.0213	0.0182	0.0156
	B	0.0427	0.0371	0.0337	0.0325	0.0258	0.0230	0.0212	0.0172	0.0163
	Mean	0.0427	0.0375	0.0333	0.0327	0.0258	0.0232	0.0213	0.0177	0.0160
	SD	0.0000	0.0004	0.0003	0.0002	0.0000	0.0001	0.0000	0.0005	0.0003

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 17: Total mass and aqueous concentration of fluopicolide (C_{des}) in Parcey Meslay soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	30	44	59	91	120
Total mass (µg)	A	42.75	41.87	39.36	38.97	35.19	32.18	31.69	27.65	25.72
	B	42.91	42.6	40.09	38.78	35.49	32.78	30.45	27.86	25.3
	Mean	42.83	42.24	39.73	38.88	35.34	32.48	31.07	27.76	25.51
	SD	0.08	0.37	0.37	0.09	0.15	0.30	0.62	0.11	0.21
C_{des} (µg/mL)	A	0.0521	0.0435	0.0382	0.0381	0.0288	0.0246	0.0241	0.0194	0.0175
	B	0.0522	0.0459	0.0392	0.0379	0.0293	0.0252	0.0230	0.0188	0.0178
	Mean	0.0522	0.0447	0.0387	0.0380	0.0291	0.0249	0.0235	0.0191	0.0176
	SD	0.0000	0.0012	0.0005	0.0001	0.0003	0.0003	0.0005	0.0003	0.0001

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 18: Total mass and aqueous concentration of fluopicolide (C_{des}) in Mas du Coq soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	30	44	59	91	120
Total mass (µg)	A	42.53	41.74	40.07	38.59	36.23	33.75	31.85	28.76	26.44
	B	41.84	42.04	40.57	39.51	35.47	33.00	32.07	28.63	26.24
	Mean	42.19	41.89	40.32	39.05	35.85	33.38	31.96	28.70	26.34
	SD	0.34	0.15	0.25	0.46	0.38	0.38	0.11	0.07	0.10
C_{des} (µg/mL)	A	0.0723	0.0635	0.0553	0.0543	0.0409	0.0362	0.0332	0.0270	0.0226
	B	0.0714	0.0640	0.0571	0.0555	0.0408	0.0356	0.0333	0.0259	0.0235
	Mean	0.0718	0.0637	0.0562	0.0549	0.0408	0.0359	0.0333	0.0264	0.0231
	SD	0.0004	0.0002	0.0009	0.0006	0.0001	0.0003	0.0001	0.0006	0.0004

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 19: Total mass and aqueous concentration of fluopicolide (C_{des}) in Albaro soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	30	44	59	91	120
Total mass (µg)	A	41.33	42.18	38.2	38.29	34.21	32.27	30.18	25.82	23.33
	B	42.49	41.34	38.94	38.81	34.84	32.65	29.6	25.57	22.99
	Mean	41.91	41.76	38.57	38.55	34.53	32.46	29.89	25.70	23.16
	SD	0.58	0.42	0.37	0.26	0.32	0.19	0.29	0.13	0.17
C_{des} (µg/mL)	A	0.0512	0.0493	0.0436	0.0429	0.0346	0.0305	0.0283	0.0231	0.0196
	B	0.0531	0.0478	0.0434	0.0437	0.0350	0.0310	0.0268	0.0221	0.0179
	Mean	0.0522	0.0486	0.0435	0.0433	0.0348	0.0307	0.0275	0.0226	0.0187
	SD	0.0009	0.0007	0.0001	0.0004	0.0002	0.0003	0.0007	0.0005	0.0008

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 20: Total mass and aqueous concentration of fluopicolide (C_{des}) in Vilobi d'Ònyar soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	7	10	30	44	59	91	120
Total mass (µg)	A	41.50	40.00	36.55	35.15	26.34	23.44	20.41	16.35	13.25
	B	41.62	40.37	35.83	34.10	25.96	23.55	20.65	16.07	13.39
	Mean	41.56	40.19	36.19	34.63	26.15	23.50	20.53	16.21	13.32
	SD	0.06	0.18	0.36	0.52	0.19	0.05	0.12	0.14	0.07
C_{des} (µg/mL)	A	0.0620	0.0555	0.0459	0.0436	0.0290	0.0239	0.0201	0.0144	0.0113
	B	0.0620	0.0571	0.0452	0.0427	0.0283	0.0245	0.0209	0.0141	0.0113
	Mean	0.0620	0.0563	0.0456	0.0431	0.0286	0.0242	0.0205	0.0143	0.0113
	SD	0.0000	0.0008	0.0003	0.0005	0.0003	0.0003	0.0004	0.0002	0.0000

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

B: Extraction efficiency

Total extractable residues (i.e. residues desorbed by aqueous 0.01 M $CaCl_2$ solution and residues in organic soil extracts) at DAT 0 were 97.6%, 96.2%, 97.3%, 97.0%, 95.2% and 96.7% AR in H[redacted], Great Chishill, Parcey Meslay, Mas du Coq, Albaro and Vilobi d'Onyar soils, respectively, which represented 97.9%, 97.1%, 99.1%, 99.1%, 97.7% and 99.3% of recovered radioactivity. The extraction efficiency of the method is ≥ 95% at DAT 0 in all soils. It is concluded all residues of fluopicolide potentially available for leaching were extracted by this method.

C: Time-Dependent Sorption

The sorption of fluopicolide in soil increased steadily with time. Apparent sorption coefficients ($K_{d,app}$) increased from DAT-0 to DAT-120 in all six soils. The values are summarised in Table 7.1.3.2- 21.

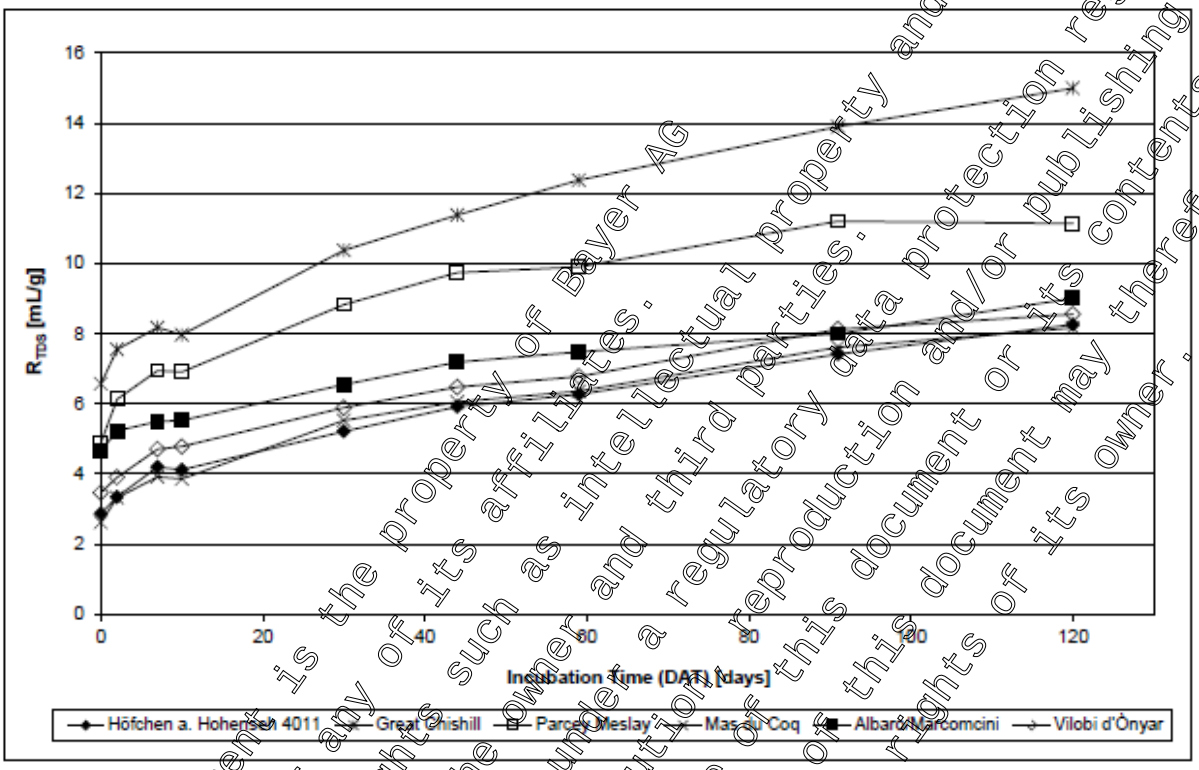
Table 7.1.3.2- 21: Apparent sorption coefficients ($K_{d, app}$) (expressed as mL/g)

DAT	Mean SD	H[redacted]	Great Chishill	Parcey Meslay	Mas du Coq	Albaro	Vilobi d'Onyar
0	Mean	2.87	6.57	4.89	2.62	4.66	3.46
	SD	± 0.01	± 0.00	± 0.00	± 0.01	± 0.01	± 0.00
2	Mean	3.34	7.55	6.13	3.32	6.23	3.90
	SD	± 0.00	± 0.01	± 0.03	± 0.00	± 0.01	± 0.02
7	Mean	4.20	8.17	6.95	3.92	5.49	4.70
	SD	± 0.01	± 0.00	± 0.01	± 0.02	± 0.02	± 0.00
10	Mean	4.12	7.96	6.91	3.86	5.52	4.78
	SD	± 0.01	± 0.01	± 0.00	± 0.00	± 0.00	± 0.01
30	Mean	5.21	10.38	8.83	5.52	6.55	5.89
	SD	± 0.03	± 0.01	± 0.01	± 0.01	± 0.01	± 0.01
44	Mean	5.92	11.38	9.74	6.05	7.19	6.47
	SD	± 0.01	± 0.01	± 0.00	± 0.00	± 0.01	± 0.01
69	Mean	6.26	12.37	9.90	6.36	7.48	6.77
	SD	± 0.03	± 0.00	± 0.00	± 0.00	± 0.02	± 0.02
91	Mean	7.41	13.90	11.21	7.61	8.00	8.12
	SD	± 0.02	± 0.04	± 0.03	± 0.03	± 0.02	± 0.01
120	Mean	8.26	15.00	11.14	8.16	9.00	8.55
	SD	± 0.01	± 0.02	± 0.02	± 0.03	± 0.05	± 0.01
Factor [A]		2.88	2.28	2.28	3.12	1.93	2.47
Mean Factor		2.49					

Apparent Sorption Coefficients ($K_{d, app}$) are called Time-Dependent Sorption Ratios (R_{TDS}) in the report.
[A] Calculated as $K_{d, app}$ DAT-120 divided by $K_{d, app}$ DAT-0

A plot of the apparent sorption coefficients ($K_{d, app}$) with time in the six soils is shown in Figure 7.1.3.2-2.

Figure 7.1.3.2- 2: Apparent sorption coefficients ($K_{d, app}$) with time



Apparent Sorption Coefficients ($K_{d, app}$) are called Time-Dependent Sorption Ratios (R_{TDS}) in the report.

III. Conclusion

The adsorption of fluopicolide to soil increased significantly with time under laboratory conditions at 20 °C and a soil moisture content of 53.9% of the maximum water holding capacity. Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.93 to 3.12 (mean 2.49).

Assessment and conclusion by applicant:

The study is considered valid to assess the aged sorption of fluopicolide in soil and meets current requirements for conducting aged sorption studies for use in regulatory assessments (CRD, 2019).

Data Point:	KCA 7.1.3.2/05
Report Author:	[redacted]
Report Year:	2019
Report Title:	[phenyl-UL-14C]fluopicolide: Degradation and time - Dependent sorption in 6 soils
Report No:	EnSa-16-0983
Document No:	M-655056-01-1
Guideline(s) followed in study:	- OECD Test Guideline 307 (April, 2002) - US EPA OCSPP Test Guideline No. 835.4100 / 835.4200 - OECD Test Guideline No. 106 (January, 2000; only in parts) - Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in the regulatory process; Food and Environmental Agency, York, UK, 2012
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The degradation and time-dependence of sorption of fluopicolide was studied in six soils under aerobic conditions in the laboratory in the dark at 20 ± 1 °C and 53.9% of the maximum water holding capacity for 120 days, with the exception of Lignieres soil which was unintentionally incubated at a soil moisture content equivalent to 72.9% of the maximum water holding capacity. In addition, the rate of degradation of fluopicolide was determined in the study.

Full details of this study are described under Point CA 7.1.2.1.1 (KCA 7.1.2.1.1/08).

Soil	Texture (USDA)	pH (CaCl2)	% Organic Carbon
Lamberton	loam	5.6	2.6
Sarotti	silty clay loam	6.9	1.4
Münster	loamy sand	5.6	1.2
Pikeville	loamy sand	4.5	1.8
Abington	sandy loam	7.3	2.6
Lignieres	sandy loam	5.7	0.8

[Phenyl-U-^{14}C]-labelled fluopicolide was applied to soil samples at an application rate of ca. 0.41 mg/kg dry weight. The radiochemical purity and specific activity were > 98% and 5.50 MBq/mg, respectively.

Samples of Lamberton and Münster soils were taken for extraction and analysis immediately after treatment (day 0) and 2, 8, 13, 30, 44, 56, 90 and 119 days of incubation and samples of Sarotti, Pikeville, Abington and Lignieres soils after 0, 2, 8, 10, 28, 45, 59, 85 (86, Lignieres soil) and 120 days of incubation. Soil samples were first desorbed with 0.01M calcium chloride solution for 24 hours at 20 °C at a soil : solution ratio of 1:3 (w/w) to determine the desorbable portion of the test item from aged soil. The soil residue was then exhaustively extracted with three further successive extractions with acetonitrile/water 4/1 (v/v) at ambient temperature, followed by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 70 °C. Desorption supernatants and concentrated soil extracts were analysed by reverse phase high performance liquid chromatography (HPLC).

The effect of aged sorption to soil was determined for fluopicolide and showed a significant increase with time. Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.62 to 2.53 (mean 2.22).

I. Materials and Methods

Full details of this study are described under Point CA 7.1.2.1.1 (KCA 7.1.2.1.1/08).

Standard OECD 106 batch equilibrium tests were performed on the same batches of soils to derive the equilibrium sorption parameters (see KCA 7.1.3.1.1/05, M-595721-01-1). Five of the soils were collected from the same sites as earlier laboratory aerobic soil studies (Lamberton, Sarotti, Minster, Pikeville and Abington soils) and one soil was from a terrestrial field dissipation site used for fluopicolide (Lignieres soil).

The extraction method used in the study was the same as that used in two other aged sorption studies (see KCA 7.1.3.2/03, M-555570-01-1 and KCA 7.1.3.2/04, M-559687-01-1). Soil samples were first shaken with 0.01M calcium chloride solution for 24 hours at 20 °C on an overhead shaker at 16 rpm to determine the desorbable portion of the test item from aged soil. A soil-to-solution ratio of 1:3 was used for all soils. Soil samples were then extracted three times with acetonitrile/water (4/1, *v/v*) at ambient temperature followed by a microwave extraction with acetonitrile/water (4/1, *v/v*) at 70 °C. After each extraction step, extract and soil were separated by centrifugation.

II. Results and Discussion

The datasets used for the fluopicolide kinetic sorption evaluation are summarised below in Table 7.1.3.2- 22 to Table 7.1.3.2- 27.

Table 7.1.3.2- 22: Total mass and aqueous concentration of fluopicolide (C_{des}) in Lamberton soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	8	13	30	44	56	90	119
Total mass (µg)	A	36.16	34.31	32.88	30.71	27.88	27.79	27.40	23.57	21.5
	B	35.91	34.68	31.97	31.79	28.82	27.03	27.25	22.94	21.24
	Mean	36.04	34.50	32.43	31.75	28.35	27.41	27.33	23.26	21.37
	SD	0.13	0.18	0.46	0.04	0.47	0.38	0.07	0.32	0.13
C_{des} (µg/mL)	A	0.0200	0.0167	0.0156	0.0160	0.0103	0.0097	0.0083	0.0060	0.0048
	B	0.0196	0.0162	0.0151	0.0144	0.0105	0.0083	0.0084	0.0052	0.0052
	Mean	0.0198	0.0165	0.0153	0.0152	0.0104	0.0090	0.0083	0.0056	0.0050
	SD	0.0002	0.0002	0.0003	0.0008	0.0001	0.0007	0.0001	0.0004	0.0002

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 23: Total mass and aqueous concentration of fluopicolide (C_{des}) in Sarotti soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	8	10	28	45	59	85	120
Total mass (µg)	A	39.80	37.74	34.58	34.30	32.88	30.38	27.25	23.64	22.55
	B	38.60	38.18	35.67	35.54	31.74	30.97	27.72	25.08	22.88
	Mean	39.20	37.96	35.13	34.92	32.31	30.68	27.49	24.36	22.72
	SD	0.60	0.22	0.55	0.62	0.57	0.30	0.23	0.72	0.16
C_{des} (µg/mL)	A	0.0565	0.0470	0.0417	0.0387	0.0324	0.0291	0.0245	0.0287	0.0186
	B	0.0548	0.0489	0.0445	0.0420	0.0320	0.0295	0.0250	0.0300	0.0202
	Mean	0.0556	0.0480	0.0431	0.0403	0.0322	0.0293	0.0247	0.0294	0.0194
	SD	0.0009	0.0010	0.0014	0.0017	0.0002	0.0002	0.0002	0.0007	0.0008

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 24: Total mass and aqueous concentration of fluopicolide (C_{des}) in Münster soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	8	13	30	44	56	90	119
Total mass (µg)	A	37.33	37.33	33.58	32.39	30.49	28.7	27.49	23.16	20.75
	B	37.18	37.56	34.69	33.43	30.27	28.22	27.05	22.43	20.53
	Mean	37.26	37.45	34.14	32.91	30.38	28.46	27.27	22.80	20.64
	SD	0.07	0.12	0.56	0.52	0.11	0.24	0.22	0.37	0.11
C_{des} (µg/mL)	A	0.0439	0.0396	0.0330	0.0297	0.0261	0.0254	0.0218	0.0154	0.0125
	B	0.0441	0.0401	0.0329	0.0304	0.0266	0.0226	0.0199	0.0161	0.0125
	Mean	0.0440	0.0398	0.0330	0.0301	0.0264	0.0240	0.0208	0.0157	0.0125
	SD	0.0001	0.0003	0.0001	0.0004	0.0002	0.0014	0.0010	0.0004	0.0000

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 25: Total mass and aqueous concentration of fluopicolide (C_{des}) in Pikeville soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	8	10	28	45	59	85	120
Total mass (µg)	A	39.04	39.39	33.28	33.14	30.74	28	24.14	23.62	19.47
	B	39.22	39.01	33.87	33.68	30.27	29.11	23.4	23.72	20.66
	Mean	39.13	39.20	33.58	33.41	30.51	28.56	23.77	23.67	20.07
	SD	0.09	0.19	0.29	0.27	0.23	0.56	0.37	0.05	0.60
C_{des} (µg/mL)	A	0.0321	0.0279	0.0220	0.0210	0.0161	0.0124	0.0108	0.0088	0.0080
	B	0.0318	0.0284	0.0231	0.0211	0.0167	0.0132	0.0114	0.0084	0.0081
	Mean	0.0319	0.0282	0.0225	0.0211	0.0164	0.0128	0.0111	0.0086	0.0081
	SD	0.0001	0.0003	0.0005	0.0000	0.0003	0.0004	0.0003	0.0002	0.0001

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 26: Total mass and aqueous concentration of fluopicolide (C_{des}) in Abington soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	8	10	28	45	59	85	120
Total mass (µg)	A	38.42	37.29	33.59	35.27	30.56	28.3	26.52	24.5	19.29
	B	38.67	38.07	33.66	34.08	31.49	28.77	24.12	24.07	19.25
	Mean	38.55	37.68	33.63	34.68	31.03	28.54	25.32	24.29	19.27
	SD	0.13	0.39	0.03	0.60	0.47	0.29	1.20	0.22	0.02
C_{des} (µg/mL)	A	0.0332	0.0314	0.0249	0.0267	0.0215	0.0193	0.0180	0.0150	0.0148
	B	0.0340	0.0304	0.0277	0.0260	0.0219	0.0190	0.0156	0.0147	0.0113
	Mean	0.0336	0.0309	0.0263	0.0263	0.0217	0.0191	0.0168	0.0149	0.0116
	SD	0.0004	0.0005	0.0014	0.0004	0.0002	0.0001	0.0012	0.0002	0.0002

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

Table 7.1.3.2- 27: Total mass and aqueous concentration of fluopicolide (C_{des}) in Lignieres soil

Fluopicolide	Replicate	Incubation time (DAT)								
		0	2	8	10	30	45	59	86	120
Total mass (µg)	A	38.20	37.62	34.07	34.92	29.95	29.88	28.44	23.46	19.62
	B	38.63	36.08	33.76	34.14	30.70	29.49	26.78	22.90	20.37
	Mean	38.42	36.85	33.92	34.53	30.33	29.69	27.61	23.18	20.00
	SD	0.22	0.77	0.16	0.39	0.38	0.20	0.83	0.28	0.38
C_{des} (µg/mL)	A	0.0523	0.0494	0.0390	0.0385	0.0284	0.0311	0.0247	0.0157	0.0154
	B	0.0530	0.0472	0.0410	0.0385	0.0297	0.0294	0.0241	0.0148	0.0153
	Mean	0.0527	0.0483	0.0400	0.0385	0.0291	0.0303	0.0244	0.0153	0.0153
	SD	0.0003	0.0011	0.0010	0.0000	0.0007	0.0008	0.0003	0.0004	0.0001

DAT: days after treatment, C_{des}: Liquid concentration of fluopicolide, SD: standard deviation

B: Extraction efficiency

Total extractable residues (i.e. residues desorbed by aqueous 0.01 M $CaCl_2$ solution and residues in organic soil extracts) at DAT 0 were 89.1%, 96.4%, 93.8%, 95.1%, 94.3% and 101.0% AR in Lamberton, Sarotti, Munster, Pikeville, Abington and Lignieres soils, respectively, which represented 95.9%, 96.4%, 99.2%, 98.2%, 97.3% and 98.6% of the recovered radioactivity. The extraction efficiency of the method is > 95% at DAT 0 in all soils. It is concluded all residues of fluopicolide potentially available for leaching were extracted by this method.

C: Time-Dependent Sorption

The sorption of fluopicolide in soil increased steadily with time. Apparent sorption coefficients ($K_{d,app}$) increased from DAT-0 to DAT-120 in all six soils. The values are summarised in Table 7.1.3.2- 28.

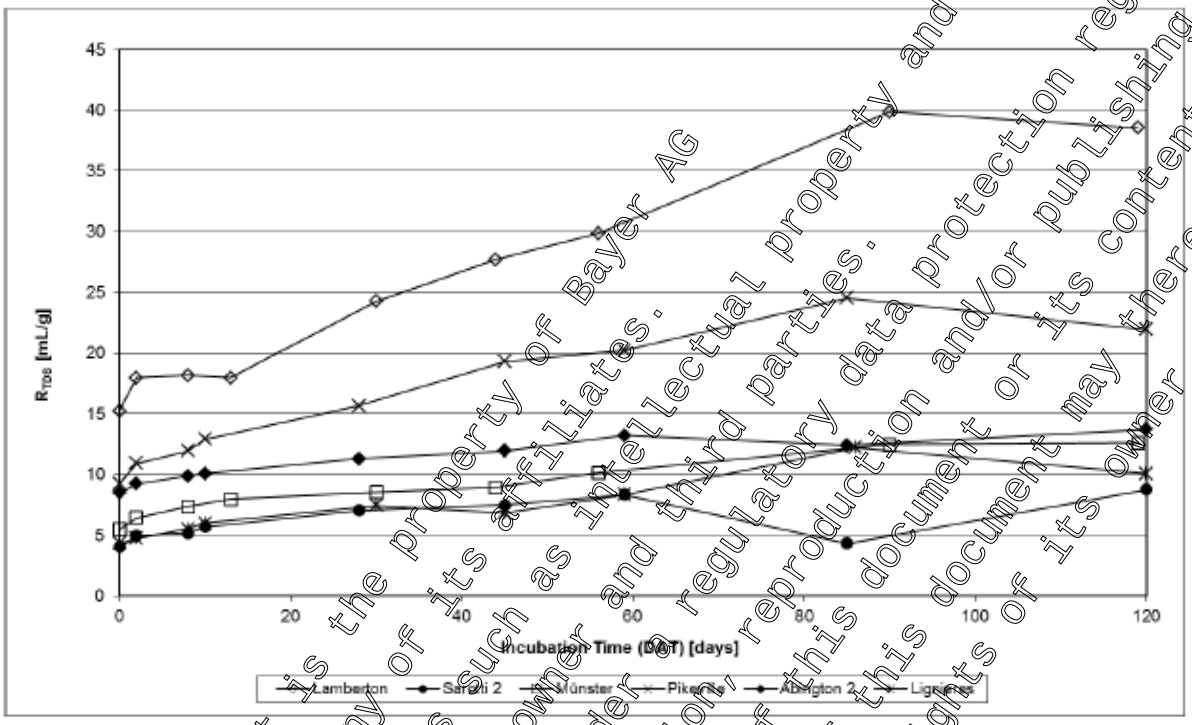
Table 7.1.3.2- 28: Apparent sorption coefficients ($K_{d, app}$) (expressed as mL/g)

DAT	Mean SD	Lamberton	Sarotti	Münster	Pikeville	Abington	Lignieres
0	Mean	15.21	4.05	5.47	9.25	8.46	4.29
	SD	± 0.01	± 0.00	± 0.01	± 0.01	± 0.01	± 0.00
2	Mean	17.95	4.91	6.4	10.92	9.71	4.7
	SD	± 0.02	± 0.02	± 0.01	± 0.02	± 0.04	± 0.00
8	Mean	18.16	5.16	7.35	11.91	9.84	5.49
	SD	± 0.00	± 0.03	± 0.03	± 0.02	± 0.07	± 0.05
10/13	Mean	17.93	5.67	7.95	12.87	10.06	5.97
	SD	± 0.06	± 0.04	± 0.00	± 0.01	± 0.01	± 0.02
28/30	Mean	24.24	7.03	8.53	15.62	11.28	7.43
	SD	± 0.01	± 0.02	± 0.02	± 0.03	± 0.01	± 0.01
44/45	Mean	27.66	7.46	8.9	19.27	11.91	6.82
	SD	± 0.08	± 0.00	± 0.07	± 0.01	± 0.02	± 0.03
56/59	Mean	29.85	8.32	10.11	20.2	13.19	8.32
	SD	± 0.01	± 0.01	± 0.05	± 0.03	± 0.07	± 0.03
85/90	Mean	39.86	4.3	12.44	24.52	12.36	12.2
	SD	± 0.07	± 0.01	± 0.04	± 0.02	± 0.01	± 0.02
119/120	Mean	38.54	8.72	12.57	21.94	13.7	10.05
	SD	± 0.05	± 0.04	± 0.01	± 0.02	± 0.02	± 0.03
Factor [A]		2.53	2.15	2.30	2.37	1.62	2.34
Mean Factor		2.22					

Apparent Sorption Coefficients ($K_{d, app}$) are called Time-Dependent Sorption Ratios (R_{TDS}) in the report.
[A] Calculated as $K_{d, app}$ DAT-119/120 divided by $K_{d, app}$ DAT-0.

A plot of the apparent sorption coefficients ($K_{d, app}$) with time in the six soils is shown in Figure 7.1.3.2-3.

Figure 7.1.3.2- 3: Apparent sorption coefficients ($K_{d, app}$) with time



Apparent Sorption Coefficients ($K_{d, app}$) are called Time-Dependent Sorption Ratios (R_{TDS}) in the report.

III. Conclusion

The adsorption of fluopicolide to soil increased significantly with time under laboratory conditions at 20 °C and a soil moisture content of 53.9% of the maximum water holding capacity (72.9% MWHC for Lignieres soil). Apparent sorption coefficients ($K_{d, app}$) increased with time in all soils by a factor of 1.62 to 2.53 (mean 2.22).

Assessment and conclusion by applicant:

The study is considered valid to assess the aged sorption of fluopicolide in soil and meets current requirements for conducting aged sorption studies for use in regulatory assessments (CRD, 2019).

Data Point:	KCA 7.1.3.2/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide: Evaluation of aged-sorption parameters
Report No:	VC/19/041C
Document No:	M-685678-02-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The data from three aerobic degradation and time-dependent sorption studies in sixteen soils for fluopicolide (KCA 7.1.3.2/03, M-555570-01-1, [redacted] 2016, KCA 7.1.3.2/04, M-550687-01-1, [redacted] 2016 and KCA 7.1.3.2/05, M-655056-01-1, [redacted] 2019) was evaluated according to current guidance on aged sorption (Morris *et al.*, 2019 and EFSA, 2018).

Optimised parameter results are summarised in the table below. Comparison of the non-equilibrium and equilibrium K_{d_app} fits showed that strong aged-sorption effects occurred for all soils, as shown by equilibrium fits not being able to adequately describe the increased observed in K_d with time. Parameter estimations of f_{NE} and k_{des} are robust for all sixteen soils and are suitable for use in exposure modelling.

#	Soil	f_{NE} (-)	k_{des} (1/d)	DT_{50},eq (d)	DT_{50},eq 20°C pF2 (d)	Evidence of aged sorption	Robust parameters
1	L[redacted]	0.553	0.0432	80.5	80.5	Yes	Yes
2	Dollendorf	0.271	0.0433	98.6	98.6	Yes	Yes
3	H[redacted]	0.632	0.0420	69.8	69.8	Yes	Yes
4	[redacted]	0.785	0.0467	45.4	45.4	Yes	Yes
5	H[redacted]	0.506	0.0507	76.2	76.2	Yes	Yes
6	Great Chishill	0.571	0.0248	170.9	170.9	Yes	Yes
7	Parcey Meslay	0.493	0.0524	111.0	111.0	Yes	Yes
8	Mas du Coq	0.514	0.0310	121.2	108.4	Yes	Yes
9	Albaro	0.303	0.0287	112.2	112.2	Yes	Yes
10	Vilobi d'Ònyar	0.435	0.0575	52.2	52.2	Yes	Yes
11	Abington	0.289	0.0855	97.5	97.5	Yes	Yes
12	Lamberton	0.830	0.0145	111.2	91.6	Yes	Yes
13	Munster	0.524	0.0163	103.1	75.4	Yes	Yes
14	Pikeville	0.710	0.0319	80.2	66.8	Yes	Yes
15	Sarrotti*	0.484	0.0534	111.5	99.3	Yes	Yes
16	Lignieres	0.638	0.0158	96.8	96.8	Yes	Yes
	Geometric mean	**0.508**	**0.0356**		**86.6**		

* Excluding outlier

The adsorption of fluopicolide increased significantly with time under laboratory conditions, with mean aged-sorption parameters of F_{ne} 0.508 and k_{des} 0.0356.

I. Materials and Methods

1. TDS data

The aerobic degradation and time-dependent sorption of fluopicolide has been measured in sixteen soils (KCA 7.1.3.2/03, M-555570-01-1, [redacted] 2016; KCA 7.1.3.2/04, M-550687-01-1, [redacted] 2016 and KCA 7.1.3.2/05, M-655056-01-1, [redacted] 2019). Table 7.1.3.2- 29 summarises the physico-chemical properties of the test soils used.

Table 7.1.3.2- 29: Test soils used for the time-dependent sorption testing of fluopicolide

#	Soil	Source	Texture (USDA)	pH	OC [%]
1	L[redacted]	Germany	Sandy loam	6.5	1.5
2	Dollendorf	Germany	Clay loam	7.3	4.8
3	H[redacted]	Germany	Loam	5.0	1.8
4	[redacted]	Germany	Silt loam	6.1	1.9
5	H[redacted]	Germany	Silt loam	6.1	0.7
6	Great Chishill	UK	Clay	7.3	2.1
7	Parcey Meslay	France	Loam	6.7	1.3
8	Mas du Coq	France	Clay loam	7.6	0.9
9	Albaro	Italy	Silty clay	7.2	2.1
10	Vilobi d'Onyar	Spain	Sandy loam	6.3	0.8
11	Abington	UK	Sandy loam	7.3	2.6
12	Lamberton	USA	Loam	5.6	2.6
13	Munster	Germany	Loamy sand	5.6	1.2
14	Pikeville	USA	Loamy sand	4.5	1.8
15	Sarrou	Germany	Silty clay loam	6.9	1.4
16	Lignères	France	Sandy loam	5.7	0.8

Soil samples (100g ode) were incubated (20oC and 55% MWHC) for up to 126 days. At each sampling timepoint, soils were extracted with 0.01M $CaCl_2$ (300mL) for 24 hours.

Table 7.1.3.2- 30 summarises important study data for each soil for use in the time-dependent sorption parameter evaluations.

Table 7.1.3.2- 30: Time-dependent sorption study data summary

Parameter	Description	Soil			
		[redacted]	Dollendorf	[redacted]	[redacted]
TimStart (d)	Start time	0	0	0	0
TimEnd (d)	End time	126	126	126	126
MasIni (µg)	Initial guess of total mass	44.4	44.4	44.4	44.4
MasSol (g)	Dry mass of soil	100	100	100	100
VolLiqSol (mL)	Vol liq in moist soil	27.8	52.4	29.3	37.9
VolLiqAdd (mL)	Vol liq ($CaCl_2$) added	300	300	300	300
CntOm (kg/kg)	Organic Matter	0.026	0.083	0.033	0.031
Temp (°C)	Incubation temperature	20	20	20	20

Parameter	Description	Soil			
		[redacted]	Great Chishill	Parcey Meslay	Mas du Coq
TimStart (d)	Start time	0	0	0	0
TimEnd (d)	End time	120	120	120	120
MasIni (µg)	Initial guess of total mass	44.0	44.0	44.0	44.0
MasSol (g)	Dry mass of soil	100	100	100	100
VolLiqSol (mL)	Vol liq in moist soil	29.8	35.9	34.9	25.6
VolLiqAdd (mL)	Vol liq ($CaCl_2$) added	300	300	300	300
CntOm (kg/kg)	Organic Matter	0.012	0.036	0.022	0.016
Temp (°C)	Incubation temperature	20	20	20	20

Parameter	Description	Soil			
		Albaro	Vilobi d'Onyar	Abington	Lamberton
TimStart (d)	Start time	0	0	0	0
TimEnd (d)	End time	120	120	120	119
MasIni (µg)	Initial guess of total mass	44.0	44.0	41.0	41.0
MasSol (g)	Dry mass of soil	100	100	100	100
VolLiqSol (mL)	Vol liq in moist soil	27.5	24.1	32.8	32.7
VolLiqAdd (mL)	Vol liq ($CaCl_2$) added	300	300	300	300
CntOm (kg/kg)	Organic Matter	0.036	0.014	0.045	0.045
Temp (°C)	Incubation temperature	20	20	20	20

Parameter	Description	Soil			
		Munster	Pikeville	Sarrotti	Lignieres
TimStart (d)	Start time	0	0	0	0
TimEnd (d)	End time	119	120	120	120
MasIni (µg)	Initial guess of total mass	41.0	41.0	41.0	41.0
MasSol (g)	Dry mass of soil	100	100	100	100
VolLiqSol (mL)	Vol liq in moist soil	18.1	24.5	30.1	30.7
VolLiqAdd (mL)	Vol liq ($CaCl_2$) added	300	300	300	300
CntOm (kg/kg)	Organic Matter	0.021	0.031	0.024	0.014
Temp (°C)	Incubation temperature	20	20	20	20

2. Adsorption data

Three adsorption-desorption batch-equilibrium studies were performed with [^{14}C]-fluopicolide in the same batches of the sixteen soils (KCA 7.1.3.1.1/03, M-544194-02-1, [redacted] 2015; KCA 7.1.3.1.1/04, M-550735-01-1, [redacted] 2016 and KCA 7.1.3.1.1/05, M-595721-01-1, [redacted], 2017). The studies were evaluated by fitting the Freundlich equation, $C_{sorb} = K_f C_{aqu}^{1/n}$, to the measured equilibrium concentrations.

Soil properties as well as the Freundlich coefficients are given in Table 7.1.3.2- 31.

Table 7.1.3.2- 31: Soil adsorption data of fluopicolide

#	Soil	Texture (USDA)	pH	OC [%]	K_f (mL/g)	$K_{f,oc}$ (mL/g)	1/n (-)
1	L[redacted]	Sandy loam	6.5	1.5	4.04	269.3	0.8561
2	Dollendorf	Clay loam	7.3	4.8	11.71	244.1	0.8596
3	H[redacted]	Loam	5.0	1.8	6.22	337.5	0.8741
4	[redacted]	Silt loam	6.1	1.9	4.65	258.6	0.9258
5	H[redacted]	Silt loam	6.1	0.7	2.12	303.3	0.8868
6	Great Chishill	Clay	7.3	2.1	5.40	257.0	0.9076
7	Parcey Meslay	Loam	6.7	1.3	3.33	257.4	0.8992
8	Mas du Coq	Clay loam	7.6	0.9	1.84	204.9	0.8668
9	Albaro	Silty clay	7.2	2.1	3.93	187.0	0.9110
10	Vilobi d'Onyar	Sandy loam	6.3	0.8	2.34	292.0	0.8818
11	Abington	Sandy loam	7.3	2.6	5.6	214.7	0.868
12	Lamberton	Loam	5.6	2.6	8.6	331.9	0.844
13	Munster	Loamy sand	5.6	1.2	3.4	282.6	0.916
14	Pikeville	Loamy sand	4.5	1.8	6.2	342.6	0.873
15	Sarrotti	Silty clay loam	6.9	1.4	2.6	185.6	0.851
16	Lignieres	Sandy loam	5.7	0.8	2.9	363.1	0.888

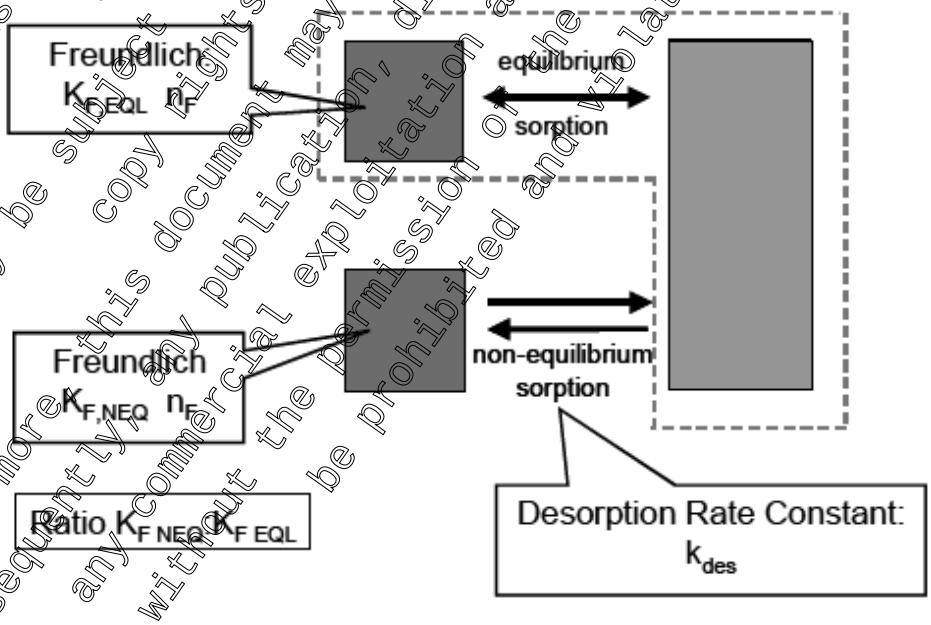
3. Optimisation procedures

The evaluations were conducted according to the guidance document proposed by UK CRD (Morris *et al.*, 2019) in combination with the EFSA PPR Panel Opinion (EFSA, 2018). The guidance document provides details of a number of data requirement and handling checks to be considered:

- The incubation study should follow the guidance given by OECD 307 and the sorption study should follow OECD 106
- The system must be well characterised. The mass and water content of the soil during incubation, the volume of water added during extraction, the duration and intensity of the extraction should be stated. Information on the texture, organic carbon content, pH and water retention or maximum water holding capacity of the sieved soil should also be available
- Data on total mass and aqueous concentration must be available. Experimental studies must provide sufficient and adequate sampling points to ensure a robust estimation of parameters. The pattern of decline in mass and concentration must be well established
- Incubation studies should be carried out with at least two true, independent replicates. Experimental results often include measurements below the limit of quantification (LOQ). Measurements below the limit of quantification (LOQ) are uncertain and these should be discarded
- A robust optimisation of parameters is only possible if the number of observations is appreciably larger than the number of model parameters. The total number of sampling dates remaining after the elimination of measurements below the limit of quantification or outliers must not be smaller than six

The data was evaluated using PEARLNEQ v5.1 which combines the two-site sorption model with the optimisation software PEST. The two-site kinetic sorption model is illustrated schematically in Figure 7.1.3.2- 4. The model is simultaneously fitted against the total mass of the fluopicolide in soil (µg) and the concentration of fluopicolide in the liquid ($CaCl_2$ extract) phase (µg/mL) as these are directly measured experimental data. The datasets used in the evaluations are provided in the study summaries for KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05.

Figure 7.1.3.2- 4: Two-site kinetic sorption model description



f_{NE} is defined as $K_{F,NEQ}$ / $K_{F,EQL}$

Five parameters (M_{ini}, DT_{50eq}, $K_{om,eq}$, f_{NE} and k_{des}) were fitted during the optimisations. M_{ini} is the initial mass dosed into the system (µg). DT_{50eq} is the DT_{50} (d) in the equilibrium domain and is only valid in combination with f_{NE} and k_{des}. $K_{om,eq}$ is the sorption coefficient in the equilibrium domain (mL/g). f_{NE} and k_{des} are the aged-sorption parameters, with f_{NE} being the ratio of sorption coefficients in the non-equilibrium and equilibrium domains and k_{des} the desorption rate constant (d^{-1}).

The Freundlich 1/n values were available from OECD 106 studies conducted in the same batches of soils and were used directly. Starting parameters for f_{NE} and k_{des} used in the evaluations are listed below in Table 7.1.3.2- 32. The starting parameter combination that resulted in the lowest objective function value during the PEST optimisation were selected and are provided in this summary. The optimized parameter results for the four sets of starting values are provided in the report.

Table 7.1.3.2- 32: Starting values for f_{NE} and k_{des}

f_{NE} (-)	k_{des} (1/d)
0.2	0.004
0.2	0.05
1.5	0.004
1.5	0.05

A fifth evaluation with f_{NE} and k_{des} set to zero was conducted to test whether equilibrium sorption could account for the apparent increase in sorption with time.

Apparent K_d (K_{d_app}) values were calculated as the ratio of fluopicolide sorbed to soil (µg/g) to the concentration of fluopicolide in the liquid 0.01M $CaCl_2$ supernatant phase (µg/ml) at each timepoint.

4. Acceptance criteria

The decision on whether a model fit is acceptable or not was based on:

- An assessment of the visual fit of a model with and without time-dependent sorption
- A χ^2-test to assess the goodness of fit and to compare a model with and without time-dependent sorption
- An assessment of the confidence in the parameter estimates

Plots of total mass and liquid phase concentration with time were plotted with plots of residuals and evaluated to allow a visual assessment of the evaluations.

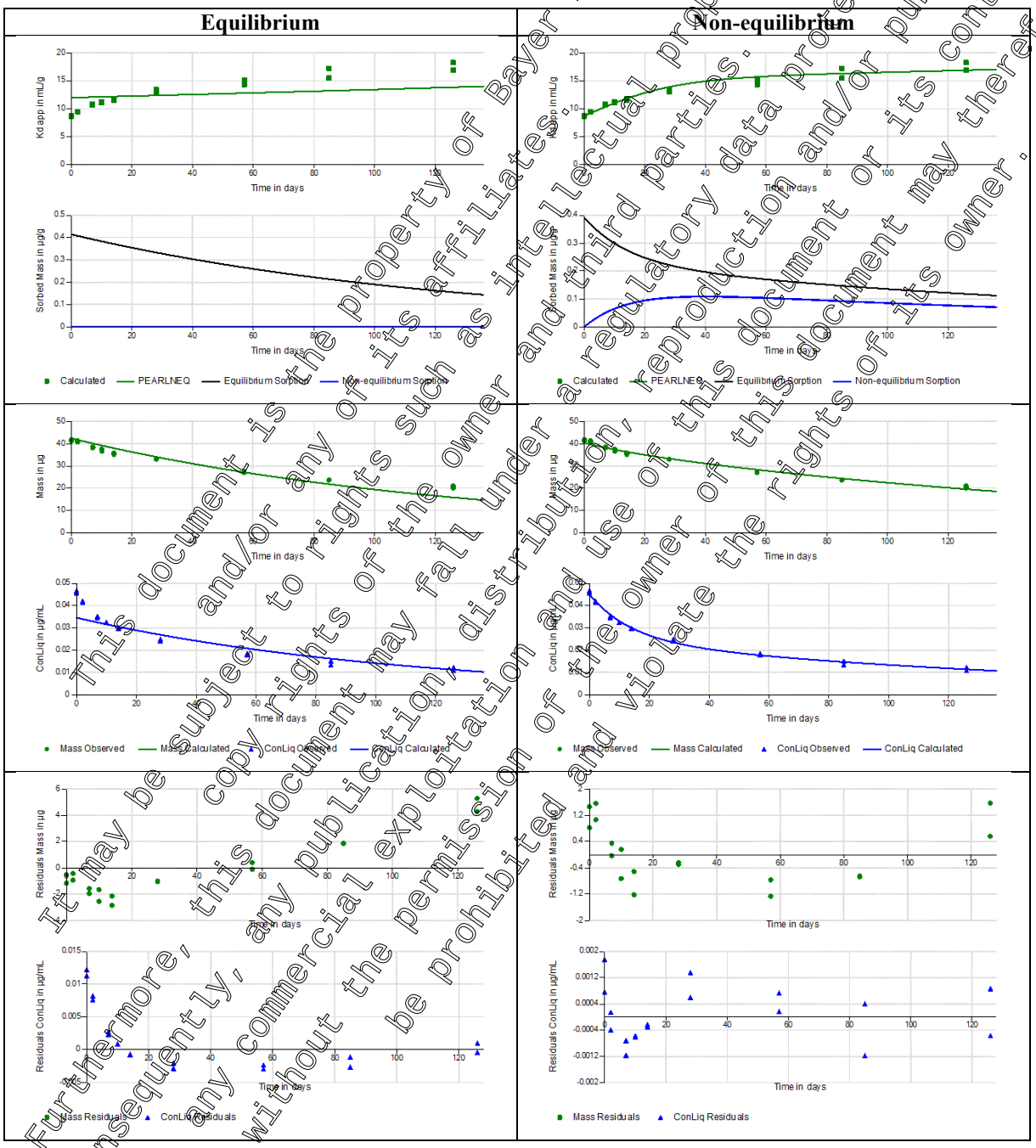
Apparent K_d (K_{d_app}) values calculated from the measured data and from the simulated concentrations were plotted against time. The apparent K_d values were evaluated for both equilibrium and non-equilibrium sorption to check that the increase in apparent K_d could not be accounted for by the effect of concentration and the Freundlich coefficient.

Model fits were also be evaluated using chi-square (χ^2) error statistics and the relative standard error (RSE) was calculated for each optimised parameter. It is recommended the RSE should be ≤ 0.4.

II. Results and Discussion

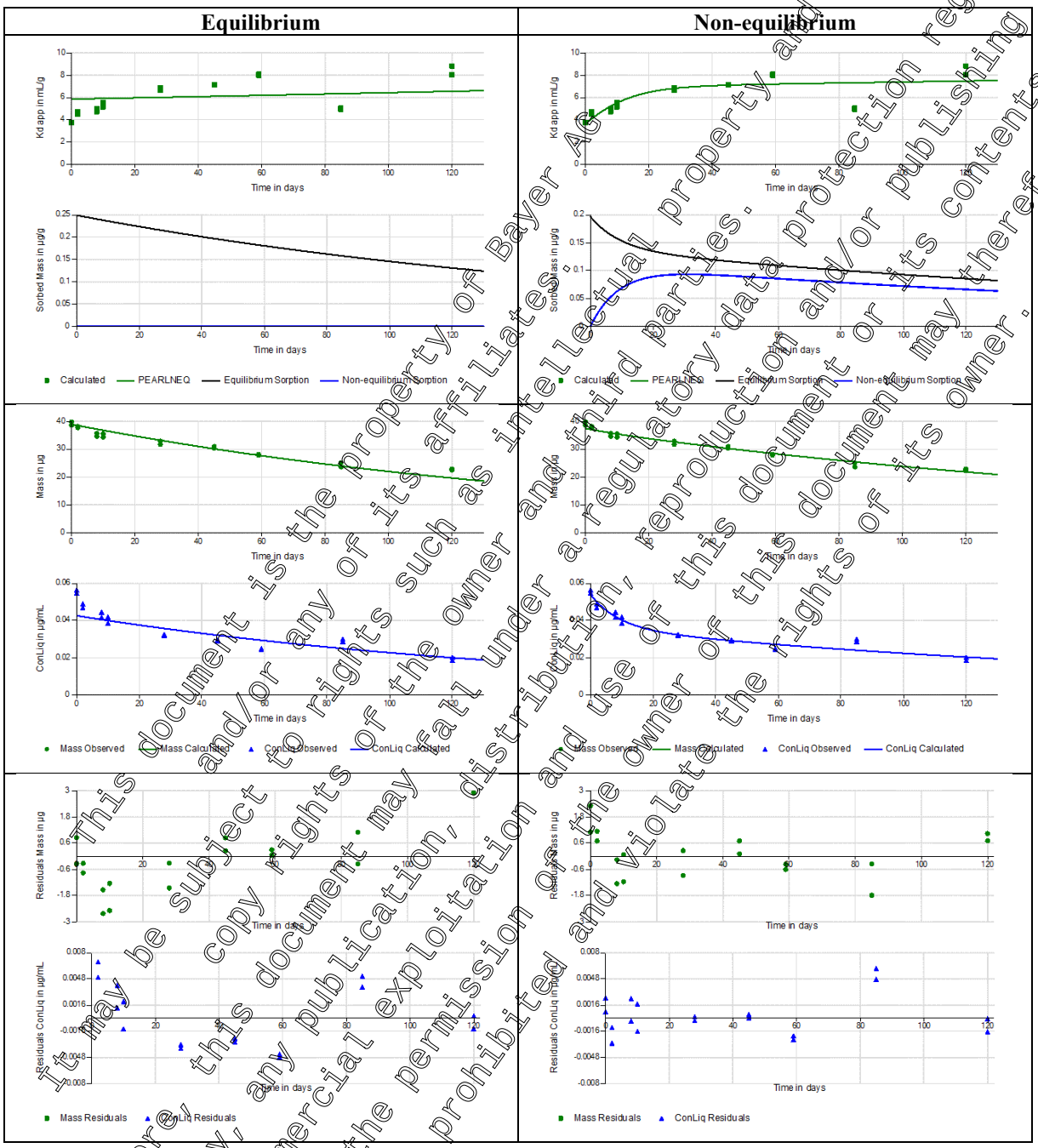
Comparison of the non-equilibrium and equilibrium K_{d_app} fits showed that strong aged-sorption effects occurred for all soils, as evidenced by the equilibrium fit not being able to adequately describe the increased observed in K_d with time. Plots for all soils are provided in the report. Example comparison plots are provided in Figure 7.1.3.2- 5 for L[redacted] soil and in Figure 7.1.3.2- 6 and Figure 7.1.3.2- 7 for Sarrotti soil.

Figure 7.1.3.2- 5: L[redacted] - optimised model fits for equilibrium and non-equilibrium sorption



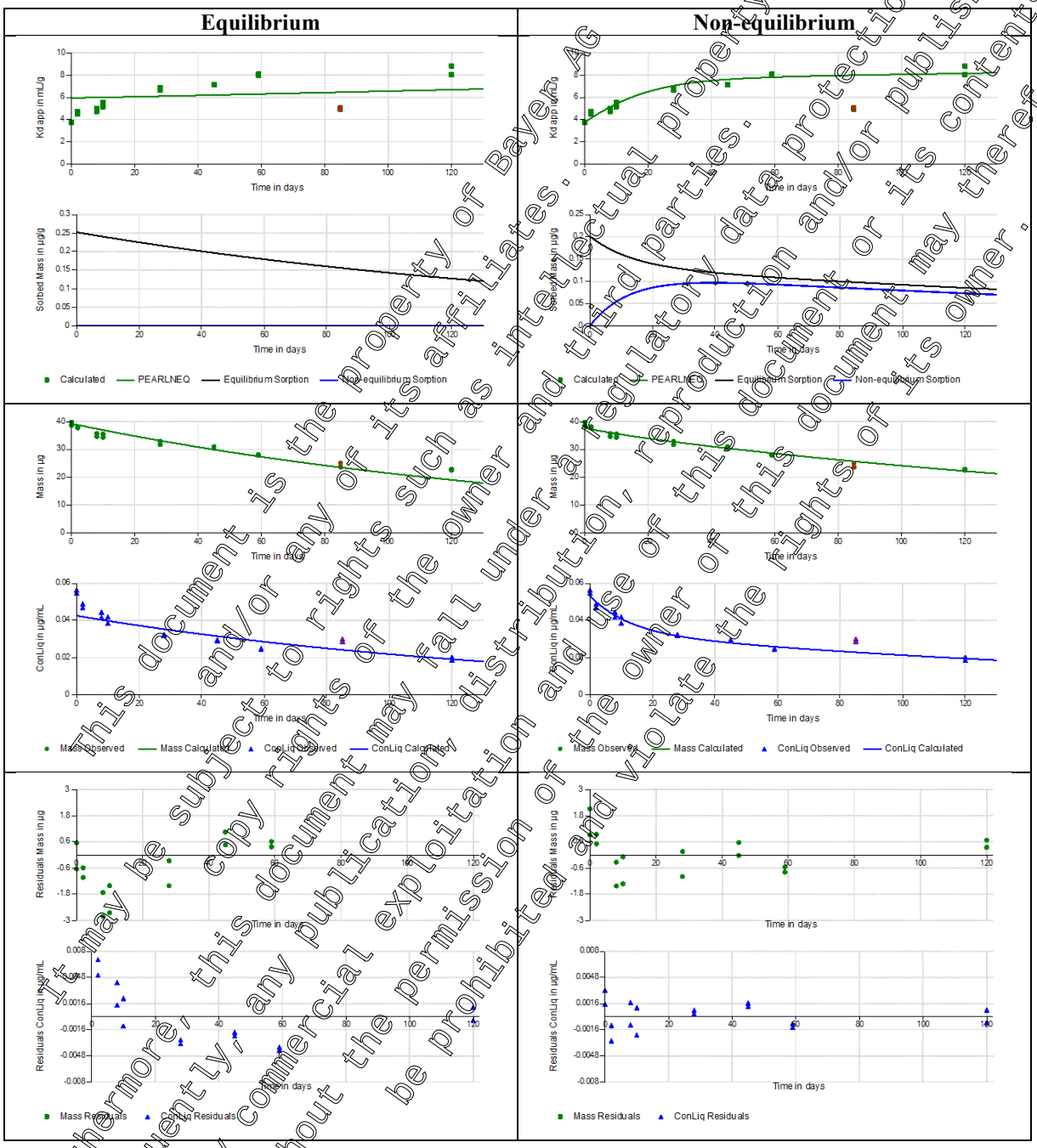
In Sarrotti soil, comparison of the apparent K_d plots also indicated that equilibrium sorption was not able to adequately describe the data and that significant aged-sorption was occurring (see Figure 7.1.3.2- 6).

Figure 7.1.3.2- 6: Sarrotti - optimised model fits for equilibrium and non-equilibrium sorption



However, it is evident that data at DAT 85 is out of alignment with the remaining data. Thus, an additional evaluation was conducted with DAT 85 excluded, resulting in a significant improvement in the fits as shown in Figure 7.1.3.2- 7. The results with DAT 85 excluded were selected as the endpoints for this soil.

Figure 7.1.3.2- 7: Sarrotti - optimised model fits for equilibrium and non-equilibrium sorption – DAT 85 outlier excluded



Model fits were evaluated using chi-square (χ^2) error statistics. In each soil the calculated minimum χ^2 error values for were lower for the non-equilibrium sorption model compared to equilibrium sorption. The relative standard error (RSE) calculated for each optimised parameter was ≤ 0.4.

The optimised parameter results are summarised in Table 7.1.3.2- 33. Parameter estimations of f_{NE} and k_{des} were robust for all sixteen soils and are suitable for use in exposure modelling.

Table 7.1.3.2- 33: Optimised parameter results

#	Soil	M_{ini} (µg)	$K_{om,eq}$ (mL/g)	f_{NE} (-)	k_{des} (1/d)	$DT_{50,eq}$ (d)	$DT_{50,eq}$ 20°C pF2 (d)	Evidence of aged sorption	Robust parameters
1	L[redacted]	40.4	224.8	0.553	0.0432	80.5	80.5	Yes	Yes
2	Dollendorf	38.1	141.6	0.271	0.0433	98.6	98.6	Yes	Yes
3	H[redacted]	39.3	232.9	0.632	0.0420	69.8	69.8	Yes	Yes
4	[redacted]	37.5	233.2	0.785	0.0467	45.4	45.4	Yes	Yes
5	H[redacted]	39.9	348.2	0.506	0.0507	76.2	76.2	Yes	Yes
6	Great Chishill	40.1	201.1	0.571	0.0248	170.9	170.9	Yes	Yes
7	Parcey Meslay	41.6	262.7	0.493	0.0524	111.0	111.0	Yes	Yes
8	Mas du Coq	41.8	256.5	0.514	0.0310	121.2	108.4	Yes	Yes
9	Albaro	41.2	166.5	0.303	0.0287	112.2	112.2	Yes	Yes
10	Vilobi d'Ònyar	38.7	312.4	0.435	0.0575	52.2	52.2	Yes	Yes
11	Abington	37.2	111.7	0.289	0.0855	97.5	97.5	Yes	Yes
12	Lamberton	34.6	184.4	0.830	0.0145	111.2	91.6	Yes	Yes
13	Munster	36.8	219.5	0.524	0.0163	103.1	75.4	Yes	Yes
14	Pikeville	37.2	188.1	0.710	0.0319	80.2	66.8	Yes	Yes
15	Sarrotti*	37.7	101.7	0.484	0.0534	111.5	99.3	Yes	Yes
16	Lignieres	37.3	222.7	0.638	0.0158	96.8	96.8	Yes	Yes
	Geometric mean			**0.508**	**0.0356**		**86.6**		

* Excluding outlier

II. Conclusion

The adsorption of fluopicolide to soil is known to increase significantly with time under laboratory conditions. Data from three aerobic degradation and time-dependent sorption studies in sixteen soils (KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05) was evaluated according to the draft guidance document on aged sorption (Morris *et al.* 2019) and EFSA PPR Panel Opinion (EFSA, 2018), resulting in mean aged-sorption parameters of F_{ne} 0.508 and k_{des} 0.0356 for fluopicolide.

Assessment and conclusion by applicant:

The modelling report was conducted according to current guidance on aged sorption and is considered valid to assess the aged sorption of fluopicolide in soil.

Data Point:	KCA 7.1.3.2/07
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide: Aged-sorption parameters - Evaluation of tier-1 DegT50EQ values from laboratory and field studies
Report No:	VC/19/041D
Document No:	M-687157-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

For inclusion in PEC_{gw} exposure assessments, lower-tier degradation study data (laboratory and field) must be combined with the aged-sorption data following re-evaluation to estimate $DegT_{50eq}$ values (Morris *et al.*, 2019).

Data from three aerobic degradation and time-dependent sorption studies in sixteen soils for fluopicolide (KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05) was evaluated according to the draft guidance document on aged sorption (Morris *et al.*, 2019) and EFSA PPR Panel Opinion (EFSA, 2018), resulting in mean aged-sorption parameters of: F_{ne} 0.508; k_{des} 0.0356 (see KCA 7.1.3.2/06).

Lower-tier degradation study data for fluopicolide from laboratory studies (KCA 7.1.1.1/01, KCA 7.1.1.1/02, KCA 7.1.2.1.1/01, KCA 7.1.2.1.1/02) and field studies (KCA 7.1.2.2.1/01, KCA 7.1.2.2.1/02, KCA 7.1.2.2.1/03, KCA 7.1.2.2.1/04, KCA 7.1.2.2.1/08, KCA 7.1.2.2.1/09, KCA 7.1.2.2.1/12 and KCA 7.1.2.2.1/13) have been evaluated to derive $DegT_{50eq}$ values, which when combined with the higher-tier aged-sorption values yield an overall geometric mean $DegT_{50eq}$ of 121 days for use in exposure modelling (in combination with the mean aged-sorption parameters: F_{ne} 0.508; k_{des} 0.0356).

I. Materials and Methods

1. Laboratory data

The aerobic degradation and time-dependent sorption of fluopicolide has been measured in sixteen soils (KCA 7.1.3.2/03, M-555570-01-1, [redacted] 2016; KCA 7.1.3.2/04, M-550687-01-1, [redacted] 2016 and KCA 7.1.3.2/05, M-655056-01-1, [redacted] 2019). Table 7.1.3.2- 28 summarises the physico-chemical properties of the test soils used.

Table 7.1.3.2- 34: Test soils used for the time-dependent sorption testing of fluopicolide

#	Soil	Source	Texture (USDA)	pH	OC [%]
1	L[redacted]	Germany	Sandy loam	6.5	1.5
2	Dollendorf	Germany	Clay loam	7.3	4.8
3	H[redacted]	Germany	Loam	5.0	1.8
4	[redacted]	Germany	Silt loam	6.1	1.9
5	H[redacted]	Germany	Silt loam	6.1	0.7
6	Great Chishill	UK	Clay	7.3	2.1
7	Parcey Meslay	France	Loam	6.7	1.3
8	Mas du Coq	France	Clay loam	7.6	0.9
9	Albaro	Italy	Silty clay	7.2	2.1
10	Vilobi d'Ònyar	Spain	Sandy loam	6.3	0.8
11	Abington	UK	Sandy loam	7.3	2.6
12	Lamberton	USA	Loam	5.6	2.6
13	Munster	Germany	Loamy sand	5.6	1.2
14	Pikeville	USA	Loamy sand	4.5	1.8
15	Sarrotti	Germany	Silty clay loam	6.9	1.4
16	Lignieres	France	Sandy loam	5.7	0.8

The aerobic degradation of fluopicolide has been measured in five soils (see KCA 7.1.1.1/01, KCA 7.1.1.1/02, KCA 7.1.2.1/01, KCA 7.1.2.1.1/02). Table 7.1.3.2- 35 summarises the test soils used.

Table 7.1.3.2- 35: Test soils used for the aerobic degradation testing of fluopicolide

#	Soil	Source	Texture (USDA)	pH	OC [%]
1	Abington	UK	Sandy loam	7.5	2.2
2	Lamberton	USA	Loam	5.6	3.3
3	Lamberton	USA	Sandy clay loam	5.9	3.5
4	Munster	Germany	Loamy sand	4.9	0.7
5	Pikeville	USA	Loamy sand	5.7	1.6
6	Sarrotti	Germany	Silty clay loam	7.4	0.9

2. Field data

The behaviour of fluopicolide was investigated in twelve terrestrial field soil dissipation studies designed to determine the dissipation under representative European field conditions (see KCA 7.1.2.2.1/01, KCA 7.1.2.2.1/02, KCA 7.1.2.2.1/03, KCA 7.1.2.2.1/04, KCA 7.1.2.2.1/08, KCA 7.1.2.2.1/09, KCA 7.1.2.2.1/12 and KCA 7.1.2.2.1/13). Table 7.1.3.2- 36 summarises the locations and soils used in the trials and normalised DegT50 values (KCA 7.1.2.2.1/22, M-685676-01-1, [redacted] 2020; KCA 7.1.2.2.1/23, M-685675-01-1, [redacted] 2020).

Table 7.1.3.2- 36: Test soils used for the field dissipation testing of fluopicolide

#	Soil	Source	Texture (USDA)	pH	OC [%]	$DegT_{50\ matrix}$ (d) norm
1	Burscheid	Germany	Silty loam	5.9	1.2	211.9
2	Great Chishill	UK	Clay	7.4	2.1	216.9
3	Lignieres de Touraine	France	Sandy loam	6.2	0.8	158.6
4	St. Etienne du Grès	France	Clay loam	8.0	0.8	303.2
5	Albaro	Italy	Clay loam	7.4	1.8	237.3
6	Vilobi	Spain	Loam	6.2	0.8	166.8
7	Philippsburg	Germany	Loamy sand	6.4	0.27	199.6
8	Rödelsee	Germany	Sandy clay loam	7.4	1.5	146.4
9	Huntlosen	Germany	Sand	4.9	1.9	168.4
10	Valencia	Spain	Loamy sand	7.3	1.87	317.4
11	Appilly	France	Sandy silt	7.1	1.51	144.2
12	Senas	France	Sandy silt loam	7.6	1.65	136.5

Five adsorption-desorption batch-equilibrium studies, (KCA 7.1.3.1.1/01, KCA 7.1.3.1.1/02, KCA 7.1.3.1.1/03, KCA 7.1.3.1.1/04 and KCA 7.1.3.1.1/05) with twenty-four soils were performed with [^{14}C] fluopicolide. The studies were evaluated by fitting the Freundlich equation, $C_{sorb} = K_f C_{aqu}^{1/n}$, to the measured equilibrium concentrations.

Soil properties as well as the Freundlich coefficients are given in Table 7.1.3.2- 37.

Table 7.1.3.2- 37: Soil adsorption data of fluopicolide

#	Soil	Texture (USDA)	pH	OC [%]	$K_{f,oc}$ (mL/g)	$K_{f,om}$ (mL/g)	1/n (-)
1	L[redacted]	Sandy loam	6.5	1.5	269.3	156.2	0.8561
2	Dollendorf	Clay loam	7.3	4.8	244.1	141.6	0.8596
3	H[redacted]	Loam	5.0	1.8	327.5	190.0	0.8741
4	[redacted]	Silt loam	6.1	1.9	258.6	150.0	0.9258
5	H[redacted]	Silt loam	6.1	0.7	303.3	175.9	0.8868
6	Great Chishill	Clay	7.3	2.1	257.0	149.1	0.9076
7	Parcey Meslay	Loam	6.7	1.3	257.4	149.3	0.8992
8	Mas du Coq	Clay loam	7.6	0.9	204.9	118.9	0.8668
9	Albaro	Silty clay	7.2	2.1	187.0	108.5	0.9110
10	Vilobi d'Ònyar	Sandy loam	6.3	0.8	292.0	169.4	0.8818
11	Abington	Sandy loam	7.3	2.6	214.7	124.5	0.868
12	Lamberton	Loam	5.6	2.6	331.9	192.5	0.844
13	Munster	Loamy sand	5.6	1.2	282.6	163.9	0.916
14	Pikeville	Loamy sand	4.5	1.8	342.6	198.7	0.873
15	Sarrotti	Silty clay loam	6.9	1.4	185.6	107.7	0.851
16	Lignieres	Sandy loam	5.7	0.8	363.1	210.6	0.888
17	Philippsburg	Sandy loam	6.3	0.6	248	143.9	0.841
18	Senas	Clay loam	7.5	1.5	239	138.6	0.882
19	Huntlosen	Loamy sand	5.2	1.6	580	336.4	0.953
20	Rodelsee	Clay	7.1	1.5	172	99.8	0.859
21	North Carolina	Sand	4.7	0.5	283	164.2	0.924
22	Abbington	Sandy loam	7.5	2.21	151.6	87.9	0.882
23	Sarotti	Silty clay loam	7.4	0.9	356	206.5	0.905
24	Munster	Loamy sand	4.9	0.7	349	202.4	0.929
	Geometric mean				**267.6**	**155.3**	
	Arithmetic mean						**0.888**

3. Aged sorption data

Data from three aerobic degradation and time-dependent sorption studies in sixteen soils for fluopicolide (KCA 7.1.3.2/03, KCA 7.1.3.2/04 and KCA 7.1.3.2/05) was evaluated according to the draft guidance document on aged sorption (Morris *et al.*, 2019) and EFSA PPR Panel Opinion (EFSA, 2018), resulting in mean aged-sorption parameters of: F_{ne} 0.508; k_{des} 0.0356 (see KCA 7.1.3.2/06).

Table 7.1.3.2- 38: Aged-sorption data of fluopicolide

#	Soil	f_{NE} (-)	k_{des} (1/d)	$DT_{50,eq}$ (d)	$DT_{50,eq}$ 20°C pF2 (d)	Evidence of aged sorption	Robust parameters
1	L[redacted]	0.553	0.0432	80.5	80.5	Yes	Yes
2	Dollendorf	0.271	0.0433	98.6	98.6	Yes	Yes
3	H[redacted]	0.632	0.0420	69.8	69.8	Yes	Yes
4	[redacted]	0.785	0.0467	45.4	45.4	Yes	Yes
5	H[redacted]	0.506	0.0507	76.2	76.2	Yes	Yes
6	Great Chishill	0.571	0.0248	170.9	170.9	Yes	Yes
7	Parcey Meslay	0.493	0.0524	111.0	111.0	Yes	Yes
8	Mas du Coq	0.514	0.0310	121.2	108.4	Yes	Yes
9	Albaro	0.303	0.0287	112.2	112.2	Yes	Yes
10	Vilobi d'Onyar	0.435	0.0573	52.2	52.2	Yes	Yes
11	Abington	0.289	0.0855	97.5	97.5	Yes	Yes
12	Lamberton	0.830	0.0145	111.2	91.6	Yes	Yes
13	Munster	0.524	0.0163	103.1	75.4	Yes	Yes
14	Pikeville	0.710	0.0319	80.2	66.8	Yes	Yes
15	Sarrotti*	0.484	0.0534	111.3	99.3	Yes	Yes
16	Lignieres	0.638	0.0158	96.8	96.8	Yes	Yes
	Geometric mean	**0.508**	**0.0356**		**86.6**		

* Excluding outlier

4. Estimating $DegT_{50eq}$ values from lower-tier $DegT_{50}$ values

The evaluations were conducted according to the guidance document proposed by UK CRD (Morris *et al.*, 2019) in combination with the EFSA PPR Panel Opinion (EFSA, 2018). Before combining and averaging the degradation endpoints, $DegT_{50eq}$ values need to be calculated for Tier-1 degradation endpoints. Three methods are available to calculate the $DegT_{50eq}$; Refit of residue data, Scaling Factor 1 and Scaling Factor 2. A refit of the aged sorption model to the original data (total mass only) is always the preferred option for the conversion of lower-tier degradation endpoints. Scaling factor method 1 is recommended where there is insufficient information to perform an inverse optimization but soil moisture is available or can be derived from soil texture, and finally scaling factor method 2 is to be used when there is insufficient information to perform scaling factor method 1.

Refit of residue data

The PEARLNEQ model is used to estimate $DegT_{50eq}$ values for the lower-tier degradation study data (without $CaCl_2$ extractions), in an analogous way to a full aged sorption evaluation but using the geometric mean F_{ne} and k_{des} parameters derived from the aged sorption studies.

Scaling Factor 1

The lower-tier modelling endpoint $DegT_{50}$value is corrected using a scaling factor based on the geometric mean F_{ne} derived from the aged sorption experiment evaluations, *w* (incubation moisture content, cm^3 / cm^3), batch K_{OM} and f_{OM} (organic matter fraction) according to the following equation:

$$DegT50_{EQ} = DegT50 * \frac{1.1 * (w + K_{OM} * f_{OM})}{w + (1 + f_{NE}) * K_{OM} * f_{OM}}$$

with the limitation that the calculated $DegT_{50eq}$ <= $DegT_{50}$. If the estimated $DegT_{50eq}$ is greater than the measured $DegT_{50}$, then set $DegT_{50eq}$ = $DegT_{50}$

If a batch OECD 106 K_{OC} value is available for the soil, then the soil-specific K_{OM} should be used directly in the estimation (K_{OM} = K_{OC}/1.724). Where no soil-specific K_{OC} data is available, K_{OM} can be calculated from the overall geometric mean K_{OC}.

The incubation soil moisture content (*w*) should be available from the soil degradation study reports and used directly. Where no information is available for *w*, an alternative is to select the FOCUS default pF2 soil moisture content based on soil texture.

Scaling Factor 2

The lower-tier modelling endpoint $DegT_{50}$ value is corrected using a simplified scaling factor based on the geometric mean F_{ne} derived from the aged sorption experiment evaluations according to the following equation:

$$DegT50_{EQ} = DegT50 * \frac{1.2}{(1 + f_{NE})}$$

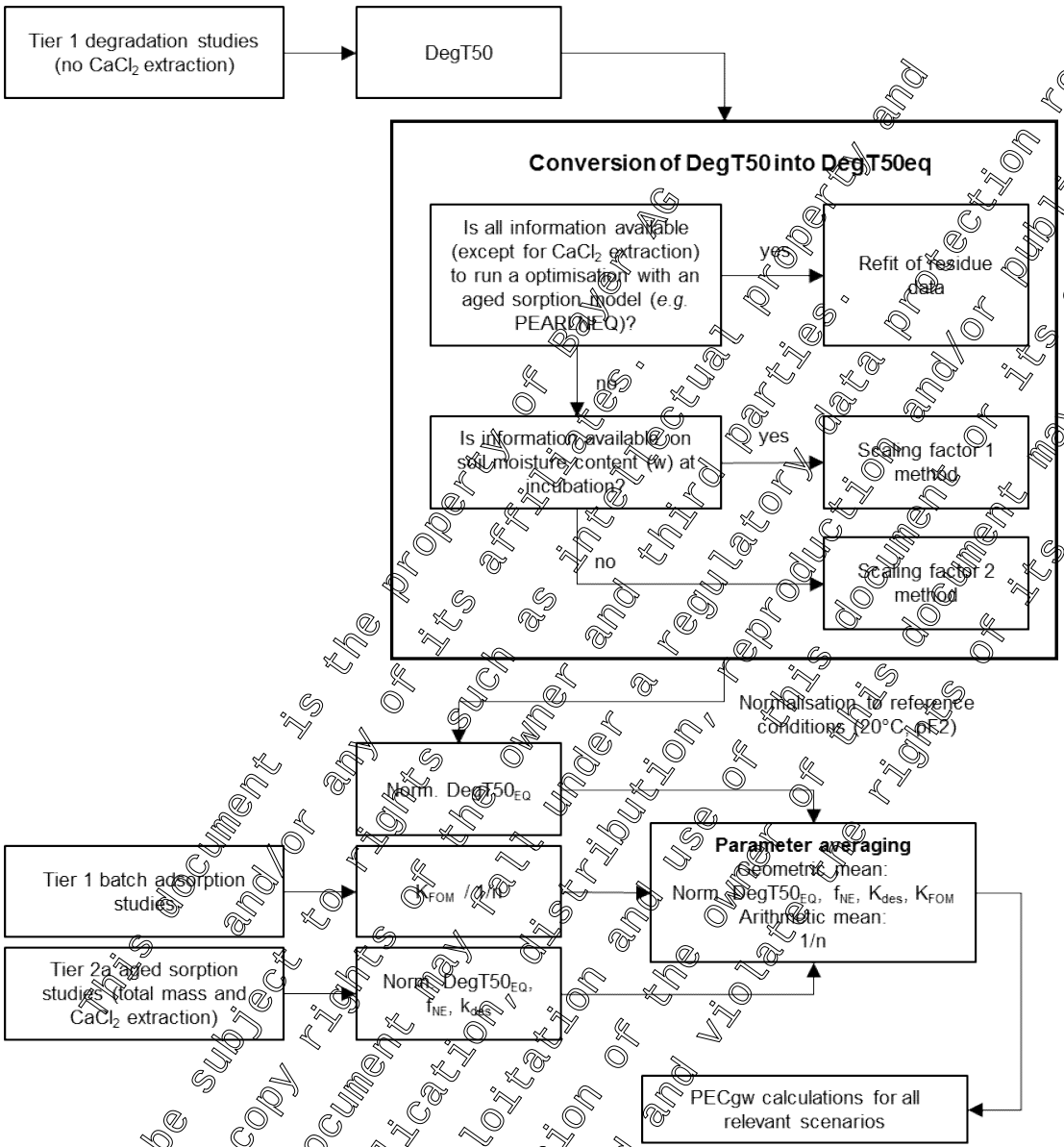
with the limitation that the calculated $DegT_{50eq}$ <= $DegT_{50}$.

For this conservative approach, F_{ne} values < 0.2 will result in the unrealistic situation that the estimated $DegT_{50eq}$ is > the measured $DegT_{50}$. For F_{ne} values < 0.2 the $DegT_{50eq}$ should therefore be set to the measured $DegT_{50}$. Use of scaling factor 2 was not required for fluopicolide studies.

5. Combining Tier 1 and aged sorption studies

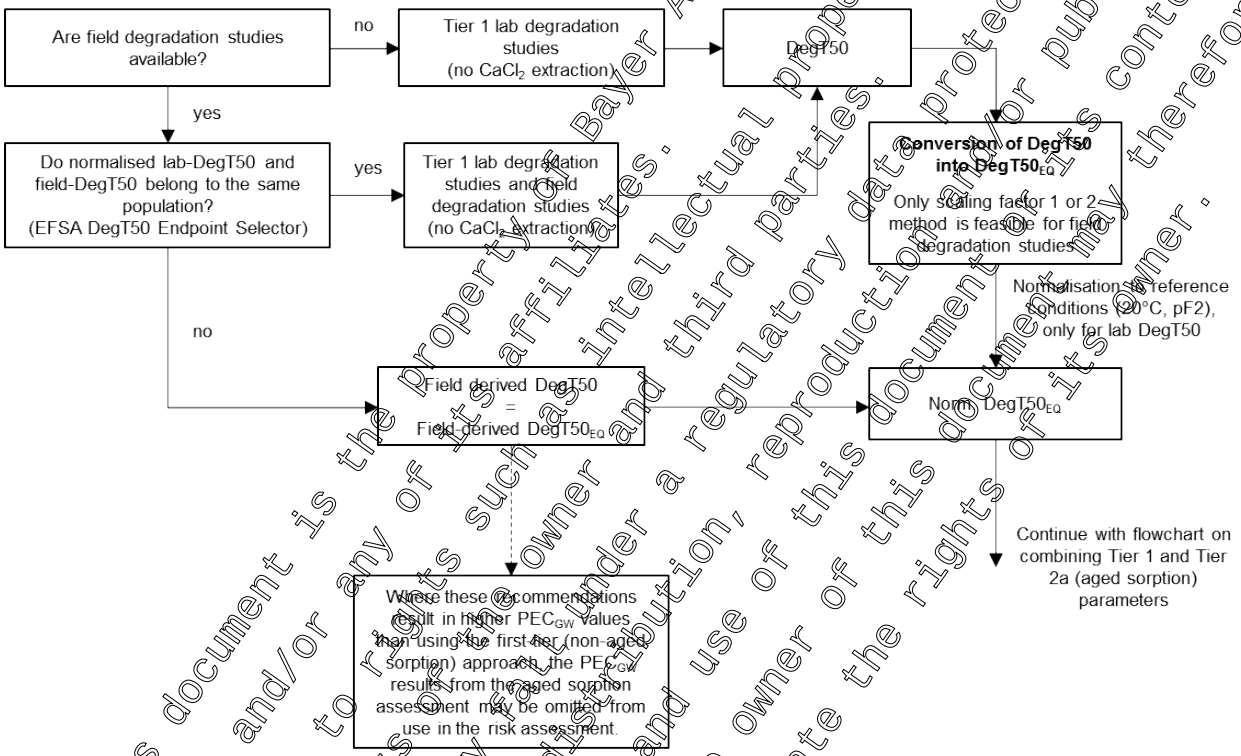
All available lower-tier degradation and adsorption parameters should be combined with the parameters from the aged sorption studies obtained at Tier 2a for use in groundwater leaching assessments. Figure 7.1.3.2- 8 illustrates the flow chart to combine parameters from Tier 1 and aged sorption studies (Tier 2a). This chart assumes all soils in the higher-tier experiments were extracted with the same procedure for the determination of total mass as was the case for fluopicolide studies.

Figure 7.1.3.2- 8: Flow chart for combining Tier 1 and Tier 2a (aged sorption) parameters for groundwater risk assessment



It is recommended that soil bulk matrix $DegT_{50}$ values from field studies should be accounted for in the leaching assessment, including checking whether laboratory and field degradation data are from different populations. For consistency with EFSA (2014), this check should be carried out based on soil bulk matrix $DegT_{50}$ values rather than $DegT_{50eq}$ values. Laboratory $DegT_{50}$ values derived from aged sorption experiments are combined with other lower tier laboratory $DegT_{50}$ values and compared with matrix $DegT_{50}$ values from field studies. The procedure is illustrated in Figure 7.1.3.2- 9.

Figure 7.1.3.2- 9: Flow chart for combining Tier 1 and Tier 2a (aged sorption) parameters for groundwater risk assessment



If laboratory and field $DegT_{50}$ are shown to be from the same population it is recommended field $DegT_{50}$ values are converted to appropriate $DegT_{50eq}$ values using the one of the scaling factors; scaling factor 1 where specific water holding capacity (measured at pF 2) is available for the soil or scaling factor 1 where soil moisture data is not available.

If field $DegT_{50}$ values represent a different population, and the field $DegT_{50}$ values are statistically shorter than the laboratory $DegT_{50}$ values, the EFSA PPR panel (2018) considers that rescaling the field $DegT_{50}$ data on the basis of laboratory TDS data is not justifiable because there is no experimental evidence that the extent of aged sorption in the laboratory and in the field is the same. In this case EFSA PPR panel (2018) recommends using the field $DegT_{50}$ values together with the laboratory aged sorption data in the leaching assessment without scaling the field $DegT_{50}$ values as a conservative approach.

Field $DegT_{50}$ values should not be statistically longer than the laboratory $DegT_{50}$ values and should be investigated further.

II. Results and Discussion

1. Kinetic sorption evaluation of lower-tier data

Refit of residue data

Aerobic degradation data of fluopicolide for the five soils summarised in Table 7.1.3.2- 35 were evaluated according to the recommended refit procedures. Fluopicolide data reported as % applied radioactivity (%AR) was converted to total µg by multiplying the %AR by the dosed mass of fluopicolide (20.5 or 41.0 µg). The input data are summarised in Table 7.1.3.2- 39 to Table 7.1.3.2- 44.

Measured K_{oc} data were available for all of the soils (see Table 7.1.3.2- 37) and used directly in the refit procedure. Where multiple K_{oc} measurements were available (Abington, Munster and Sarrotti) the mean parameters were calculated first. The mean aged-sorption parameters of Fne 0.508 and kdes 0.0356 (Table 7.1.3.2- 38) were used in the refit procedure. VolLiq (mL) is the volume of water in the soil during incubation ie soil moisture.

Table 7.1.3.2- 39: Input data for lower-tier refit - Munster

Weight soil (g)		100
Dose (µg)		41.0
VolLiq (mL)		9.2
OM (%)		1.2
K_{om} (mL/g)		182.2
1/n (-)		0.923
Time (d)	**Fluopicolide (%AR)**	**Total mass (µg)**
0	102.1	41.86
1	90.32	37.03
14	90.56	37.13
22	86.48	35.46
34	82.46	33.81
49	79.38	32.55
64	76.63	31.42
78	82.24	33.72
98	72.36	29.67
120	68.03	27.89
160	64.12	26.29
200	54.79	22.46

Table 7.1.3.2- 40: Input data for lower-tier refit - Sarrotti

Weight soil (g)		100
Dose (µg)		41.0
VolLiq (mL)		28.3
OM (%)		1.6
K_{om} (mL/g)		149.1
1/n (-)		0.878
Time (d)	**Fluopicolide (%AR)**	**Total mass (µg)**
0	98.18	40.25
7	81.52	33.42
14	76.19	31.24
22	79.44	32.57
34	75.60	31.00
49	72.33	29.66
64	75.68	31.03
78	70.15	28.76
98	64.48	26.44
120	52.82	21.66

Table 7.1.3.2- 41: Input data for lower-tier refit - Abington

Weight soil (g)		50
Dose (µg)		20.5
VolLiq (mL)		9.1
OM (%)		3.8
K_{om} (mL/g)		104.6
1/n (-)		0.875
Time (d)	**Fluopicolide (%AR)**	**Total mass (µg)**
0	95.1	19.50
0	98.4	20.17
14	97.0	19.89
14	93.3	19.13
28	92.2	18.90
28	90.9	18.63
42	80.7	16.54
42	82.7	16.95
56	85.5	17.53
56	79.6	16.32
77	83.2	17.06
77	83.8	17.18
98	77.4	15.87
98	81.5	16.71
120	77.2	15.83
120	80.2	16.44

Table 7.1.3.2- 42: Input data for lower-tier refit – Lamberton (20°C)

Weight soil (g)		50
Dose (µg)		20.5
VolLiq (mL)		15.1
OM (%)		5.6
K_{om} (mL/g)		192.5
1/n (-)		0.844
Time (d)	**Fluopicolide (%AR)**	**Total mass (µg)**
0	96.2	19.72
0	94.0	19.27
14	82.7	16.95
14	86.4	17.71
28	80.4	16.48
28	88.3	18.10
42	91.1	18.68
42	90.3	18.51
56	90.0	18.45
56	91.7	18.80
77	89.9	18.43
77	86.5	17.73
98	86.8	17.79
98	84.5	17.32
120	90.9	18.63
120	81.6	16.73

Table 7.1.3.2- 43: Input data for lower-tier refit – Lamberton (25°C)

Weight soil (g)		50
Dose (µg)		20.5
VolLiq (mL)		11.6
OM (%)		6.1
K_{om} (mL/g)		192.5
1/n (-)		0.844
Time (d)	**Fluopicolide (%AR)**	**Total mass (µg)**
0	83.9	17.2
0	94.4	19.4
14	82.6	16.9
14	85.0	17.4
31	81.0	16.6
31	82.3	16.9
60	81.3	16.7
60	85.8	17.6
94	69.2	14.2
94	65.3	13.4
116	68.0	13.9
116	69.4	14.2
188	63.4	13.0
188	64.2	13.2
273	54.6	11.2
273	63.0	12.9
369	45.3	9.3
369	40.4	8.3

Table 7.1.3.2- 44: Input data for lower-tier refit – Pikeville (25°C)

Weight soil (g)		50
Dose (µg)		20.5
VolLiq (mL)		4.3
OM (%)		2.8
K_{om} (mL/g)		198.7
1/n (-)		0.873
Time (d)	**Fluopicolide (%AR)**	**Total mass (µg)**
0	96.8	19.8
0	97.6	20.0
14	89.1	18.3
14	91.1	18.7
31	82.4	16.9
31	81.7	16.7
60	81.3	16.7
60	80.3	16.5
94	75.5	15.5
94	68.6	14.1
116	67.2	13.8
116	68.6	14.1
188	74.2	15.2
188	78.5	16.1
273	58.3	12.0
273	57.3	11.7
369	53.5	11.0
369	49.3	10.1

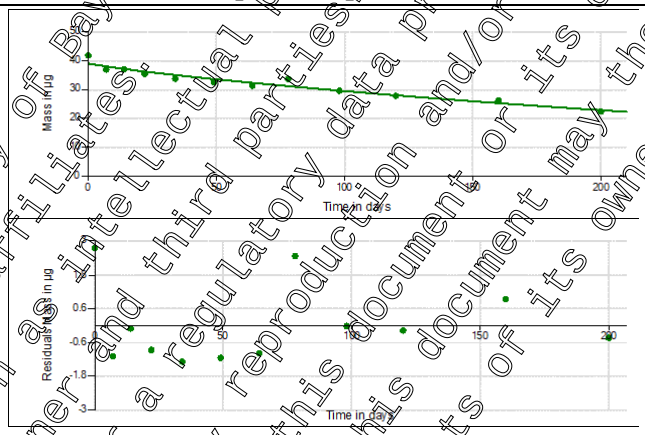
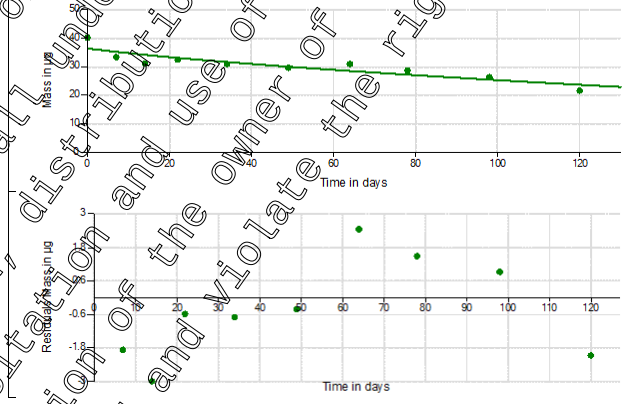
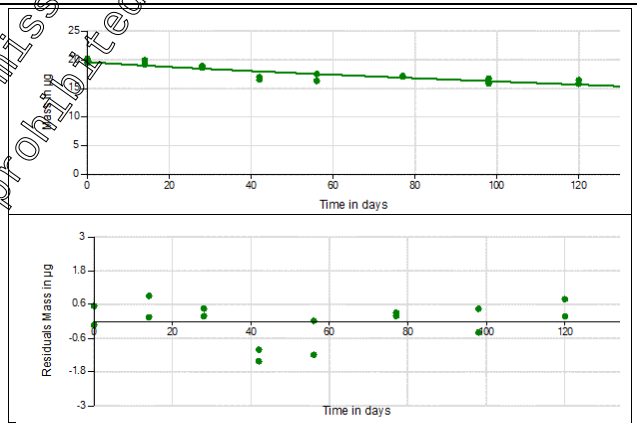
The calculated $DegT_{50eq}$ refit values for the lower-tier laboratory degradation studies are summarised in Table 7.1.3.2- 45 and visual fits shown in Table 7.1.3.2- 46. PEARLneq output files are included in the report.

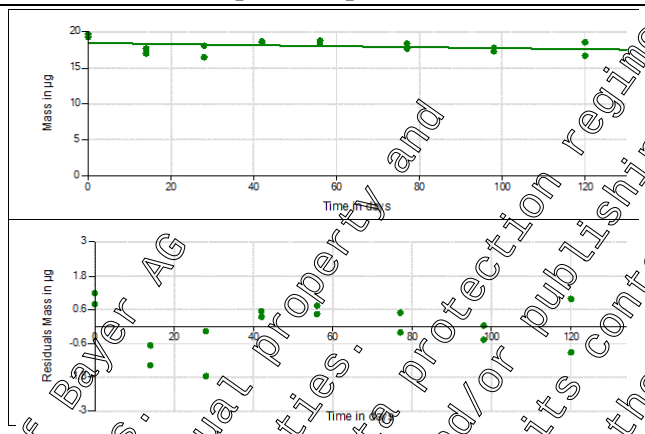
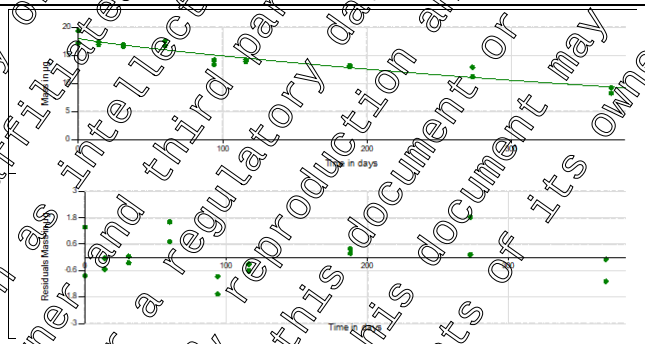
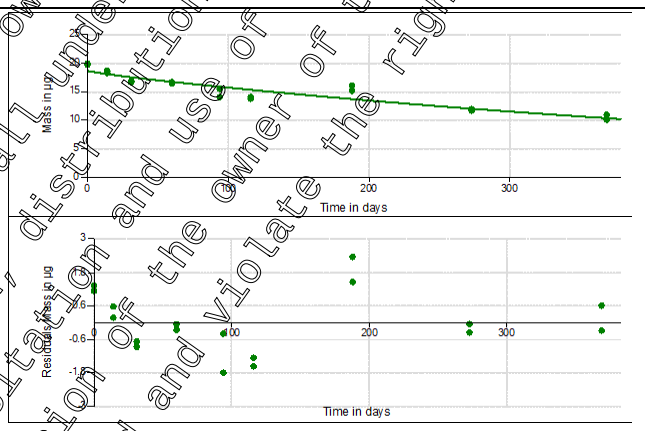
Table 7.1.3.2- 45: $DegT_{50eq}$ refit values for lower-tier degradation studies

#	Soil	Temperature (°C)	FOCUS correction factor	$DegT_{50eq}$ (d)	$DegT_{50eq}$ 20°C pF2 (d)
1	Munster	20	1.000	178.1	178.1
2	Sarrotti	20	1.000	138.6	138.6
3	Abington	20	0.978	262.3	256.4
4	Lamberton	20	0.805	1210.7	974.1
5	Lamberton	25	1.105	263.4	291.1
6	Pikeville	25	1.005	293.7	295.2

The rate of degradation of fluopicolide was investigated twice in Lamberton soil in separate studies. The two studies were conducted in the same laboratory by the same study director shortly after each other. However, degradation in the 20°C study appeared significantly slower than the study conducted at 25°C, which cannot be accounted for by temperature/moisture effects. The calculated DT_{50} of 1210 days is therefore not consistent with neither the replicate study, nor the overall behaviour of fluopicolide in all other soils and may be considered as an outlier. As a conservative approach the geometric mean value for the two Lamberton datasets of 532.5 days is taken forward for use in the overall $Deg1_{50eq}$ determination.

Table 7.1.3.2- 46: Graphical representation of PEARLneq refits

Soil	Graphical representation
Munster	
Sarrotti	
Abington	

Soil	Graphical representation
Lamberton 20°C	
Lamberton 25°C	
Pikeville	

Field $DegT_{50}$ evaluations

Normalised field $DegT_{50}$ values are summarised in Table 7.1.3.2- 36 for the 12 trials. Full details are provided in KCA 7.1.2.2.1/22, M-685675-01-1 and KCA 7.1.2.2.1/23, M-685676-01-1.

A comparison of these $DegT_{50}$ values with laboratory data (KCA 7.1.2.1.1/10, M-685680-01-1) indicated that they were part of the same distribution and thus the correction to derive $DegT_{50eq}$ values for field trials was conducted.

Soil-specific K_{om} and F_{ne} values were used where available, and where they were not available the overall geometric mean K_{om} (155.3 mL/g) and F_{ne} (0.508) values were used.

The incubation soil moisture content (w) was derived from the PEARL simulations conducted for the timestep normalisation procedure (KCA 7.1.2.2.1/22, M-685675-01-1 and KCA 7.1.2.2.1/23, M-685676-01-1) as the average over the two years of the study.

Table 7.1.3.2- 47 summarises the $DegT_{50eq}$ values for the 12 field trials calculated with scaling factor 1.

Table 7.1.3.2- 47: Normalised field $DegT_{50eq}$ values for fluopicolide

Location (country)	$DegT_{50}$ matrix (d) norm	K_{om} (mL/g)	f_{om}	w (cm^3/cm^3)	F_{ne} (-)	Scaling factor 1	$DegT_{50eq}$ (d)
Burscheid (Germany)	111.9	175.9	0.021	0.365	0.506	0.753	84.3
Great Chishill (UK)	216.9	149.1	0.036	0.402	0.571	0.718	155.8
Lignieres de Touraine (France)	158.6	210.6	0.014	0.239	0.638	0.692	109.8
St.Etienne du Grès (France)	303.2	118.9	0.014	0.348	0.514	0.772	234.2
Albaro di Ronco all'Adige (Italy)	237.3	108.5	0.031	0.391	0.303	0.865	205.3
Vilobi d'Onyar (Spain)	166.8	169.4	0.014	0.303	0.435	0.794	132.5
Philippsburg (Germany)	199.6	155.3	0.005	0.240	0.508	0.796	158.9
Rödelsee (Germany)	146.4	155.3	0.029	0.299	0.508	0.745	109.0
Huntlosen (Germany)	168.4	155.3	0.033	0.234	0.508	0.740	124.7
Valencia (Spain)	317.4	155.3	0.032	0.191	0.508	0.739	234.4
Appilly (France)	144.2	155.3	0.026	0.292	0.508	0.746	107.6
Senas (France)	136.5	155.3	0.028	0.238	0.508	0.742	101.3

Overall $DegT_{50eq}$ summary

The final results for all $DegT_{50eq}$ evaluations are summarised in Table 7.1.3.2- 48, with an overall geometric mean value of 124 days calculated. This $DegT_{50eq}$ is only valid for use in combination with the mean aged-sorption parameters of F_{ne} 0.508 and k_{des} 0.0356 for FOCUS model exposure calculations.

Table 7.1.3.2- 48: Overall $DegT_{50eq}$ evaluation results

#	Soil	$DegT_{50eq}$ (days)	Derivation
1	[redacted]	80.5	TDS - PEARLneq
2	Dollendorf	98.6	TDS - PEARLneq
3	[redacted]	69.8	TDS - PEARLneq
4	[redacted]	45.4	TDS - PEARLneq
5	[redacted]	76.2	TDS - PEARLneq
6	Great Chishill	170.9	TDS - PEARLneq
7	Parcey Meslay	111.0	TDS - PEARLneq
8	Mas du Coq	108.4	TDS - PEARLneq
9	Albaro	112.2	TDS - PEARLneq
10	Vilobi d'Onyar	52.2	TDS - PEARLneq
11	Abington	97.5	TDS - PEARLneq
12	Lamberton	91.6	TDS - PEARLneq
13	Munster	75.4	TDS - PEARLneq
14	Pikeville	66.8	TDS - PEARLneq
15	Sarrotti	99.3	TDS - PEARLneq
16	Lignieres	96.8	TDS - PEARLneq
17	Munster	178.1	Lab Tier-1 Refit
18	Sarrotti	138.6	Lab Tier-1 Refit

#	Soil	$DegT_{50eq}$ (days)	Derivation
19	Abington	256.4	Lab Tier-1 Refit
20	Lamberton	532.5	Lab Tier-1 Refit
21	Pikeville	295.2	Lab Tier-1 Refit
22	Burscheid (Germany)	84.3	Field Scaling factor 1
23	Great Chishill (UK)	155.8	Field Scaling factor 1
24	Lignieres de Touraine (France)	109.8	Field Scaling factor 1
25	St.Etienne du Grès (France)	234.2	Field Scaling factor 1
26	Albaro di Ronco all'Adige (Italy)	205.3	Field Scaling factor 1
27	Vilobi d'Onyar (Spain)	132.5	Field Scaling factor 1
28	Philippsburg (Germany)	1589	Field Scaling factor 1
29	Rödelsee (Germany)	109.0	Field Scaling factor 1
30	Huntlosen (Germany)	124.7	Field Scaling factor 1
31	Valencia (Spain)	234.4	Field Scaling factor 1
32	Appilly (France)	107.6	Field Scaling factor 1
33	Senas (France)	101.3	Field Scaling factor 1
	Geometric mean	**121**	

III. Conclusion

Lower-tier degradation data (laboratory and field) must be combined with the aged-sorption data following re-evaluation to estimate $DegT_{50eq}$ values for inclusion in PECgw exposure assessments. Data for fluopicolide from laboratory studies and field studies have been evaluated to derive $DegT_{50eq}$ values, which when combined with the higher-tier aged-sorption values yield an overall geometric mean $DegT_{50eq}$ of 121 days for use in exposure modelling (in combination with the mean aged-sorption parameters: F_{ac} 0.508; K_{des} 0.0356).

Assessment and conclusion by applicant:

The modelling report was conducted according to current guidance on aged sorption and is considered valid to assess the aged sorption of fluopicolide in soil.

CA 7.1.4 Mobility in soil

The uptake of fluopicolide and its metabolite M-01 (AE C 653711) has been investigated in potato plants and used to determine the transpiration stream concentration factors (TSCF) for the substances. KCA 7.1.4/01 and KCA 7.1.4/02 are provided as new studies not yet reviewed.

	Report reference	Author, Year	Comment
Fluopicolide	KCA 7.1.4/01 M-688372-01-1	[redacted] 2020	New data not yet reviewed.
M-01	KCA 7.1.4/02 M-688374-01-1	[redacted] 020	New data not yet reviewed.

The studies confirm experimentally the predicted TSCF values calculated for fluopicolide and M-01 with the Briggs equation and justify the use of a default TSCF value of 0.5 in FOCUS surface water and groundwater modelling.

Data Point:	KCA 7.1.4/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the plant uptake of AE C638206 in potato plants
Report No:	EnSa-18-0217
Document No:	M-688372-01-1
Guideline(s) followed in study:	"Interim study design to determine uptake of chemicals by plant roots"; ECPA/IVA Working Group "plant uptake factor" (2019-07-25)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The uptake of fluopicolide was investigated in potato plants and used to determine the transpiration stream concentration factor (TSCF). The study was conducted in compliance with the "Interim study design to determine uptake of chemicals by plant roots" ECPA/IVA Working Group "Plant uptake factor" (2019).

An application rate of 107.4 µg [phenyl-U-^{14}C]-fluopicolide per test system was used, equivalent to final concentration of 109.1 µg/L of nutrient solution.

The test was performed in hydroponic systems consisting of brown glass bottles each containing either approximately 1000 mL test solution (nutrient solution plus test item) or pure nutrient solution. The nutrient solutions were adjusted to a pH value of 5.47 at the start of the experiments. The oxygen content of the nutrient solutions was recorded at the start and the sampling dates. Pre-grown potato plants (BBCH 11-12) were equilibrated in nutrient solution for 11 days and then from BBCH 18-19 exposed to the test solution for up to 6 days.

During the study, the plants were incubated in a climatic chamber in the greenhouse under controlled temperature, humidity and light conditions (22/16 °C (day/night), 60% humidity and a day/night cycle of 16 h/8 h). Potato plants remained healthy in both treated and untreated test systems throughout the study. Potato plants grew from BBCH 18-19 at t_{start} (application) to BBCH 22-24 at t_{end} (end of study at DAT-6).

Mean material balances were 98.0% AR for t_1 (range from 96.4 to 99.4% AR), 98.8% AR for t_2 (range from 97.3 to 100.0% AR) and 98.8% AR for t_{end} (range from 88.9 to 105.9% AR).

The transpiration stream concentration factor (TSCF) of fluopicolide in potato plants amounted to ± at t_1, 0.75 ± 0.03 at t_2 and 0.82 ± 0.23 at t_{end}. The four plants with the strongest biomass growth were chosen for the calculation of the average TSCF. The individual values for all single tests are shown in the following table.

Replicate No.	$TSCF_{potato}$		
	t_{start}-t_1	t_{start}-t_2	t_{start}-t_{end}
1	0.90	~~0.69*~~	1.15
2	0.69	~~0.62*~~	~~0.67*~~
3	0.68	0.77	0.52
4	~~0.64*~~	0.72	~~0.81*~~
5	0.60	0.79	0.90
6	~~0.70*~~	0.74	0.72
Mean ± SD	**0.71 ± 0.11**	**0.75 ± 0.03**	**0.82 ± 0.23**

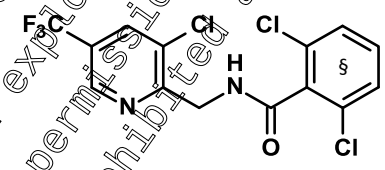
t_{start} = start of uptake at DAT-0
t_1 = interim sampling at DAT-2
t_2 = interim sampling at DAT-4
t_{end} = end of study at DAT-6
*These test systems were not considered for the calculation of the average TSCF

I. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-fluopicolide (called [phenyl-U-^{14}C]-AE C638206 in the report)



§ = position of radiolabel.

Batch number: MXM 20028

Specific radioactivity: 3.53 MBq/mg

Radiochemical purity: ≥ 99% (HPLC with radiodetector)

2. Test system

Nutrient solution: Plants were maintained hydroponically in a nutrient solution (Hoagland No. 2 basal salt mixture containing 2-(N-morpholino)-ethanesulfonic acid (MES) buffer, pH 5.5).

Plants: Potato tubers (variety: Annabelle) were grown in perlite in a greenhouse until reaching BBCH 11-12. At growth stage BBCH 11-12, plants were carefully transferred to the hydroponic system. Perlite was removed from the root system by washing with untreated nutrient solution and the tuber carefully removed from the plant (shoot and root). The nutrient solutions were adjusted to a pH value of 5.47 at the start of the experiments. The oxygen content of the nutrient solutions was recorded at the start and the sampling dates.

B. Study design

1. Experimental conditions:

The study was conducted in compliance with the latest guidance "Interim study design to determine uptake of chemicals by plant roots" ECPA/TVA Working Group "Plant uptake factor" (2019).

The test was performed in hydroponic systems consisting of brown glass bottles each containing either approximately 1000 mL test solution (nutrient solution plus test item) or pure nutrient solution with one plant per vessel. The root system of the plants was fully submerged. Each bottle was closed with a polyurethane (PU) foam plug and aerated in a stream of air.

Pre-grown potato plants (BBCH 11-12) were equilibrated in nutrient solution for 11 days and then from BBCH 18-19 exposed to the test solution for a maximum period of 6 days. The incubation time considered to calculate the transpiration stream concentration (TSCF) factor was over 6 days in total starting from the day of application of the test item.

The nominal target test item concentration in test solution was 100 µg/L which corresponds to 0.261 µmol/L. The test item was dissolved in methanol and 107.4 µg [phenyl-U-^{14}C]-fluopicolide applied per test system (volume of methanol 93.5 µg per system). The final concentration was 109.1 µg fluopicolide /L nutrient solution.

Plant controls were treated with 93.5 ug methanol per system.

During the test, plants were grown in a greenhouse under controlled growth conditions with a light:dark cycle of 16 hrs:8 hrs, a temperature range of day/night of approx. 22/16 °C and a relative humidity approx. 50-85%. During the definitive test, the light intensity was measured on each sampling day (range 28000 to 44000 Lux).

Potato plants appeared healthy over the study duration both in treated and untreated test systems. No differences between treated and untreated test systems were detected. During the study the potato plants grew from BBCH 18-19 at t_{start} (application and start of equilibration) to BBCH 22-24 at t_{end} (end of study at DAT-6).

Parameter		Description
Duration of test		1st interim sampling: DAT-2 (t_1); 2nd interim sampling: DAT-4 (t_2); 3rd harvest / end of the experiment: DAT-6 (t_{end})
Test concentrations	Nominal	100 µg/L
	Real	109.1 µg/L at study start
Number of replications		4 replicate vessels and 2 back-up vessels for harvesting on DAT-2, DAT-4 or DAT-6
Preparation of test medium	Volume used per vessel	1000 mL
	Method of preparation	For 1 litre nutrient solution 0.8 g Hoagland's No. 2 basal salt mixture, 2.06 g MES buffer and 0.75 ml of 1.5% ferric EDTA solution are solved in an appropriate amount of demineralized water.
	Target pH	5.5
Test material application	Solvent	Methanol
	Volume of application solution	93.5 µL per vessel
	Application method	Pipette
Test apparatus		1000 mL brown glass flasks
Is there any indication of the test item adsorbing to the walls of the test apparatus?		No, the amount of dissolved radioactivity in the vessel with nutrient solution and test item (no plant) stays constant with time.
Experimental conditions	Temperature	22 °C (day) 16 °C (night)
	Humidity	Set to 60%
	Lighting	Climatic chamber in the greenhouse with day night cycle (16 h light & 8 h dark)
Other Details		Additional vessels with nutrient solution only to determine pH level, dissolved oxygen and loss of nutrient solution via evaporation. Additional vessel with nutrient solution and test item (no plant) to determine the stability of the test item at test end and to show no indication of the test item adsorbing to the walls of the test system. Additional vessels with nutrient solution and plants to determine the growth of the plant during the experiment.

Three additional plants were treated with [phenyl-UL-^{14}C] fluopicolide in a separate non-GLP experiment and grown under the same conditions as plants used to determine the TSCF. Plants were subjected to imaging plates designed for radioluminography and the resulting plates were scanned with Fuji BAS-5000® image analyzer. Quantitative evaluation was based on radiographic images of the plant sections using the image analysis software "AIDA®" (Elysia, Straubenhard, Germany).

2. Sampling

The mass of test substance, volume of test solution in the test vessels, pH and dissolved oxygen levels in the treated nutrient solution were determined in all vessels on day 0 (immediately after application), DAT 2, 4 and 6 (t_{start}, t_1, t_2 and t_{end}).

Treated plants were processed and analysed on DAT 2, 4 and 6 (t_1, t_2 and t_{end}). 4 replicates plus 2 additional back-up replicates were taken at each timepoint. The biomass development of plant roots and shoots in the presence of test item was evaluated when sampled.

Control plants grown in systems treated with solvent (but no test item) were processed and analysed on DAT 0, 2, 4 and 6 (t_{start}, t_1, t_2 and t_{end}) to determine biomass development in roots and shoots. 3 replicates (4 replicates at DAT 0) were taken at each timepoint.

Three vessels containing nutrient solution only (without plants) were used to measure pH, oxygen saturation and evaporation during incubation. One vessel was prepared without plants but treated with test item to monitor stability of the test item as well as pH level and evaporation during incubation. These 4 vessels were inoculated by dipping the roots of a test plant into the nutrient solution for 10 seconds.

3. Analytical procedures

At each sampling date the volume of the test solution in individual test systems was determined by weighing. The plants were removed from the test vessels and any compound associated with the root surface was removed by gently shaking the roots in approximately 200 mL acetonitrile/water 4/1 (v/v) for 5 minutes. Roots were patted dry, plants divided into shoot and roots and the fresh weight biomasses were determined by weighing.

The pH and oxygen saturation of the test solution remaining in the test vessel were measured, and the radioactivity determined by LSC. The radioactivity in air dried roots and shoots was determined by combustion. Root samples were combusted completely, whereas shoot samples were first homogenized by UltraTurax in liquid nitrogen and aliquots taken for combustion.

Plant controls were divided into shoot and roots and the fresh weight biomasses were determined by weighing as described above.

The volume of the test solution in the stability control was determined at t_{start}, t_1, t_2 and t_{end} by weighing the test vessels. Aliquots were taken and analysed by LSC. At the end of the study (t_{end}, DAT-6), an aliquot was analysed by HPLC with radiodetection to confirm the test item stability.

pH, oxygen saturation and evaporation during incubation (by weighing) were monitored on each sampling day in the three control vessels without plants or test item. Aerobic conditions were maintained throughout the experimental period. The mean pH values of the test solutions increased within 0.4 pH units of the system initially buffered to pH 5.5.

The identity of the test item was confirmed in stock solution by HPLC-MS(/MS) including accurate mass determination.

II. Results and Discussion

A. Data

The transpiration stream concentration factor (TSCF) for fluopicolide was calculated from 4 replicates and amounted to 0.71 ± 0.11 at t_1, 0.75 ± 0.03 at t_2 and 0.82 ± 0.23 at t_{end} (see Table 7.1.4- 1). The four plants with the strongest biomass growth were chosen for the calculation of TSCF.

Table 7.1.4- 1: Transpiration stream concentration factor (TSCF) for fluopicolide

Timepoint t_1						
Replicate	**V_{start} [mL]**	**m_{start} [µg]**	**V_{sol} [mL]**	**m_{sol} [µg]**	**m_{shoot} [µg]**	**TSCF**
MK18PT14	986.28	108.05	908.44	94.99	7.26	0.90
MK18PT15	983.95	108.17	914.74	95.31	4.89	0.69
MK18PT16	940.61	102.80	870.02	92.98	5.07	0.68
~~MK18PT17~~	~~979.52~~	~~106.67~~	~~910.03~~	~~96.08~~	~~4.61~~	~~0.64~~
MK18PT18	972.48	105.50	890.94	95.20	5.11	0.69
~~MK18PT19~~	~~991.66~~	~~107.85~~	~~922.95~~	~~98.81~~	~~5.21~~	~~0.70~~
Mean	970.8	106.1	896.03	94.62	5.58	**0.71**
SD	18.21	2.20	17.37	0.95	0.97	0.11
CV %	1.88	2.07	1.94	1.01	17.40	15.41
Timepoint t_2						
Replicate	**V_{start} [mL]**	**m_{start} [µg]**	**V_{sol} [mL]**	**m_{sol} [µg]**	**m_{shoot} [µg]**	**TSCF**
~~MK18PT20~~	~~990.50~~	~~107.77~~	~~916.41~~	~~96.18~~	~~5.34~~	~~0.69~~
~~MK18PT21~~	~~981.06~~	~~108.90~~	~~923.24~~	~~98.51~~	~~3.76~~	~~0.62~~
MK18PT22	989.81	107.42	812.76	88.03	14.34	0.77
MK18PT23	996.17	103.96	773.04	84.94	16.98	0.72
MK18PT24	986.33	108.80	815.92	89.02	14.42	0.79
MK18PT25	953.65	106.21	794.91	87.93	12.71	0.74
Mean	981.5	106.6	799.16	87.48	14.61	**0.75**
SD	16.46	1.77	17.07	1.53	1.53	0.03
CV %	1.68	1.67	2.14	1.75	10.45	3.59
Timepoint t_{end}						
Replicate	**V_{start} [mL]**	**m_{start} [µg]**	**V_{sol} [mL]**	**m_{sol} [µg]**	**m_{shoot} [µg]**	**TSCF**
MK18PT26	1009.51	108.80	846.07	90.56	19.26	1.15
~~MK18PT27~~	~~975.90~~	~~106.21~~	~~915.02~~	~~97.81~~	~~4.33~~	~~0.67~~
MK18PT28	997.19	108.55	630.40	70.57	18.80	0.52
~~MK18PT29~~	~~996.46~~	~~108.70~~	~~905.94~~	~~97.03~~	~~7.76~~	~~0.81~~
MK18PT30	1007.43	110.43	788.63	85.46	21.16	0.90
MK18PT31	990.70	108.11	743.07	80.84	18.56	0.72
Mean	999.0	109.0	752.04	81.05	19.45	**0.82**
SD	6.03	0.88	79.15	7.75	1.02	0.23
CV %	0.60	0.80	10.52	9.57	5.25	28.49

Two replicates with lowest biomass growth (marked by strikethrough) out of all six were excluded from TSCF calculation
V_{start}: volume of test solution at t_{start} (start of equilibration at DAT-0)
m_{start}: mass of test item at t_{start} (start of equilibration at DAT-0)
V_{sol}: volume of test solution at t_1 / t_2 / t_{end}
m_{sol}: mass of test item in test solution at t_1 / t_2 / t_{end}
m_{shoots}: mass of test item in shoots at t_1 / t_2 / t_{end}

Good plant health, as shown by the intense biomass increase and water consumption during the study, demonstrated the test system provided a reliable and robust method to calculate TSCF for fluopicolide.

B. Material Balance

Mean material balances were 98.0% AR for t_1 (range from 96.4 to 99.4% AR), 98.8% AR for t_2 (range from 97.3 to 100.0% AR) and 98.8% AR for t_{end} (range from 88.9 to 105.9% AR).

There were no signs for losses of radioactivity from the test systems or during sample processing. No adsorption of fluopicolide to the walls of the test systems was observed throughout the test.

C. Translocation of Parent Compound

During the incubation period 75.5 to 330.2 mL of the test solution was taken up by the plants. About 9.0 mL of the test solutions were lost due to evaporation after 6 days.

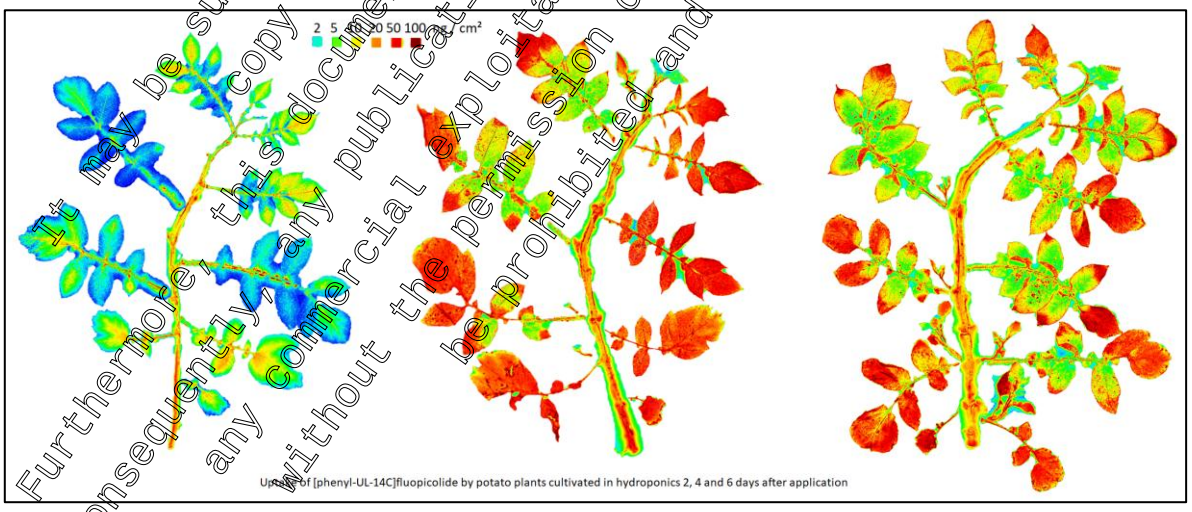
The mean initial concentrations (t_{start}, DAT-0) of [phenyl-UL-^{14}C]-fluopicolide in test solutions was 109.1 µg/L, decreasing to 103.1 µg/L at t_1 (first interim sampling at DAT 2) and slightly increasing to 103.4 µg/L at t_2 (second interim sampling at DAT 4) and 104.0 µg/L at t_{end} (end of study at DAT-6). Root wash desorbed 3.2 µg of the test item from the roots at t_1, 3.8 µg at t_2 and 3.9 µg at t_{end}. The separate analysis of roots and shoots showed that at t_1 60.3%, at t_2 67.5% and at t_{end} 71.1% of radioactivity taken up by the plants was translocated from roots into shoots.

No degradation of fluopicolide was observed at the end of the study in test solution from the stability control (without plants).

D. Autoradiography

Autoradiographs of three additional plants treated with [phenyl-UL-^{14}C] fluopicolide, grown under the same conditions as plants used to determine the TSCF and harvested at day t_1, t2, and t_{end} (DAT 2, 4 and 6) are shown below. The images provide supportive data for the plant uptake of fluopicolide and give a detailed picture of the distribution of radioactivity at different time points.

Figure 7.1.4- 1: Autoradiographs of plants treated with [phenyl-UL-^{14}C] fluopicolide harvested at day t_1, t_2, and t_{end} (DAT 2, 4 and 6)



III. Conclusion

Fluopicolide was translocated into the shoots of potato plants, with 60.3, 67.5 and 71.1% of the radioactivity taken up by the plants translocated from roots into shoots after 2, 4 and 6 days, respectively. The TSCF for fluopicolide amounted to 0.71 ± 0.11 after 2 days, 0.75 ± 0.03 after 4 days and 0.82 ± 0.23 after 6 days.

This study confirms experimentally the predicted TSCF of 0.47 calculated for fluopicolide with the Briggs equation based on the Log P_{ow} value of 2.9.

Assessment and conclusion by applicant:

The study is considered valid to assess the TSCF of fluopicolide in plants.

Data Point:	KCA 7.1.4/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of the plant uptake of AE C653711 in potato plants
Report No:	EnSa-20-0590
Document No:	M-688374-01-1
Guideline(s) followed in study:	"Interim study design to determine uptake of chemicals by plant roots"; ECPA/IVA Working Group "plant uptake factor" (2019-07-25)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The uptake of M-01 (called AE C653711 in the report) was investigated in potato plants and used to determine the transpiration stream concentration factor (TSCF). The study was conducted in compliance with the "Interim study design to determine uptake of chemicals by plant roots" ECPA/IVA Working Group "Plant uptake factor" (2019).

An application rate of 100.0 µg [phenyl-U-^{14}C]-M-01 per test system was used, equivalent to final concentration of 103.0 µg/L of nutrient solution.

The test was performed in hydroponic systems consisting of brown glass bottles each containing either approximately 1000 mL test solution (nutrient solution plus test item) or pure nutrient solution. The nutrient solutions were adjusted to a pH value of 5.5 at the start of the experiments. The oxygen content of the nutrient solutions was recorded at the start and the sampling dates. Pre-grown potato plants (BBCH 10-12) were equilibrated in nutrient solution for 10 days and then from BBCH 23 exposed to the test solution for up to 6 days.

During the study, the plants were incubated in a climatic chamber in the greenhouse under controlled temperature, humidity and light conditions (22/16 °C (day/night), 60% humidity and a day/night cycle of 16 h/8 h). Potato plants remained healthy in both treated and untreated test systems throughout the study. Potato plants grew from BBCH 23 at t_{start} (application) to BBCH 24 at t_{end} (end of study at DAT-6).

Mean material balances were 101.6% AR for t_1 (range from 100.6 to 103.4% AR), 100.1% AR for t_2 (range from 98.9 to 101.1% AR) and 99.2% AR for t_{end} (range from 97.7 to 103.4% AR).

The transpiration stream concentration factor (TSCF) of fluopicolide in potato plants amounted to 0.86 ± 0.09 at t_1, 0.75 ± 0.05 at t_2 and 0.72 ± 0.05 at t_{end}. At t_1 two of six plants showed almost no or negative growth therefore the four plants with the strongest biomass growth were chosen for the calculation of the average TSCF for the first sampling date. For the second and last sampling dates (t_2 and t_{end}), all six plants were considered for calculation. The individual values for all single tests are shown in the following table.

Replicate No.	$TSCF_{potato}$		
	t_{start}-t_1	t_{start}-t_2	t_{start}-t_{end}
1	1.05	0.67	0.70
2	0.78	0.80	0.75
3	0.83	0.80	0.69
4	0.88*	0.77	0.66
5	0.80	0.74	0.71
6	0.79*	0.74	0.81
Mean ± SD	**0.86 ± 0.09**	**0.75 ± 0.05**	**0.72 ± 0.05**

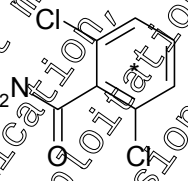
t_{start} = start of uptake at DAT-0
t_1 = interim sampling at DAT-2
t_2 = interim sampling at DAT-4
t_{end} = end of study at DAT-6
*These test systems were not considered for the calculation of the average TSCF

I. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-M-01 (referred to as AE C653711 in the report)



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

Chemical name (IUPAC): 2,6-dichlorobenzamide

Specific activity: 3.91 MBq/mg

Batch number: MXM 20218

Radiochemical purity: ≥ 99% (HPLC with radiodetector)

2. Test system

Nutrient solution: Plants were maintained hydroponically in a nutrient solution (Hoagland No. 2 basal salt mixture containing 2-(N-morpholino)-ethanesulfonic acid (MES) buffer, pH 5.5).

Plants: Potato tubers (variety: Annabelle) were grown in perlite in a greenhouse until reaching BBCH 10-12. At growth stage BBCH 10-12, plants were carefully transferred to the hydroponic system. Perlite was removed from the root system by washing with untreated nutrient solution and the tuber carefully removed from the plant (shoot and root). The nutrient solutions were adjusted to a pH value of 5.5 at the start of the experiments. The oxygen content of the nutrient solutions was recorded at the start and the sampling dates.

B. Study design

1. Experimental conditions:

The study was conducted in compliance with the latest guidance "Interim study design to determine uptake of chemicals by plant roots" ECPA/TVA Working Group "Plant uptake factor" (2019).

The test was performed in hydroponic systems consisting of brown glass bottles each containing either approximately 1000 mL test solution (nutrient solution plus test item) or pure nutrient solution with one plant per vessel. The root system of the plants was fully submerged. Each bottle was closed with a polyurethane (PU) foam plug and aerated in a stream of air.

Pre-grown potato plants (BBCH 10-12) were equilibrated in nutrient solution for 10 days and then from BBCH 23 exposed to the test solution for a maximum period of 6 days. The incubation time considered to calculate the transpiration stream concentration (TSCF) factor was over 6 days in total starting from the day of application of the test item.

The nominal target test item concentration in test solution was 100 µg/L which corresponds to 0.526 µmol/L. The test item was dissolved in methanol and 100.0 µg [phenyl-U-^{14}C]-M-01 applied per test system (volume of methanol 89.2 µg per system). The final concentration was 103.0 µg M-01 / L nutrient solution.

Plant controls were treated with 89.2 ug methanol per system.

During the test, plants were grown in a greenhouse under controlled growth conditions with a light:dark cycle of 16 hrs:8 hrs, a temperature range of day/night of approx. 22/16 °C and a relative humidity approx. 50-85%. During the definitive test, the light intensity was measured on each sampling day (range 27000 to 48000 Lux).

Potato plants appeared healthy over the study duration both in treated and untreated test systems. No differences between treated and untreated test systems were detected. During the study the potato plants grew from BBCH 23 at t_{start} (application and start of equilibration) to BBCH 24 at t_{end} (end of study at DAT-6).

Parameter		Description
Duration of test		1st interim sampling: DAT-2 (t_1); 2nd interim sampling: DAT-4 (t_2); 3rd harvest / end of the experiment: DAT-6 (t_{end})
Test concentrations	Nominal	100 µg/L
	Real	103.0 µg/L at study start
Number of replications		4 replicate vessels and 2 back-up vessels for harvesting on DAT-2, DAT-4 or DAT-6
Preparation of test medium	Volume used per vessel	1000 mL
	Method of preparation	For 1 litre nutrient solution 0.8 g Hoagland's No. 2 basal salt mixture, 2.06 g MES buffer and 0.75 ml of 1.5% ferric EDTA solution are solved in an appropriate amount of demineralized water.
	Target pH	5.5
Test material application	Solvent	Methanol
	Volume of application solution	89.2 µL per vessel
	Application method	Pipette
Test apparatus		1000 mL brown glass flasks
Is there any indication of the test item adsorbing to the walls of the test apparatus?		No, the amount of dissolved radioactivity in the vessel with nutrient solution and test item (no plant) stays constant with time.
Experimental conditions	Temperature	22 °C (day), 16 °C (night)
	Humidity	Set to 60%
	Lighting	Climatic chamber in the greenhouse with day/night cycle (16 h light & 8 h dark)
Other Details		Additional vessels with nutrient solution only to determine pH level, dissolved oxygen and loss of nutrient solution via evaporation. Additional vessel with nutrient solution and test item (no plant) to determine the stability of the test item at test end and to show no indication of the test item adsorbing to the walls of the test system. Additional vessels with nutrient solution and plants to determine the growth of the plant during the experiment.

2. Sampling

The mass of test substance, volume of test solution in the test vessels, pH and dissolved oxygen levels in the treated nutrient solution were determined in all vessels on day 0 (immediately after application), DAT 2, 4 and 6 (t_{start}, t_1, t_2 and t_{end}).

Treated plants were processed and analysed on DAT 2, 4 and 6 (t_1, t_2 and t_{end}). 4 replicates plus 2 additional back-up replicates were taken at each timepoint. The biomass development of plant roots and shoots in the presence of test item was evaluated when sampled.

Control plants grown in systems treated with solvent (but no test item) were processed and analysed on DAT 0, 2, 4 and 6 (t_{start}, t_1, t_2 and t_{end}) to determine biomass development in roots and shoots. 3 replicates (4 replicates at DAT 0) were taken at each timepoint.

Three vessels containing nutrient solution only (without plants) were used to measure pH, oxygen saturation and evaporation during incubation. One vessel was prepared without plants but treated with test item to monitor stability of the test item as well as pH level and evaporation during incubation. These 4 vessels were inoculated by dipping the roots of a test plant into the nutrient solution for 10 seconds.

3. Analytical procedures

At each sampling date the volume of the test solution in individual test systems was determined by weighing. The plants were removed from the test vessels and any compound associated with the root surface was removed by gently shaking the roots in approximately 200 mL acetonitrile/water 4/1 (v/v) for 5 minutes. Roots were patted dry, plants divided into shoot and roots and the fresh weight biomasses were determined by weighing.

The pH and oxygen saturation of the test solution remaining in the test vessel were measured, and the radioactivity determined by LSC. The radioactivity in air dried roots and shoots was determined by combustion. Root samples were combusted completely, whereas shoot samples were first homogenized by UltraTurax in liquid nitrogen and aliquots taken for combustion.

Plant controls were divided into shoot and roots and the fresh weight biomasses were determined by weighing as described above.

The volume of the test solution in the stability control was determined at t_{start}, t_1, t_2 and t_{end} by weighing the test vessels. Aliquots were taken and analysed by LSC. At the end of the study (t_{end}, DAT-6), an aliquot was analysed by HPLC with radiodetection to confirm the test item stability.

pH, oxygen saturation and evaporation during incubation (by weighing) were monitored on each sampling day in the three control vessels without plants or test item. Aerobic conditions were maintained throughout the experimental period. The mean pH values of the test solutions increased within 0.4 pH units of the system initially buffered to pH 5.5.

The identity of the test item was confirmed in stock solution by HPLC-MS(/MS) including accurate mass determination.

II. Results and Discussion

A. Data

The transpiration stream concentration factor (TSCF) for M-01 was calculated and amounted to 0.86 ± 0.09 at t_1, 0.75 ± 0.05 at t_2 and 0.72 ± 0.05 at t_{end}. At t_1 two of six plants had shown almost no or negative growth therefore the four plants with the strongest biomass growth were chosen for the calculation of the average TSCF for the first sampling date. For the second and last sampling dates (t_2 and t_{end}), all six plants were considered for calculation.

Good plant health, as shown by the intense biomass increase and water consumption during the study, demonstrated the test system provided a reliable and robust method to calculate TSCF for M-01.

Table 7.1.4- 2: Transcription stream concentration factor (TSCF) for M-01

Timepoint t_1						
Replicate	**V_{start} [mL]**	**m_{start} [µg]**	**V_{sol} [mL]**	**m_{sol} [µg]**	**m_{shoot} [µg]**	**TSCF**
MK19PT14	929.59	95.66	838.23	88.21	10.14	1.05
MK19PT15	970.14	100.16	880.77	92.88	7.32	0.78
MK19PT16	937.99	96.43	838.86	88.58	8.56	0.83
~~MK19PT17~~	~~1001.28*~~	~~103.06*~~	~~938.76*~~	~~97.89*~~	~~5.71*~~	~~0.88*~~
MK19PT18	952.74	98.32	882.56	93.30	5.85	0.80
~~MK19PT19~~	~~966.35*~~	~~99.19*~~	~~890.70*~~	~~94.02*~~	~~6.27*~~	~~0.79*~~
Mean	947.6	97.6	860.10	90.74	7.97	**0.86**
SD	15.42	1.74	21.57	3.31	1.60	0.09
CV %	1.63	1.79	2.51	3.65	20.06	10.83
Timepoint t_2						
Replicate	**V_{start} [mL]**	**m_{start} [µg]**	**V_{sol} [mL]**	**m_{sol} [µg]**	**m_{shoot} [µg]**	**TSCF**
MK19PT20	983.53	100.94	756.93	81.70	17.29	0.67
MK19PT21	992.64	102.29	774.20	83.89	18.53	0.80
MK19PT22	959.25	98.75	758.38	82.01	16.88	0.80
MK19PT23	986.48	101.76	778.33	83.84	16.69	0.77
MK19PT24	984.73	101.50	774.22	84.16	16.42	0.74
MK19PT25	959.46	99.13	784.58	84.91	13.61	0.74
Mean	977.7	100.7	767.77	83.42	16.57	**0.75**
SD	13.27	1.33	15.90	1.16	1.49	0.05
CV %	1.36	1.32	2.07	1.40	8.96	6.11
Timepoint t_{end}						
Replicate	**V_{start} [mL]**	**m_{start} [µg]**	**V_{sol} [mL]**	**m_{sol} [µg]**	**m_{shoot} [µg]**	**TSCF**
MK19PT26	982.79	101.04	678.10	76.82	22.79	0.70
MK19PT27	991.16	102.24	606.25	69.01	30.73	0.75
MK19PT28	990.27	101.99	636.80	73.53	26.23	0.69
MK19PT29	972.61	100.18	676.54	74.92	20.33	0.66
MK19PT30	968.05	99.83	648.90	72.78	23.73	0.71
MK19PT31	976.23	100.57	593.26	68.63	33.99	0.81
Mean	980.2	101.0	639.98	72.62	26.30	**0.72**
SD	8.65	0.89	32.15	2.97	4.71	0.05
CV %	0.88	0.88	5.02	4.08	17.93	6.58

Two replicates with lowest biomass growth (marked by strikethrough) out of all six were excluded from TSCF calculation on DAT-2. For the other two timepoints, no major differences between biomasses were observed and all six replicates were used for the calculation.
V_{start}: volume of test solution at t_{start} (start of equilibration at DAT-0)
m_{start}: mass of test item at t_{start} (start of equilibration at DAT-0)
V_{sol}: volume of test solution at t_1 / t_2 / t_{end}
m_{sol}: mass of test item in test solution at t_1 / t_2 / t_{end}
m_{shoots}: mass of test item in shoots at t_1 / t_2 / t_{end}

B. Material Balance

Mean material balances were 101.6% AR for t_1 (range from 100.6 to 103.4% AR), 100.1% AR for t_2 (range from 98.9 to 101.1% AR) and 99.2% AR for t_{end} (range from 97.7 to 103.4% AR).

There were no signs for losses of radioactivity from the test systems or during sample processing. No adsorption of fluopicolide to the walls of the test systems was observed throughout the test.

C. Translocation of Parent Compound

During the incubation period 79.9 to 422.0 mL of the test solution was taken up by the plants. About 10.0 mL of the test solutions were lost due to evaporation after 6 days.

The mean initial concentrations (t_{start}, DAT-0) of [phenyl-UL-^{14}C]-M-01 in test solutions was 103.0 µg/L, increasing to 104.5 µg/L at t_1 (first interim sampling at DAT 2), to 107.8 µg/L at t_2 (second interim sampling at DAT 4) and then to 113.4 µg/L at t_{end} (end of study at DAT-6). Root wash desorbed 1.0 µg of the test item from the roots at t_1, 0.9 µg at t_2 and 1.1 µg at t_{end}. The separate analysis of roots and shoots showed that at t_1 92.7%, at t_2 95.0% and at t_{end} 95.5% of radioactivity taken up by the plants was translocated from roots into shoots.

No degradation of M-01 was observed at the end of the study in test solution from the stability control (without plants).

III. Conclusion

M-01 (AE C653711) was translocated into the shoots of potato plants, with 92.7, 95.0 and 95.5% of the radioactivity taken up by the plants translocated from roots into shoots after 2, 4 and 6 days, respectively. The TSCF for M-01 amounted to 0.86 ± 0.09 after 2 days, 0.75 ± 0.05 after 4 days and 0.72 ± 0.05 after 6 days.

This study confirms experimentally the predicted TSCF of 0.48 calculated for M-01 with the Briggs equation based on the Log P_{ow} value of 0.7.

Assessment and conclusion by applicant:

The study is considered valid to assess the TSCF of M-01 in plants.

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

A comprehensive range of adsorption/desorption studies have been conducted with fluopicolide and therefore column leaching studies would provide no further useful information to determine mobility and are considered unnecessary.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

In the light of the comprehensive range of adsorption/desorption studies with the soil metabolites reported under Point CA 7.1.2.1.2, column leaching studies would provide no further information and are considered unnecessary.

CA 7.1.4.2 Lysimeter studies

A lysimeter study with [2,6-pyridyl-14C]-labelled fluopicolide (KCA 7.1.4.2/01) and a related report in which the metabolites were identified from leachate samples (KCA 7.1.4.2/02) were evaluated during the previous EU review and are still considered as reliable to assess the fate and mobility of fluopicolide and its metabolites in soil.

A short letter report describing attempts to devise synthetic routes to the metabolites M-11, M-12 and M-13 in 2003 (KCA 7.1.4.2/03) was also evaluated during the previous EU review and is still considered valid. Two similar reports describing attempts to devise synthetic routes to M-11, M-12 (KCA 7.1.4.2/04) in 2016 and M-13 (KCA 7.1.4.2/05) in 2017 are provided as new data not yet reviewed.

A modelling report deriving a formation fraction for the metabolite M-15 (AE 1413903) in lysimeter leachate data was submitted as part of the Confirmatory Data (KCA 7.1.4.2/05) for the first submission of fluopicolide. This has been superseded by KCA 7.1.4.2/08 and consequently is not summarised in this dossier. KCA 7.1.4.2/08 and KCA 7.1.4.2/09 are provided as new data not yet reviewed. KCA 7.1.4.2/08 and KCA 7.1.4.2/09 are similar modelling reports deriving formation fractions for the metabolite M-15 and the metabolites M-11, M-12 and M-13, respectively in lysimeter leachate data.

Finally, for procedural reasons a statement addressing the non-relevance of the fluopicolide metabolites in lysimeter and field leaching studies (KCA 7.1.4.2/04) has to be included in the current dossier. This position paper has been superseded by Document N4 in the current dossier and consequently is not summarised in this dossier.

Report reference	Author, Year	Comment
KCA 7.1.4.2/01 M-218506-01-1	[redacted] 2004	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
KCA 7.1.4.2/02 M-218465-01-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
KCA 7.1.4.2/03 M-227129-01-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
KCA 7.1.4.2/04 M-227293-01-1	[redacted] 2004	Submitted and reviewed for first approval of fluopicolide, 2005. Superseded by Document N4.
KCA 7.1.4.2/05 M-628814-01-1	[redacted] 2018	Submitted as additional information regarding fluopicolide confirmatory data on metabolite M-15, 2018. Reviewed and accepted by RMS Austria. Superseded by M-687165-01-1.
KCA 7.1.4.2/06 M-584253-01-1	[redacted] 2016	New data not yet reviewed.
KCA 7.1.4.2/07 M-638433-01-1	[redacted] 2017	New data not yet reviewed.
KCA 7.1.4.2/08 M-687165-01-1	[redacted] 2020	New data not yet reviewed.
KCA 7.1.4.2/09 M-687853-01-1	[redacted] 2020	New data not yet reviewed.

Data Point:	KCA 7.1.4.2/01
Report Author:	[redacted]
Report Year:	2004
Report Title:	(14C)AE C638206: Leaching in outdoor lysimeters following spring/summer applications
Report No:	C035403
Document No:	M-218506-01-1
Guideline(s) followed in study:	BBA: IV, 4-3, (1990)
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The leaching potential of [2,6-pyridyl-^{14}C]-fluopicolide was investigated in two square stainless steel lysimeters (L32 and L33) containing undisturbed soil monoliths (silty sand, Gleyic Cambisol) of 1 m^2 soil-surface and 110 cm depth under outdoor conditions for a period of two years after the last application.

The applications were performed at the maximum proposed annual application rate of 400 g active ingredients (a.i.)/ha for both lysimeters. Four equal amounts of formulated [2,6-pyridyl-^{14}C]-fluopicolide were applied, in the same growing season, to the lysimeters cropped with potatoes.

In lysimeter L33, following the first years' potato harvest, potatoes were again planted in the second vegetation period and treated with a second four-fold application. In lysimeter L32, winter wheat was sown and grown to maturity instead of potatoes. The further crop rotation for both lysimeters was winter wheat and winter barley.

The potatoes and all subsequent crops in the following years were maintained and harvested according to Good Agricultural Practice (GAP) as far as possible in the small plot size. The surrounding area was cultivated with the same crop in order to avoid edge effects and to achieve an identical microclimate consistent with the field situation. Air temperature, air humidity, wind speed, solar radiation and precipitation were recorded at the test facility and supplementary irrigation carried out as appropriate to ensure a total precipitation of approximately 800 mm per year. One additional lysimeter at the test facility was designated as a control lysimeter. It remained untreated and was reserved to monitor soil temperature and soil humidity.

Following the first application of fluopicolide in May 1999, the production of leachate was monitored routinely in bi-weekly intervals and the leachate was collected if the volume exceeded one litre.

The radioactivity in the leachate was determined by liquid scintillation counting (LSC). Samples containing > 0.1 µg a.i. equivalents/L were analysed by LSC following acidification to pH < 2 to determine the amounts of $^{14}CO_2$ present. Further analysis of leachate water samples was performed by HPLC on samples with the concentration of a.i. equivalents > 0.10 µg/L after removal of $^{14}CO_2$.

The overall mass balances of 50.24% and 53.98% of applied radioactivity (% AR) in harvested plants, leachate and the soil monoliths for L32 and L33, respectively, indicated significant mineralisation to $^{14}CO_2$ under outdoor conditions.

The amount of leachate collected each year ranged from 333.5 to 469.2 L resulting in a mean total volume of leachate water of 1123.8 L (41% of the total of the water that reached the plots).

The radioactivity recovered in the leachate samples during the entire monitoring period accounted for 6.35 and 3.81% AR in lysimeters L32 and L33, respectively. The majority of residues was recovered from the soil with 43.11% AR in L32 and 48.91% AR in L33 after three years with the majority of the radioactivity located in the top 20 cm of the soil profile.

The concentration of radioactivity in leachate reached a peak of 5.05 and 4.45 µg a.i. equivalents/L for L32 and L33, respectively, in the first year (week 41 after the first application). The leaching of the bromide tracer monitored during the first year started at the same time, reached its maximum slightly earlier (week 31 to 33) and decreased again to values close to the LOD of 1.0 mg/L. Thereafter, the concentrations of radioactivity decreased to temporary minimum values of 0.77 and 0.47 µg a.i. equivalents/L for L32 and L33, respectively. During the second year of monitoring the concentration of radioactivity in leachate water reached maximum values of 3.26 µg a.i. equivalents/L for L32 and 6.47 µg a.i. equivalents/L for L33. Similarly to the previous year, the concentration declined to a temporary minimum (0.54 and 1.37 µg a.i. equivalents/L for L32 and L33). Within the third year, the concentrations of radioactivity increased again to maxima of 2.48 µg a.i. equivalents/L for L32 and 6.52 µg a.i. equivalents/L for L33, before slightly decreasing towards the end of the study to 2.28 and 5.83 µg a.i. equivalents/L when the last leachate samples were collected.

The parent compound, fluopicolide, did generally not occur in single leachate samples in amounts > LOQ (0.008 to 0.028 µg/L). Nevertheless, it appeared in total 3 times > LOQ each in the first leachate after treatment from one lysimeter, L33, at a maximum concentration of 0.76 µg/L, together with the conservative tracer bromide. These observations were considered to be a specific, artificial effect in L33 resulting from fast flow transport in e.g. macropores. The annual average concentration of fluopicolide in pooled annual leachate samples from L33 was below LOD in all years (LOD ranged from ≤ 0.004 to ≤ 0.014 µg/L). No parent was detected in L32.

The metabolites M-05 (AE 1344122, P1x in the report) and M-10 (AE 1344123, P4 in the report) were found to reach maximum annual average concentrations of 0.902 and 0.831 µg/L, respectively. No other single metabolite was detected in leachate at annual concentrations > 0.75 µg/L. The metabolites M-14 (AE 1388273, P7 in the report), M-11 (P2a in the report), M-12 (P2b in the report) and M-13 (P3 in the report) were found to reach maximum annual average concentrations of 0.194, 0.546, 0.364 and 0.137 µg/L, respectively. No other metabolite was detected in leachate at annual concentrations > 0.10 µg/L. The metabolites M-15 (AE 1413903, P8 in the report) and M-16 (P9 in the report) were found to reach maximum annual average concentrations of 0.095 and 0.080 µg/L, respectively.

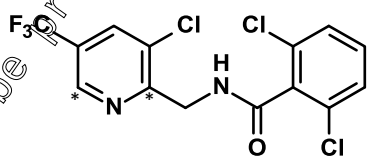
This study showed that even under worst-case conditions for leaching, fluopicolide will not pose a risk to groundwater at a depth of one metre or more.

I. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-fluopicolide



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

Specific Activity:	at or close to 4.87 MBq/mg
Radiochemical Purity:	≥ 97.8% for individual formulations (HPLC and TLC analyses)

For L32, an average specific radioactivity of 4.871 MBq/mg was used in all quantifications. For L33, a specific radioactivity of 4.873 MBq/mg was used for the first and of 4.871 MBq/mg for the second and third monitoring year.

Batch 903AE-1 was used for applications 1 and 2 in 1999 and batch 903AE-3 was used for applications 3 and 4 in 1999 and for all applications in 2000.

2. Test Soil

The study was performed using one test soil as characterized in Table 7.1.4.2- 1. Undisturbed soil monoliths of 1 m2 surface and 110 cm depth were taken on 11 September 1998, approximately seven months prior to study initiation, from an agriculturally used field in Münster-Handorf (Germany) and transported to the lysimeter station at Covance. The stainless steel lysimeter cores were inserted in watertight outer containers, which are located in the ground of the lysimeter station so that the surface of the lysimeter soil was level with the surrounding soil surface.

Table 7.1.4.2- 1: Physico-chemical properties of test soil

Parameter	Soil		
Soil Designation	Gleyic Cambisol		
Horizon	Ap	GoBv	Go
Depth [cm]	0-30	30-85	85-130
Geographic Location City Country	 Münster-Handorf Germany		
Textural Classification (USDA)	silty sand		
Sand [50 - 2000 µm] (%) Silt [2 – 50 µm] (%) Clay [< 2 µm] (%)	78 18 4	86 12 2	86 12 2
pH in $CaCl_2$	5.2[1]	5.7	5.7
Organic Matter (%) [2]	1.9	0.2	0.09
Organic carbon (%)	1.10	0.1	0.05

[1] The historical average pH in the top soil was typically pH 5.0 to 5.5. The pH of the top soil determined on 7 December 1998 ranged from pH 6.09 to 6.17 due to liming performed on 22 September 1998.

[2] Calculated as organic carbon content * 1.72

B. Study Design

1. Experimental Conditions

The tests were performed in two square stainless steel lysimeters (L32 and L33) containing undisturbed soil monoliths (silty sand, Gleyic Cambisol) of 1 m^2 soil-surface and 110 cm depth under outdoor conditions for a period of two years after the last application. The lysimeter station was located on the site of Covance Laboratories GmbH, Münster, Germany (geographical location: 51°55'07" north and 7°35'40" east, 61 m above sea level).

The applications were performed using formulated [2,6-pyridyl-^{14}C]-fluopicolide at the maximum proposed annual application rate of 400 g active ingredient (a.i.)/ha for both lysimeters. Therefore, four equal amounts of [2,6-pyridyl-^{14}C]-fluopicolide were applied, in the same growing season, to the lysimeters cropped with potatoes using a pneumatic sprayer. The test item was first applied on 27 May 1999 to potatoes at growth stage 17, at an application rate of 103.1 g a.i./ha for L32 and 105.6 g a.i./ha for L33 (nominal rate 100 g ai/ha). The three following applications were performed on 17 June 1999 (106.1 g and 108.4 g ai/ha), on 20 July 1999 (106.8 g and 108.1 g ai/ha) and on 30 July 1999 (106.2 g

and 107.4 g a.i./ha for lysimeter L32 and L33, respectively). On 25 May 1999, two days before the first application, the conservative tracer bromide was applied at a nominal application rate of 100 kg/ha.

The potatoes and all subsequent crops in the following years were maintained and harvested according to Good Agricultural Practice (GAP) as far as possible in the small plot size. The surrounding area was cultivated with the same crop in order to avoid edge effects and to achieve an identical microclimate consistent with the field situation. Air temperature, air humidity, wind speed, solar radiation and precipitation were recorded at the test facility and supplementary irrigation carried out as appropriate to ensure a total precipitation of approximately 800 mm per year. One additional lysimeter at the test facility was designated as a control lysimeter. It remained untreated and was reserved to monitor soil temperature and soil humidity. Fertilizer and pesticides to control weeds, pests or diseases were applied according to Good Agricultural Practice during the vegetation periods.

In lysimeter L33, following the first years' potato harvest (13 August 1999), potatoes were again planted in the second vegetation period (in 2000) and treated with a second four-fold application of [2,6-pyridyl-^{14}C]-fluopicolide according to the same regime as in the first year (105.0 g a.i./ha, 108.5 g a.i./ha, 92.8 g a.i./ha and 105.7 g a.i./ha on 06 May 2000, 19 May 2000, 23 June 2000 and 10 July 2000). In lysimeter L32, winter wheat was sown on 21 October 1999 and grown to maturity instead of potatoes. The further crop rotation for both lysimeters was winter wheat (sown on 20 October 2000) and winter barley (sown on 25 September 2001) with the latter harvested in summer on 08 July 2002. Full details are shown in table below.

Vegetation periods	Lysimeter 32		Lysimeter 33	
	Crop	Planting date	Crop	Planting date
First	Potatoes cv. *Cilena*	12 April 1999	Potatoes cv. '*Cilena*'	12 April 1999
Second	Winter wheat cv. '*Greif*' commercially treated with Arena C (25.04 g/L fludioxonil + 5.03 g/L tebuconazole)	21 October 1999	Potatoes cv. '*Sieglinde*'	07 April 2000
Third	Winter wheat cv. '*Asketis*' commercially treated with Arena C (25.04 g/L fludioxonil + 5.03 g/L tebuconazole	20 October 2000	Winter wheat cv. '*Asketis*' commercially treated with Arena C (25.04 g/L fludioxonil + 5.03 g/L tebuconazole	20 October 2000
Fourth	Winter barley cv. '*Candesse* commercially treated with SolitSr (25 g/L cyprodinil, 25 g/L fludioxonil, 10 g/L tebuconazole)	25 September 2001	Winter barley cv. '*Candesse*' commercially treated with SolitSr (25 g/L cyprodinil, 25 g/L fludioxonil, 10 g/L tebuconazole)	25 September 2001

2. Sampling

Following the first application of fluopicolide in May 1999, the production of leachate was monitored routinely in bi-weekly intervals and the leachate was collected if the volume exceeded one litre. Leachate was collected for the first time on 16 November 1999 (25 weeks after the first application) from L32 and on 19 October 1999 (21 weeks after the first application) from L33 after a period of no leachate production during the summer 1999. Leachate was sampled until 18 April 2000 for L32 and 02 May 2000 in L33. The leaching period for lysimeters L32 and L33 started again on 08 August 2000 and 19 September 2000 and lasted until 01 May 2001. The last leaching period started much earlier for L32 on 10 July 2001 than for L33 (18 September 2001) and the last leachate was collected on 14 May 2002 for the entire monitoring period.

Soil cores (3.5 cm diameter) were taken from the lysimeters to a depth of 15 cm after each crop harvest (25 August 1999, 21 July 2000 (L32), 14 August 2000 (L33) and 27 July 2001). The soil cores were separated in 5 cm segments, combined according to depth and analysed to characterise the nature of the radioactivity present in the soil at these time points. At the end of the study on 29 July 2002 the lysimeters were sectioned. The upper 30 cm of the soil monoliths was excavated and segmented into 10 cm layers. Eight 5 cm diameter cores were taken from the subsoil to a depth of 110 cm and the cores sectioned into 10 cm layers. The respective 10 cm layers of all cores were combined for analysis.

3. Analytical Procedures

The radioactivity in the leachate was determined by liquid scintillation counting (LSC). Samples containing > 0.1 µg a.i. equivalents/L were analysed by LSC following acidification to pH < 2 to determine the amounts of $^{14}CO_2$ present. Further analysis of leachate water samples was performed by HPLC on samples with the concentration of a.i. equivalents > 0.10 µg/L after removal of $^{14}CO_2$.

Two different reverse phase HPLC systems were used routinely in the analysis of leachate samples. The initial HPLC method was used to analyse leachate samples from the first year, while those from year 2 and 3 and the pooled samples were analysed using a second different HPLC method developed to provide better resolution of the radioactive components detected in leachate. In order to enable direct comparison of leachate each year, pooled annual samples were prepared in the ratio of the leachate volumes collected on individual sampling occasions for each lysimeter. These samples were analysed using the second HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) was calculated for each individual analysis and each compound. The LOD ranged between 0.004 to 0.014 µg/L while the LOQ ranged between 0.008 to 0.028 µg/L.

Subsamples of leachate removed for analysis were stored at -20 ± 4 °C in the dark.

Soil samples of each layer were analysed for distribution of total radioactivity by combustion/LSC. Samples containing significant levels of radioactivity were extracted at ambient temperature once using acetonitrile/water (1/1, v/v) and twice using acetonitrile followed by a Soxhlet extraction step. Radioactivity in soil extracts was determined by LSC. Finally, all soil extracts were combined and analysed by the initial HPLC method using a different gradient program. Radioactivity associated with soil bound residues was quantified after extraction by combustion/LSC.

The total radioactive residue (TRR) of the crops was determined separately for the characteristic fractions (e.g. grain and straw) by combustion analysis and LSC.

II. Results and Discussion

The leachate data for lysimeters L32 and L33 is presented in Table 7.1.4.2- 2. The annual average concentration of fluopicolide and its metabolites determined in pooled annual samples for each year of the lysimeter study are summarised in Table 7.1.4.2- 3 and Table 7.1.4.2- 4.

A.Data

Table 7.1.4.2- 2: Leachate data for lysimeters L32 (winter wheat) and L33 (potatoes)

Year	Precipitation & irrigation (mm)	Lysimeter L32		Lysimeter L33	
		Application: May to July 1999 422.2 g/ha		Application: May to July 1999 429.5 g/ha	
		Application: -		Application: May to July 2000 412.1 g/ha	
		Leachate		Leachate	
		Radioactivity (% of applied)	Annual average concentration (µg parent equivalents/L)	% of applied	Annual average concentration (µg/L)
1	850.1	2.67	3.36	1.20	2.80
2	875.7	1.83	2.06	1.04	2.62
3	1008.0	1.85	1.66	1.57	3.53
Total	2738.8	6.35	n.a.	3.81	n.a.

n.a. = not applicable

Table 7.1.4.2- 3: Annual average concentrations of [2,6-pyridyl-^{14}C]-fluopicolide and its degradation products in leachate of lysimeter L32

Compound	Year		
	1	2	3
Fluopicolide (parent)	<LOD	<LOD	<LOD
M-05 (AE 1344122, P1x)	0.372	0.331	0.310
M-10 (AE 1344123, P4)	0.831	0.067	0.097
M-11 (P2a)	0.546	0.450	0.301
M-12 (P2b)	0.364	0.300	0.200
M-13 (P3)	0.052	0.078	0.066
M-14 (AE 1388273, P7)	0.035	0.080	0.080
M-15 (AE 1413903, P8)	0.032	0.041	<LOQ
M-16 (P9)	0.025	0.041	<LOD
unknown M54 [1]	0.024	0.020	<LOD
unknown P6A [1]	0.018	0.018	<LOD
unknown P6 [1]	<LOD	0.039	0.056
unknown M3 [1]	0.015	0.033	0.017
unknown M29 [1]	0.016	<LOD	<LOD

n.d. = not detected

LOD = Limit of detection (0.004 to 0.014 µg/L)

LOQ = Limit of quantification (0.008 to 0.028 µg/L)

[1] not identified due to amounts of < 0.10 µg/L for all monitoring years and in both lysimeters, nomenclature of these metabolites in this table is identical to study report

Table 7.1.4.2- 4: Annual average concentrations of [2,6-pyridyl-^{14}C]-fluopicolide and its degradation products in leachate of lysimeter L33

Compound	Year		
	1	2	3
Fluopicolide (parent)	<LOD	<LOD	<LOD
M-05 (AE 1344122, P1x)	0.305	0.455	0.902
M-10 (AE 1344123, P4)	0.679	0.229	0.287
M-11 (P2a)	0.466	0.468	0.337
M-12 (P2b)	0.311	0.312	0.225
M-13 (P3)	0.042	0.083	0.137
M-14 (AE 1388273, P7)	0.045	0.061	0.194
M-15 (AE 1413903, P8)	<LOD	0.090	0.095
M-16 (P9)	<LOD	0.053	0.080
unknown M54 [1]	<LOD	0.025	<LOQ
unknown P6A [1]	<LOD	0.032	<LOQ
unknown P6 [1]	<LOD	0.029	0.071
unknown M3 [1]	<LOD	0.016	0.040
unknown M29 [1]	0.085	<LOQ	<LOQ

LOD = Limit of detection (0.004 to 0.014 µg/L)

LOQ = Limit of quantification (0.008 to 0.028 µg/L)

[1] not identified due to amounts of ≤ 0.10 µg/L for all monitoring years and in both lysimeters, nomenclature of these metabolites in this table is identical to study report

B. Leachate

The total amount of precipitation and irrigation in the first monitoring year (27 May 1999 to 22 May 2000) was 850.1 mm for L32 and 830.1 mm for L33 and the total amount of leachate water collected was 335.6 L and 360.3 L for L32 and L33, respectively. Within the second monitoring year (23 May 2000 to 04 June 2001), the sum of precipitation and irrigation was 875.7 mm and the amount of leachate water collected was 374.2 L and 333.5 L for L32 and L33, respectively. The total precipitation and irrigation in the third monitoring year (05 June 2001 to 09 July 2002) was 1008.0 mm and the respective leachate volumes were 469.2 L for L32 and 374.9 L for L32, respectively. The total of precipitation and irrigation over the three monitoring years was 2733.8 mm (2713.8 mm for L33) and the mean total volume of leachate water 1123.8 L (41% of the total of the water that reached the plots).

C. Material balance

The overall mass balances of 50.24% and 53.98% of applied radioactivity in harvested plants, leachate and the soil monoliths for L32 and L33, respectively, indicated significant mineralisation to $^{14}CO_2$ under outdoor conditions.

Table 7.1.4.2- 5: Mass balance in Lysimeter L32 and L33 after 3 years

Matrix	Fraction	Lysimeter L32	Lysimeter L32
Soil (after 3 years)	0-10 cm	20.84	26.50
	10-20cm	16.72	16.90
	20-30 cm	3.43	2.97
	30-40 cm	0.87	0.85
	40-50 cm	0.30	0.41
	50-60 cm	0.19	0.23
	60-70 cm	0.12	0.21
	70-80 cm	0.12	0.19
	80-90 cm	0.13	0.20
	90-100 cm	0.16	0.21
	100-105 cm	0.24	0.25
Sum in soil		43.11	48.91
Crop residues	Years 1- 3	0.78	1.26
Leachate	Year 1	2.67	1.20
	Year 2	1.83	1.04
	Year 3	1.85	1.57
Total		50.24	53.98

D. Residues in leachate and soil

On average 41% of the precipitation (including supplemental irrigation) received by the lysimeters over the three years of the study was collected as leachate (range 37% to 47%). Radioactivity was detected in both lysimeters during the first year, beginning 21 weeks (L33) and 25 weeks (L32) after the first application, respectively, on the 19 October 1999 and 16 November 1999. The concentration of radioactivity in leachate reached a peak of 5.05 and 4.45 µg a.i. equivalents/L for L32 and L33, respectively, in the first year (7 March 2000, week 41 after the first application). The leaching of the bromide tracer monitored during the first year started at the same time, reached its maximum slightly earlier (week 31 to 35) and decreased again to values close to the LOD of 1.0 mg/L. Thereafter, the concentrations of radioactivity decreased to temporary minimum values of 0.77 and 0.47 µg a.i. equivalents/L for L32 and L33, respectively, in autumn 2000. During the second year of monitoring the concentration of radioactivity in leachate water reached maximum values of 3.26 µg a.i. equivalents/L for L32 on 03 April 2001 and 6.47 µg a.i. equivalents/L for L33 on 01 May 2001. Similarly to the previous year, the concentration declined to a temporary minimum (0.54 and 1.37 µg a.i. equivalents/L for L32 and L33) in winter 2001. Within the third year, the concentrations of radioactivity increased again to maxima of 2.48 µg a.i. equivalents/L for L32 on the 5 March 2002 and 6.52 µg a.i. equivalents/L for L33 on the 2 April 2002, before slightly decreasing towards the end of the study to 2.28 and 5.83 µg a.i. equivalents/L in May 2002 when the last leachate samples were collected. The radioactivity recovered in the leachate samples during the entire monitoring period accounted for 6.35% and 3.81% of the applied radioactivity (% AR) in lysimeters L32 and L33, respectively.

The majority of residues was recovered from the soil with 43.11% AR in L32 and 48.91% AR in L33 after three years with the majority of the radioactivity located in the top 20 cm of the soil profile. Radioactivity decreased in the 20-30 cm layer to ≤ 3.43 and ≤ 0.87% AR in the 30-40 cm layer. Below 40 cm depth, levels of radioactivity were ≤ 0.41% AR in all of the deeper soil layers. Of the ca. 26% AR that was extractable from the top 20 cm, virtually all was identified as fluopicolide. Approximately 10% AR was non-extractable from the soil profile after three years.

A total of 0.78% and 1.26% of applied radioactivity was recovered from the four crop harvests grown on L32 and L33, respectively.

E. Volatile radioactivity

No significant quantities of dissolved $^{14}CO_2$ were detected in leachate.

F. Degradation of parent compound

The parent compound, fluopicolide, was detected in the first two leachate samples collected from L33 in the first year of monitoring. The concentrations amounted to 0.76 µg/L and 0.18 µg/L on 19 October and 16 November 1999. In all other samples analysed throughout the three monitoring years, the test item was not present in quantifiable concentrations except in L33 on 19 September 2000 at a concentration of 0.055 µg/L. These observations were considered to be a specific effect in L33 resulting from artificial fast flow transport in macropores. The annual average concentration of fluopicolide in pooled annual leachate samples from L33 was below LOD in all years (LOD ranged from ≤ 0.004 to ≤ 0.014 µg/L). No parent was detected in L32.

The chromatographic pattern obtained from the individual and the pooled annual leachate samples demonstrated that a number of degradation products were present throughout the monitoring period. The degradation products M-05 (called Plx in the report), M-11 and M-12 (P2a and P2b in the report) exceeded 0.10 µg/L in the mean of all three monitoring years in both lysimeters with 0.305 µg/L to 0.902 µg/L for M-05, 0.301 µg/L to 0.546 µg/L for M-11 and 0.200 µg/L to 0.364 µg/L for M-12 in the annual average. M-10 (P4 in the report) was determined in amounts above 0.1 µg/L in both lysimeters during the first year and only in L33 in the second and third year ranging from 0.229 µg/L to 0.831 µg/L. M-14 (P7 in the report) was usually below 0.10 µg/L in the annual average except in L33 in the third year (0.194 µg/L). M-13 (P3 in the report) remained ≤ 0.083 µg/L in both lysimeters and in all monitoring years except for 0.137 µg/L in L33 during the third year. The annual mean values were ≤ 0.095 µg/L for M-15 (P8 in the report) and ≤ 0.080 µg/L for M-16 (P9 in the report) during the entire study. A number of smaller components (called M54, P6A, P6, M3 and M29 in the study report) and unresolved radioactivity were below 0.10 µg/L for all monitoring years and in both lysimeters and, thus, was not identified.

Metabolites, M-05, M-10, M-11, M-12, M-13, M-14, M-15 and M-16 were not observed in laboratory soil metabolism studies conducted with the parent and were identified from leachate samples in a separate study [KCA 7.1.4.2/02, M-218465-01-1]. In that study the metabolite P2 was shown to consist of 2 isomers, named P2a now called M-11 and P2b now called M-12, present in leachate at a ratio of 6:4. As such, the concentrations of P2 were subdivided to calculate the annual average concentrations of M-11 (P2a) and M-12 (P2b).

Soil laboratory degradation studies conducted with leachate metabolites [see Point CA 7.1.2.1.2] have shown that the slowest rate of degradation of these metabolites was observed in the Münster soil. Thus the concentrations of these metabolites observed in the lysimeter study represented worst-case values based on the degradation rate observed in other soils investigated in laboratory studies.

III Conclusion

The parent compound, fluopicolide, did generally not occur in single leachate samples at amounts > LOQ. Nevertheless, it appeared in total 3 times > LOQ each in the first leachate after treatment from one lysimeter, L33, at a maximum concentration of 0.76 µg/L, together with the conservative tracer bromide. These observations were considered to be a specific, artificial effect in L33 resulting from fast flow transport in e.g. macropores. The annual average concentration of fluopicolide in pooled annual leachate samples from L33 was below LOD in all years (LOD ranged from ≤ 0.004 to ≤ 0.014 µg/L). No parent was detected in L32.

The metabolites M-05 and M-10 were found to reach maximum annual average concentrations of 0.902 and 0.831 µg/L, respectively. No other single metabolite was detected in leachate at annual concentrations > 0.75 µg/L. The metabolites M-11, M-12, M-13 and M-14 were found to reach maximum annual average concentrations of 0.546, 0.364, 0.137 and 0.194 µg/L, respectively. No other metabolite was detected in leachate at annual concentrations > 0.10 µg/L. The metabolites M-15 and M-16 were found to reach maximum annual average concentrations of 0.095 and 0.080 µg/L, respectively

This study has shown that even under worst-case conditions for leaching, fluopicolide will not pose a risk to groundwater at a depth of one metre or more. A number of further studies on M-05, M-10 and M-14 have been conducted as part of the work undertaken to prove the non-relevance of all metabolites present in leachate at annual concentrations in excess of 0.1 µg/L.

Assessment and conclusion by applicant:

The study was conducted in accordance with BBA IV, 4-3, (1990). The study is considered valid to aid assessment of the leaching potential of fluopicolide and its metabolites in the environment. Definitive assessment of the leaching potential of fluopicolide and its metabolites is provided by FOCUS groundwater assessment (see Document MCP-9).

Data Point:	KCA 7.1.4.2/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	Investigation on leachate samples from lysimeters treated with (2,6-pyridinyl-14C)-AE C638206
Report No:	C035262
Document No:	M-248465-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

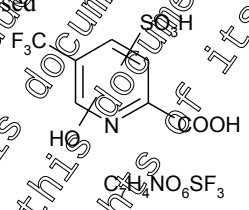
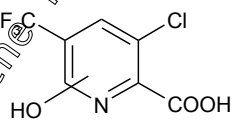
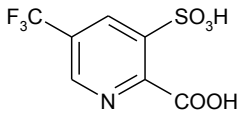
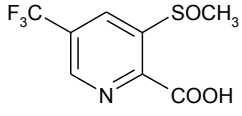
Executive Summary

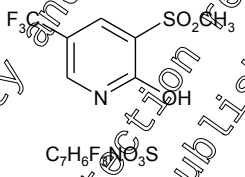
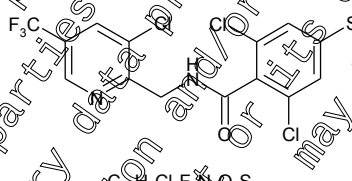
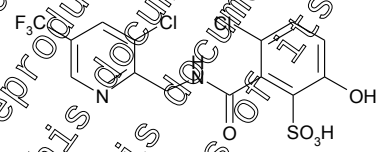
Leachates of lysimeters treated with [2,6-pyridyl-^{14}C]-fluopicolide at a dose rate of 400 g/ha, were selected for peak concentrations of radioactivity in order to identify metabolites present. Samples collected in spring from year 2000-2002 from 2 soil cores (lysimeter 23 and 33) and three aliquots of autumn percolates from year 2001 (lysimeter 33) were concentrated and analysed by reversed-phase HPLC for the metabolic pattern present. The seven components that have been reported to be around or above the maximum annual average concentration of 0.1 ug/L were detectable in all of the investigated leachates in this study. These radioactive metabolites were named P2, P3, P4, P1x, P7, P8 and P9 in the original report (now called M-11/M-12, M-13, M-10, M-05, M-14, M-15 and M-16) and were observed at approximately 4, 7, 10, 21, 30, 56, and 57 min, respectively. Their maximum annual concentrations determined within the lysimeter trial were 0.92 ug/L (M-11/M-12), 0.14 ug/L (M-13), 0.84 ug/L (M-10), 0.90 ug/L (M-05), 0.19 ug/L (M-14), 0.09 ug/L (M-15) and 0.08 ug/L (M-16). During isolation of P2 (M-11/M-12) it was shown that this fraction consisted of 2 subfractions, named M-11 (P2a) and

M-12 (P2b), of which M-11 contributed with ca. 60% to the radioactive peak within the chromatogram and M-12 with approximately 40%. As such, the maximum annual average concentrations of M-11 and M-12 were calculated to be 0.55 ug/L and 0.37 ug/L, respectively.

HPLC profiles of individual leachate samples confirmed the presence of each of the metabolites of interest and thus purified fractions were combined as appropriate and subjected to further purification to achieve structure elucidation by LC-MS/MS, FT-MS and 1H-NMR. All metabolites present within the leachate at or above an annual average concentration of 0.1 ug/L were isolated with the intention to identify their structure.

As far as technically feasible structural proposals were made for each metabolite, although for three metabolites it was not possible to define the exact position of one or two substituent groups within the structures using mass spectroscopy techniques. For the majority of metabolites unequivocal structures were proposed and confirmed by comparison with certified reference standards, including all metabolites which exceeded annual concentrations of 0.75 µg/L in the lysimeter study.

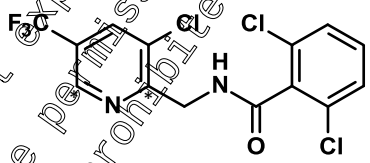
Metabolite	Characterisation by HPLC and LC-MS/MS	Maximum Annual Average Concentration	Structure
M-11/M-12 (P2) [P2a/P2b]	Retention time ca. 4 min Molecular mass: 287 Splits into 2 fractions M-11 (P2a), M-12 (P2b) by chromatography using a diol-phase. Isomers of the structure given are suggested	0.91 µg/L M-11 (P2a), 0.55 µg/L M-12 (P2b), 0.36 µg/L	Proposed  $C_7H_4NO_6SF_3$
M-13 (P3)	Retention time ca. 7 min Molecular mass: 241	0.14 µg/L	Proposed  $C_7H_3ClF_3NO_3$
M-10 (P4)	Retention time ca. 10 min Molecular mass: 271	0.83 µg/L	Confirmed: AE 1344123  $C_7H_4F_3NO_5S$
M-05 (P1x)	Retention time ca. 21 min Molecular mass: 253	0.90 µg/L	Confirmed: AE 1344122  $C_8H_6F_3NO_3S$

Metabolite	Characterisation by HPLC and LC-MS/MS	Maximum Annual Average Concentration	Structure
M-14 (P7)	Retention time ca.30 min Molecular mass: 241	0.19 µg/L	Confirmed: AE 1388273  $C_7H_6F_3NO_3S$
M-15 (P8)	Retention time ca. 56 min Molecular mass: 462	0.095 µg/L	Confirmed: AE 1413903  $C_{14}H_8Cl_3F_3N_2O_4S$
M-16 (P9)	Retention time ca. 57 min Molecular mass: 444	0.08 µg/L	Proposed unequivocally  $C_{14}H_8Cl_2F_3N_2O_5S$

I. Materials and Methods

Seven radioactive components were detected in leachate samples from a lysimeter study conducted with [2,6-pyridyl-14C]-fluopicolide at concentrations at or close to 0.1 µg/L. That study has been summarized previously [KCA 7.1.4.2.01, M-218506-01-1]. Leachate samples collected from two lysimeters (Lysimeter 32 and 33) in the spring of the years 2000 to 2002 and from lysimeter 33 in the autumn of 2001 were transferred to this study in order to isolate and identify these radioactive components. Leachate samples containing peak concentrations of the radioactive components were selected and the details of the samples are summarized in Table 7.1.4.2- 6.

[2,6-Pyridyl-^{14}C]-fluopicolide



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

Table 7.1.4.2- 6: Details of leachate samples used in the study

Date of sampling [dd/mm/yy]	Concentration [µg Fluopicolide equivalents/L]	Lysimeter	Volume [L]	Sample ID
22.02.00	4.4	32	1.0	L3
07.03.00	5.1	32	1.0	L4
21.03.00	4.8	32	1.0	L5
04.04.00	4.4	32	0.9	L6
08.02.00	3.4	33	1.0	L7
22.02.00	4.1	33	1.0	L8
07.03.00	4.4	33	1.0	L9
21.03.00	3.9	33	1.0	L10
04.04.00	3.7	33	1.0	L11
18.04.00	3.5	33	1.0	L12
03.04.01	5.8	33	10	L16
17.04.01	6.4	33	10	L17
01.05.01	6.5	33	1.0	L1
01.05.01	6.5	33	1.0	L2
01.05.01	6.5	33	6	L18
18.09.01	3.6	33	6	L19
02.10.01	2.9	33	9	L20
16.10.01	2.4	33	8	L21
05.03.02	5.5	33	10	L22
19.03.02	6.4	33	6.6	L13
02.04.02	6.5	33	7.3	L14
14.05.02	5.8	33	9.2	L15

Isolation of metabolites:

Initially, from a total of 12 litres of leachate the 5 predominant metabolites, M-11/M-12, M-13, M-10, M-05, and M-14 were isolated by fractionation on a polar reversed-phase column.

M-05 and M-14 were further purified by chromatography on a Nucleodur C18 Gravity column, followed by solid phase extraction using silica gel for M-05 and polar reversed-phase chromatography for M-14.

Sufficient purification for structure elucidation was achieved for M-13 and M-10 by repeated isolation and re-analysis by reversed-phase chromatography. For M-13, additional attempts were made to confirm the initial structural proposals. Further isolation was carried out from leachate samples L13-L15 and L16-L22 which combined consisted of a total of 83 litres of leachate. A sequence of chromatographic separations with different selectivities was used to finally obtain a fraction of sufficient purity for structure analysis. In total, M-13 was isolated from three leachate batches and the structural information obtained from each batch was in agreement.

Isolation of M-11/M-12 required the application of a series of different analytical methods because the majority of impurities present in leachate, such as soil matrix and salts co-eluted with this polar metabolite. Purification was finally obtained by rigorous selection of the peak from each chromatographic run performed. As a result, recoveries of M-11/M-12 were limited.

Initial purification of M-11/M12 was achieved following repeated solid-phase extraction on various modified silica and polymer based phases, followed by selective organic extraction and finally, chromatography on a diol-phase column prior to mass spectrometry. The diol-phase chromatography separated M-11/M-12 into 2 subfractions, named M-11 and M-12. A second scaled-up purification of M-11/M-12 undertaken to allow for FT-MS and ^{1}H-NMR spectrometry was achieved using reversed-phase chromatography, selective organic extraction, followed by HPLC analysis involving the use of an ion pairing reagent and then normal phase diol-chromatography. M-11/M-12 was again separated into two fractions M-11 and M-12, by diol-phase chromatography in the ratio 60 : 40, respectively. The radioactive fractions were collected individually and required an additional purification step prior to mass spectrometry. A hydrophilic, zwitterionic ZIC®-HILIC column was used for the final purification step. Further fractionation of M-12 was observed which was thought to be a chromatographic effect of the Hilic column.

M-15 and M-16 were detected within the leachate with a maximum annual concentration of 0.095 µg/L and 0.080 µg/L, respectively. These metabolites were isolated initially from 23 litres of leachate and in a second larger scale purification from *ca.* 60 litres of leachate. Purification was achieved by organic extraction, followed by reverse-phase chromatography on an Aqua C18 column using a variety of elution conditions.

Structure elucidation:

M-05 was identified by LC-MS/MS showing fragments in the positive and negative ion mode with a molecular mass of 253. The absence of chlorine was indicated. The structure of M-05 was proposed as 3-methylsulfinyl-5-trifluoromethylpyridine-2-carboxylic acid and subsequently confirmed by co-chromatography with the reference standard of M-05 (AE 1344122).

M-11/M-12 (P2) was characterised by weak retention on polar reversed-phase HPLC columns. A molecular mass of 287 was obtained from LC-MS/MS analyses run after the first small-scale preparation. The absence of chlorine was indicated. Daughter fragments of m/z 222 and m/z 242 were characteristic. Analyses for the isolated components from the second batch, M-11 and M-12, confirmed the fragmentation pattern detected in the first preparation and, additionally, FT-MS analysis suggested the same molecular formula $C_7H_4NO_6SF_3$ for each of the subpeaks. The structures of P2a and P2b were proposed as isomers of *x*-hydroxy-*y*-sulfo-5-trifluoromethylpyridin-2-carboxylic acid. Further attempts were made to define the structural arrangement by means of ^{1}H-NMR spectrometry but no further information on the substitution pattern was obtained.

In summary, M-11/M-12 present within the crude leachate concentrates was not considered to be a homogeneous fraction and was proposed to be composed of two isomers, M-11 and M-12. Every technically feasible effort was undertaken to elucidate the final structures, but the exact substitution pattern remained unknown.

M-13 was characterised by LC-MS/MS and FT-MS resulting in a structural formula of $C_7H_3ClF_3NO_3$ with a molecular mass of 241. The structure was proposed as 3-chloro-5-trifluoromethyl-*x*-hydroxypyridine-2-carboxylic acid. The reference standard of M-13 (RPA 433654, 3-chloro-6-hydroxy-5-trifluoromethylpyridine-2-carboxylic acid) did not coelute with M-13 although ^{1}H-NMR spectra of M-13 confirmed the presence of the hydroxy-group attached to the pyridine ring. Spectra generated from the reference standard of M-13 demonstrated the same fragmentation pattern but due to limited amounts of M-13 available the hydroxylation site could not be confirmed by additional investigations.

M-10 was identified by LC-MS/MS showing a mass of 271 and specific daughter ion spectra indicating fragmentation of CO_2^- and SO_2^- from the molecular ion. ^{1}H-NMR spectrometry resulted in a structure proposal of 2-carboxy-5-trifluoromethylpyridine-3-sulfonic acid as M-10. The structure was confirmed by co-chromatography with the standard of M-10 (AE 1344123).

LC-MS/MS analysis of M-14 within the negative ion mode indicated loss of -CH_3, -SO_2-CH_3 and -CO as significant fragments from a molecular mass of 241. The structure was suggested as 2-hydroxy-3-mesyl-5-trifluoromethylpyridine and the proposal was verified by co-chromatography with the synthesis standard of M-14 (AE 1388273).

M-15 was identified by the combination of LC-MS/MS, FT-MS and ^{1}H-NMR. Accurate mass determination indicated a molecular formula of $C_{14}H_8Cl_3F_3N_2O_4S$ and interpretation of the negative and positive MS-daughter spectra, as well as ^{1}H-NMR-spectrometry, suggested the structure of M-15 to be 3,5-dichloro-4-[(3-chloro-5-trifluoromethyl-pyridine-2-ylmethyl)carbamoyl]benzene sulfonic acid. The structure proposal was confirmed by co-elution with the reference standard of M-15 (AE 1413903).

Structure elucidation of M-16 was obtained by LC-MS/MS, FT-MS and ^{1}H-NMR spectrometry. The LC-MS/MS results revealed a molecular mass of 444. Negative daughter fragments were detected at m/z 425, 407, 232 and 206, positive daughter ions were detected at m/z 427, 235, 211 and 194. Accurate mass determination suggested $C_{14}H_9Cl_2F_3N_2O_5S$ as the molecular formula. Interpretation of the ^{1}H-NMR spectra indicated that the sulfonic acid group was in the ortho-position of the hydroxyl-group and the structure was therefore defined as 3-chloro-2-[({[3-chloro-5-(trifluoro-methyl)pyridin-2-yl]methyl}amino)carbonyl]-6-hydroxy-benzenesulfonic acid.

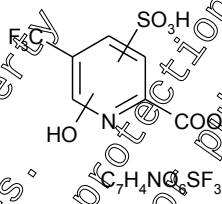
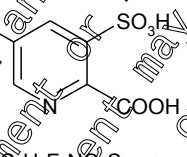
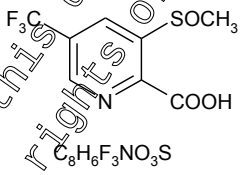
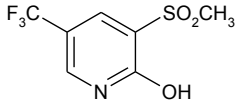
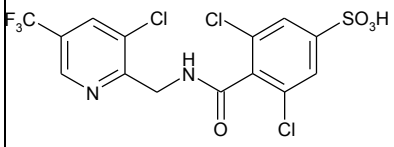
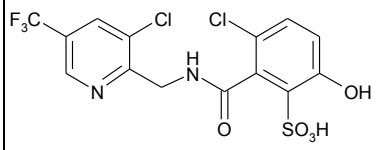
II. Results and Discussion

All metabolites present in leachate at or above an annual average concentration of 0.1 µg/L were isolated with the intention of identifying their structures. As the HPLC profiles of individual leachate samples confirmed the presence of each of the metabolites of interest, purified fractions were combined as appropriate and subjected to further purification to achieve structure elucidation by LC-MS/MS, FT-MS and ^{1}H-NMR.

The metabolites were named M-11/M-12, M-13, M-10, M-05, M-14, M-15 and M-16 and had HPLC retention times of approximately 4.7, 9.21, 30, 56, and 58 minutes, respectively. Their maximum annual average concentrations in leachate were determined within the lysimeter trial as 0.91 µg/L (M-11/M-12), 0.14 µg/L (M-13), 0.83 µg/L (M-10), 0.90 µg/L (M-05), 0.194 µg/L (M-14), 0.095 µg/L (M-15) and 0.08 µg/L (M-16). During isolation of M-11/M-12 it was shown that this fraction consisted of 2 subfractions, named M-11 and M-12 present in a ratio of approximately 60:40. As such, the maximum annual average concentrations of M-11 and M-12 were calculated to be 0.55 µg/L and 0.36 µg/L, respectively. The values quoted for M-11/M12, M-10, M-14 and M-11 in the report are slightly different. The values given above were taken from the final lysimeter report [KCA 7.1.4.2/01, M-218506-01-1] which are considered to be definitive.

The structures and molecular masses of metabolites are summarised in Table 7.1.4.2- 7.

Table 7.1.4.2- 7: Characterisation and identification of metabolites of [2,6-pyridyl-^{14}C]-fluopicolide detected in lysimeter leachates

Metabolite	Characterisation by HPLC and LC-MS/MS	Maximum Annual Average Concentration	Structure
M-11/M-12 (P2) [P2a/P2b]	Retention time ca. 4 min Molecular mass: 287 Splits into 2 fractions M-11 (P2a), M -12 (P2b) by chromatography using a diol-phase. Isomers of the structure given are suggested	0.91 µg/L M-11 (P2a), 0.55 µg/L M-12 (P2b), 0.36 µg/L	Proposed  $C_7H_4NO_6SF_3$
M-10 (P4)	Retention time ca.10 min Molecular mass: 241	0.83 µg/L	Confirmed: AE 1344123  $C_7H_4F_3NO_5S$
M-05 (P1x)	Retention time ca.21 min Molecular mass: 223	0.90 µg/L	Confirmed: AE 1344122  $C_8H_6F_3NO_3S$
M-14 (P7)	Retention time ca.30 min Molecular mass: 241	0.19 µg/L	Confirmed: AE 1388273  $C_7H_6F_3NO_3S$
M-15 (P8)	Retention time ca. 56 min Molecular mass: 462	0.095 µg/L	Confirmed: AE 1413903  $C_{14}H_8Cl_3F_3N_2O_4S$
M-16 (P9)	Retention time ca. 57 min Molecular mass: 444	0.08 µg/L	Proposed unequivocally  $C_{14}H_9Cl_2F_3N_2O_5S$

III. Conclusion

Seven radioactive regions were isolated from leachate samples originating from a lysimeter study conducted with fluopicolide. One of the regions was shown to be composed of two metabolites. Molecular masses and structural formulas were determined for each of the eight metabolites which enabled actual concentrations in leachate to be reported [KCA 7.1.4.2/01, M-218506-01-1].

As far as technically feasible structural proposals were made for each metabolite, although for three metabolites it was not possible to define the exact position of one or two substituent groups within the structures using mass spectroscopy techniques. For the majority of metabolites unequivocal structures were proposed and confirmed by comparison with certified reference standards, including all metabolites which exceeded annual concentrations of 0.75 µg/L in the lysimeter study.

The structural proposals enabled further risk assessments of each metabolite to be made. A number of further studies with M-05, M-10 and M-14 were conducted as part of the work undertaken to assess the relevance of all metabolites present in leachate at annual concentrations in excess of 0.1 µg/L.

Assessment and conclusion by applicant:

Metabolites of [2,6-pyridyl-^{14}C]-fluopicolide were isolated and identified from samples of leachate. The study is considered valid to aid assessment of the leaching potential of fluopicolide and its metabolites in the environment.

Data Point:	KCA 7.1.4.2/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	Synthesis of leachate metabolites of AE C638206
Report No:	C039695
Document No:	M-227129-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Seven radioactive components were detected in leachate samples from a lysimeter study conducted with [2,6-pyridyl-^{14}C]-fluopicolide at concentrations at or close to 0.1 µg/L. Metabolites were isolated from leachate samples and the radioactive components identified by mass spectrometry and nuclear magnetic resonance (NMR) methods. As far as technically feasible structural proposals were made for each metabolite, although for three metabolites (previously called P2a, P2b and P3 and now called M-11, M-12 and M-13) it was not possible to define the exact position of one or two substituent groups within the structures using spectroscopic techniques.

Following extensive mass spectrometric analysis, the structure of M-11/M-12 (P2) was partially assigned as *x*-hydroxy-*y*-sulfo-5-(trifluoromethyl)pyridine-2-carboxylic acid [KCA 7.1.4.2/02, M-218465-01-1]. In that study the metabolite M-11/M-12 (P2) was shown to consist of 2 isomers, named M-11 (P2a) and M-12 (P2b), present in leachate at a ratio of 6:4.

From that work the positions of the sulfonic and hydroxy substituents on the pyridine ring could not be determined. Extensive past experience in metabolic chemistry suggested that the most likely position for the sulfonic group was the pyridine 3-position. Synthetic experience also suggested that the chlorine atom in 3-chloro substituted pyridines such as M-02 (AE C657188) could fairly easily be replaced by sulfur-containing groups (as was done, for example, in the synthesis of M-05 (AE 1344122) and M-10 (AE 1344123). In addition, such groups could easily be oxidised. This left only 3 positions for an oxygen atom: at positions 1 (ie the N-oxide), 4 or 6.

Loss of the group at position 2 was noticed both during attempts to make the N-oxide during attempts to make the N-oxide of M-13, and in one of the synthetic routes to M-14 (AE 1388273). It is probable therefore that such pyridine-2-carboxylic acid N-oxides or alkyl esters thereof are prone to decarboxylation or dealkoxycarbonylation.

These observations would perhaps explain why, despite sustained intensive efforts including trying no less than twelve routes to these isomers, no success was achieved in synthesising them.

Following similar analysis to that conducted on M-11/M-12, the structure of M-13 was partially assigned as 3-chloro-5-trifluoromethyl-x-hydroxypyridine-2-carboxylic acid.

The N-oxide was ruled out on the basis of NMR evidence on the leachate (the aromatic region contained only a singlet, as would be expected for the 4- or 6-hydroxy derivatives but not for the N-oxide). Also, decarboxylation again accompanied an attempt using a very mild method to de-esterify an ester of the N-oxide, suggesting that the P3 N-oxide structure is unstable. The M-13, 6-hydroxy derivative structure was also ruled out, as the compound was synthesised and its structure established beyond doubt by X-ray analysis. It did not co-elute with the corresponding leachate metabolite. Attempts to synthesise the 4-hydroxy isomer failed, whether attempted directly or as an intermediate in the synthesis of M-11/M12 4-hydroxy isomer. It is therefore not possible at present to confirm the position of the hydroxyl group.

By virtue of the nature and juxtaposition of the functional groups in the structures proposed for the leachate metabolites named 4- or 6-hydroxy M-13, 4-hydroxy P2 and 6-hydroxy P2, the metabolites represent highly complex chemical substances for which no precedent exists in fluopicolide chemistry or in the published literature on pyridine chemistry.

I. Materials and Methods

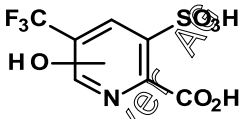
Seven radioactive components were detected in leachate samples from a lysimeter study conducted with [2,6-pyridyl-^{14}C]-fluopicolide at concentrations at or close to 0.1 µg/L. That study has been summarized previously [KCA 7.1.4.2/01, M-218506-01-1]. Metabolites were isolated from leachate samples and the radioactive components identified by mass spectrometry and nuclear magnetic resonance methods. As far as technically feasible structural proposals were made for each metabolite, although for three metabolites (previously called P2a, P2b and P3 and now called M-11, M-12 and M-13) it was not possible to define the exact position of one or two substituent groups within the structures using spectroscopic techniques [KCA 7.1.4.2/02, M-218465-01-1].

The attempts to synthesise the most probable structures of M-11, M-12 and M-13 to enable final confirmation of the exact structures were summarised in this letter/report.

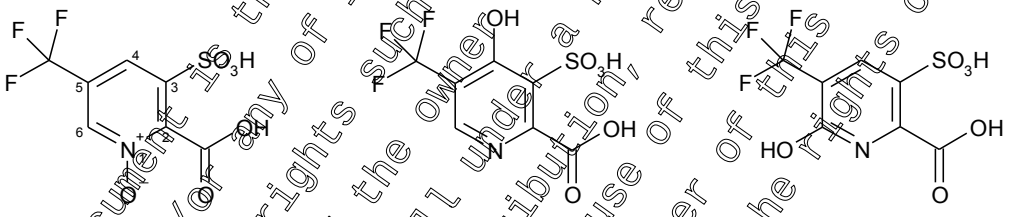
II. Results and Discussion

M-11/M-12 (P2, P2a/P2b)

Following extensive mass spectrometric analysis, the structure of M-11/M-12 (P2) was partially assigned as *x*-hydroxy-*y*-sulfo-5-(trifluoromethyl)pyridine-2-carboxylic acid [Annex II, Section 7, point 7.1.3.3, Report C035362]. In that study the metabolite M-11/M-12 (P2) was shown to consist of 2 isomers, named M-11 (P2a) and M-12 (P2b), present in leachate at a ratio of 6:4.



From that work the positions of the sulfonic and hydroxy substituents on the pyridine ring could not be determined. Extensive past experience in metabolic chemistry suggested that the most likely position for the sulfonic group was the pyridine 3-position. Synthetic experience also suggested that the chlorine atom in 3-chloro substituted pyridines such as M-02 (AE C657188) could fairly easily be replaced by sulfur-containing groups (as was done for example in the synthesis of M-05 (AE 1344122) and M-10 (AE 1344123). In addition, such groups could easily be oxidised. This left only 3 positions for an oxygen atom: at positions 1 (ie the N-oxide), 4 or 6. The corresponding structures were as follows:



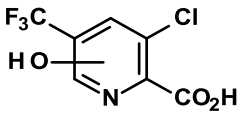
M-11/M-12 N-oxide M-11/M-12 4-hydroxy derivative M-11/M-12 6-hydroxy derivative

Loss of the group at position 2 was noticed both during attempts to make the N-oxide, during attempts to make the N-oxide of M-13 (below), and in one of the synthetic routes to M-14 (AE 1388273). It is probable therefore that such pyridine-2-carboxylic acid N-oxides or alkyl esters thereof are prone to decarboxylation or dealkoxycarbonylation. Support for this assertion was provided by computational chemistry considerations. Ab initio calculations indicated that the stability of the anionic species formed during decarboxylation / dealkoxycarbonylation of compounds such as the N-oxide would greatly be enhanced by sulfonate anion formation leading to the decarboxylated / dealkoxycarbonylated product. This would result from capture of a proton by the carbanion first formed on loss of carbon dioxide, and lead to formation of the above-mentioned sulfonate anion. Calculations based on the same carbanion intermediate hypothesis showed similarly that the 4- and 6-hydroxy isomers also lacked stability.

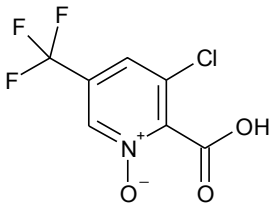
These observations would perhaps explain why, despite sustained intensive efforts including trying no less than twelve routes to these isomers, no success was achieved in synthesising them.

M-13

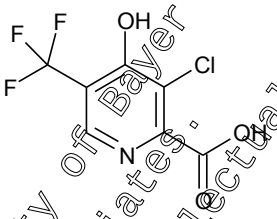
Following similar analysis to that conducted on M-11/M-12, the structure of M-13 was partially assigned as 3-chloro-5-trifluoromethyl-x-hydroxypyridine-2-carboxylic acid:



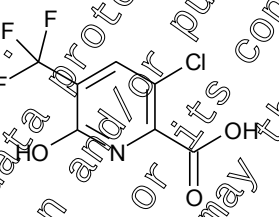
Once again 3 structures were possible:



M-13 N-oxide



M-13 4-hydroxy derivative



M-13 6-hydroxy derivative

The N-oxide was ruled out on the basis of nmr evidence on the leachate (the aromatic region contained only a singlet, as would be expected for the 4- or 6-hydroxy derivatives but not for the N-oxide). Also, decarboxylation again accompanied an attempt using a very mild method to de-esterify an ester of the N-oxide, suggesting that the P3 N-oxide structure is unstable. The M-13, 6-hydroxy derivative structure was also ruled out, as the compound was synthesised and its structure established beyond doubt by X-ray analysis. It did not co-elute with the corresponding leachate metabolite. Attempts to synthesise the 4-hydroxy isomer failed, whether attempted directly or as an intermediate in the synthesis of M-11/M12 4-hydroxy isomer. It is therefore not possible at present to confirm the position of the hydroxyl group.

III. Conclusion

By virtue of the nature and juxtaposition of the functional groups in the structures proposed for the leachate metabolites named 4- or 6-hydroxy M-13, and 4-hydroxy and 6-hydroxy M-11/M-12, the metabolites represent highly complex chemical substances for which no precedent exists in fluopicolide chemistry or in the published literature on pyridine chemistry. Neither are any suitably tetrasubstituted intermediates available from chemical suppliers, or via synthesis from suitable available starting materials. Extensive programs of research were undertaken with the object of devising synthetic routes to these substances. No success was achieved in these programs, and it was concluded the synthetic problems cannot be solved without further extensive fundamental research for which, based on present experience, little or no success is envisaged.

Assessment and conclusion by applicant:

This statement documents the original attempts to devise synthetic routes to M-11, M-12 and M-13 in 2003 and is considered valid, providing justification for the need for bridging studies with other pyridyl ring metabolites to meet the data requirements for these three leachate metabolites.

Data Point:	KCA 7.1.4.2/04
Report Author:	[redacted]
Report Year:	2004
Report Title:	The non-relevance of the metabolites of AEC 638206 found in lysimeter leachate and field leaching studies - Position paper
Report No:	C039866
Document No:	M-227293-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	No

In the previous submission (DAR, 2005), this statement (KCA 7.1.4.2/04, M-227293-01-1) was evaluated and accepted as valid to assess the non-relevance of the metabolites of fluopicolide found in lysimeter leachate and field leaching studies. However additional studies have been conducted and the groundwater exposure assessments have changed, thus the statement is no longer considered as valid. For procedural reasons it is included in the current dossier but it has been superseded by Document N4 Relevance of Metabolites in Groundwater and hence a summary is not presented in this dossier.

Data Point:	KCA 7.1.4.2/05
Report Author:	[redacted]
Report Year:	2018
Report Title:	Fluopicolide: Estimation of the formation fraction of the metabolite M-15 (AE 1413903) by inverse modelling of a lysimeter study
Report No:	VC18009C
Document No:	M-628814-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted by RMS Austria in 2018
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

In the Fluopicolide Confirmatory Data (2018) this modelling report (KCA 7.1.4.2/05, M-628814-01-1) was accepted as valid to derive formation fractions for M-15 to use in groundwater modelling. For procedural reasons it is included in the current dossier. However additional studies have been conducted and the endpoints used to derive formation fractions for M-15 have changed. This report has been superseded by the latest modelling report (see KCA 7.1.4.2/08, M-687165-01-1) and hence a summary is not presented in this dossier.

Data Point:	KCA 7.1.4.2/06
Report Author:	[redacted]
Report Year:	2016
Report Title:	Object: Synthesis of leachate metabolites M-11 and M-12 of Fluopicolide (AE C638206=BCS-AM59797)
Report No:	M-584253-01-1
Document No:	M-584253-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The compounds M-11 and M-12 represent tentatively assigned leachate metabolites of the fungicide fluopicolide. The structures were based exclusively on extensive mass spectroscopic analyses, as nuclear magnetic resonance spectra was not available.

M-11 and M-12 represent highly complex chemical compounds for which no precedent exist in published literature on pyridine chemistry. Suitable tetra-substituted intermediates which could function as precursors for M-11 and M-12 are not available from chemical suppliers or via synthesis from other suitable available starting materials.

Extensive research has been undertaken in the past but failed to synthesize M-11 and M-12. There have been more recent attempts to synthesize M-11 and M12 via multi-step synthesis.

Please refer to confidential document JCA for details.

Despite all the efforts taken and particularly due to the unstable nature of M-11 and M-12 under laboratory synthesis conditions it was not possible to synthesize metabolite M-11 or M-12.

Assessment and conclusion by applicant:

This statement documents further attempts to devise synthetic routes to M-11 and M-12 in 2016 and is considered valid, providing justification for the need for bridging studies with other pyridyl ring metabolites to meet the data requirements for these leachate metabolites.

Data Point:	KCA 7.1.4.2/07
Report Author:	[redacted]
Report Year:	2017
Report Title:	Synthesis of leachate metabolite M-13 (4-Hydroxy-P3) of fluopicolide (AE C638206= BCS-AM59797)
Report No:	M-638433-01-1
Document No:	M-638433-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

This paper discusses the ease of synthesis of M-13 (4-hydroxy-P3) a tentatively assigned leachate metabolite of the fungicide fluopicolide. The structure is based exclusively on mass spectroscopic analyses and proton nuclear magnetic resonance spectra (only one signal).

M-13 (4-hydroxy-P3) is a highly complex tetra-substituted chemical compound for which no precedent exists in the published literature on pyridine chemistry. Suitable tetra-substituted intermediates which could function as precursors for M-13 (4-hydroxy-P3) are not available from chemical suppliers or via synthesis from other suitable available starting materials.

Extensive research has been undertaken in the past but failed to synthesize M-13 (4-hydroxy-P3). Recent attempts to synthesize M-13 (4-hydroxy-P3) showed that the precursor M-13-methyl ester (4-hydroxy-P3) could be obtained but only in non-isolatable traces along with the main component M-13-methyl ester (6-hydroxy-P3). Following hydrolysis of the ester mixture even under mild basic or acidic conditions, the only product that could be isolated was M-13 6-hydroxy-P3. No 4-hydroxy-P3 was detected by LC-MS.

Please refer to confidential document JCA for details.

The results from the hydrolysis studies hypothesize that picolinic acids bearing a hydroxy substituent in 4-position of the heterocycle are unstable under laboratory conditions and cannot be synthesized.

Assessment and conclusion by applicant:

This statement documents further attempts to devise synthetic routes to M-13 in 2017 and is considered valid, providing justification for the need for bridging studies with other pyridyl ring metabolites to meet the data requirements for this leachate metabolite.

Data Point:	KCA 7.1.4.2/08
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide: - Estimation of the formation fraction of the metabolite M-15 (AE 1413903) by inverse modelling of a lysimeter study
Report No:	VC/19/041H
Document No:	M-687165-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A formation fraction for the metabolite M-15 (AE 1413903) from fluopicolide in soil was derived by inverse modelling data from a lysimeter study conducted with the parent compound. [2,6-pyridyl-^{14}C]-fluopicolide had been applied at a rate of 400 g/ha per year to lysimeters cropped with potatoes, followed by winter cereals rotations. Leachate flows and concentrations of conservative tracer (bromide), parent substance and its metabolites were monitored for three years (1999-2002). On site daily rain and air temperature were recorded.

A modelling evaluation of hydrology and solute transport was carried out to simulate the water balance in the two lysimeters and the chemical dynamic of fluopicolide and its metabolites.

Once an acceptable simulation of the lysimeter hydrology was obtained, after calibrating predicted evapotranspiration and soil parameters, inverse modelling of fluopicolide and its metabolite M-15 was carried out to derive a formation fraction for M-15 from parent material in each lysimeter. Inverse modelling was conducted to estimate formation fractions only, with DT_{50} and K_{oc} inputs fixed to those considered for forward groundwater modelling.

The optimal estimated formation fractions were only slightly different between the two lysimeters. M-15 showed best fitting between measured and simulated cumulated leached mass in lysimeter 32 with a formation fraction of 0.15% (ffm 0.0015). In lysimeter 33 best fitting was obtained with a formation fraction of 0.175% (ffm 0.00175). This resulted in a mean value of the two lysimeters of 0.16%. (ffm 0.0016).

I. Materials and Methods

The aim of this evaluation was to derive formation fractions for the metabolite M-15 from fluopicolide for use in groundwater exposure assessments. This was achieved by first simulating the water balance and hydrology of lysimeter soil cores and then subsequently estimating by inverse modelling a formation fraction for M-15 from fluopicolide.

A. Details of the Lysimeter Study

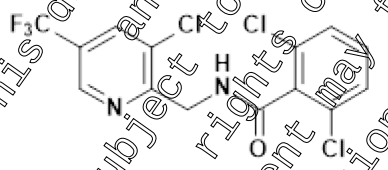
The lysimeter study was conducted under Northern European conditions in Germany with [2,6-pyridyl-^{14}C]- fluopicolide. The lysimeters contained an acidic silty sand soil (pH 5.2) with low organic carbon content selected to conform to BBA lysimeter soil requirements. The study was conducted over a three year period with two lysimeters treated at the maximum annual application rate (400 g/ha) in the first year (1999) and one lysimeter re-treated in the second year (2000). All applications were made to potatoes. Subsequent crop rotations were with winter cereals. On 25th May 1999, two days before the first application of fungicide, a conservative tracer, potassium bromide, was applied to both lysimeters at a rate of 100 kg/ha.

Air and soil temperatures were recorded continuously for the whole study period. All precipitation and any additional irrigation of the lysimeters were recorded.

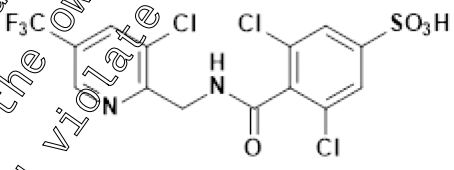
Leachate was collected regularly and analysed by liquid scintillation counting (LSC) to detect and quantify total radioactivity. Samples containing > 0.1 µg a.s. equivalents/L were concentrated by vacuum-evaporation prior to analysis by HPLC, and the concentration of fluopicolide and its metabolites, including M-15 measured in leachate samples.

B. Substance data

In this report inverse modelling was conducted to estimate formation fractions for M-15 only, with DT_{50} and K_{oc} inputs fixed to those considered for forward groundwater modelling.



Fluopicolide



M-15 (AE 1413903)

The parameters for fluopicolide are the same as those used in the groundwater modelling provided in Document MCP 9.

For M-15, DT_{50} and K_{oc} values were taken from studies conducted with the metabolite. The degradation endpoint for M-15 in each soil was selected from one of the three kinetic model fits, based on criteria following FOCUS (2014) guidance, and normalised to 20 °C and a moisture content of pF 2. The normalised DT_{50} values varied from 132.4 to 172.5 days and the geometric mean DT_{50} of 144.8 days was selected for modelling.

Table 7.1.4.2- 8: Summary of DegT_{50} values derived for M-15 (AE 1413903) under laboratory conditions

Applied compound	Study	Soil	Model selected	DT_{50} un-normalised (d)	DT_{50} normalised (d)
M-15	KCA 7.1.2.1.2/07 M-585202-01-1 [redacted] 2016	Dollendorf II	DFOP	172.5[a]	172.5[a]
		H[redacted]	DFOP	137.9[a]	137.9[a]
		I[redacted]	DFOP	139.6[a]	139.6[a]
		L[redacted]	DFOP	132.4[a]	132.4[a]
				Geometric mean	**144.8**

[a] Pseudo-SFO value based on slow phase of decline (calculated as $\ln(2)/k_2$)

The following input parameters were used for modelling fluopicolide and M-15.

Table 7.1.4.2- 9: Modelling parameters for fluopicolide and M-15

Parameter	Unit	Fluopicolide	M-15
Molecular weight	g mol^{-1}	383.59	463.3
DT_{50} in soil	d	182	145
K_{oc}	mL g^{-1}	267.7	18.8
K_{om}	mL g^{-1}	155.3	10.9
Freundlich exponent		0.888	0.94
Plant uptake factor	-	0.5	0

C. Hydrological Modelling

An accurate simulation of the water balance for the lysimeters was carried out as a prerequisite to the modelling evaluation of the behaviour of fluopicolide and M-15 using PEARL 4.4.4.

The soil hydrological characteristics were estimated from the lysimeter soil texture and organic carbon content. PEARL 4.4.4 meteorology files were based on-site weather measurements integrated with data from DWD (Deutscher WetterDienst, Münster/Osnabrücto meteo stat 1776) and MARS (Monitoring Agricultural ResourceS). On-site rainfall and supplementary irrigation from the lysimeter study was transferred to the PEARL scenario. In order to simulate the lysimeter water balance correctly it was necessary to optimise predicted evapotranspiration (PET) losses and to implement the different cropping and management regimes of the study. The cropping information from the lysimeter study was used to set up crop rotation scenarios in PEARL.

For solute modelling of fluopicolide and M-15, application scenarios were set up with dates and application rates detailed in the report for each lysimeter. Measured concentrations of M-15 in leachate were available for all three years of the lysimeter study. For Year 1 (and also Year 2 and 3) of the study the concentration of M-15 was available for pooled annual samples for each lysimeter (prepared in the ratio of leachate volumes collected on individual sampling occasions). In addition, for Year 2 and 3, but not for Year 1, measured concentrations of M-15 in individual leachate samples were available. These measured concentrations were compared to the simulated concentrations of M-15. The annual average concentrations in Year 1 (0.032 µg/L for lysimeter 32, <LOD of 0.005 µg/L for lysimeter 33) were used to calculate a cumulative mass of M-15 in the first year which was used to calibrate the output of the simulated concentrations for lysimeter 32.

II. Results and Discussion

The results of water balance modelling and simulations are shown in the figures below for lysimeters 32 and 33. The PEARL model, after being calibrated for soil, potential evapo-transpiration (PET) and crop rotations, predicted leachate volumes Figure 7.1.4.2- 1 with mean square error (MSE) values of 30.71 and 36.39 compared to measured leachate volumes for lysimeters 32 and 33, respectively. The corrected hydrological setting obtained was validated by simulating the concentrations of the applied bromide measured in the leachate samples Figure 7.1.4.2- 2.

Figure 7.1.4.2- 1: Measured and Simulated Leachate Amounts

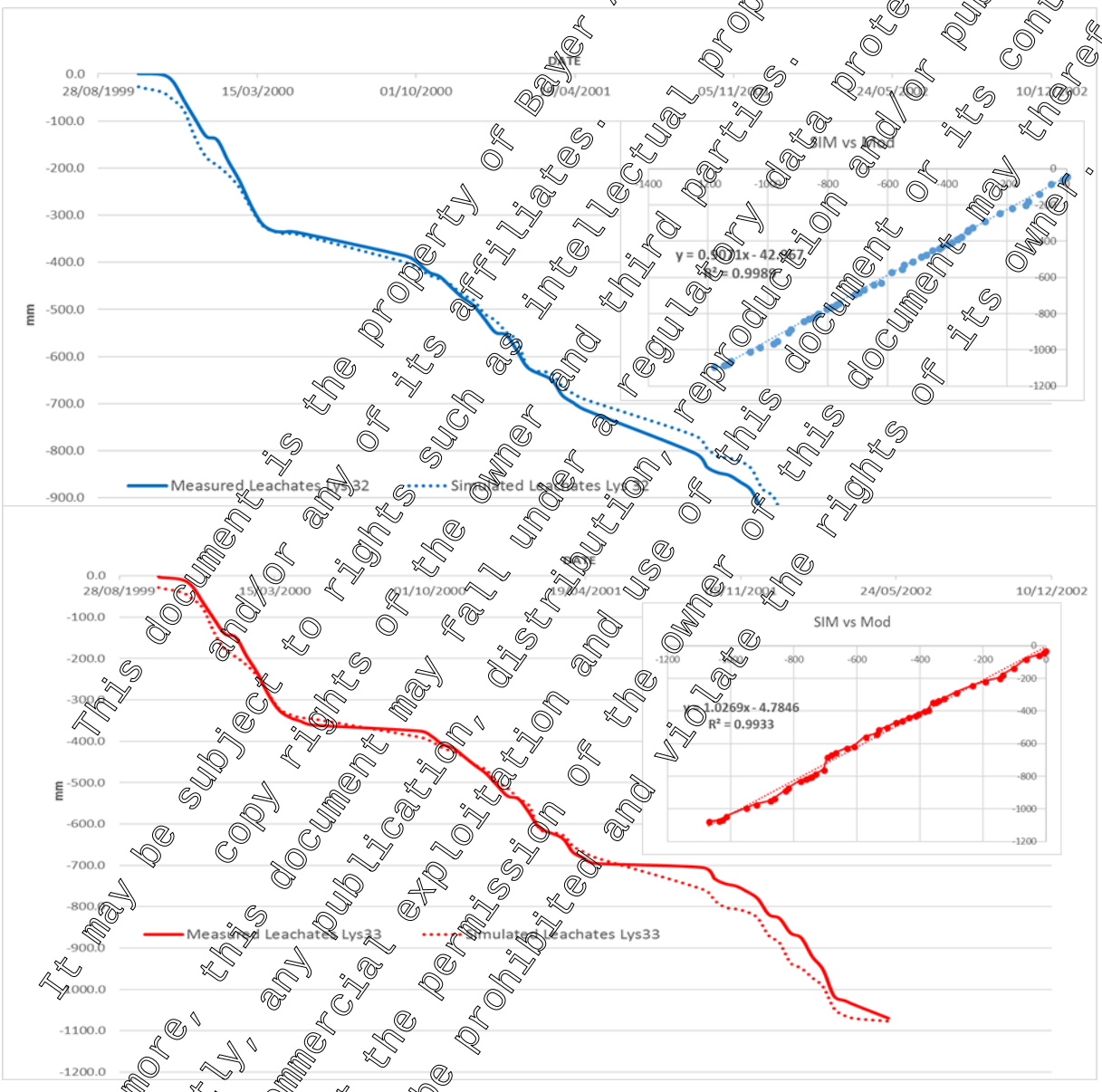
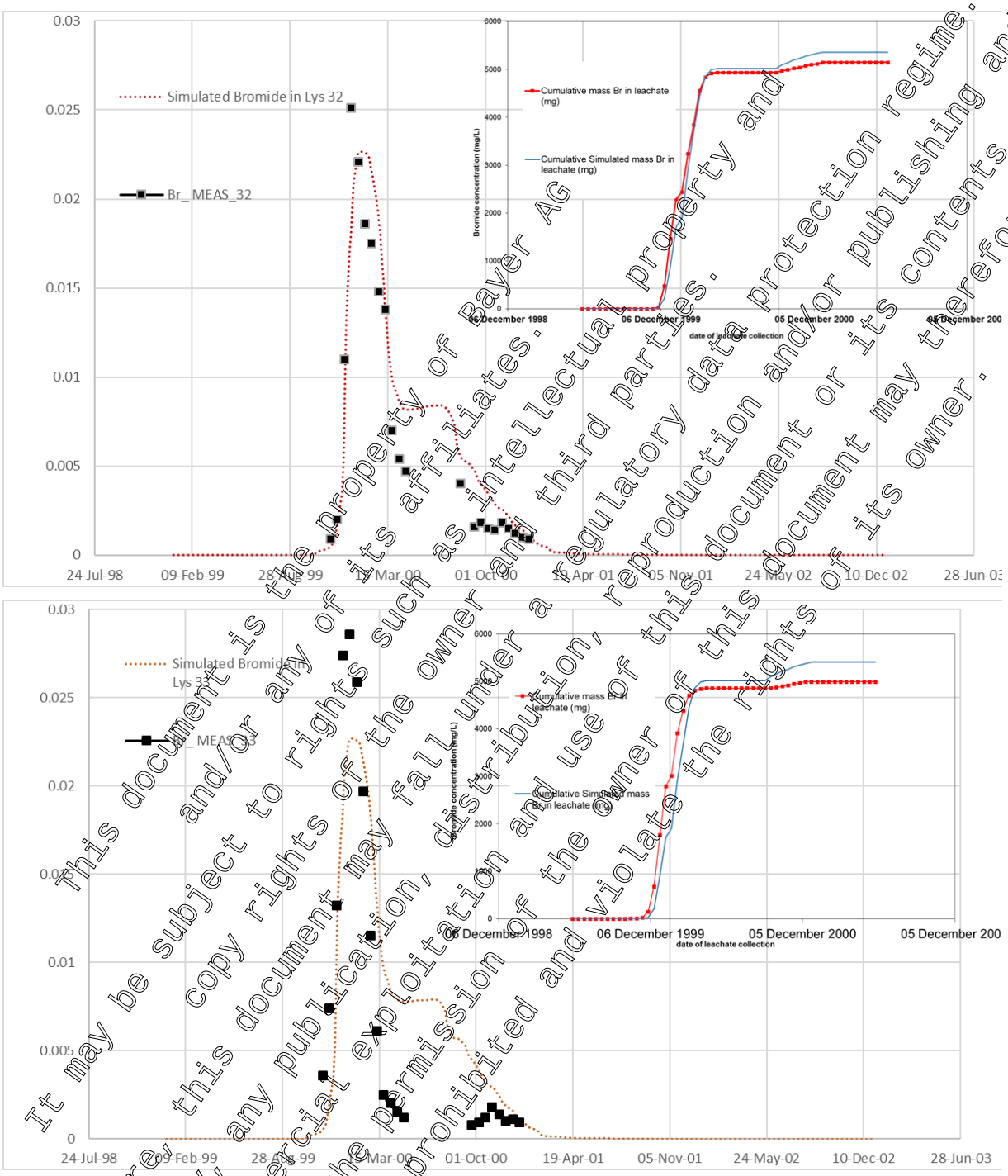


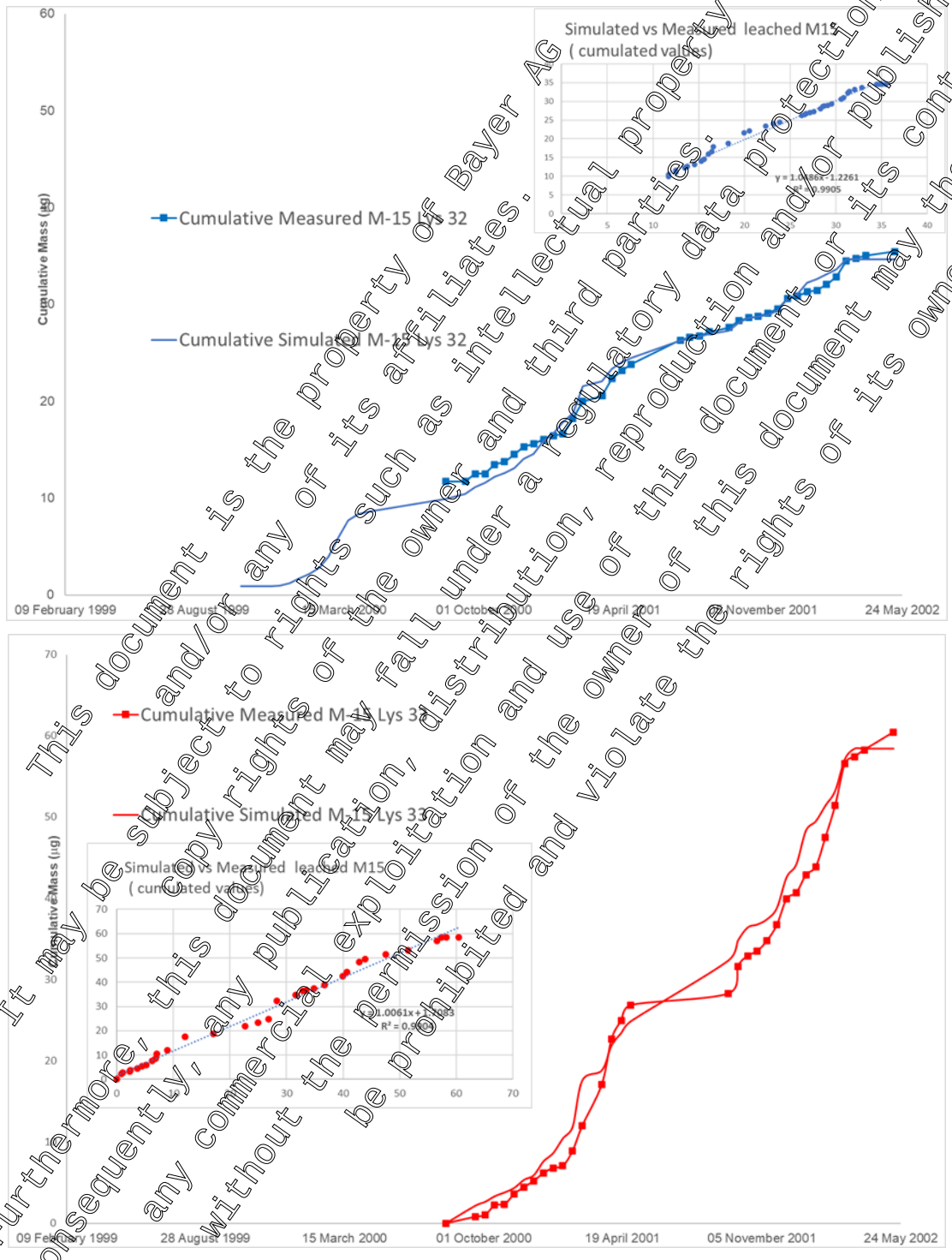
Figure 7.1.4.2- 2: Bromide Simulations in Lysimeters 32 and 33



After calibration, the simulated leaching flows were strongly correlated to measured data (R^2 >0.99), in the two lysimeters used in the study (Figure 7.1.4.2- 1). The concentration and cumulated leached mass of the applied tracer bromide from both the lysimeters were also adequately simulated by the calibrated input sets (Figure 7.1.4.2- 2). Thus validated, the inputs sets were applied to simulate the cumulative mass/concentrations of fluopicolide and M-15 in each lysimeter, assuming different formation fractions from the parent compound.

The best modelling fits of M-15 were obtained with formation fractions of 0.15% (0.00135) and 0.175% (0.00175) in lysimeters 32 and 33 respectively (R^2 >0.99, see Figure 7.1.4.2- 3). The relative mean square error (RMSE) between measured and simulated leachate concentrations of M-15 in the two lysimeters were 0.88 and 2.7 respectively, indicating robust simulation of the data.

Figure 7.1.4.2- 3: M-15 Simulations in Lysimeter 32 and 33 – Cumulative Load



III. Conclusion

Inverse modelling of the lysimeter study conducted with [^{14}C]- fluopicolide was performed with the model PEARL 4.4.4 using locally measured meteorological data, integrated with DWD and MARS meteo data.

The aim was to evaluate and compare modelled and measured datasets in order to i) simulate the water balance and hydrology of the soil cores and then ii) to subsequently estimate a suitable formation fraction of M-15 from fluopicolide.

After calibration, the simulated leaching flows were strongly correlated to measured data (R^2 >0.99) in the lysimeters used in the study. The concentration and cumulated leached mass of bromide, applied as a tracer, from both lysimeters were also adequately simulated by the calibrated input sets. Thus validated, the inputs sets were applied to simulate the cumulative mass concentrations of fluopicolide and M-15 in each lysimeter, assuming different formation fractions from the parent compound.

The best modelling fits of M-15 were obtained with formation fractions of 0.15% (0.00135) and 0.175% (0.00175) in lysimeters 32 and 33, respectively with a mean value of 0.16% (0.0016).

Assessment and conclusion by applicant:

This modelling report is considered valid to assess the formation fraction of M-15 (AE 1413903) from fluopicolide in soil.

Data Point:	KCA 7.1.4.2/09
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide: Estimation of the formation fraction of the metabolites M-11/12 and M-13 by inverse modelling of a lysimeter study
Report No:	VC/190411
Document No:	M-687853-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A formation fraction for the M-11&12 (P2) and M-13 (P3) in soil from the primary metabolite M-02 (AE C657188) derived with fixed formation fractions from the parent fluopicolide, either directly (0.47) or through the metabolite M-03 (AE 0608000) (0.53). The formation fractions were derived by inverse modelling data from a lysimeter study conducted with the parent compound. [2,6-pyridyl-^{14}C]-fluopicolide had been applied at a rate of 400 g/ha per year to lysimeters cropped with potatoes, followed by winter cereals rotations. Leachate flows and concentrations of conservative tracer (bromide), parent substance and its metabolites were monitored for three years (1999-2002). On site daily rain and air temperature were recorded.

A modelling evaluation of hydrology and solute transport was carried out to simulate the water balance in the two lysimeters and the chemical dynamic of fluopicolide and its metabolites.

Once an acceptable simulation of the lysimeter hydrology was obtained, after calibrating predicted evapotranspiration and soil parameters, inverse modelling of fluopicolide and its metabolites M-11&12 and M-13 was carried out to derive their formation fraction from M-02 metabolites in each lysimeter in each lysimeter. The modelling was set through the degradation chain detailed as above (FLC to M-03 ffm 0.53; FLC to M-02 ffm 0.47; M-03 to M-02 ffm 1). Inverse modelling was conducted to estimate formation fractions only from M-02 to M-11&M-12 and M-13, with DT_{50} and K_{oc} inputs fixed to those considered for forward groundwater modelling.

The optimal estimated formation fractions were only slightly different between the two lysimeters. M-11&12 and M-13 showed best fitting between measured and simulated cumulated leached mass in lysimeter 32 with a formation fraction from M-02 of 6.8% (ffm 0.068) and 2.7% (0.027), respectively. In Lysimeter 33 the best fitting was obtained with a formation fraction of 4 % (ffm 0.040) for M-11&12 and of 1.95% (ffm 0.195) for M13.This gives mean values of ffm 0.054 for M-11&12 and of ffm 0.0233 for M-13.

1. Materials and Methods

The aim of this evaluation was to derive formation fractions for the metabolites M-11&12 and M-13 from the fluopicolide metabolite M-02 (AE C657188) for use in groundwater exposure assessments. This was achieved by first simulating the water balance and hydrology of lysimeter soil cores and then subsequently estimating by inverse modelling formation fractions for M-11&12 and M-13 from M-02.

A. Details of the Lysimeter Study

The lysimeter study was conducted under Northern European conditions in Germany with [2,6-pyridyl-^{14}C]- fluopicolide. The lysimeters contained an acidic silty sand soil (pH 5.2) with low organic carbon content selected to conform to BBA lysimeter soil requirements. The study was conducted over a three year period with two lysimeters treated at the maximum annual application rate (400 g/ha) in the first year (1999) and one lysimeter re-treated in the second year (2000). All applications were made to potatoes. Subsequent crop rotations were with winter cereals. On 25th May 1999, two days before the first application of fungicide, a conservative tracer potassium bromide, was applied to both lysimeters at a rate of 100 kg/ha.

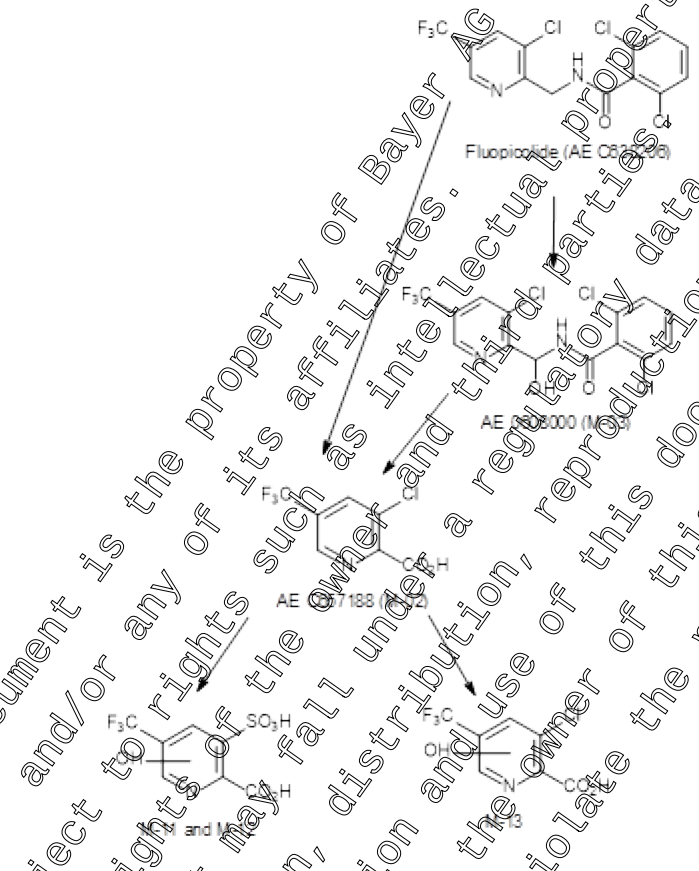
Air and soil temperatures were recorded continuously for the whole study period. All precipitation and any additional irrigation of the lysimeters were recorded.

Leachate was collected regularly and analysed by liquid scintillation counting (LSC) to detect and quantify total radioactivity. Samples containing > 0.1 µg a.s. equivalents/L were concentrated by vacuum-evaporation prior to analysis by HPLC, and the concentration of fluopicolide and its metabolites, including M-11&12 and M-13 measured in leachate samples.

B. Substance data

The objective of this study was to derive formation fractions for the secondary fluopicolide metabolites M-11&12 (P2) and M-13 (P3) in soil from the primary metabolite M-02 derived with fixed formation fractions from the parent fluopicolide, either directly (0.47) either through the metabolite M-03 (0,53), see below reaction scheme:

Figure 7.1.4.2- 4: Reaction scheme to derive formation fraction for M-11/M-12 and M-13



In this report inverse modelling was conducted to estimate formation fractions for M-11/M-12 and M-13 only, with DT_{50} and K_{oc} inputs fixed to those considered for forward groundwater modelling. The parameters for fluopicolide, M-02 and M-03 are the same as those used in the groundwater modelling provided in Document MCP 9.

The metabolite P2 consists of two isomers P2a (M-11) and P2b (M-12). This metabolite P2 has been detected in a M-02 soil degradation study at a maximum of 6.55 % (sum for both isomers) of the applied amount (KCA 7.1.2.1.2/02, M-219824-01-1).

Table 7.1.4.2- 10: Summary of DegT$_{50}$ values derived for M-11/M-12 under laboratory conditions

Applied compound	Study	Soil	Model selected	DT$_{50}$ un-normalised (d)	DT$_{50}$ normalised (d)	ffm from M-02 (PCA)
M-02 (PCA)	[redacted] 2003	Abington	SFO	31.7	31.7	0.0177
		Münster	SFO	242.5	242.5	0.0711
				Geometric mean	**87.6**	-
				Arithmetic mean		**0.044**

The metabolite M-13 was also detected in the M-02 soil degradation study at a maximum of 4.38 % of the applied amount (KCA 7.1.2.1.2//02, M-210824-01-1).

Table 7.1.4.2- 11: Summary of DegT$_{50}$ values derived for M-13 under laboratory conditions

Applied compound	Study	Soil	Model selected	DT$_{50}$ un-normalised (d)	DT$_{50}$ normalised (d)	ffm from M-02 (PCA)
M-02 (PCA)	[redacted] 2003	Abington	SFO	13.3	13.3	0.0667
		Münster	SFO	48.4	48.4	0.0286
		Sarotti	SFO	44.8	13.8	0.0507
				Geometric mean	**20.7**	-
				Arithmetic mean		**0.049**

The sorption behaviour of the metabolites M-11/12 and M-13 have been investigated using samples of the metabolites isolated from lysimeter leachate. No reliable K_{oc} could be derived for these metabolites. Therefore, K_{oc} values of 0 L/kg was assumed as a worst case.

The following input parameters were used for modelling fluopicolide and its metabolites.

Table 7.1.4.2- 12: Modelling parameters for fluopicolide and metabolites

Parameter	Unit	Fluopicolide	M-02	M-03	M-11/12	M-13
Molecular weight	g mol^{-1}	383.59	225.56	399.58	287.17	241.55
DT$_{50}$ in soil	d	482	1.6	17.9	87.6	20.7
Koc	mL g^{-1}	267.7	5.7	106.9	0	0
Kom	mL g^{-1}	155.3	3.3	62.0	0	0
Freundlich exponent	-	0.888	0.889	0.971	1.0	1.0
Plant uptake factor	-	0.5	0	0	0	0
ffm to M-02		0.47		1		
ffm to M-03		0.53				

C. Hydrological Modelling

An accurate simulation of the water balance for the lysimeters was carried out as a prerequisite to the modelling evaluation of the behaviour of fluopicolide and its metabolites using PEARL 4.4.4.

The soil hydrological characteristics were estimated from the lysimeter soil texture and organic carbon content. PEARL 4.4.4 meteorology files were based on-site weather measurements integrated with data from DWD (Deutscher WetterDienst, Munster/Osnabrücto meteo stat 1776) and MARS (Monitoring Agricultural ResourceS). On-site rainfall and supplementary irrigation from the lysimeter study was transferred to the PEARL scenario. In order to simulate the lysimeter water balance correctly it was necessary to optimise predicted evapotranspiration (PET) losses and to implement the different cropping and management regimes of the study. The cropping information from the lysimeter study was used to set up crop rotation scenarios in PEARL.

For solute modelling of fluopicolide, M-11/M-12 and M-13, application scenarios were set up with dates and application rates detailed in the report for each lysimeter. Measured concentrations of M-11/M-12 and M-13 in leachate were available for all three years of the lysimeter study. For Year 1 (and also Year 2 and 3) of the study the concentration of the metabolites was available for pooled annual samples for each lysimeter (prepared in the ratio of leachate volumes collected on individual sampling occasions). In addition, for Year 2 and 3, but not for Year 1, measured concentrations of the metabolites in individual leachate samples were available. These measured concentrations were compared to the simulated concentrations of M-11/M-12 and M-13.

II. Results and Discussion

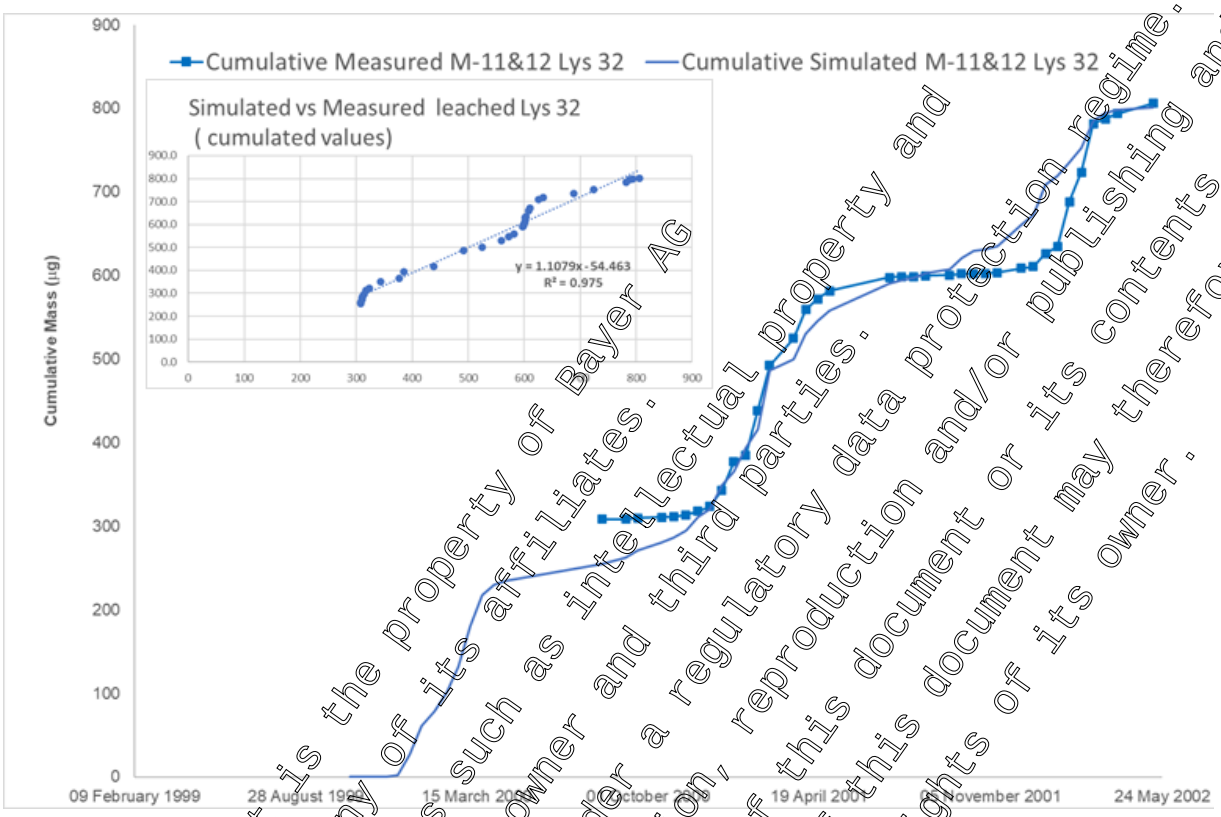
After calibration, the simulated leaching flows were strongly correlated to measured data ($R^2 > 0.99$), in the two lysimeters used in the study. The concentration and cumulated leached mass of the applied tracer bromide from both the lysimeters were also adequately simulated by the calibrated input sets. Thus validated, the inputs sets were applied to simulate the cumulative mass/concentrations of two secondary fluopicolide metabolites M-11/12 (P2) and M-13 (P3) in each lysimeter, assuming different formation fractions from the commune precursor primary metabolite M-02.

After calibration, the simulated leaching flows were strongly ($R^2 > 0.99$) correlated to measured data, in the two lysimeters used in the study (32 and 33). The concentration and cumulated leached mass of the applied trace bromide from both the lysimeters were also adequately simulated by the calibrated input sets. Thus validated, the inputs sets were applied to simulate the cumulative mass/concentrations of two secondary fluopicolide metabolites M-11/12 (P2) and M-13 (P3) in each lysimeter, assuming different formation fractions from the commune precursor primary metabolite M-02.

The best modelling fits of M-11/M-12 were obtained with formation fractions of 6.8% (0.068) and 4% (0.04) in lysimeters 32 and 33 respectively ($R^2 > 0.97$, see Figure 7.1.4.2- 5) with a mean value of 5.4% (0.054). The relative mean square error (RMSE) between measured and simulated leachate concentrations of M-11/12 in the two lysimeters were 32.7 and 80.5, respectively indicating robust simulation of the data.

The best modelling fits of M-13 were obtained with formation fractions of 2.7% (0.027) and 1.95% (0.195) in lysimeters 32 and 33 respectively ($R^2 > 0.97$ and >93, see Figure 7.1.4.2- 6) with a mean value of 2.33% (0.0233). The RMSE between measured and simulated leachate concentrations of M-13 in the two lysimeters were 9.07 and 9.36, respectively indicating robust simulation of the data.

Figure 7.1.4.2- 5: M-11/M-12 Simulations in Lysimeter 32 and 33 – Cumulative Load



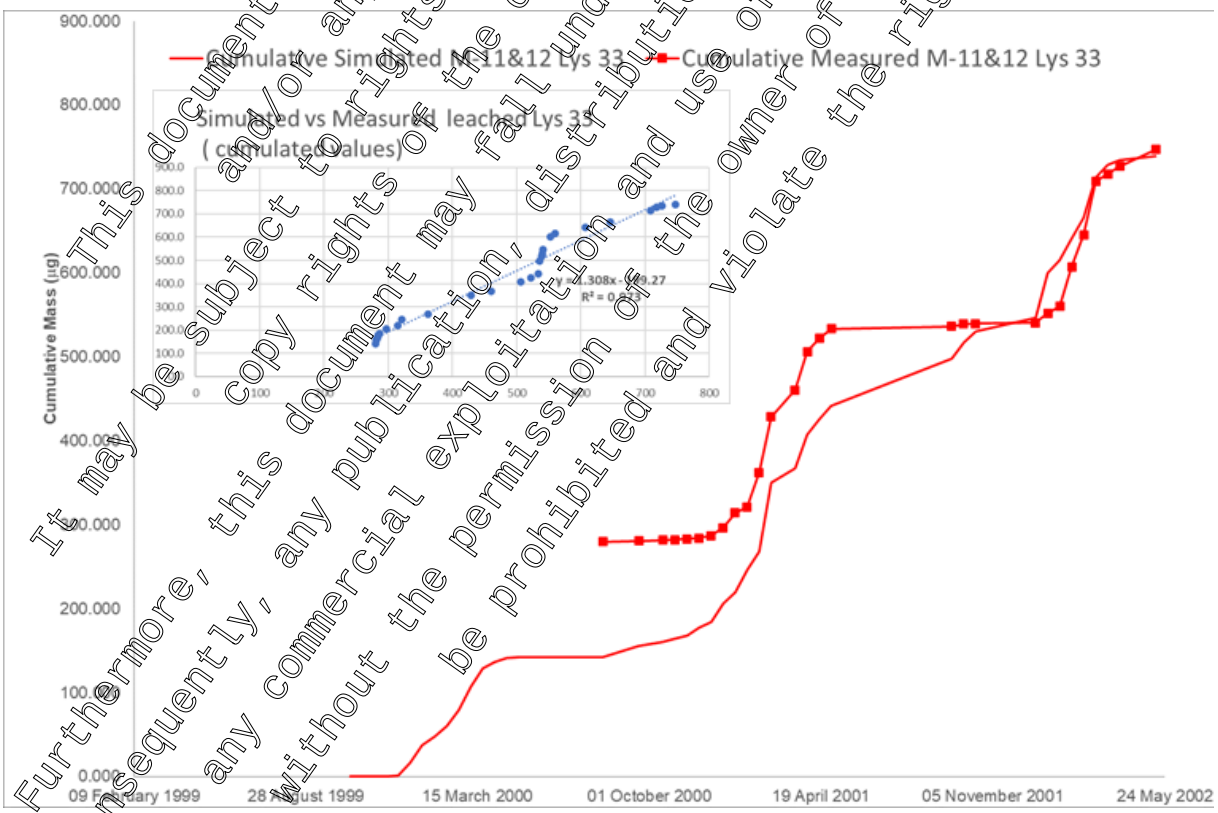
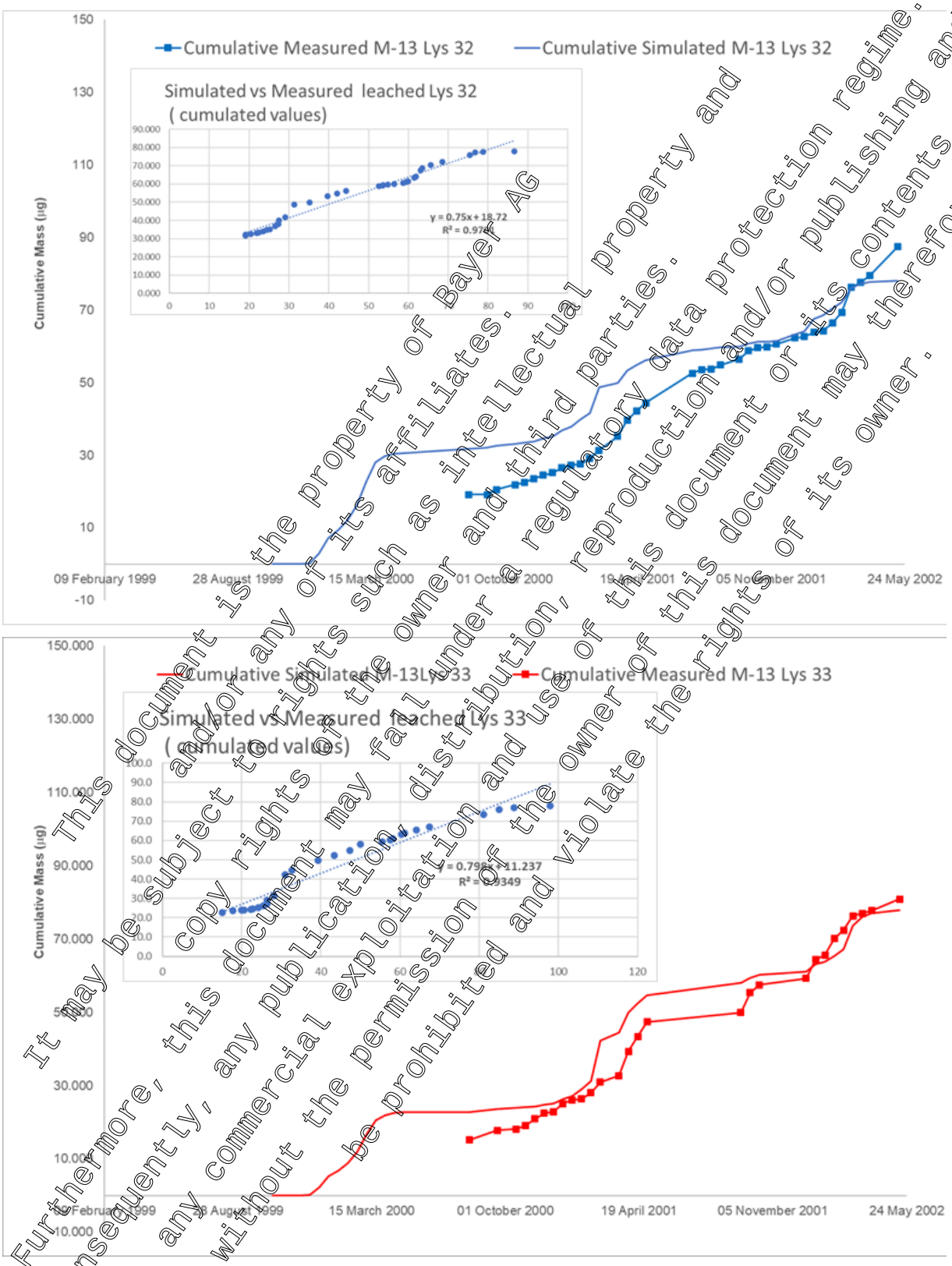


Figure 7.1.4.2- 6: M-13 Simulations in Lysimeter 32 and 33 – Cumulative Load



III. Conclusion

Inverse modelling of the lysimeter study conducted with [^{14}C]- fluopicolide was performed with the model PEARL 4.4.4 using locally measured meteorological data, integrated with DWD and MARS meteo data.

The aim was to evaluate and compare modelled and measured datasets in order to i) simulate the water balance and hydrology of the soil cores and then ii) to subsequently estimate a suitable formation fraction two secondary fluopicolide metabolites M-11/12 and M-13 from the primary metabolite M-02 (AE C657188).

After calibration, the simulated leaching flows were strongly correlated to measured data (R^2 >0.99) in the lysimeters used in the study. The concentration and cumulated leached mass of bromide, applied as a tracer, from both lysimeters were also adequately simulated by the calibrated input sets. Thus validated, the inputs sets were applied to simulate the cumulative mass concentrations of two secondary fluopicolide metabolites M-11/12 and M-13 in each lysimeter, assuming different formation fractions from the communal precursor primary metabolite M-02.

The best modelling fits of M-11/M-12 were obtained with formation fractions of 6.8% (0.068) and 4% (0.04) in lysimeters 32 and 33 respectively, with a mean value of 5.4% (0.054). The best modelling fits of M-13 were obtained with formation fractions of 2.7% (0.027) and 1.95% (0.195) in lysimeters 32 and 33 respectively, with a mean value of 2.33% (0.0233).

Assessment and conclusion by applicant:

This modelling report is considered valid to assess formation fractions for the secondary fluopicolide metabolites M-11/M-12 and M-13 in soil from the primary metabolite M-02 (AE C657188) in soil.

CA 7.1.4.3 Field leaching studies

A field leaching study with [fluopicolide (KCA 7.1.4.3/01) was evaluated during the previous EU review and are still considered as reliable to assess the fate and mobility of fluopicolide and its metabolites in soil.

Report reference	Author; Year	Comment
KCA 7.1.4.3/01 M-223180-01-2	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.

Data Point:	KCA 7.1.4.3/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Field leaching study for AE C638206 on a sandy soil in vegetables
Report No:	M-223180-01-2
Document No:	M-223180-01-2
Guideline(s) followed in study:	EU (=EEC): ; IVA: Beutel et al., (1993); SETAC: Lynch et al., (1995)
Deviations from current test guideline:	Method: Deviations from current guideline SANCO/3029/99 rev. 4: The mean recovery for M-03 at the LOQ level is 71%. However, as this is just outside guideline requirements, the %RSD for the LOQ level is < 20% and the 100xLOQ level meets all guideline requirements, this is a minor deviation and the method is fit for purpose. For all other analytes, there are no guideline deviations. Study: none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary:

The fate and mobility of fluopicolide and its primary metabolites in soil, were investigated in a field leaching study conducted at a site near Philippsburg, Baden-Württemberg, South West Germany. The top soil at the test site was a low organic carbon, silty sand overlying sand subsoil. Fluopicolide, formulated as a suspo-emulsion containing 97.9 g/L fluopicolide, was applied to lettuce at growth stage BBCH 14 to 19 between May and October 2000, at the rate required to achieve a total application of 400 g a.i. /ha. In order to determine the leaching potential of fluopicolide and its metabolites in soil, a total of 45 suction samplers were employed to collect soil water at 2 different depths throughout the soil profile at depths down to 150 cm.

The test site was planted with four successive crops of lettuce in 2000. In 2001 and 2002 summer oil seed rape was grown. Winter wheat was planted in October 2002. During periods where no crop was present the plots were maintained as bare soil by manual weeding or the periodic application of the herbicide glyphosate.

Throughout the study a permanent low pressure of approximately –0.200 hPa was applied to the suction samplers. The permanent suction pressure enabled the continuous collection of soil water and ensured no peak concentrations were missed, while the low pressure applied avoided the sampling of immobile soil water. Samples were collected at regular intervals (*ca.* 70 timepoints) throughout the study from each of the 45 suction samplers (15 per plot).

Samples of soil water were collected at intervals over a three year period and analysed by a LC/MS/MS method to determine the levels of fluopicolide and its metabolites M-01 (AE C653711), M-02 (AE C657188) and M-03 (AE 0608000) present in the samples. The analytical method involved acidification of the water sample with formic acid and extraction with ethyl acetate, evaporation and analysis by LC/MS/MS. The limit of quantification was 0.075 µg/L for each analyte.

Fluopicolide residues were detected in soil water at 30 cm depth initially in September 2000 and reached peak concentrations of *ca.* 1 to 10 µg/L in April 2001 in the individual suction samplers. The concentrations were significantly attenuated with depth with peak concentrations of *ca.* 0.08 to 0.8 µg/L at 50 cm depth. Annual average concentrations throughout the study in the deeper soil layers were all below < 0.1 µg/L at 85 cm and < LOQ at 120 and 150 cm depth.

Metabolite M-01 is mobile and moderately degraded in soil and consequently residues were detected in soil water at all soil depths. At 30 cm depth M-01 residues were detected initially in July 2000 and reached a maximum peak concentration of 19 µg/L in September 2001 in the individual suction samplers. Maximum annual average concentrations out of the three years of the study in the deeper soil layers were 4.4 µg/L at 85 cm (second year), 2.9 µg/L at 120 cm (third year) and 2.4 µg/L at 150 cm depth (third year).

As a consequence of the moderate degradation of fluopicolide in soil, the metabolite M-02 was present in soil water at 30 cm depth throughout the study. M-02 was rapidly degraded in soil and therefore during the first year was only occasionally detected at 50 cm depth at concentrations > 0.1 µg/L and once at 85 cm at a concentration equal to the LOQ (0.075 µg/L). It was not detected below 85 cm depth in the first year at concentrations greater than the LOQ. In subsequent years it was not detected below 30 cm depth except for a single finding which was concluded to be an artefact.

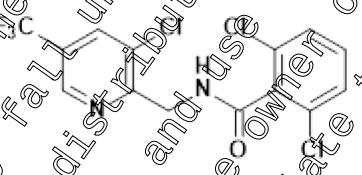
M-03 was rapidly degraded in soil to form M-01 and M-02 and was detected only once throughout the three years of the study at a concentration of 0.067 µg/L at 30 cm depth.

This study has shown that under worst case conditions for leaching, fluopicolide and its metabolites M-02 and M-03 would not be expected to reach groundwater at 1 m depth at concentrations exceeding 0.1 µg/L. Residues of the metabolite M-01 below 120 cm will not exceed an annual average concentration of 5 µg/L and concentrations in groundwater would be expected to be lower based on the observed decline in concentrations at soil depths of 120 to 150 cm.

I. Materials and Methods

1. Test Item

Fluopicolide formulated as SE containing 97.9 g/L fluopicolide.



Product Code:	AE C638206 00 SE10 A3
Batch Number:	QP200271
Content of a.i.	97.9 mg/L
Density:	1.002 mg/L

2. Trial Location & Soil

The test site was located in the Rhine valley near Philippsburg, Baden-Wurttemberg, South West Germany. This area is part of the former floodplain area of the river Rhine. The field was level and the soil surface visually homogeneous.

The average annual rainfall in this region is 769.9 mm.

The top soil at the test site was a low organic carbon, silty sand overlying sand subsoil. The soil properties of the test site were significantly more stringent than lysimeter guideline requirements (OECD 1999, BBA 1990) with a sand content of > 79 %, clay content < 5% and an organic carbon content of < 0.7 % in the 0 to 30 cm soil horizon.

The characteristics of the soil at each horizon are summarised in Table 7.1.4.3- 1.

Table 7.1.4.3- 1: Location of field site and soil characterisation

Characteristic	Units	Philippsburg, Baden-Württemberg, D-76661, Germany				
Soil depth	m	0.25 – 0.35	0.45 – 0.55	0.80 – 0.90	1.15 – 1.25	1.45 – 1.55
pH	$CaCl_2$	5.8	5.8	7.3	7.6	7.5
Total organic carbon	%	0.47	0.16	0.11	0.04	0.18
Cation exchange capacity	mval/100 g	4.5	3.8	3.5	3.6	2.0
Particle size distribution (DIN)						
Clay < 0.002 mm	%	2.8	4.1	4.0	1.9	0.8
Total silt 0.002 - 0.063 mm	%	13.7	10.7	7.0	5.1	3.9
Total sand 0.063 - 2 mm	%	83.5	85.2	89.0	93.0	95.3
Textural class	DIN	Silty sand (Su)	Silty sand (Su)	Sand (S)	Silty sand (Su)	Sand (S)

B. Study Design

1. Experimental Conditions

Fluopicolide, formulated as a suspo-emulsion containing 97.9 g/L fluopicolide, was applied to lettuce at growth stage BBCH 14 to 19 between May and October 2000, at the rate required to achieve a total application of 400 g a.i. /ha. In order to determine the leaching potential of fluopicolide and its metabolites in soil, a total of 45 suction samplers were employed to collect soil water at 5 different depths throughout the soil profile at depths down to 150 cm.

Throughout the study different crops were cultivated according to the normal agricultural practice of the region. The test site was planted with four successive crops of lettuce in 2000. In 2001 and 2002 summer oil seed rape was grown. Winter wheat was planted in October 2002. During periods where no crop was present the plots were maintained as bare soil by manual weeding or the periodic application of the herbicide glyphosate.

The test site was divided into three plots, each measuring 23.1 m^2. Plots T1 and T2 were treated with the test substance while Plot C remained untreated and served as a control. Each plot was equipped with 3 suction samplers at 30, 50, 85, 120 and 150 cm soil depth. In Plots C and T2 TDR (Time Domain Reflectrometery) probes were installed at the same depths to measure the hydrological status of the soil. Extensive investigations were carried out to further characterise the soil hydrological properties of each plot. Undisturbed soil cores were used to measure soil moisture release curves, saturated hydraulic conductivity, soil bulk density and soil porosity at each depth of soil. A weather station was mounted approximately 1 km away from the test site in order to gather site specific weather data. Rainfall, air temperature, soil temperature, wind speed, wind direction, relative air humidity and global radiation were measured on a daily basis. The long term mean precipitation of the region (Karlsruhe, 769.9 mm) was supplemented by irrigation to reach 879.5 mm in the first year, 867.0 mm in the second and 723.6 mm in the third.

A pre-test with a dye tracer and lithium chloride was successfully carried out to check the proper function and installation of the equipment. The test item fluopicolide was first applied on 23 May 2000, at an incorrect application rate of 9.52 g ai/ha to Plot T1 and 9.96 g ai/ha to Plot T2. The intended nominal application rate was 100 g/ha and therefore this application was repeated on 6 June 2000 at an application rate of 97.84 g ai/ha to Plot T1 and 93.94 g ai/ha to Plot T2. Three following applications were performed on 6 July 2000 (115.4 and 99.6 g ai/ha), 28 August 2000 (98.8 and 102.4 g ai/ha) and 4 October 2000 (100.9 and 99.6 g ai/ha) to Plots T1 and T2, respectively. On the same day as the initial application (23 May 2000), potassium bromide was applied to all three plots as a conservative tracer at a nominal rate equivalent to 100 kg/ha.

2. Sampling

Samples of soil water were collected at intervals over a three year period to determine the levels of fluopicolide and its metabolites M-01, M-02 and M-03 present in the samples.

Throughout the study a permanent low pressure of approximately –0.200 hPa was applied to the suction samplers. The permanent suction pressure enabled the continuous collection of soil water and ensured no peak concentrations were missed, while the low pressure applied avoided the sampling of immobile soil water. Samples were collected at regular intervals (*ca.* 70 timepoints) throughout the study from each of the 45 suction samplers (15 per plot). Immediately after sampling the water samples were transported to GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany, where a subsample was removed for bromide analysis. The remaining sample was retained for residue analysis and was stored at < -18 ºC, prior to being dispatched frozen to the analytical laboratory at Bayer CropScience, France. The samples were then stored at -18 ºC until required for analysis.

3. Analytical Procedures

The analytical method used in this study involved acidification of the water sample with formic acid and extraction with ethyl acetate, followed by evaporation and re-dissolving in a mixture of water : acetontirile : formic acid (95 : 5 : 0.1 by volume) with final analysis by LC-MS/MS. The limit of quantification was 0.075 µg/L for each analyte. The method was successfully validated according to EU requirements for each analyte.

II. Results and Discussion

A. Analytical Methodology

Full details and acceptable validation data to support this method are presented in Document M-CA 4, Section 4.1.2. The method complies with the EU regulatory requirements outlined within SANCO/3029/99 rev. 4 and is suitable for the determination of fluopicolide and its metabolites M-01 (AE C653711), M-02 (AE C657188) and M-03 (AE 0608000) in soil samples by HPLC-MS/MS.

B. Data

The arithmetic mean annual concentration of fluopicolide and it metabolites at different soil depths are summarised in Table 7.1.4.3- 2. In order to calculate mean values, concentrations <LOQ were assumed to be 0.0375 mg/kg.

Table 7.1.4.3- 2: Annual average concentrations in soil water at 30 cm, 50 cm, 85 cm, 120 cm and 150 cm depth

Time Period	Arithmetic mean annual concentration (µg/L)				Depth
	Fluopicolide	M-01	M-02	M-03	
Year 1	1.145	5.320	0.100	<LOQ	30 cm
Year 2	1.688	6.691	0.079	<LOQ	
Year 3	1.189	2.930	0.054	<LOQ	
Year 1	0.078	3.257	0.058	<LOQ	50 cm
Year 2	0.151	5.764	<LOQ	<LOQ	
Year 3	0.173	3.378	<LOQ	<LOQ	
Year 1	<LOQ	0.845	<LOQ	<LOQ	85 cm
Year 2	0.081	4.361	<LOQ	<LOQ	
Year 3	0.050	3.346	<LOQ	<LOQ	
Year 1	<LOQ	0.282	<LOQ	<LOQ	120 cm
Year 2	<LOQ	2.548	<LOQ	<LOQ	
Year 3	<LOQ	2.928	<LOQ	<LOQ	
Year 1	<LOQ	0.085	<LOQ	<LOQ	150 cm
Year 2	<LOQ	1.302	<LOQ	<LOQ	
Year 3	<LOQ	2.415	<LOQ	<LOQ	

LOQ = Limit of quantification (0.075 µg/L)

C. Residues

Soil leaching conditions were prevalent between May and July 2000 and from September 2000 to May 2001 in the first year of the study. Soil hydrology measurements and the early breakthrough of bromide ions in soil water at 30 cm depth in June 2000 (1 month after application) and at 85 cm depth in July 2000 confirmed rapid downward movement of water through the soil profile at the time of application. These unusually wet conditions caused immediate downward movement of bromide ions and of the organic test items. In drier, more typical years a significant portion of the test item would be retained and metabolised in the microbially active upper soil layer throughout the summer period, when evapotranspiration would normally exceed precipitation and consequently no net downward movement of water through the soil profile would occur. In the second year the leaching period was from September 2001 to June 2002 and in the third year from October 2002 to March 2003.

Peak concentrations of bromide ions were detected at 30, 50 and 85 cm depth between June and August 2000, indicating significant leaching during this period. The first bromide residues were detected at 120 cm depth 56 days after application (18 July 2000).

Fluopicolide residues were detected in soil water at 30 cm depth initially in September 2000 and reached peak concentrations of *ca.* 1 to 10 µg/L in April 2001 in the individual suction samplers. The concentrations were significantly attenuated with depth with peak concentrations of *ca.* 0.08 to 0.8 µg/L at 50 cm depth. At 85 cm depth fluopicolide was detected only in two of the suction samplers in Plot T1 at concentrations greater than the limit of quantification (LOQ, 0.075 µg/L) and not at all in Plot T2 throughout the study. At 120 cm and 150 cm depth fluopicolide was not detected throughout the study except a single finding which was concluded to be an artefact. Annual average concentrations throughout the study in the deeper soil layers were all below < 0.1 µg/L at 85 cm and < LOQ at 120 and 150 cm depth.

The metabolite M-03 (referred to as AE 0608000 in the study report) was rapidly degraded in soil to form M-01 (referred to as AE C653711 in the study report) and M-02 (referred to as AE C657188 in the study report) and was detected only once throughout the three years of the study at a concentration of 0.067 µg/L at 30 cm depth.

The metabolite M-01 is mobile and moderately degraded in soil and consequently residues were detected in soil water at all soil depths. At 30 cm depth M-01 residues were detected initially in July 2000 and reached a maximum peak concentration of 19 µg/L in September 2001 in the individual suction samplers. The concentrations were attenuated with depth with a maximum peak concentration of 3.7 µg/L at 150 cm depth in August 2002. Maximum annual average concentrations out of the three years of the study in the deeper soil layers were 4.4 µg/L at 85 cm (second year), 2.9 µg/L at 120 cm (third year) and 2.4 µg/L at 150 cm depth (third year).

As a consequence of the moderate degradation of fluopicolide in soil, the metabolite M-02 was present in soil water at 30 cm depth throughout the study. M-02 was rapidly degraded in soil and therefore during the first year was only occasionally detected at 50 cm depth at concentrations > 0.1 µg/L and once at 85 cm at a concentration equal to the LOQ (0.075 µg/L). It was not detected below 85 cm depth in the first year at concentrations greater than the LOQ. In subsequent years it was not detected below 30 cm depth except for a single finding which was concluded to be an artefact.

III. Conclusions

This study has shown that under worst case conditions for leaching, fluopicolide and its metabolites M-02 and M-03 would not be expected to reach groundwater at 1 m depth at concentrations exceeding 0.1 µg/L.

Residues of the metabolite M-01 below 120 cm will not exceed an annual average concentration of 5 µg/L and concentrations in groundwater would be expected to be lower based on the observed decline in concentrations at soil depths of 120 to 150 cm. Further studies on M-01 have been conducted as part of the work undertaken to assess the relevance of this metabolite.

Assessment and conclusion by applicant:

The study is considered valid to aid assessment of the leaching potential of fluopicolide and its metabolites in the environment. Definitive assessment of the leaching potential of fluopicolide and its metabolites is provided by FOCUS groundwater assessment (see Document MCP-9).

Data Point:	KCA 7.1.4.3/02
Report Author:	[redacted]
Report Year:	2004
Report Title:	The non-relevance of the metabolites of AEC 638206 found in lysimeter leachate and field leaching studies - Position paper
Report No:	C039866
Document No:	M-227293-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	No

In the previous submission (DAR, 2005), this statement (KCA 7.1.4.3/02, M-227293-01-1) was evaluated and accepted as valid to assess the non-relevance of the metabolites of fluopicolide found in lysimeter leachate and field leaching studies. However additional studies have been conducted and the groundwater exposure assessments have changed, thus the statement is no longer considered as valid. For procedural reasons it is included in the current dossier but it has been superseded by Document N4 Relevance of Metabolites in Groundwater and hence a summary is not presented in this dossier.

CA 7.2 Fate and behaviour in water and sediment

The following summary provides an overview of the behaviour of fluopicolide in aquatic systems.

Surface Water

The fate and behaviour of fluopicolide in surface water has been investigated under abiotic and biotic conditions.

In aerobic water/sediment systems fluopicolide was relatively stable with total system DT_{50} values ranging from 856.3 to 1340.0 days. The compound was dissipated in the water by a combination of degradation and partitioning to sediment. The dissipation rates from the water phase in the two aerobic systems were very different with DT_{50} values of 5.6 and 228.9 days. These differences were reported to be due to differences in sorption properties of the sediment. Rapid dissipation of fluopicolide from the water phase was observed in the sediment with higher organic carbon content and cation exchange capacity.

Fluopicolide showed similar stability in abiotic and biotic water sediment systems, indicating that fluopicolide degradation was not enhanced by microbial activity.

Fluopicolide undergoes cleavage to form the metabolites M-01 (AE C653711) containing the phenyl ring and M-02 (AE C657188) containing the pyridyl ring. M-01 was detected as a major metabolite in the water phase of aerobic water sediment systems and was also observed as a minor metabolite in the sediment phase. No other major metabolites (>10 %) were detected. M-02 was detected as a minor metabolite in the water phase not exceeding 8 % and was observed at detectable levels in the sediment (<1%).

The aerobic mineralisation of fluopicolide was investigated in a 'pelagic' natural water system at pH 7.25. No significant degradation of fluopicolide was observed in aerobic surface water over 63 days. The results indicated that fluopicolide was stable in both low and high concentration tests (10 and 100 µg/L) and did not significantly mineralise (<0.1% AR) over the study duration.

Aquatic metabolites formed from fluopicolide

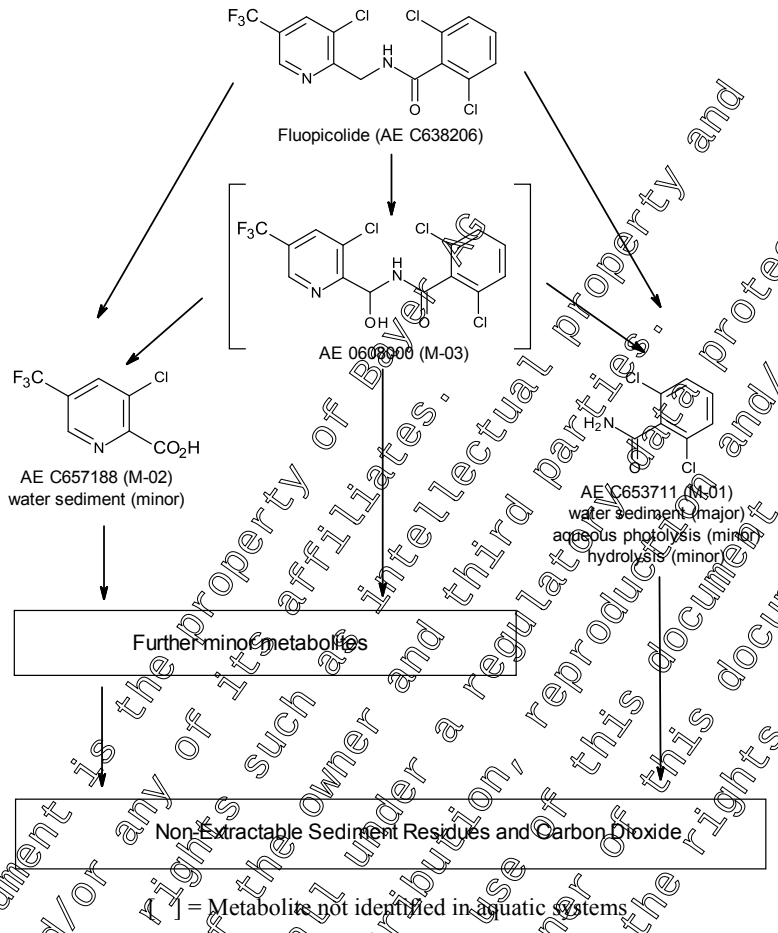
Maximum observed in laboratory studies (as % of applied fluopicolide)	Metabolite	Hydrolysis	Photolysis	Water Sediment		
				Total	Water	Sediment
	M-01 (AE C653711)	4.0	4.1	20.3	18.2	3.9
	M-02 (AE C657188)	- [A]	not detected	8.2 [B]	7.4 [B]	0.8
	M-03 (AE 0608000)	not detected	not detected	not detected	not detected	not detected

[A] Fluopicolide was stable to hydrolysis and this study was conducted with [phenyl-U-^{14}C]-fluopicolide only. Consequently M-02 would not be detected.

[B] Significant minor metabolite, >5% at 3 consecutive timepoints and increasing at final timepoint

No significant metabolites were formed in the aerobic mineralisation study

Table 7.2- 1: Metabolic pathway for fluopicolide (AE C638206) in surface water



Fluopicolide was stable to hydrolysis at pH 4, 7 and 9 at 50 °C and pH 5, 7 and 9 at 25 ºC with ≥ 95% recovered at the end of the study (5 days at 50 ºC and 30 days at 25 ºC respectively). The degradation product M-01 was detected in small amounts in the 50 °C and 25 °C tests. It reached a maximum of 3% of applied radioactivity in the 50 °C test at pH 9 after 5 days and 4% of applied in the 25 °C test at pH 7 after 30 days.

The photolytic degradation of fluopicolide in water has been investigated under sterile conditions in natural water and in phosphate buffer solution at pH 7. [phenyl-U-^{14}C]-fluopicolide exhibited slow degradation when irradiated under 12 light / 12 dark cycles in sterile pH 7 buffer solution at 25 ºC, with up to 83 % of applied radioactivity was still recovered as parent at the end of the study after 31 days. No photolytic degradation of fluopicolide was observed in sterile natural water and a second study conducted in sterile buffer at 25 °C with [2,6-pyridyl-^{14}C]-labelled fluopicolide irradiated continuously for 16 and 10 days, respectively.

As M-01 is a major metabolite in the water phase of water / sediment systems, the hydrolysis and aqueous photolysis of this compound were investigated. At pH 4, 7 and 9 at 50 ºC and pH 5, 7 and 9 at 25 ºC M-01 was shown to be stable to hydrolysis at all pHs and both temperatures tested. No significant photolysis or degradation of M-01 was observed in acetate buffer at pH 5 in either the light exposed samples or the dark control samples where the test item represented 99% and 98% of applied radioactivity respectively at the end of the incubation period (31 days).

In addition, the hydrolysis of the soil metabolite M-03 (AE 0608000), which was not observed in aquatic systems, was investigated to provide further information on its fate in the environment. AE 0608000 was shown to be hydrolytically labile under acidic, neutral and alkaline conditions at 20°C. The rate of hydrolysis of M-03 was strongly dependent on pH with half-lives (DT_{50}) of 0.1, 0.7, 4.4 and 45.5 hours at pH 8, 7, 6 and 5, respectively.

A new kinetic evaluation of the experimental data generated in the water sediment study has been conducted according to FOCUS kinetics guidance with the aim of deriving DT_{50} values for use as modelling and trigger endpoints (KCA 7.2.2.3/03, M-685681-01-1, [redacted] 2020). The geometric mean modelling endpoints $DegT_{50}$ values for fluopicolide are summarised in the table below.

Table 7.2- 2: Summary of modelling endpoint DT_{50} values for fluopicolide in aquatic sediment systems

Compound	Laboratory modelling endpoint DT_{50} (20 °C)		
	DT_{50} range (days)	Number of datasets (n)	Geometric mean DT_{50} (days) for exposure assessment
Total system	856.3 – 1340.0	2	1071.2
Water phase	57.0 [A] – 594.5 [B]	2	184.1

After removal of data points prior to the maximum in sediment (76.2% AR at DAT 89), only three data points remained for each system, which was insufficient to provide a robust, meaningful fit.
[A] Pseudo-SFO DT_{50} value derived from the FOMC DT_{90}/3.32
[B] Pseudo-SFO DT_{50} values derived from the slow phase of the DFOP fit

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

A statement addressing the nature of the residues formed during water treatment processing of ground and surface water for drinking water will be provided in November 2020.

CA 7.2.1.1 Hydrolytic degradation

The hydrolytic degradation of fluopicolide and its metabolites M-01 and M-03 have been investigated in Study KCA 7.2.1.1/01, KCA 7.2.1.1/02 and KCA 7.2.1.1/03 respectively. These studies were evaluated during the previous EU review and are still considered acceptable.

Test item	Report reference	Author, Year	Phenyl Label	Pyridyl Label	Comment
Fluopicolide	KCA 7.2.1.1/01 M-241162-01-2	[redacted] 2002	✓	×	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-01	KCA 7.2.1.1/02 M-223750-01-1	[redacted] 2003	✓	NA	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-03	KCA 7.2.1.1/03 M-236241-01-2	[redacted] 2004	-	-	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.

Data Point:	KCA 7.2.1.1/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	Hydrolysis of [14C]- AE C638206 at pH 4,5,7 and 9
Report No:	M-241162-01-2
Document No:	M-241162-01-2
Guideline(s) followed in study:	EU (=EEC): Guideline EC.C7; USEPA (=EPA): Subdivision N, E-Fate 161-1
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The hydrolysis of [phenyl-U-^{14}C]-labelled fluopicolide was investigated at 50 ± 0.1 °C at pH 4, 7 and 9 and at 25 ± 1 °C at pH 5, 7 and 9, in the dark, under sterile conditions. [Phenyl-U-^{14}C]-labelled fluopicolide was dissolved in sterile buffer at a nominal concentration of 1 mg/L.

Samples incubated at 50 °C were removed immediately after treatment and after 5 days. Samples at 25 °C were removed immediately after treatment and after 3, 6, 13, 20 and 30 days. Duplicate replicates of samples incubated at 50 °C and 25 °C were removed at each time point. Sterility of test samples was confirmed at the beginning and end of the 25 °C test.

Radiochemical balances were quantitative in all samples. Buffer samples were analysed by HPLC to identify the compounds present, based on comparison of retention times with analytical standards. Select samples were analysed by TLC.

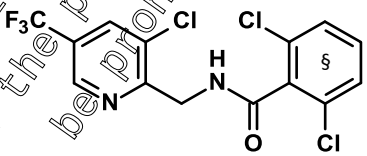
Fluopicolide was relatively stable to hydrolysis at all pHs and both temperatures tested. HPLC analysis confirmed that fluopicolide was the major compound detected. A degradation product, M-01 (referred to as AE C653711 in the study report) was detected at a maximum of 3% of applied radioactivity in the 50 °C test at pH 9 after 5 days and 4% of applied in the 25 °C test at pH 7 after 30 days.

I. Materials and Methods

A. Materials

1. Test Item

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



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

Specific activity: 155 µCi/mg (344,100 dpm/ng)

Batch number: 901CU-2

Radiochemical purity: 95.7% (HPLC analysis)

2. Sterile Buffers

The buffers used were: pH 4 (0.05M citrate), pH 5 (0.01M acetate), pH 7 (0.005M phosphate) and pH 9 (0.005M borate) and were prepared as follows:

pH4 Sodium citrate - sodium hydroxide: 50 mL of 0.1 M sodium citrate were added to 9.0 mL of 0.1 M NaOH and HPLC grade water was added to a final volume of 100 mL.

pH 5 Acetic acid - sodium acetate: 14.6 mL of 0.1 M acetic acid were added to 10.0 mL of 0.1 M NaOH and then HPLC grade water was added to a final volume of 100 mL.

pH 7 Potassium dihydrogen phosphate - disodium hydrogen phosphate: 2.24 mL of 0.1 M KH_2PO_4 solution (1.36 g KH_2PO_4/100 mL HPLC water) were combined with 2.58 mL 0.1 M Na_2HPO_4, solution and then HPLC grade water was added to a final volume of 100 mL.

pH 9 Sodium borate - hydrochloric acid: 4.6 mL of 0.04 M HCl were added to 50 mL of 0.01 M $Na_2B_4O_7.10H_2O$ and then HPLC grade water was added to a final volume of 100 mL.

All buffer solutions were sterilized by filtration through a 0.22 µm filter prior to use.

B. Study Design

1. Experimental Conditions

The hydrolysis of [phenyl-U-^{14}C]-labelled fluopicolide was investigated at 50 ± 0.1 °C at pH 4, 7 and 9 and at 25 ± 1 °C at pH 5, 7 and 9, in the dark, under sterile conditions. [Phenyl-U-^{14}C]-labelled fluopicolide was dissolved in sterile buffer at a nominal concentration of 1 mg/L. The aqueous solubility of fluopicolide has been determined to be 2.8 mg/L at 20 ºC.

2. Sampling

Samples incubated in amber glass vials in a water bath at 50 °C were removed immediately after treatment and after 5 days. Samples incubated in amber glass vials in a water bath at 25 °C were removed immediately after treatment and after 3, 6, 13, 20 and 30 days. Duplicate replicates of samples incubated at 50 °C and 25 °C were removed at each time point. Sterility of test samples was confirmed at the beginning and end of the 25 °C test.

3. Analytical procedures

Immediately after sampling, the pH of the test sample was measured and triplicate aliquots were removed for analysis by LSC. HPLC was used to identify compounds present, based on comparison of retention times with analytical standards. Select samples were analysed by TLC to confirm the presence of fluopicolide and M-01.

4. Determination of degradation kinetics

The reported hydrolysis half-life of fluopicolide in pH 5, 7 and 9 aqueous buffers was calculated based on the percent in solution using single-exponential first order kinetics.

The degradation kinetics determined in the report were conducted prior to the issuing of the FOCUS guidance document on degradation kinetics and are no longer considered valid. DT_{50} and DT_{90} values for the degradation of fluopicolide have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1).

An initial comparison was performed between the SFO and FOMC fits for fluopicolide. The data was best fit by the simple first order (SFO) model all buffers, with an $\chi 2$ error of 0.401, 0.172 and 0.984%.

II. Results and Discussion

A. Data

The results of the HPLC analysis of aqueous buffer solution are summarised in Table 7.2.1.1- 1 to Table 7.2.1.1- 2.

Table 7.2.1.1- 1: Characterisation of radioactivity in aqueous buffer solution incubated at 50 ºC (mean of duplicate values)

pH 4

Compound	Mean SD	DAT 0	DAT 5
Fluopicolide (% in solution)	Mean	98.0	96.2
	SD	± 0.2	± 0.1
M-01 (% in solution)	Mean	1.0	2.4
	SD	± 0.1	± 0.0
Total (% AR)	Mean	100.0	100.1
	SD	± 0.0	± 0.0

DAT: days after treatment, SD: standard deviation

pH 7

Compound	Mean SD	DAT 0	DAT 5
Fluopicolide (% in solution)	Mean	98.1	97.0
	SD	± 0.4	± 0.2
M-01 (% in solution)	Mean	1.2	2.2
	SD	± 0.0	± 0.1
Total (% AR)	Mean	100.0	94.9
	SD	± 0.0	± 0.5

DAT: days after treatment, SD: standard deviation

pH 9

Compound	Mean SD	DAT 0	DAT 5
Fluopicolide (% in solution)	Mean	98.0	95.2
	SD	± 0.1	± 0.4
M-01 (% in solution)	Mean	1.9	3.4
	SD	± 0.2	± 0.0
Total (% AR)	Mean	100.0	93.5
	SD	± 0.0	± 0.1

DAT: days after treatment, SD: standard deviation

Table 7.2.1.1- 2: Characterisation of radioactivity in aqueous buffer solution incubated at 25 °C (mean of duplicate values)

pH 5

Compound	Mean SD	DAT					
		0	3	6	13	20	30
Fluopicolide (% in solution)	Mean	97.3	95.8	96.6	96.4	95.8	96.4
	SD	± 0.1	± 0.4	± 0.1	± 0.1	± 1.3	± 0.6
M-01 (% in solution)	Mean	1.1	2.5	2.7	2.7	2.9	3.1
	SD	± 0.9	± 0.1	± 0.1	± 0.4	± 0.4	± 0.4
Others (% in solution)	Mean	1.7	1.8	0.8	0.9	1.4	0.6
	SD	± 1.0	± 0.5	± 0.1	± 0.3	± 0.9	± 0.4
Total (% AR)	Mean	98.7	98.2	98.1	99.0	95.7	97.3
	SD	± 1.8	± 1.9	± 1.1	± 1.6	± 0.4	± 0.0

DAT: days after treatment, SD: standard deviation

pH 7

Compound	Mean SD	DAT					
		0	3	6	13	20	30
Fluopicolide (% in solution)	Mean	96.5	95.8	95.9	95.8	96.0	95.6
	SD	± 0.8	± 0.1	± 0.1	± 0.3	± 0.4	± 0.1
M-01 (% in solution)	Mean	2.4	3.4	3.2	3.0	2.9	4.0
	SD	± 0.6	± 0.0	± 0.0	± 0.1	± 0.1	± 0.1
Others (% in solution)	Mean	1.2	0.8	0.9	1.2	1.1	0.5
	SD	± 0.1	± 0.1	± 0.1	± 0.4	± 0.6	± 0.2
Total (% AR)	Mean	100.2	96.9	98.0	103.8	101.4	99.3
	SD	± 0.6	± 2.7	± 0.0	± 0.4	± 0.6	± 1.3

DAT: days after treatment, SD: standard deviation

pH 9

Compound	Mean SD	DAT					
		0	3	6	13	20	30
Fluopicolide (% in solution)	Mean	96.9	95.4	96.3	95.7	95.8	96.5
	SD	± 0.1	± 0.5	± 0.5	± 0.5	± 0.1	± 0.1
M-01 (% in solution)	Mean	2.0	3.0	2.9	3.0	2.9	3.0
	SD	± 0.2	± 0.3	± 0.1	± 0.3	± 0.1	± 0.2
Others (% in solution)	Mean	1.2	1.4	0.9	1.4	0.4	0.6
	SD	± 0.4	± 0.8	± 0.4	± 0.5	± 0.1	± 0.1
Total (% AR)	Mean	98.1	99.5	97.7	98.7	94.9	96.2
	SD	± 0.7	± 0.7	± 1.7	± 0.8	± 1.6	± 2.7

DAT: days after treatment, SD: standard deviation

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 100.1% for pH 4, 96.6% for pH 7 and 95.7% for pH 9 samples incubated at 50°C. The mean recoveries of applied radioactivity for samples incubated at 25 °C were 97.8% for pH 4, 99.9% for pH 7 and 97.5% for pH 9 samples. The pH values and sterility of samples was maintained throughout the study.

C. Transformation of test substance

Fluopicolide was relatively stable to hydrolysis at all pHs and both temperatures tested. HPLC analysis confirmed that fluopicolide was the major compound detected. A degradation product, M-01 was detected in small amounts in the 50 °C and 25 °C tests. It reached a maximum of 3% of applied radioactivity in the 50 °C test at pH 9 after 5 days and 4% of applied in the 25 °C test at pH 7 after 30 days.

D. Degradation Kinetics

In the report hydrolysis half-lives of 368, 330, and 365 days in pH 5, 7 and 9 buffers were calculated by extrapolation using single-exponential first order kinetics. The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold.

Parameter confidence was low for SFO fits, especially pH 5 and pH 9, due to extremely slow decline in residues. However FOMC fits for pH 5 and pH 9 buffers did not generate a full set of statistics. Thus SFO fits were accepted.

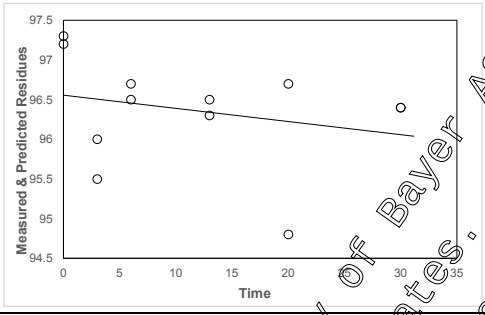
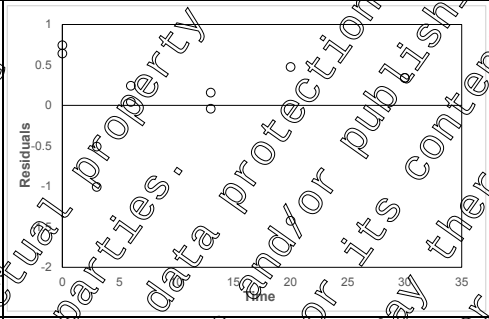
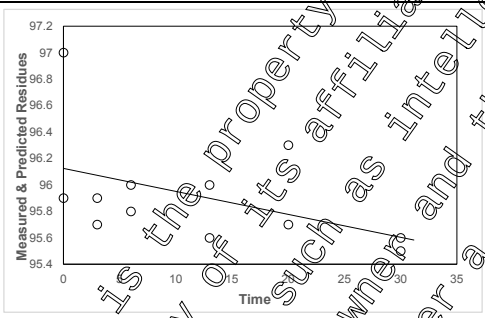
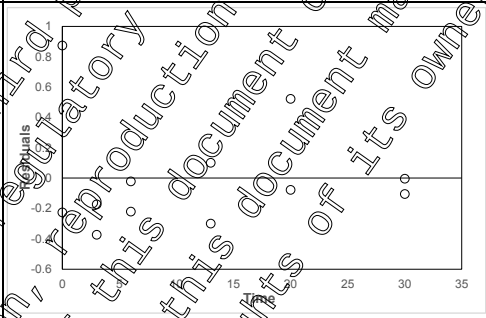
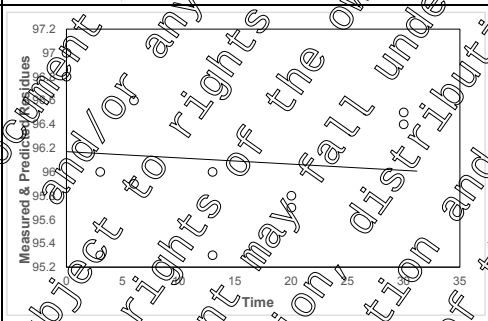
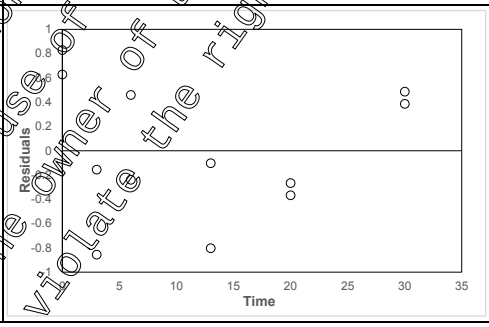
Table 7.2.1.1- 3: Hydrolysis rate of fluopicolide in sterile buffer at 25 ºC

Buffer	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
pH 5, M-241162-01-2	**SFO**	**96.56**	**k 0.0001721**	**0.401**	**0.205**	**-0.0002197**	**0.001**	**4029**	**>10000**
	FOMC	97.25	α 0.0001801 β 2.56E-26	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000
pH 7, M-241162-01-2	**SFO**	**96.13**	**k 0.0001814**	**0.172**	**0.0639**	**-3.27E-05**	**0.00E+00**	**3821**	**>10000**
	FOMC	96.45	α 0.0005771 β 0.0001045	0.119	n.r. n.r.	-0.002164 -0.005709	0.003 0.006	>10000	>10000
pH 9 M-241162-01-2	**SFO**	**96.17**	**k 5.46E-05**	**0.384**	**0.375**	**-0.000273**	**0.00E+00**	**>10000**	**>10000**
	FOMC	96.9	α 0.0001196 β 2.38E-35	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000

Best fit model highlighted in bold

A graphical representation of the final kinetic fit is shown below.

Table 7.2.1.1- 4: Hydrolysis of fluopicolide in sterile buffer at 25 °C (best-fit DT_{50} values)

Buffer Model Reference	Modelled vs observed	Residuals
pH5 SFO M-241162-01-2		
pH7 SFO M-241162-01-2		
pH9 SFO M-241162-01-2		

III. Conclusion

The hydrolysis of [^{14}C]-fluopicolide was studied using sterile aqueous buffer solutions at pH 4, 7 and 9 incubated at 50 °C over a 5 day period and at pH 5, 7 and 9 incubated at 25 °C over a 30 day period.

Fluopicolide did not degrade significantly in any of the sterile buffer solutions after 5 days at 50 °C or 30 days at 25 °C, indicating that fluopicolide is hydrolytically stable.

Assessment and conclusion by applicant:

The study is considered valid to assess the hydrolysis of fluopicolide as a function of pH.

Data Point:	KCA 7.2.1.1/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE C653711: Hydrolysis at pH 4, 5, 7 and 9
Report No:	C037891
Document No:	M-223750-01-1
Guideline(s) followed in study:	USEPA (=EPA): N, 161-1
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The hydrolysis of [Phenyl-U-^{14}C]-M-01 (AE C653711) was investigated in the dark under sterile conditions, at 50 ± 2 °C in pH 4, 7 and 9 buffer solutions and at 25 ± 1 °C in pH 5, 7 and 9 buffer solutions. [Phenyl-U-^{14}C]-M-01 was dissolved in sterile buffers at a nominal concentration of 1.75 g/L.

Samples incubated at 50 °C were removed immediately after treatment and after 5 days. Samples at 25 °C were removed immediately after treatment and after 3, 6, 13, 20 and 30 days. Duplicate replicates of samples incubated at 50 °C and 25 °C were removed at each time point. Sterility of test samples was confirmed at the beginning and end of the 25 °C test.

Radiochemical balances were quantitative in all samples. Buffer samples were analysed by HPLC to identify the compounds present, based on comparison of retention times with analytical standards. . Select samples were analysed by LCMS to confirm the presence M-01.

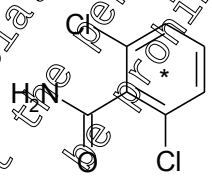
M-01 was relatively stable to hydrolysis at all pHs and both temperatures tested. M-01 accounted for ≥95% of applied radioactivity in every sample analysed. Unidentified components were detected in samples incubated at both 25 °C and 50 °C. Two of these unidentified components were present initially and the levels remained constant throughout the incubation period (at *ca.* 0.3% and *ca.* 0.6%). A third component was observed after 14 days in samples incubated at 25 °C (maximum 0.2%).

I. Materials and Methods

A. Materials

1. Test Item

[Phenyl-U-^{14}C]-M-01 (referred to as AE C653711 in the report)



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

Chemical name (IUPAC):	2,6-dichlorobenzamide
Specific activity:	9.51 MBq/mg
Batch number:	SEL/1169
Radiochemical purity:	99% (HPLC analysis)

2. Sterile Buffers

The buffers used were; pH 4 (0.05M citrate), pH 5 (0.015M acetate), pH 7 (0.05M phosphate) and pH 9 (0.005M disodium tetraborate) and were prepared as follows:

pH4 - Sodium citrate solution (500 mL, 0.1M) and sodium hydroxide solution (90 mL, 0.1M) were mixed and diluted to 1 litre with water.

pH 5 - Acetic acid solution (146 mL, 0.1M) and sodium hydroxide solution (100 mL, 0.1M) were mixed and diluted to 1 litre with water.

pH 7 - Potassium dihydrogen orthophosphate solution (22.4 mL, 0.1M) and disodium hydrogen orthophosphate solution (25.8 mL, 0.1M) were mixed and diluted to 1 litre with water.

pH 9 - Sodium tetraborate solution (500 mL, 0.01M) and hydrochloric acid solution (46 mL, 0.04M) were mixed and diluted to 1 litre with water.

The buffer solutions were sterilised by autoclaving at 126°C for 20 minutes.

B. Study Design

1. Experimental Condition

The hydrolysis of [^{14}C]-M-01 was investigated in the dark, under sterile conditions, at 50 ±2°C in pH 4, 7 and 9 buffer solutions and at 25 ±1°C in pH 5, 7 and 9 buffer solutions. [^{14}C]-M-01 was dissolved in sterile buffers at a nominal concentration of 1.75 g/L. The aqueous solubility of M-01 has been determined to be 2.22 g/L at 20 °C.

2. Sampling

Buffer samples incubated in glass vessels in a water bath at 50°C in the dark were removed immediately, after 2.4 hours and after 5 days. Buffer samples incubated in glass vessels in a temperature-controlled room at 25°C were removed immediately and after 5, 9, 14, 20, 26 and 30 days. Duplicate samples incubated at 50°C and 25°C were removed at each time point, except at time zero when a single sample was taken for analysis. Sterility of test samples was confirmed throughout the study.

3. Analytical procedures

Immediately after sampling triplicate aliquots were removed for analysis by LSC. HPLC was used to identify compounds present, based on comparison of retention times with analytical standards. Select samples were analysed by LCMS to confirm the presence M-01.

4. Determination of degradation kinetics

No degradation kinetics were determined in the report. DT_{50} and DT_{90} values for the degradation of M-01 have been calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1).

An initial comparison was performed between the SFO and FOMC fits for M-01. The data was best fit by the simple first order (SFO) model all buffers, with an $\chi 2$ error of 0.529, 0.953 and 1.09%.

II. Results and Discussion

A. Data

The results of the HPLC analysis of aqueous buffer solution are summarised in Table 7.2.1.1- 5 to Table 7.2.1.1- 6.

Table 7.2.1.1- 5: Characterisation of radioactivity in aqueous buffer solution incubated at 50 °C (mean of duplicate values, % AR)

pH 4

Compound	Mean SD	DAT		
		0	0.1	5
M-01 (AE C653711)	Mean	99.2	100.9	97.7
	SD	± 0.0	± 1.1	± 0.1
Unidentified 1 (RRT 1.6)	Mean	0.3	0.3	0.3
	SD	± 0.0	± 0.0	± 0.0
Unidentified 2 (RRT 1.8)	Mean	n.d.	n.d.	n.d.
	SD	± 0.0	± 0.0	± 0.0
Unidentified 3 (RRT 1.9)	Mean	0.5	0.4	0.6
	SD	± 0.0	± 0.3	± 0.1
Total	Mean	100.0	101.6	98.7
	SD	± 0.0	± 0.9	± 0.05

n.d.: not detected, DAT: days after treatment, SD: standard deviation, RRT; relative retention time

pH 7

Compound	Mean SD	DAT		
		0	0.1	5
M-01 (AE C653711)	Mean	99.2	99.4	98.7
	SD	± 0.0	± 0.2	± 3.5
Unidentified 1 (RRT 1.6)	Mean	0.3	0.3	0.3
	SD	± 0.0	± 0.0	± 0.0
Unidentified 2 (RRT 1.8)	Mean	n.d.	n.d.	n.d.
	SD	± 0.0	± 0.0	± 0.0
Unidentified 3 (RRT 1.9)	Mean	0.6	0.6	0.5
	SD	± 0.0	± 0.3	± 0.1
Total	Mean	100.0	100.3	99.5
	SD	± 0.0	± 0.2	± 3.6

n.d.: not detected, DAT: days after treatment, SD: standard deviation, RRT; relative retention time

pH 9

Compound	Mean SD	DAT		
		0	0.1	5
M-01 (AE C653711)	Mean	99.2	96.9	96.9
	SD	± 0.0	± 2.3	± 0.6
Unidentified 1 (RRT 1.6)	Mean	0.2	0.3	0.3
	SD	± 0.0	± 0.0	± 0.1
Unidentified 2 (RRT 1.8)	Mean	n.d.	n.d.	n.d.
	SD	± 0.0	± 0.0	± 0.0
Unidentified 3 (RRT 1.9)	Mean	0.6	0.6	0.7
	SD	± 0.0	± 0.0	± 0.0
Total	Mean	100.0	97.8	97.8
	SD	± 0.0	± 0.9	± 0.05

n.d.: not detected, DAT: days after treatment, SD: standard deviation, RRT; relative retention time

Table 7.2.1.1- 6: Characterisation of radioactivity in aqueous buffer solution incubated at 25 °C (mean of duplicate values, % AR)

pH 5

Compound	Mean SD	DAT						
		0	5	9	14	20	26	30
M-01 (AE C653711)	Mean	99.1	97.4	97.6	98.2	96.6	97.3	98.0
	SD	± 0.0	± 0.2	± 0.2	± 0.3	± 0.1	± 1.2	± 0.4
Unidentified 1 (RRT 1.6)	Mean	0.3	0.3	0.2	0.3	0.3	0.3	0.3
	SD	± 0.0	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1
Unidentified 2 (RRT 1.8)	Mean	n.d.	n.d.	n.d.	0.1	0.1	0.2	0.1
	SD	± 0.0	± 0.0	± 0.0	± 0.0	± 0.1	± 0.0	± 0.1
Unidentified 3 (RRT 1.9)	Mean	0.6	0.6	0.7	0.6	0.6	0.7	0.7
	SD	± 0.0	± 0.1	± 0.1	± 0.1	± 0.1	± 0.0	± 0.1
Total	Mean	100.0	98.3	98.5	99.2	97.7	98.5	99.1
	SD	± 0.0	± 0.3	± 0.3	± 0.5	± 0.2	± 1.2	± 0.5

n.d.: not detected, DAT: days after treatment, SD: standard deviation, RRT; relative retention time

pH 7

Compound	Mean SD	DAT						
		0	5	9	14	20	26	30
M-01 (AE C653711)	Mean	99.3	95.7	95.6	95.3	95.6	95.0	96.6
	SD	± 0.1	± 0.2	± 0.1	± 0.8	± 0.3	± 0.4	± 0.7
Unidentified 1 (RRT 1.6)	Mean	0.2	0.2	0.3	0.2	0.2	0.2	0.3
	SD	± 0.0	± 0.0	± 0.1	± 0.1	± 0.0	± 0.0	± 0.1
Unidentified 2 (RRT 1.8)	Mean	n.d.	n.d.	n.d.	0.2	0.1	0.1	0.1
	SD	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Unidentified 3 (RRT 1.9)	Mean	0.5	0.7	0.6	0.6	0.6	0.5	0.7
	SD	± 0.0	± 0.1	± 0.1	± 0.1	± 0.0	± 0.0	± 0.1
Total	Mean	100.0	96.6	96.5	96.3	96.5	95.9	97.7
	SD	± 0.0	± 0.0	± 1.9	± 0.3	± 0.4	± 0.3	± 1.0

n.d.: not detected, DAT: days after treatment, SD: standard deviation, RRT; relative retention time

pH 9

Compound	Mean SD	DAT						
		0	5	9	14	20	26	30
M-01 (AE C653711)	Mean	99.2	95.9	96.4	96.5	97.4	95.7	99.2
	SD	± 0.0	± 1.3	± 0.8	± 1.0	± 0.1	± 0.3	± 0.9
Unidentified 1 (RRT 1.6)	Mean	0.3	0.2	0.3	0.3	0.3	0.3	0.2
	SD	± 0.0	± 0.1	± 0.0	± 0.0	± 0.1	± 0.1	± 0.2
Unidentified 2 (RRT 1.8)	Mean	n.d.	n.d.	n.d.	0.1	0.2	0.1	0.1
	SD	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.1
Unidentified 3 (RRT 1.9)	Mean	0.5	0.7	0.6	0.6	0.6	0.7	0.7
	SD	± 0.0	± 0.0	± 0.0	± 0.1	± 0.1	± 0.1	± 0.2
Total	Mean	100.0	96.8	97.3	97.5	98.5	96.8	100.2
	SD	± 0.0	± 1.6	± 0.8	± 1.2	± 0.1	± 0.4	± 0.4

n.d.: not detected, DAT: days after treatment, SD: standard deviation, RRT; relative retention time

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 100.1% for pH 4, 99.9% for pH 7 and 97.8% for pH 9 samples incubated at 50 °C. The mean recoveries of applied radioactivity for samples incubated at 25 °C were 98.5% for pH 4, 96.6% for pH 7 and 97.8% for pH 9 samples. The pH values and sterility of samples was maintained throughout the study.

C. Transformation of test substance

M-01 was relatively stable to hydrolysis at all pHs and both temperatures tested. M-01 accounted for ≥95% of applied radioactivity in every sample analysed. Unidentified components were detected in samples incubated at both 25 ºC and 50 ºC. Two of these unidentified components were present initially and the levels remained constant throughout the incubation period (at *ca.* 0.3% and *ca.* 0.6%). A third component was observed after 14 days in samples incubated at 25 ºC (maximum 0.2%).

D. Degradation Kinetics

The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold.

Parameter confidence was low for SFO fits due to the extremely slow decline in residues. FOMC fits did not generate a full set of statistics.

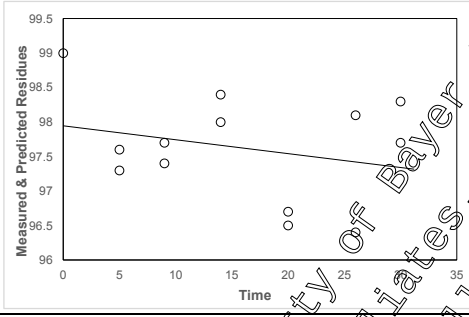
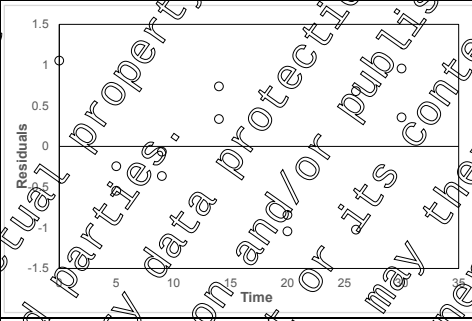
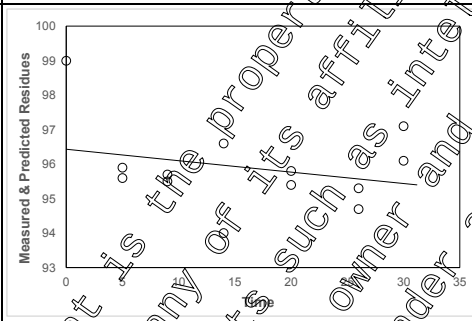
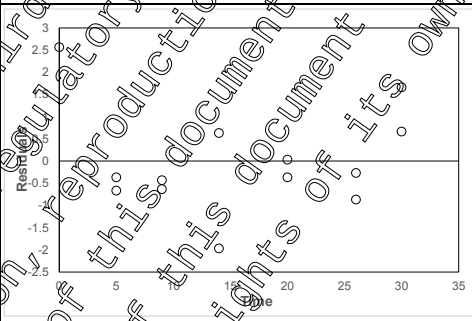
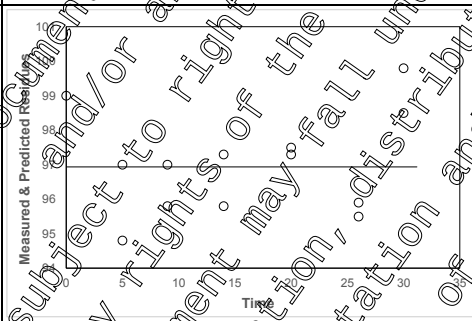
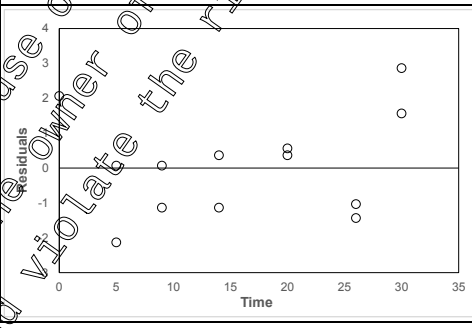
Table 7.2.1.1- 7: Hydrolysis rate of M-01 (AE C653711) in sterile buffer at 25 ºC

Buffer	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob > t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
pH 5, M-223750-01-1	**SFO**	**97.94**	**k 0.0002058**	**0.529**	**0.192**	**-0.0002383**	**0.001**	**3368**	**>10000**
	FOMC	99	α 0.000459 β 6.23E-14	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000
pH 7, M-223750-01-1	**SFO**	**96.43**	**k 0.0003463**	**0.953**	**0.179**	**-0.0003614**	**0.001**	**2002**	**6649**
	FOMC	99	α 0.0004208 β 3.53E-35	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000
pH 9, M-223750-01-1	**SFO**	**96.93**	**k 0.00E+00**	**1.09**	**0.5**	**-0.0008834**	**0.001**	**>10000**	**>10000**
	FOMC	98.99	α 0.0001621 β 5.59E-58	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000

Best fit model highlighted in bold

A graphical representation of the final kinetic fit is shown below.

Table 7.2.1.1- 8: Hydrolysis of M-01 (AE C653711) in sterile buffer at 25 ºC (best-fit DT_{50} values)

Buffer Model Reference	Modelled vs observed	Residuals
pH 5 SFO M-223750-01-1		
pH 7 SFO M-223750-01-1		
pH 9 SFO M-223750-01-1		

III. Conclusion

The hydrolysis of [^{14}C]-M-01 was studied using sterile aqueous buffer solutions at pH 4, 7 and 9 incubated at 50 °C over a 5 day period and at pH 5, 7 and 9 incubated at 25 °C over a 30 day period.

M-01 did not degrade significantly in any of the sterile buffer solutions after 5 days at 50 °C or 30 days at 25 °C, indicating that the metabolite is hydrolytically stable.

Assessment and conclusion by applicant:

The study is considered valid to assess the hydrolysis of M-01 (AE C653711) as a function of pH.

Data Point:	KCA 7.2.1.1/03
Report Author:	[redacted]
Report Year:	2004
Report Title:	AE 0608000: Hydrolytic degradation
Report No:	M-236241-01-2
Document No:	M-236241-01-2
Guideline(s) followed in study:	OECD 111, Draft proposal October 2002
Deviations from current test guideline:	Deviations from SANCO/3029/99-rev. 4: Method: The method was not conducted according to this guideline and as such does not have accuracy and precision data presented. However, the results show that the method is suitable for analysing M-03 (AE 0608000) residues in aqueous buffer solutions via HPLC-UV and is fit for purpose. Study: none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The hydrolysis of M-03 (referred to as AE 0608000 in the study report) was investigated at 20 ± 0.03 °C at pH 5, 6, 7 and 8 in the dark under sterile conditions. Non-radiolabelled M-03 was dissolved in sterile buffer at a nominal concentration of 1 mg/L.

Buffer samples incubated in 10-mL glass crimp-top vials (pH 5, 6, and 7) and volumetric flask (pH 8) were closed with Teflon-faced septa and a plug, respectively and were placed in a temperature-controlled water bath at 20°C in the dark. Duplicate samples were removed at the following timepoints:

- pH 5 0, 3, 9, 24, 31, 48, 57, 72 and 144 hours
- pH 6 0, 0.25, 0.5, 1, 3, 5, 7, 9 and 24.5 hours
- pH 7 0, 0.25, 0.5, 1, 1.5, 2, 3 and 4 hours
- pH 8 0, 5, 10, 15, 20, 25, 30, 40 and 50 minutes

Immediately after sampling, aliquots were acidified with formic acid and analysed by HPLC directly to determine the amount of M-03 remaining in solution. Selected samples were analysed by LC-MS and NMR to confirm the presence of M-03.

M-03 was shown to be hydrolytically labile under acidic, neutral and alkaline conditions at 20°C.

A re-evaluation of the hydrolysis kinetics has been conducted in accordance with FOCUS guidance document on degradation kinetics (2014). The rate of hydrolysis of M-03 was strongly dependent on pH with best-fit DT_{50} values of 0.1, 0.7, 4.4 and 45.5 hours at pH 8, 7, 6 and 5, respectively.

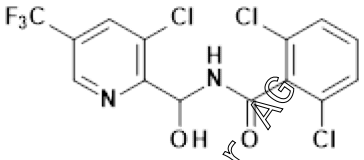
From these results it is concluded that hydrolysis is a major route for the degradation of M-03 in the environment.

I. Materials and Methods

A. Materials

1. Test Item

M-03 (referred to as AE 0608000 in the study report)



Batch number:	MOY4622M
Chemical purity:	96.9% according to HPLC-UV

2. Sterile Buffers

The buffers used were; pH 5 (0.01M acetate), pH 6 (0.01M acetate), pH 7 (0.01M phosphate) and pH 8 (0.01M phosphate) and were prepared as follows: -

pH 5 - sodium acetate solution (250 mL, 0.01M) was adjusted to pH 5.0 using glacial acetic and diluted to 1 litre with water.

pH 6 - sodium acetate solution (250 mL, 0.01M) was adjusted to pH 6.0 using glacial acetic and diluted to 1 litre with water.

pH 7 - 0.1 M tris(hydroxymethyl)aminomethane solution was mixed with 0.1 N hydrochloric acid solution and diluted to 100 mL with purified water. The pH of this solution was measured with a pH electrode and then adjusted to pH 7.0 using 0.1 N HCl solution. The buffer stock solution was diluted to 500 mL with purified water.

pH 9 - 0.1 M tris(hydroxymethyl)aminomethane solution was mixed with 0.1 N hydrochloric acid solution and diluted to 100 mL with purified water. The pH of this solution was measured with a pH electrode and then adjusted to pH 9.0 using 0.1 N NaOH solution. The buffer stock solution was diluted to 500 mL with purified water.

The buffer solutions were sterilised by autoclaving prior to use.

B. Study Design

1. Experimental Condition

The hydrolysis of M-03 was investigated at 20 ± 0.03 °C at pH 5, 6, 7 and 8 in the dark, under sterile conditions. Non-radiolabelled M-03 was dissolved in sterile buffer at a nominal concentration of 1 mg/L. The aqueous solubility of M-03 has been determined to be 10 mg/L at 20 ºC.

Buffer samples incubated in 10-mL glass crimp-top vials (pH 5, 6, and 7) and volumetric flask (pH 8) were closed with Teflon-faced septa and a plug, respectively, and were placed in a temperature-controlled water bath at 20°C in the dark.

2. Sampling

Duplicate samples were removed at the following timepoints:

- pH 5 0, 3, 9, 24, 31, 48, 57, 72 and 144 hours
- pH 6 0, 0.25, 0.5, 1, 3, 5, 7, 9 and 24.5 hours
- pH 7 0, 0.25, 0.5, 1, 1.5, 2, 3 and 4 hours
- pH 8 0, 5, 10, 15, 20, 25, 30, 40 and 50 minutes

Sterility and pH of test samples was confirmed at the beginning and end of the incubation periods. Duplicate samples of each pH buffer were removed at each time point.

3. Analytical procedures

Immediately after sampling, aliquots were acidified with formic acid and analysed by HPLC directly to determine the amount of M-03 remaining in solution. The limit of detection was determined as <30 µg/L (<3% of applied test substance). Selected samples were analysed by LC-MS and NMR to confirm the presence of M-03. Transformation / hydrolysis products were not identified.

4. Determination of degradation kinetics

The reported rate of hydrolysis of M-03 at each pH was calculated assuming simple first order (SFO) kinetics.

The degradation kinetics determined in the report were conducted prior to the issuing of the FOCUS guidance document on degradation kinetics and are no longer considered valid. DT_{50} and DT_{90} values for the degradation of M-03 have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1). An initial comparison was performed between the SFO and FOMC fits for fluopicolide. The comparison of the SFO and FOMC fits suggested bi-phasic decline, and so the DFOP and HS models were therefore also fitted.

The data was best fit by the simple first order (SFO) model at pH 5, with an $\chi 2$ error of 0.423% and by double first order in parallel (DFOP) model at pH 6, 7 and 8 with $\chi 2$ error of 0.491, 1.24 and 1.12%.

II. Results and Discussion

A. Data

The decline of M-03 with time in each buffer is presented in Table 7.2.1.1- 9. The pH values and sterility of samples was maintained throughout the study.

Table 7.2.1.1- 9: Decline of M-03 in aqueous buffer at 20 ºC (mean of duplicate values)

pH 5

M-03	Time (hours)								
	0	3	9	24	31	48	57	72	144
Concentration (mg/L)	1.00	0.95	0.87	0.69	0.63	0.48	0.42	0.33	0.11
% of applied	100	95	87	69	63	48	42	33	11

pH 6

M-03	Time (hours)								
	0	0.25	0.5	1	3	5	7	9	24.5
Concentration (mg/L)	1.01	0.93	0.88	0.82	0.61	0.47	0.35	0.27	0.03
% of applied	100	92	87	81	60	46	34	27	3

pH 7

M-03	Time (hours)							
	0	0.25	0.5	1	1.5	2	3	4
Concentration (mg/L)	0.94	0.70	0.53	0.36	0.24	0.16	0.08	0.04
% of applied	100	75	56	39	26	17	9	4

pH 8

M-03	Time (minutes)								
	0	5	10	15	20	25	30	40	50
Concentration (mg/L)	0.96	0.57	0.39	0.28	0.20	0.13	0.09	0.05	0.03
% of applied	100	60	41	29	20	14	10	5	3

B. Degradation Kinetics

In the report the rate of hydrolysis of M-03 at each pH was calculated assuming first order kinetics and is presented below.

Table 7.2.1.1- 10: Hydrolysis rate of M-03 at 20 ºC

pH	DT_{50}	DT_{90} (days)	k	Fitting criteria (r^2)
5.1	45.5 hours	151.2 hours	$1.52 \pm 0.02 \times 10^{-2}$ h^{-1}	1.00
6.1	4.7 hours	15.7 hours	$1.47 \pm 0.12 \times 10^{-1}$ h^{-1}	0.997
7.1	44.9 minutes	2.5 hours	$9.25 \pm 0.12 \times 10^{-1}$ h^{-1}	0.994
8.1	8.4 minutes	27.9 minutes	$1.38 \pm 0.14 \times 10^{-3}$ s^{-1}	0.994

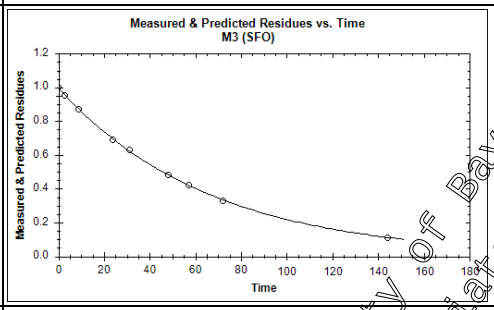
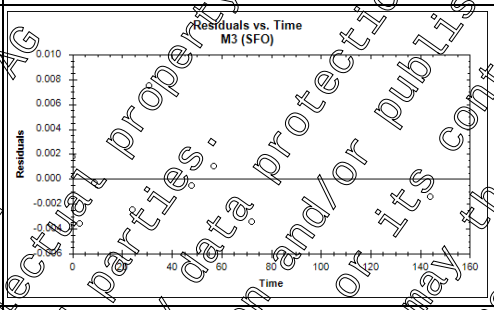
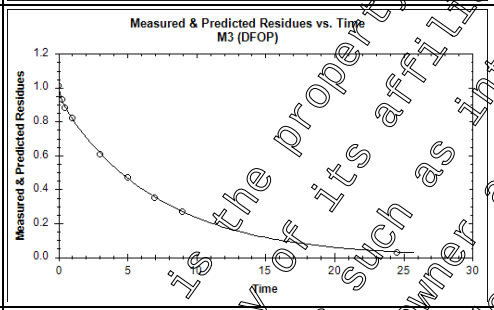
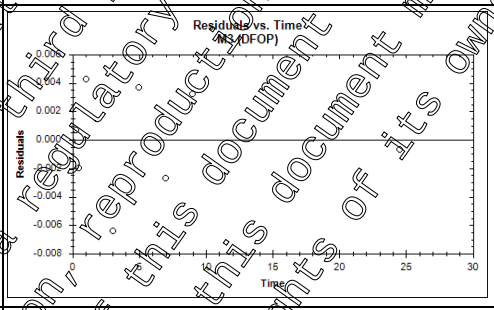
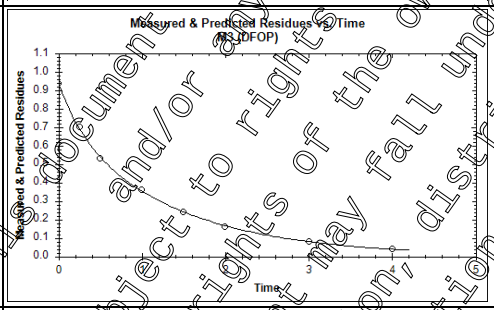
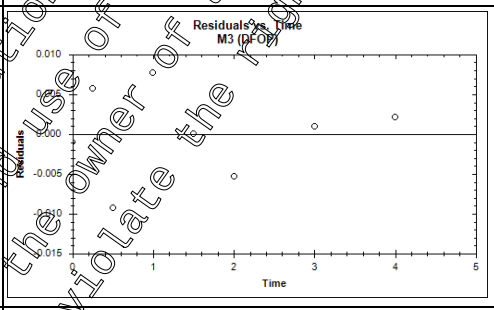
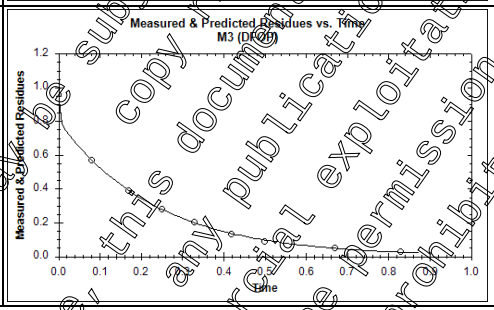
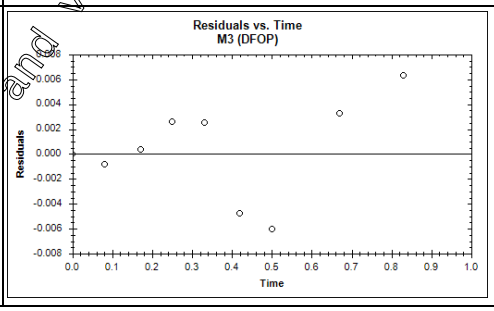
The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold.

Table 7.2.1.1- 11: Hydrolysis rate of M-03 (AE 0608000) in sterile buffer at 20 °C

Buffer	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [hours]	DT_{90} [hours]
pH 5	**SFO**	**0.9982**	**k 0.01523**	**0.423**	**3.34E-14**	**0.01505**	**0.015**	**45.5**	**151.2**
	FOMC	0.9982	α 14690 β 9.65E+05	0.448	n.r. n.r.	14690 9.65E+05	14690 9.65E+05	45.5	151.2
	DFOP	1	k1 10 k2 0.0152 g 0.003055	0.463	<2e-16 2.39E-10 n.r.	10 0.01498 -0.00749	10 0.015 0.014	45.4	151.3
	HS	0.9967	k1 0.01504 k2 0.01545 tb 31	0.414	9.38E-09 2.10E-08 n.r.	0.01458 0.01489 -25.81	0.016 0.016 87.81	45.7	149.8
pH 6	SFO	0.9696	k 0.147	2.35	7.33E-09	0.1371	0.157	4.7	15.7
	FOMC	0.9757	α 6.969 β 43.74	2.24	n.r. n.r.	4.526 -34.16	18.46 121.7	4.6	17
	DFOP	**1.01**	**k1 4.353 k2 0.1395 g 0.07256**	**0.491**	**0.00342 1.60E-09 n.r.**	**2.426 0.1365 0.05916**	**6.28 0.143 0.086**	**4.4**	**16**
	HS	1.01	k1 0.3301 k2 0.1406 tb 0.3677	0.81	2.96E-05 7.03E-10 n.r.	0.2782 0.138 0.2808	0.382 0.143 0.455	4.4	15.9
pH 7	SFO	0.9068	k 0.9248	4.97	7.07E-07	0.829	1.021	0.7	2.5
	FOMC	0.9337	α 3.257 β 2.787	3.7	n.r. n.r.	1.879 1.335	4.636 4.24	0.7	2.9
	DFOP	**0.941**	**k1 3.356 k2 0.7363 g 0.2352**	**1.24**	**0.00725 3.04E-05 n.r.**	**1.763 0.6545 0.1247**	**4.949 0.818 0.346**	**0.7**	**2.8**
	HS	0.9068	k1 0.9248 k2 0.00E+00 tb 2705	5.72	5.14E-05 NA n.r.	0.8074 0.00E+00 2705	1.042 0.00E+00 2705	0.7	2.5
pH 8	SFO	0.9285	k 4.945	6.53	7.27E-08	4.481	5.409	0.1	0.5
	FOMC	0.9517	α 3.666 β 0.5931	3.98	n.r. n.r.	1.599 0.1846	5.733 1.002	0.1	0.5
	DFOP	**0.96**	**k1 257.5 k2 4.244 g 0.165**	**1.12**	**<2e-16 5.24E-09 n.r.**	**257.5 4.127 0.1446**	**257.5 4.361 0.185**	**0.1**	**0.5**
	HS	0.9285	k1 4.945 k2 0.5534 tb 1.547	6.36	3.36E-06 <2e-16 n.r.	4.396 0.5534 1.547	5.494 0.553 1.547	0.1	0.5

A graphical representation of the final kinetic fit is shown below.

Table 7.2.1.1- 12: Hydrolysis of M-03 (AE 0608000) in sterile buffer at 20 °C (best-fit DT_{50} values)

Buffer Model Reference	Modelled vs observed	Residuals
pH 5 SFO		
pH 6 DFOP		
pH 7 DFOP		
pH 8 DFOP		

III. Conclusion

The hydrolysis of M-03 was investigated to provide further information on its fate in the aquatic environment. M-03 was shown to be hydrolytically labile under acidic, neutral and alkaline conditions at 20°C.

A re-evaluation of the hydrolysis kinetics has been conducted in accordance with FOCUS guidance document on degradation kinetics (2014). The rate of hydrolysis of M-03 was strongly dependent on pH with best-fit DT_{50} values of 0.1, 0.7, 4.4 and 45.5 hours at pH 8, 7, 6 and 5, respectively.

From these results it is concluded that hydrolysis is a major route for the degradation of M-03 in the environment.

Assessment and conclusion by applicant:

The hydrolysis of the soil metabolite M-03 (AE 0608000), which was not observed in water sediment systems, was investigated to provide further information on its fate in the environment. The study is considered valid to assess the hydrolysis of M-03 as a function of pH.

CA 7.2.1.2 Direct photochemical degradation

The aqueous photolysis of fluopicolide and its metabolite M-01 in sterile buffer have been investigated in Study KCA 7.2.1.2/01, KCA 7.2.1.2/02 and KCA 7.2.1.2/03. These studies were evaluated during the previous EU review and are still considered acceptable.

Test item	Report reference	Author, Year	Phenyl Label	Pyridyl Label	Comment
Fluopicolide	KCA 7.2.1.2/01 M-241161-01-2	[redacted] 2003	✓	×	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
Fluopicolide	KCA 7.2.1.2/02 M-228807-01-1	[redacted] 2004	×	✓	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-01	KCA 7.2.1.2/03 M-234915-01-1	[redacted] 2000	✓	NA	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.

Data Point:	KCA 7.2.1.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Photolysis and quantum yield of [14C]- AE C638206 in buffered aqueous solution
Report No:	M-241161-01-2
Document No:	M-241161-01-2
Guideline(s) followed in study:	EU (=EEC): 91/414/EEC; USEPA (=EPA): Subdivision N, E-Fate 161-1
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aqueous photolysis of [phenyl-U-^{14}C]-fluopicolide was investigated after exposure to artificial light in sterile 0.005M phosphate buffer solution at pH 7 for up to 31 days in 12 hour light and dark cycles. [Phenyl-U-^{14}C]-fluopicolide was dissolved in buffer at a concentration of 0.65 mg/L with 0.2% acetonitrile present as a co-solvent.

Quartz sample tubes were irradiated in a Heraeus Suntest CPS+ apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. Light exposed samples were placed in a temperature controlled water bath (25 ± 1°C) and the temperature was monitored continuously. Dark control samples were run concurrently in Pyrex® tubes wrapped in aluminium foil to prevent irradiation. The dark control samples were placed in an incubator and maintained at 25 ± 1°C for the study period. Volatiles were continuously trapped using a series of traps containing ethylene glycol to trap organic volatiles, two 10% aqueous potassium hydroxide solutions to trap CO_2 and a solid charcoal trap.

Irradiated and dark control samples were removed after 0, 3, 7, 14, 21 and 31 days. Triplicate aliquots were taken for LSC to quantify the radioactivity present in solution. From day 7 onwards, selected test vessels were rinsed with acetonitrile and the radioactivity removed quantified by LSC. The radioactivity in the trap solutions attached to both the irradiated and non-irradiated samples were quantified by LSC. Buffer solutions were analysed by high performance liquid chromatography (HPLC) using fraction collection followed by LSC to quantify the individual radioactive peaks. Identification of radioactive peaks was achieved by co-chromatography with certified reference standards. The peak assignments of fluopicolide and M-01 were confirmed by two-dimensional thin layer chromatography (TLC).

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 99.7 ± 3.3% for light exposed samples, and 98.9 ± 4.6% for dark control samples.

Fluopicolide exhibited slow degradation in light exposed samples and represented up to 83 % of applied radioactivity after 31 days. Carbon dioxide was detected in potassium hydroxide traps and represented 4% of applied radioactivity by the end of the study in the irradiated samples. Virtually no organic volatiles were detected (≤ 0.1%). Fluopicolide did not degrade significantly in the dark control samples and represented 95% of applied radioactivity at the end of the incubation period.

M-01 (AE C653711) was a minor degradation product present in both light exposed and dark control samples at the time 0 sampling (≤ 2.0%). It increased to a maximum 4 % and 3 % of applied radioactivity in light exposed and dark control samples, respectively, by the end of the experiment. These levels are comparable to those detected as a result of hydrolysis of fluopicolide.

A heterogeneous mixture of minor components was detected throughout the chromatographic run as a result of fraction collecting the HPLC eluant from the buffer solutions. No single component in this background radioactivity exceeded 3.5% of applied radioactivity.

The quantum yield of fluopicolide was calculated to be 3.50×10^{-2} based on a concurrently irradiated [PNAP]/[PYR] actinometer.

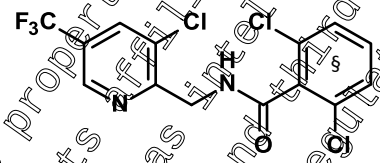
The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). The best fit DT_{50} value calculated for fluopicolide was 106.9 days summer sunlight at 30-50 °N.

I. Materials and Methods

A. Materials

1. Test Item

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



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

Specific activity:	155 µCi/mg
Batch number:	901CU-2
Radiochemical purity:	95.5% (HPLC analysis)

2. Sterile Buffer

The buffer used was pH 7 (0.005M phosphate). The electrical conductivity of the buffer solution was 722 µS/cm.

B. Study Design

1. Experimental Conditions

The aqueous photolysis of [phenyl-U-^{14}C]-fluopicolide was exposed to artificial light in sterile 0.005M phosphate buffer solution at pH 7 for up to 31 days in 12 hour light and dark cycles. [Phenyl-U-^{14}C]-fluopicolide, was dissolved in buffer at a concentration of 0.65 mg/L with 0.2% acetonitrile present as a co-solvent. Quartz sample tubes were irradiated in a Heraeus Suntest CPS+ apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. Light exposed samples were placed in a temperature controlled water bath (25 ± 1°C) and the temperature was monitored continuously. Dark control samples were run concurrently in Pyrex® tubes wrapped in aluminium foil to prevent irradiation. The dark control samples were placed in an incubator and maintained at 25 ± 1°C for the study period. Volatiles were continuously trapped using a series of traps containing ethylene glycol to trap organic volatiles, two 10% aqueous potassium hydroxide solutions to trap CO_2 and a solid charcoal trap.

2. Sampling

Irradiated and dark control samples were removed after 0, 3, 7, 14, 21 and 31 days. Duplicate samples were removed at each timepoint, and the pH and electrical conductivity measured. One of the duplicate irradiated samples from DAT 7 was partially lost and the data from this sample has not been used in any subsequent calculations.

Sterility of test samples was confirmed at day 0, DAT 14 and DAT 31.

3. Analytical procedures

Triplicate aliquots were taken for LSC to quantify the radioactivity present in solution. From DAT 7 onwards, selected test vessels were rinsed with acetonitrile and the radioactivity removed quantified by LSC. The radioactivity in the trap solutions attached to both the irradiated and non-irradiated samples were quantified by LSC. The radioactivity contained in the potassium hydroxide traps from the DAT 31 irradiated sample was confirmed as $^{14}CO_2$ by precipitation as ^{14}C barium carbonate. It was not necessary to process the charcoal traps as quantitative recoveries were achieved.

Buffer solutions were analysed by HPLC using fraction collection followed by LSC to quantify the individual radioactive peaks. Identification of radioactive peaks was achieved by co-chromatography with certified reference standards. The limit of detection was determined as 50 dpm or 0.2%. The acetonitrile rinse of one light exposed and one dark control sample from DAT 31 timepoint were analyzed by HPLC to compare profiles of acetonitrile rinses and buffer solutions. The peak assignments of fluopicolide and M-01 were confirmed by two-dimensional TLC analysis of the DAT 31 light exposed and dark control buffer solution samples.

A p-nitroacetophenone (PNAP) / pyridine (PYR) chemical actinometer was exposed concurrently to determine the quantum yield of fluopicolide in aqueous solutions. The concentration of PNAP in samples was determined by HPLC.

4. Determination of degradation kinetics

The reported half-life of fluopicolide in light exposed samples was calculated assuming single-exponential first order kinetics.

The kinetics determined in the report were conducted prior to the issuing of the FOCUS guidance document on degradation kinetics and are no longer considered valid. DT_{50} and DT_{90} values for the degradation of fluopicolide have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1).

An initial comparison was performed between the SFO and FOMC fits for fluopicolide under irradiated conditions and in the dark control samples. For the irradiated samples, FOMC provided a slight decrease in χ2-error and DFOP and HS fits were assessed. However, parameter confidence was poor for all biphasic models. The SFO model provided an acceptable fit and was selected for trigger endpoints.

For the dark control samples, the FOMC fit provided no significant improvement, and the SFO fit was therefore accepted.

II. Results and Discussion

A. Data

The recovery and distribution of radioactivity in light exposed and dark control samples is presented in Table 7.2.1.2- 1 and Table 7.2.1.2- 2. The characterisation of radioactivity in light exposed and dark control samples is presented in Table 7.2.1.2- 3 to Table 7.2.1.2- 5.

Table 7.2.1.2- 1: Recovery and distribution of radioactivity in light exposed samples expressed as % of applied radioactivity

	Replicate	Incubation time (DAT)					
		0	3	7	14	21	31
Buffer solution	1	104.1	96.5	58.8*	90.2	93.7	96.1
	2	102.6	95.1	95.4	97.8	98.3	89.8
	Mean	103.4	95.8	n.a.	94.0	96.0	93.0
	SD	± 1.0	± 1.0	n.a.	± 5.4	± 3.3	± 4.5
Acetonitrile rinse	1	n.a.	n.a.	6.4*	2.5	2.4	2.8
	2	n.a.	n.a.	n.a.	n.a.	2.1	2.7
	Mean	n.a.	n.a.	n.a.	n.a.	2.3	2.8
	SD	n.a.	n.a.	n.a.	n.a.	± 0.2	± 0.1
Carbon dioxide	1	n.a.	0.6	0.1*	4.0	3.7	3.8
	2	n.a.	0.4	1.4	3.1	3.5	3.7
	Mean	n.a.	0.5	n.a.	3.6	3.6	3.8
	SD	n.a.	± 0.1	n.a.	± 0.6	± 0.1	± 0.1
Organic Volatiles	1	n.a.	0.1	0.0*	0.1	0.1	0.1
	2	n.a.	0.1	0.0	0.1	0.1	0.1
	Mean	n.a.	0.1	n.a.	0.1	0.1	0.1
	SD	n.a.	± 0.0	n.a.	± 0.0	± 0.0	± 0.0
Total	1	104.1	97.2	n.a.*	96.8	99.9	102.8
	2	102.6	95.6	96.9	101.0	104.0	96.3
	Mean	103.4	96.4	n.a.	98.9	102.0	99.6
	SD	± 1.0	± 1.1	n.a.	± 3.0	± 2.9	± 4.6

n.a. = not applicable, *Sample leaked during exposure, data not used

Table 7.2.1.2- 2: Recovery and distribution of radioactivity in dark control samples expressed as % of applied radioactivity

	Replicate	Incubation time (DAT)					
		0	3	7	14	21	31
Buffer solution	1	104.1	98.4	96.2	99.9	101.1	100.2
	2	102.6	97.2	98.8	87.1	93.9	97.4
	Mean	103.4	97.8	97.5	93.5	97.5	98.8
	SD	± 1.0	± 0.8	± 1.8	± 9.1	± 5.1	± 2.0
Acetonitrile rinse	1	n.a.	n.a.	n.a.	n.a.	2.9	1.8
	2	n.a.	n.a.	n.a.	1.8	2.4	2.2
	Mean	n.a.	n.a.	n.a.	0.9	2.7	2.0
	SD	n.a.	n.a.	n.a.	± 1.3	± 0.4	± 0.3
Carbon dioxide	1	n.a.	0.0	0.0	0.0	0.0	0.2
	2	n.a.	0.0	0.0	0.0	0.0	0.2
	Mean	n.a.	0.0	0.0	0.0	0.0	0.2
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Organic Volatiles	1	n.a.	0.0	0.1	0.0	0.1	0.1
	2	n.a.	0.0	0.0	0.1	0.1	0.0
	Mean	n.a.	0.0	0.1	0.1	0.1	0.1
	SD	n.a.	± 0.0	± 0.1	± 0.1	± 0.0	± 0.1
Total	1	104.1	98.4	96.3	99.9	104.1	102.3
	2	102.6	97.2	98.8	89.0	96.4	99.8
	Mean	103.4	97.8	97.6	94.5	100.3	101.1
	SD	± 1.1	± 0.8	± 1.8	± 7.7	± 5.4	± 1.8

n.a. = not applicable

Table 7.2.1.2- 3: Characterisation of radioactivity in light exposed buffer samples expressed as % of applied radioactivity

	Replicate	Incubation time (DAT)					
		0	3	7	14	21	31
Buffer solution	1	104.1	96.5	58.8*	90.2	93.7	96.1
	2	102.6	95.1	95.4	97.8	98.3	89.8
	Mean	103.4	95.8	n.a.	94.0	96.0	93.0
	SD	± 1.1	± 1.0	n.a.	± 5.4	± 3.3	± 4.5
Fluopicolide	1	97.0	92.3	77.3	80.8	80.3	85.6
	2	95.5	92.3	89.6	83.2	82.0	78.9
	Mean	96.3	92.3	n.a.	82.0	81.2	81.3
	SD	± 1.1	± 0.0	n.a.	± 1.7	± 1.2	± 3.3
M-01 (AE C653711)	1	1.9	3.0	4.2	3.8	4.1	4.1
	2	2.0	2.9	3.4	4.1	4.2	4.8
	Mean	2.0	3.0	n.a.	4.0	4.2	4.5
	SD	± 0.1	± 0.1	n.a.	± 0.2	± 0.1	± 0.5
Remainder	1	1.1	4.7	18.5	15.4	15.6	12.3
	2	2.5	4.9	7.0	12.7	13.8	16.3
	Mean	1.8	4.8	n.a.	14.1	14.7	14.3
	SD	± 1.0	± 0.1	n.a.	± 1.9	± 1.3	± 2.8
Number of peaks in remainder	1	2	12	14	20	19	21
	2	5	12	12	20	20	17

n.a. = not applicable, *Sample leaked during exposure, data not used

Table 7.2.1.2- 4: Characterisation of radioactivity in dark control buffer samples expressed as % of applied radioactivity

	Replicate	Incubation time (DAT)					
		0	3	7	14	21	31
Buffer solution	1	104.1	98.4	96.2	99.9	101.1	100.2
	2	102.6	97.2	98.8	87.1	93.9	97.4
	Mean	103.4	97.8	97.5	93.5	97.5	98.8
	SD	± 1.1	± 0.8	± 1.8	± 9.1	± 5.1	± 2.0
Fluopicolide	1	97.0	96.3	95.7	95.6	96.2	96.1
	2	95.5	95.9	96.1	94.8	95.9	95.5
	Mean	96.3	96.9	95.9	95.2	96.1	95.8
	SD	± 1.1	± 0.3	± 0.3	± 0.6	± 0.2	± 0.4
M-01 (AE C653711)	1	1.9	2.7	2.9	2.8	2.8	2.9
	2	2.0	2.9	2.8	2.8	3.1	3.1
	Mean	2.0	2.8	2.9	2.8	3.0	3.0
	SD	± 0.1	± 0.1	± 0.1	± 0.0	± 0.2	± 0.1
Remainder	1	1.1	1.4	1.4	1.6	1.0	1.0
	2	2.5	1.2	1.1	2.4	1.0	1.4
	Mean	1.8	1.3	1.3	2.0	1.0	1.0
	SD	± 1.0	± 0.1	± 0.2	± 0.5	± 0.0	± 0.3
Number of peaks in remainder	1	3	5	4	5	3	4
	2	5	3	4	11	2	5

Table 7.2.1.2- 5: Characterisation of radioactivity in acetonitrile rinses

Incubation time (DAT)	**31**	
Sample	Light Exposed	Dark Control
Replicate	1	2
Acetonitrile Rinse (% AR)	2.8	2.0
Fluopicolide (% in rinse) [A]	87.4	86.8
M-01 (% in rinse)	6.3	2.7
Remainder (% in rinse)	6.3	10.5
Number of peaks in remainder	5	4

[A] % of radioactivity in acetonitrile rinse

The characterisation data in the report has been presented as percentage in solution. A summary of the distribution and characterisation expressed as percentage of applied radioactivity at the start of the incubation period and at the final timepoint is presented in Table 7.2.1.2- 6. This data has been taken from or calculated from data presented in the report.

Table 7.2.1.2- 6: Summary of distribution and characterisation of radioactivity at initiation and end of incubation period

DAT	Sample	% of applied radioactivity						
		In solution (buffer and acetonitrile rinse)				Carbon dioxide	Organic Volatiles	Total
		Total	Fluopicolide	M-01	Remainder*			
0	Mean	103.4	99.5	2.0	1.9	na	na	103.4
31	Light Exposed (Replicate 1)	98.9	82.8	4.1	12.0	3.8	0.1	102.8
31	Dark Control (Replicate 2)	99.6	94.9	3.1	1.6	0.2	0.0	99.8

* Remainder = up to 21 radioactive peaks and no single component exceeded 3.5% of applied radioactivity

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 99.7 ± 3.3% for light exposed samples, and 98.9 ± 4.6% for dark control samples.

C. Transformation of test substance

Fluopicolide exhibited slow degradation in light exposed samples and represented up to 83 % of applied radioactivity after 31 days. Carbon dioxide was detected in potassium hydroxide traps and represented 4% of applied radioactivity by the end of the study in the irradiated samples. Virtually no organic volatiles were detected (≤ 0.1%). Fluopicolide did not degrade significantly in the dark control samples and represented 95% of applied radioactivity at the end of the incubation period.

M-01 (AE C653718) was a minor degradation product present in both light exposed and dark control samples at the time 0 sampling (≤ 2.0%). It increased to a maximum 4 % and 3 % of applied radioactivity in light exposed and dark control samples, respectively, by the end of the experiment. These levels are comparable to those detected as a result of hydrolysis of fluopicolide.

A heterogeneous mixture of minor components was detected throughout the chromatographic run as a result of fraction collecting the HPLC eluant from the buffer solutions (listed as Remainder in Table 7.2.1.2- 6). At day 0 three to five radioactive peaks were detected with retention times in the HPLC ranging from 16 to 36 minutes. At DAT 31 in the Light Exposed sample 21 radioactive peaks were detected with retention times in the HPLC ranging from 3 to 38 minutes. At DAT 31 in the Dark Control sample five radioactive peaks were detected with retention times in the HPLC ranging from 13 to 38 minutes.

These minor components represented ~3 % in total in the time zero and dark control samples throughout the study. In the light exposed buffer solutions the total levels and number of heterogeneous components was greater and represented a maximum of ca. 16% of the radioactivity in solution (approximately 12% of applied radioactivity) by the end of the study. No single component in this background radioactivity exceeded 3.5% of applied radioactivity.

D. Degradation Kinetics

In the report the half-life of [^{14}C]-fluopicolide in light exposed samples was calculated to be 64.2 days (r2 = 0.996), assuming single-exponential first order kinetics and based on experimental conditions of 12 hour light/dark cycles of the Suntest unit. The quantum yield of fluopicolide was calculated to be 3.50×10^{-2} based on a concurrently irradiated [PNAP]/[PYR] actinometer. The quantum yield and average solar irradiance values at latitudes of 30º to 50º N were used to predict half-life (DT_{50}) at different seasons. The predicted half-life values for fluopicolide at 30º, 40º and 50º N latitude during summer seasons were 77, 81 and 88 days respectively. In addition to fulfil Japanese Registration requirements the half-life was estimated at 35º N latitude (Tokyo) in the spring season to be 231 days.

The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold.

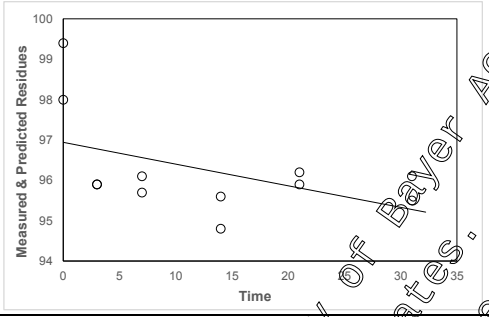
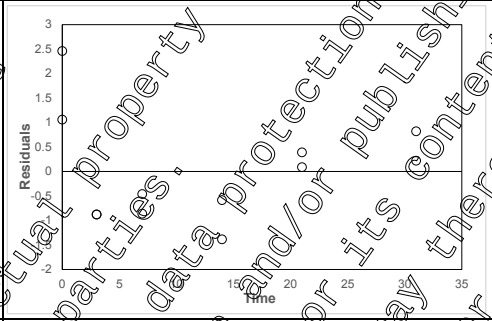
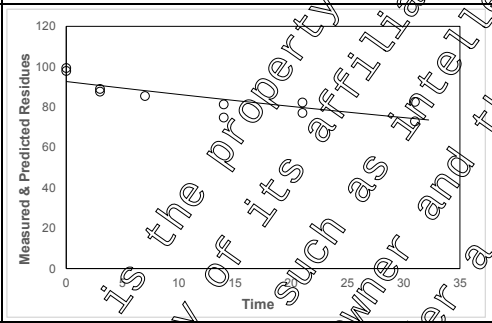
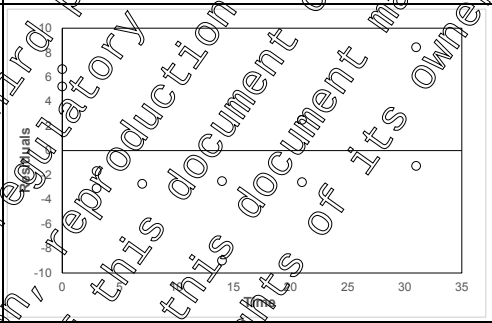
Table 7.2.1.1- 13: Degradation rate of fluopicolide in sterile buffer under irradiated conditions at 25 ºC

Test conditions	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Dark Control M-241161-01-2	**SFO**	**96.94**	**k 0.0005581**	**0.799**	**0.0543**	**-6.29E-05**	**0.001**	**1242**	**4126**
	FOMC	98.7	α 0.0006078 β 3.33E-21	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000
Irradiated, M-241161-01-2	**SFO**	**92.75**	**k 0.007173**	**3.72**	**0.00159**	**0.003644**	**0.011**	**96.6**	**321**
	FOMC	98.74	α 0.06064 β 0.5323	1.37	n.r. n.r.	0.0165 -0.9127	0.105 1.977	>10000	>10000
	DFOP	98.55	k1 0.2221 k2 0.0005059 g 0.195	1.5	0.1002 0.4492 n.r.	-0.08746 -0.006983 0.0398	0.532 0.008 0.35	941.4	4123
	HS	98.7	k1 0.03674 k2 0.002563 tb 4.821	2.05	0.0175 0.146 n.r.	0.009126 -0.001847 1.063	0.064 0.007 8.579	206.1	834

Best fit model highlighted in bold

A graphical representation of the final kinetic fit is shown below.

Table 7.2.1.1- 14: Photolysis of fluopicolide in sterile buffer at 25 ºC (best-fit DT_{50} values)

Test conditions Model Reference	Modelled vs observed	Residuals
Dark Control SFO M-241161-01-2		
Irradiated SFO M-241161-01-2		

The predicted half-life values for fluopicolide at 30º to 50º N latitude during summer was calculated in accordance with OECD guidance.

$$d = \frac{h \times Rx}{0.75 \times 12}$$

Where d = days of summer sunlight
h = hours of Suntest irradiation (12)
Rx = ratio of Suntest intensity to midday summer sunlight intensity (0.83)
0.75 = correction for diurnal variation of natural sunlight
12 = conversion of hours to days

Dark incubations did not show an appreciable decline and no correction to the photolysis degradation rate was necessary for degradation in the dark. The net adjustment factor applied was therefore 1.107, which gives DT_{50} value of 106.9 days and DT_{90} value of 355.2 days at 30º to 50º N latitude during summer.

III. Conclusion

The results of this study indicate that aqueous photolysis is not a major mode of dissipation of [^{14}C] fluopicolide under the test conditions employed.

Assessment and conclusion by applicant:

The study is considered valid to aid assessment of the photodegradation of [phenyl-U-^{14}C]-fluopicolide in the environment.

Data Point:	KCA 7.2.1.2/02
Report Author:	[redacted]
Report Year:	2004
Report Title:	Phototransformation of [pyridyl-2,6-14C]AE C638206 in sterile water buffered at pH 7
Report No:	C040386
Document No:	M-228807-01-1
Guideline(s) followed in study:	EU 94/37/EC and 95/36/EC amending 91/414/EEC; SETAC-Europe, Procedures, March 1995, Section 10; US EPA, Subdivision N, 161-2; Japanese MAFF, 12 Nousan 8147, Annex No. 2-6-2
Deviations from current test guideline:	No
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aqueous photolysis of [2,6-pyridyl-^{14}C]-labelled fluopicolide was exposed to artificial light in sterile 10 mM phosphate buffer solution at pH 7 for up to 10 days continuous irradiation. [2,6-pyridyl-^{14}C]-labelled fluopicolide was dissolved in buffer at a concentration of 0.66 mg/L with 0.16% acetonitrile present as a co-solvent.

Quartz sample tubes were irradiated in a Heraeus Suntest CPS+ apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. Light exposed samples were placed in a temperature-controlled water bath (25 ± 1°C) and the temperature was monitored continuously. Dark control samples were run concurrently in an incubator and maintained at 25 ± 1°C for the study period. Samples were incubated under static conditions in flasks equipped with a combined solid phase trap for the collection of CO_2 (soda lime) and volatile organic compounds (polyurethane plug).

Irradiated samples were removed after 0, 1, 2, 3, 6, 8 and 10 days irradiation while dark control samples were removed after 3 and 10 days.

Duplicate samples were removed at each timepoint, and the pH and oxygen content measured (of final timepoint samples only). Triplicate aliquots were taken for LSC to quantify the radioactivity present in solution. Sterility of test samples was confirmed at Day 0, 3, 6 and 10.

Buffer solutions were analysed by high performance liquid chromatography (HPLC) using a flow-through radiodetector to quantify the individual radioactive peaks. Identification of radioactive peaks was achieved by co-chromatography with certified reference standards. The peak assignment of fluopicolide was confirmed by thin layer chromatography (TLC) analysis.

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 101.4 % for light exposed samples, and 101.3 % for dark control samples. Based on these recoveries it was concluded that no volatile products were generated and volatile traps were not processed.

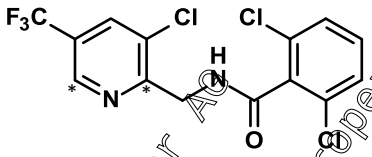
No significant degradation of fluopicolide was observed in either the light exposed samples or the dark control samples where the test item represented 102.3% and 102.8% of applied radioactivity respectively at the end of the incubation period.

I. Materials and Methods

A. Materials

1. Test Item

[2,6-Pyridyl-^{14}C]-fluopicolide



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

Batch Number:	GAR2034/4
Specific Activity:	3.7 MBq/mg, 100 µCi/mg
Radiochemical Purity:	>99.0% (HPLC analysis)

2. Sterile Buffer

The buffer used was 10 mM phosphate buffer solution at pH 7.

B. Study Design

1. Experimental Conditions

The aqueous photolysis of [2,6-pyridyl-^{14}C]-labelled fluopicolide, was exposed to artificial light in sterile 10 mM phosphate buffer solution at pH 7 for up to 10 days continuous irradiation. [2,6-pyridyl-^{14}C]-labelled fluopicolide, was dissolved in buffer at a concentration of 0.66 mg/L with 0.16% acetonitrile present as a co-solvent. Quartz sample tubes were irradiated in a Heraeus Suntest CPS+ apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. Light exposed samples were placed in a temperature-controlled water bath (25 ± 1°C) and the temperature was monitored continuously. Dark control samples were run concurrently in an incubator and maintained at 25 ± 1°C for the study period. Samples were incubated under static conditions in flasks equipped with a combined solid phase trap for the collection of CO_2 (soda lime) and volatile organic compounds (polyurethane plug).

2. Sampling

Irradiated samples were removed after 0, 1, 2, 3, 6, 8 and 10 days irradiation while dark control samples were removed after 3 and 10 days.

Sterility of test samples was confirmed at Day 0, 3, 6 and 10.

3. Analytical procedures

Duplicate samples were removed at each timepoint, and the pH and oxygen content measured (of final timepoint samples only). Triplicate aliquots were taken for LSC to quantify the radioactivity present in solution.

Buffer solutions were analysed by high performance liquid chromatography (HPLC) using a flow-through radiodetector to quantify the individual radioactive peaks. Identification of radioactive peaks was achieved by co-chromatography with certified reference standards. The limit of detection was determined as 1%. The peak assignment of fluopicolide was confirmed by thin layer chromatography (TLC) analysis.

4. Determination of degradation kinetics

No degradation kinetics were determined in the report. DT_{50} and DT_{90} values for the degradation of fluopicolide have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1).

An initial comparison was performed between the SFO and FOMC fits for fluopicolide under irradiated conditions. For the irradiated samples the FOMC fit provided no significant improvement, and the SFO fit was therefore accepted. There were insufficient data points available to obtain a robust fit for samples incubated in the dark.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity in light exposed and dark control samples is presented in Table 7.2.1.2- 7 and Table 7.2.1.2- 8.

Table 7.2.1.2- 7: Distribution and characterisation of radioactivity in light exposed samples expressed as percentage of applied radioactivity

	Replicate	Incubation time (DAT)						
		0	1	2	3	6	8	10
Aqueous Buffer	1	99.6	101.4	100.3	101.1	101.6	102.5	102.3
	2	100.4	101.9	100.2	101.8	101.7	102.4	102.2
	Mean	100.0	101.6	100.3	101.4	101.7	102.4	102.3
	SD	± 0.6	± 0.4	± 0.1	± 0.5	± 0.1	± 0.1	± 0.1
Fluopicolide	1	99.6	101.4	100.3	101.1	101.6	102.5	102.3
	2	100.4	101.9	100.2	101.8	101.7	102.4	102.2
	Mean	100.0	101.6	100.3	101.4	101.7	102.4	102.3
	SD	± 0.6	± 0.4	± 0.1	± 0.5	± 0.1	± 0.1	± 0.1

Volatile traps were not processed as recoveries were quantitative

Table 7.2.1.2- 8: Distribution and characterisation of radioactivity in dark control samples expressed as percentage of applied radioactivity

	Replicate	Incubation time (DAT)		
		0	3	10
Aqueous Buffer	1	99.6	101.6	103.0
	2	100.4	102.1	102.7
	Mean	100.0	101.8	102.8
	SD	± 0.6	± 0.4	± 0.2
Fluopicolide	1	99.6	101.6	103.0
	2	100.4	102.1	102.7
	Mean	100.0	101.8	102.8
	SD	± 0.6	± 0.4	± 0.2

Volatile traps were not processed as recoveries were quantitative

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 101.4 % for light exposed samples, and 101.5 % for dark control samples. Based on these recoveries it was concluded that no volatile products were generated and volatile traps were not processed.

C. Transformation of test substance

No significant degradation of fluopicolide was observed in either the light exposed samples or the dark control samples where the test item represented 102.3% and 102.8% of applied radioactivity respectively at the end of the incubation period.

D. Degradation Kinetics

The experimental data has been reevaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold. No significant degradation was observed for fluopicolide incubated under irradiated conditions or in dark controls.

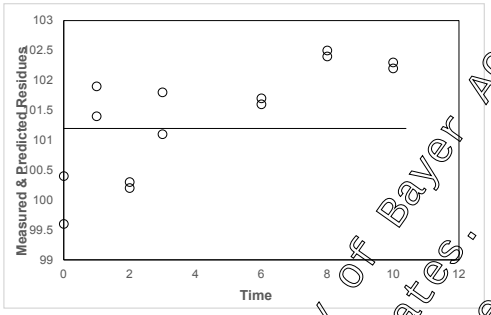
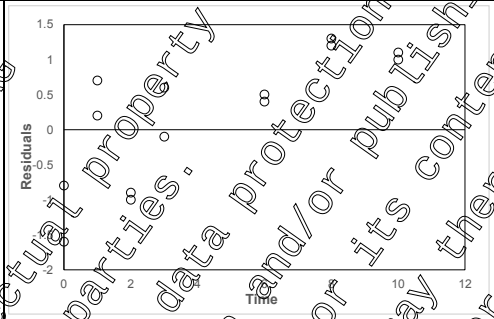
Table 7.2.1.1- 15: Degradation rate of fluopicolide in sterile buffer under irradiated conditions at 25 °C

Test conditions	Kinetic model	M0	Parameter (k, k1, k2, g, tb, α, β)	χ², %-error	Prob >t	Lower CI	Upper CI	DT50 [days]	DT90 [days]
Irradiated, M-228807-01-1[A]	**SFO**	**101.2**	**k 0**	**0.694**	**0.5**	**-0.001461**	**0.001**	**>10000**	**>10000**
	FOMC	101.2	α 0 β 0.1275	0.745	n.r. n.r.	-0.003789 0.1275	0.004 0.127	>10000	>10000

Best fit model highlighted in bold
[A] There were insufficient data points available to obtain a robust fit for samples incubated in the dark.

A graphical representation of the final kinetic fit is shown below.

Table 7.2.1.1- 16: Photolysis of fluopicolide in sterile buffer at 25 ºC (best-fit DT_{50} values)

Test conditions Model Reference	Modelled vs observed	Residuals
Irradiated SFO M-228807-01-1		

III. Conclusion

It was concluded, therefore, that there was no photolytic degradation of fluopicolide in sterile 0.01 M phosphate buffer at pH 7 at 25 ºC and under aerobic conditions.

Assessment and conclusion by applicant:

The study is considered valid to aid assessment of the photodegradation of [2,6-pyridyl-^{14}C]-fluopicolide in the environment.

Data Point:	KCA 7.2.1.2/03
Report Author:	[redacted]
Report Year:	2000
Report Title:	Photolysis of (14C) 2,6-dichlorobenzamide in water buffered at pH5
Report No:	C034064
Document No:	M-234315-01-1
Guideline(s) followed in study:	USEPA (=EPA): Subdivision N, 161-2
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

M-01 (AE C653711, referred to as 2,6-dichlorobenzamide in the report) was exposed to artificial light in sterile 0.01M acetate buffer solution at pH 5 for up to 31 days of irradiation in 12 hour light and dark cycles. [^{14}C-1-phenyl]- M-01 was dissolved in buffer at a concentration of 10 mg/L with 0.6% acetonitrile present as a co-solvent.

Pyrex glass sample tubes were irradiated in a Heraeus Suntest CPS+ apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. Dark control samples were run concurrently in Pyrex tubes wrapped in aluminium foil to prevent irradiation. The samples were maintained at 25 °C. At sampling the tubes were purged with carbon dioxide-free air drawn under vacuum through a series of traps. The traps contained polyurethane foam for neutral volatiles, 1N potassium hydroxide solution to trap CO_2 and acidic volatiles and 0.1N sulfuric acid to trap basic volatiles.

Irradiated and dark control samples were removed after 0, 3, 7, 14, 21 and 31 days.

Duplicate buffer samples were taken at each timepoint and the radioactivity present quantified by LSC and analysed by high performance liquid chromatography (HPLC).

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 100.56% for light exposed samples, and 101.09% for dark control samples.

No significant quantities of volatile products were collected in either the irradiated or the dark control experiment (<0.05% of applied radioactivity).

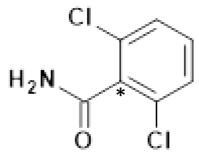
No significant degradation of M-01 was observed in either the light exposed samples or the dark control samples throughout the duration of the study, accounting for > 99% of the total applied radioactivity.

I. Materials and Methods

A. Materials

1. Test Item

[^{14}C-1-Phenyl]-M-01 (referred to as BAM, 2,6-dichlorobenzamide in the report)



* = position of radiolabel

Specific activity:	1.73 mCi/mmol
Batch number:	DUPH016
Radiochemical purity:	95.1% (HPLC analysis)

2. Sterile Buffer

The buffer used was 0.01M acetate buffer solution at pH 5.

B. Study Design

1. Experimental Condition

M-01 was exposed to artificial light in sterile 0.01M acetate buffer solution at pH 5 for up to 31 days of irradiation in 12 hour light and dark cycles. [^{14}C-1-phenyl]- M-01 was dissolved in buffer at a concentration of 10 mg/L with 0.6% acetonitrile present as a co-solvent. Pyrex glass sample tubes were irradiated in a Heraeus Suntest CPS+ apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. Dark control samples were run concurrently in Pyrex tubes wrapped in aluminum foil to prevent irradiation. The samples were maintained at 25 °C. At sampling the tubes were purged with carbon dioxide-free air drawn under vacuum through a series of traps. The traps contained polyurethane foam for neutral volatiles, 1N potassium hydroxide solution to trap CO_2 and acidic volatiles and 0.1N sulfuric acid to trap basic volatiles.

2. Sampling

Irradiated and dark control samples were removed after 0, 3, 7, 14, 21 and 31 days. Duplicate samples were removed at each timepoint.

3. Analytical procedures

Duplicate samples were removed at each timepoint. Duplicate aliquots were taken for LSC to quantify the radioactivity present in solution. The radioactivity in the trap solutions attached to both the irradiated and non-irradiated samples were quantified by LSC. The pH of test samples was confirmed at day 0, day 14 and day 31.

Buffer solutions were analysed by high performance liquid chromatography (HPLC) with a flow-through radiodetector. Validation of the HPLC integration data was obtained by fraction collection of the HPLC eluant of a day 31 light exposed sample followed by LSC. Identification of radioactive peaks was achieved by co-chromatography with certified reference standards. Replicates of the irradiated and dark control samples were also subjected to liquid chromatography/mass spectrometry (LC-MS) analysis for the purpose of confirming the identity of residual test item.

4. Determination of degradation kinetics

No degradation kinetics were determined in the report. DT_{50} and DT_{90} values for the degradation of M-01 have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1).

An initial comparison was performed between the SFO and FOMC fits for M-01 under irradiated conditions and in the dark control samples. The FOMC fit provided no significant improvement, and the SFO fit was therefore accepted. There was no significant photolytic degradation of M-01 under irradiated conditions.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity in light exposed and dark control samples is presented in Table 7.2.1.2- 9 and Table 7.2.1.2- 10.

Table 7.2.1.2- 9: Distribution and characterisation of radioactivity in light exposed samples expressed as percentage of applied radioactivity

	Replicate	Incubation time (DAT)					
		0	3	7	14	21	31
Buffer solution	1	99.83	100.74	98.75	99.24	101.57	99.67
	2	100.47	103.25	99.27	101.93	101.45	100.33
	Mean	100.15	102.00	99.01	100.59	101.15	100.00
	SD	± 0.5	± 1.8	± 0.4	± 1.9	± 0.1	± 0.5
M-01 (AE C653711)	1	99.83	100.74	98.75	99.24	101.57	99.67
	2	100.47	103.25	99.27	101.93	101.45	100.33
	Mean	100.15	102.00	99.01	100.59	101.15	100.00
	SD	± 0.5	± 1.8	± 0.4	± 1.9	± 0.1	± 0.5
Volatiles							
Polyurethane plug	1	n.a.	0.02	<0.01	<0.01	<0.01	<0.01
	2	n.a.	0.01	0.00	<0.01	<0.01	<0.01
	Mean	n.a.	0.02	<0.01	<0.01	<0.01	<0.01
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
KOH	1	n.a.	0.01	0.01	0.02	0.03	0.02
	2	n.a.	0.02	0.01	0.02	0.03	0.02
	Mean	n.a.	0.02	0.01	0.02	0.03	0.02
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
H_2SO_4	1	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01
	2	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01
	Mean	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Total	1	99.8	100.77	98.76	99.26	101.60	99.69
	2	100.47	103.28	99.28	101.95	101.48	100.35
	Mean	100.15	102.03	99.02	100.61	101.54	100.02
	SD	± 0.5	± 1.8	± 0.4	± 1.9	± 0.1	± 0.5

n.a. = not applicable

Table 7.2.1.2- 10: Distribution and characterisation of radioactivity in dark control samples expressed as percentage of applied radioactivity

	Replicate	Incubation time (DAT)					
		0	3	7	14	21	31
Buffer solution	1	99.83	103.73	101.82	101.11	100.64	100.79
	2	100.47	101.89	100.38	101.78	101.66	98.74
	Mean	100.15	102.81	101.10	101.45	101.15	99.79
	SD	± 0.5	± 1.3	± 1.0	± 0.5	± 0.7	± 1.4
M-01 (AE C653711)	1	99.83	103.73	101.82	101.11	100.64	100.79
	2	100.47	101.89	100.38	101.78	101.66	98.74
	Mean	100.15	102.81	101.10	101.45	101.15	99.79
	SD	± 0.5	± 1.3	± 1.0	± 0.5	± 0.7	± 1.4
Volatiles							
Polyurethane plug	1	n.a.	0.01	<0.01	<0.01	<0.01	<0.01
	2	n.a.	0.02	<0.01	<0.01	<0.01	0.00
	Mean	n.a.	0.02	<0.01	<0.01	<0.01	<0.01
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
KOH	1	n.a.	0.01	0.01	0.01	0.02	0.02
	2	n.a.	0.01	0.01	0.02	0.01	0.01
	Mean	n.a.	0.01	0.01	0.02	0.02	0.02
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
H_2SO_4	1	n.a.	<0.01	<0.01	0.01	<0.01	<0.01
	2	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01
	Mean	n.a.	<0.01	<0.01	0.01	<0.01	<0.01
	SD	n.a.	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Total	1	99.83	103.73	101.83	101.13	100.66	100.81
	2	100.47	101.92	100.39	101.80	101.67	98.75
	Mean	100.15	102.84	101.11	101.47	101.17	99.78
	SD	± 0.5	± 1.3	± 1.0	± 0.5	± 0.7	± 1.5

n.a. = not applicable

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 100.56% for light exposed samples, and 101.09% for dark control samples.

No significant quantities of volatile product were collected in either the irradiated or the dark control experiment (<0.05% of applied radioactivity).

C. Transformation of test substance

No significant degradation of M-01 was observed in either the light exposed samples or the dark control samples throughout the duration of the study, accounting for > 99% of the total applied radioactivity.

D. Degradation Kinetics

The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold. No significant degradation was observed for M-01 incubated under irradiated conditions or in dark controls.

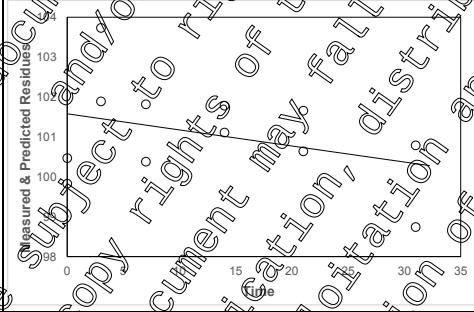
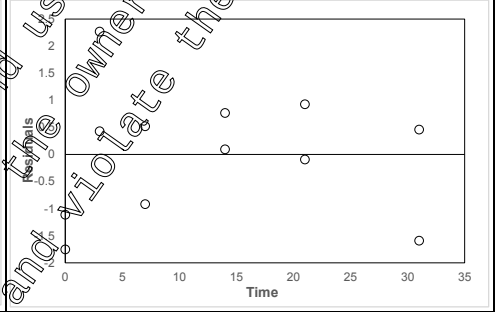
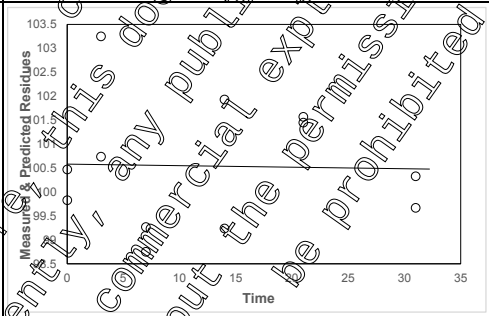
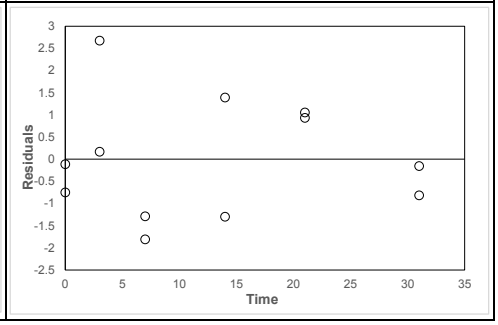
Table 7.2.1.1- 17: Degradation rate of M-01 in sterile buffer under irradiated conditions at 25 °C

Test conditions	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Dark, M-234315-01-1	**SFO**	**101.6**	**k 0.0003998**	**0.688**	**0.124**	**-0.0002385**	**0.001**	**1734**	**5759**
	FOMC	101	α 0 β 0.04452	0.847	n.r. n.r.	-0.003521 0.04452	0.004 0.045	>10000	>10000
Irradiated, M-234315-01-1	**SFO**	**100.6**	**k 3.17E-05**	**0.78**	**0.467**	**-0.0006902**	**0.001**	**>10000**	**>10000**
	FOMC	100.5	α 0 β 0.222	0.86	n.r. n.r.	-0.004957 0.222	0.005 0.222	>10000	>10000

Best fit model highlighted in bold

A graphical representation of the final kinetic fit is shown below.

Table 7.2.1.1- 18: Photolysis of M-01 in sterile buffer at 25 °C (best-fit DT_{50} values)

Test conditions Model Reference	Modelled vs observed	Residuals
Dark SFO M-234315-01-1		
Irradiated SFO M-234315-01-1		

III. Conclusion

It was concluded, therefore, that there was no photolytic degradation of M-01 in sterile buffer at 25 °C.

Assessment and conclusion by applicant: The study is considered valid to aid assessment of the photodegradation of M-01 in the environment.

CA 7.2.1.3 Indirect photochemical degradation

The aqueous photolysis of fluopicolide in natural water has been investigated in Study KCA 7.2.1.3/01 which was evaluated during the previous EU review and is still considered acceptable.

Test item	Report reference	Author, Year	Phenyl Label	Pyridyl Label	Comment
Fluopicolide	KCA 7.2.1.3/01 M-231338-01-1	[redacted] 2003	✓	✗	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.

Data Point:	KCA 7.2.1.3/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	(14C)-AE C638206: Aqueous photolysis in natural water
Report No:	C032392
Document No:	M-231338-01-1
Guideline(s) followed in study:	JMAFF 2-6-2
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Fluopicolide was exposed to artificial light in sterile natural water for up to 16 days of continuous irradiation. This period is equivalent to 73 days natural spring sunlight as defined in the relevant Japanese guidelines. [Phenyl-U-^{14}C]-fluopicolide at a specific activity of 5.5 MBq/mg (radiochemical purity 100 %), was dissolved in natural water at a concentration of 0.65 mg/L with 0.2% acetonitrile present as a co-solvent.

Glass photolysis vessels with cap containing a quartz window were irradiated in a Heraeus Suntest CPS apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. The dark control samples were placed in controlled temperature room covered to prevent irradiation. Light exposed and dark control samples were maintained at 25 °C. Volatiles were continuously trapped

using a series of traps containing ethylene glycol to trap organic volatiles followed by two aqueous potassium hydroxide solutions (2M) to trap CO_2.

Irradiated and dark control samples were removed after 0, 0.5, 1.5, 2.5, 5.5, 8.5, 13.5 and 16 days.

Single samples were removed at each timepoint. Duplicate aliquots were taken for LSC to quantify the radioactivity present in solution. Test vessels were rinsed with acetonitrile and the radioactivity removed quantified by LSC. The radioactivity in the trap solutions attached to both the irradiated and non-irradiated samples were quantified by LSC. Sterility of test samples was confirmed throughout the study period.

The acetonitrile rinse and water sample were combined prior to analysis by high performance liquid chromatography (HPLC) using a flow-through radiodetector to quantify the individual radioactive peaks. The peak assignment of fluopicolide was confirmed by thin layer chromatography (TLC) analysis of the day 16 light exposed and dark control samples.

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 98.1 % for light exposed samples, and 98.5 % for dark control samples.

No significant quantities of volatile product were collected in either the irradiated experiment (<1% of applied radioactivity) or the dark control (no volatiles).

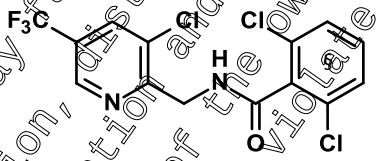
No significant degradation of fluopicolide was observed in either the light exposed samples or the dark control samples where the test item represented 99% and 98% of applied radioactivity respectively at the end of the incubation period.

I. Materials and Methods

A. Materials

1. Test Item

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



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

Specific Activity: 5.5 MBq/mg

Radiochemical Purity: 100% (HPLC analysis)

2. Water

Water collected from the river Roding, Essex, UK was used in the study. The properties of the water are summarised in Table 7.2.1.3- 1. The water was sterilised by filtration through a 0.22 µm pore filter. Electrical conductivity of the water was 738 µS/cm.

Table 7.2.1.3- 1: Characterisation of the natural water

Parameter	Water
Water Reference[a]	03/08
Organic Carbon	33.5 mg/L
Organic Matter[b]	57.8 mg/L
Total Phosphorus	0.1 mg/L
Total Nitrogen	4.2 mg/L
Nitrate	25.9 mg/L
Total Hardness	331.0 mg/L $CaCO_3$
Electrical Conductivity	738.0 µS/cm
pH	8.1
Dissolved Oxygen[d]	97%
Residue on Evaporation	0.04 % w/w
Suspended Solids[c]	<0.05 mg/L
Source	River Roding, Boarded Barns Farm, Ongar, Essex, UK.

[a] Unless stated all analyses were performed on the water after sterilisation by filtration through a 0.22µm pore filter.
[b] Calculated as organic carbon x 1.724.
[c] Measured on natural water before sterilisation.
[d] Measured on natural water before start of study.

B. Study Design

1. Experimental Conditions

Fluopicolide was exposed to artificial light in sterile natural water for up to 16 days of continuous irradiation. This period is equivalent to 73 days natural spring sunlight as defined in the relevant Japanese guidelines. Phenyl-U-^{14}C]-fluopicolide was dissolved in natural water at a concentration of 0.65 mg/L with 0.2% acetonitrile present as a co-solvent.

Glass photolysis vessels with a threaded cap containing a quartz window were irradiated in a Heraeus Suntest CPS apparatus equipped with a Xenon lamp with filters to block infrared light and irradiation below 290 nm. The dark control samples were placed in controlled temperature room covered to prevent irradiation. Light exposed and dark control samples were maintained at 25 °C. Volatiles were continuously trapped using a series of traps containing ethylene glycol to trap organic volatiles followed by two aqueous potassium hydroxide solutions (2M) to trap CO_2.

2. Sampling

Irradiated and dark control samples were removed after 0, 0.5, 1.5, 2.5, 5.5, 8.5, 13.5 and 16 days. Single samples were removed at each timepoint.

3. Analytical procedures

Duplicate aliquots were taken for LSC to quantify the radioactivity present in solution. Test vessels were rinsed with acetonitrile and the radioactivity removed quantified by LSC. The radioactivity in the trap solutions attached to both the irradiated and non-irradiated samples were quantified by LSC. Sterility of test samples was confirmed throughout the study period.

The acetonitrile rinse and water sample were combined prior to analysis by high performance liquid chromatography (HPLC) using a flow-through radiodetector to quantify the individual radioactive peaks. The limit of detection was determined as 270 dpm or 1% of the dpm typically applied to the HPLC system. To confirm quantitative recovery of radioactivity from the HPLC column recoveries were performed on selected samples. Identification of radioactive peaks was achieved by co-chromatography with certified reference standards. The peak assignment of fluopicolide was confirmed by thin layer chromatography (TLC) analysis of the day 16 light exposed and dark control samples.

4. Determination of degradation kinetics

No degradation kinetics were determined in the report. DT_{50} and DT_{90} values for the degradation of fluopicolide have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1).

An initial comparison was performed between the SFO and FOMC fits for fluopicolide under irradiated conditions and in the dark control samples. The FOMC fit provided no significant improvement, and the SFO fit was therefore accepted. There was no significant degradation of fluopicolide under irradiated conditions or in dark controls.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity in light exposed and dark control samples is presented in Table 7.2.1.3- 2 and Table 7.2.1.3-3.

Table 7.2.1.3- 2: Distribution and characterisation of radioactivity in light exposed samples expressed as percentage of applied radioactivity

Compound	Incubation time (DAT)							
	0	0.5	1.5	2.5	5.5	8.5	13.5	16
Fluopicolide	93.51	98.71	97.98	98.95	97.92	98.98	98.86	99.03
Total Extracted	93.51	98.71	97.98	98.95	97.92	98.98	98.86	99.03
Water	92.16	97.11	96.81	97.42	96.82	97.36	97.42	97.49
Acetonitrile rinse	1.35	1.6	1.17	1.53	1.1	1.62	1.44	1.53
Volatiles	na	0	0	0.04	0.06	0.18	0.25	0.19
Total	93.51	98.71	97.98	98.99	97.98	99.17	99.11	99.22

na = not applicable

Table 7.2.1.3- 3: Distribution and characterisation of radioactivity in dark control samples expressed as percentage of applied radioactivity

Compound	Incubation time (DAT)							
	0	0.5	1.5	2.5	5.5	8.5	13.5	16
Fluopicolide	96.04	99.4	97.67	97.69	98.51	101.48	99.01	97.9
Total Extracted	96.04	99.4	97.67	97.69	98.51	101.48	99.01	97.9
Water	94.15	98.43	96.38	96.51	96.45	100.08	97.79	96.45
Acetonitrile rinse	1.89	0.97	1.29	1.18	2.06	1.4	1.23	1.46
Volatiles	na	0	0	0	0	0	0	0
Total	96.04	99.4	97.67	97.69	98.51	101.48	99.01	97.9

na = not applicable

B. Material Balance

Good radiochemical balances were achieved with a mean recovery of applied radioactivity of 98.1 % for light exposed samples, and 98.5 % for dark control samples.

C. Transformation of test substance

No significant quantities of volatile product were collected in either the irradiated experiment (<1% of applied radioactivity) or the dark control (no volatiles).

No significant degradation of fluopicolide was observed in either the light exposed samples or the dark control samples where the test item represented 99% and 98% of applied radioactivity respectively at the end of the incubation period.

D. Degradation Kinetics

The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold. No degradation of fluopicolide was observed under irradiated conditions or in dark controls.

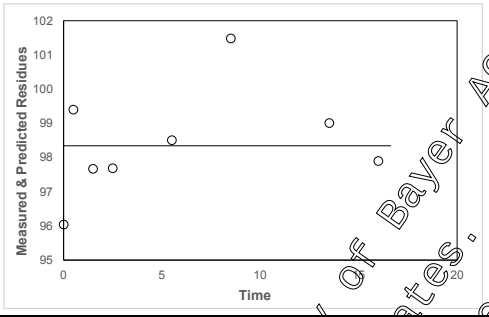
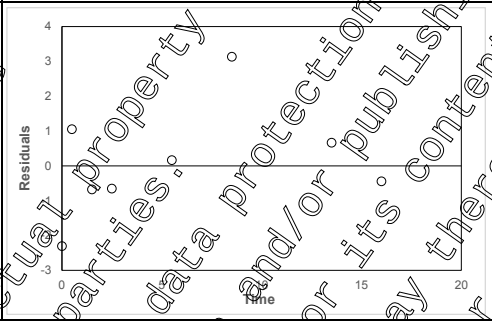
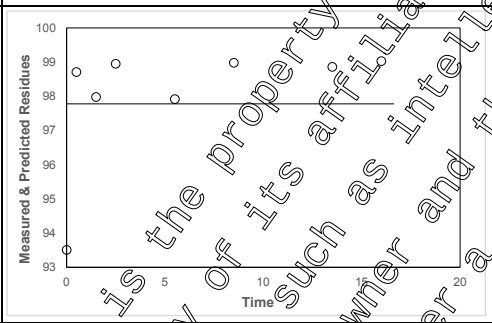
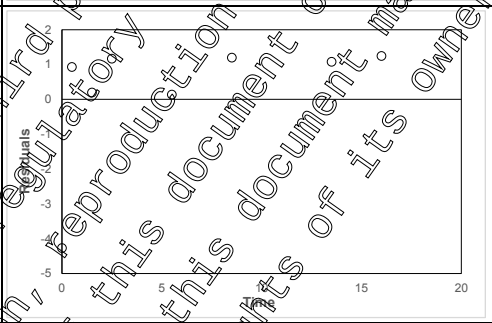
Table 7.2.1.3- 4: Degradation rate of fluopicolide in sterile natural water under irradiated conditions at 25 °C

Soil	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Dark, M-231338-01-1	**SFO**	**98.35**	**k 0**	**1.21**	**0.5**	**-0.002123**	**0.002**	**>10000**	**>10000**
	FOMC	98.26	α 0 β 0.03446	1.3	n.r. n.r.	-0.006987 0.03446	0.007 0.034	>10000	>10000
Irradiated, M-231338-01-1	**SFO**	**97.78**	**k 0**	**1.43**	**0.5**	**-0.002514**	**0.003**	**>10000**	**>10000**
	FOMC	97.65	α 0 β 0.06783	1.54	n.r. n.r.	-0.009226 0.06783	0.009 0.068	>10000	>10000

Best fit model highlighted in bold

Graphical representations of the final kinetic fits are shown below.

Table 7.2.1.3- 5: Photolysis of fluopicolide in sterile natural water at 25 ºC (best-fit DT_{50} values)

Test conditions Model Reference	Modelled vs observed	Residuals
Dark SFO M-231338-01-1		
Irradiated SFO M-231338-01-1		

III. Conclusion

It was concluded, therefore, that there was no photolytic degradation of fluopicolide under aerobic conditions in sterile natural water at 25ºC.

Assessment and conclusion by applicant:

The study is considered valid to aid assessment of the photodegradation of [^{14}C]-fluopicolide in the environment.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

The ready biodegradability of fluopicolide and its metabolite M-01 has been investigated in Study KCA 7.2.2.1/01 and KCA 7.2.2.1/02 respectively, which were evaluated during the previous EU review and are still considered acceptable.

Test item	Report reference	Author, Year	Comment
Fluopicolide	KCA 7.2.2.1/01 M-225269-01-1	[redacted] 2003	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
M-01	KCA 7.2.2.1/02 M-234316-01-1	[redacted] 1996	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.

Data Point:	KCA 7.2.2.1/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Assessment of ready biodegradability; CO2 evolution test; Code: AE C638206
Report No:	C038570
Document No:	M-225269-01-1
Guideline(s) followed in study:	OECD: 301B; USEPA (=KBA): OPPTS 835.3110
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The ready biodegradability of fluopicolide was determined according to EEC Method C.4-C (1992) and OECD Guideline 301B (1992). Fluopicolide was dissolved in aqueous culture medium and exposed to relatively low numbers of activated sewage sludge micro-organisms under aerobic conditions for a period of 28 days. The samples were incubated in the dark at 21 °C. The test systems were inoculated at a final concentration of 30 mg suspended solids/L and seven test vessels were prepared as follows:

- Control system consisting of inoculated culture medium (duplicate vessels)
- Standard material (sodium benzoate) in inoculated culture medium at a concentration of 17.1 mg/L, equivalent to 10 mg carbon/L (duplicate vessels)
- Fluopicolide in inoculated culture medium at a concentration of 22.8 mg/L, equivalent to 10 mg carbon/L (duplicate vessels)
- Toxicity control consisting of fluopicolide and standard material (sodium benzoate) in inoculated culture medium at a concentration equivalent to 20 mg carbon/L (single vessel)

Each test vessel was connected to two carbon dioxide traps containing 0.05 M sodium hydroxide.

The degradation of the test material was assessed by determining the amount of carbon dioxide produced. The first carbon dioxide trap was sampled on days 0, 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 27, 28, 29. The second trap was sampled on day 0 and day 29.

Fluopicolide attained 72 % degradation after 28 days. Under the strict terms and conditions of OECD Guideline 301B fluopicolide cannot be considered to be readily biodegradable as it failed to attain 60% degradation within 10 days of the degradation rate exceeding 10%. However the potential for rapid degradation was observed. In terms of the classification and labelling requirements (EU directive for Dangerous Substances, L110A) fluopicolide may be considered as readily biodegradable as > 70 % degradation was attained within 28 days.

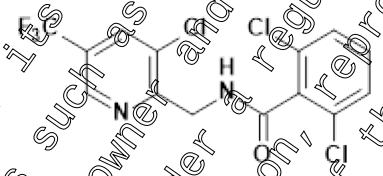
More than 60 % degradation of the control substance, sodium benzoate, was observed within 14 days which confirmed the validity of the test. The toxic control attained 90% degradation after 28 days confirming that the fluopicolide was not toxic to sewage treatment micro-organisms.

I. Materials and Methods

A. Materials

1. Test Item

Fluopicolide



Purity: 95.9% w/w

B. Study Design

1. Experimental Conditions

The ready biodegradability of fluopicolide was determined according to EEC Method C.4-C (1992) and OECD Guideline 301B (1992). Fluopicolide was dissolved in aqueous culture medium and exposed to relatively low numbers of activated sewage sludge micro-organisms under aerobic conditions for a period of 28 days. The samples were incubated in the dark at 21 °C. The test systems were inoculated at a final concentration of 30 mg suspended solids/L and seven test vessels were prepared as follows:

- Control system consisting of inoculated culture medium (duplicate vessels)
- Standard material (sodium benzoate) in inoculated culture medium at a concentration of 17.1 mg/L, equivalent to 10 mg carbon/L (duplicate vessels)
- Fluopicolide in inoculated culture medium at a concentration of 22.8 mg/L, equivalent to 10 mg carbon/L (duplicate vessels)
- Toxicity control consisting of fluopicolide and standard material (sodium benzoate) in inoculated culture medium at a concentration equivalent to 20 mg carbon/L (single vessel)

Each test vessel was connected to two carbon dioxide traps containing 0.05 M sodium hydroxide.

2. Sampling

The first carbon dioxide trap was sampled on days 0, 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 27, 28, 29. The second trap was sampled on day 0 and day 29.

3. Analytical procedures

The degradation of the test material was assessed by determining the amount of carbon dioxide produced.

II. Results and Discussion

A. Data

The percentage biodegradation of fluopicolide, the control sodium benzoate and the toxicity control are presented in Table 7.2.2.1- 1.

Table 7.2.2.1- 1: Biodegradation of fluopicolide according to the theoretical amount of carbon dioxide produced

Time (days)	% Biodegradation		
	Fluopicolide	Sodium Benzoate	Fluopicolide plus Sodium Benzoate (Toxicity Control)
0	0	0	0
1	9	29	15
2	4	51	19
3	5	64	39
5	6	66	41
7	2	62	39
9	0	65	41
13	2	64	40
15	11	67	38
19	22	77	44
21	38	76	52
24	47	75	58
27	63	77	71
28	72	78	90
29	74	79	97

B. Biodegradation of test substance

Fluopicolide attained 72 % degradation after 28 days. Under the strict terms and conditions of OECD Guideline 301B fluopicolide cannot be considered to be readily biodegradable as it failed to attain 60% degradation within 10 days of the degradation rate exceeding 10%. However the potential for rapid degradation was observed. In terms of the classification and labelling requirements (EU directive for Dangerous Substances, L119A) fluopicolide may be considered as readily biodegradable as > 70 % degradation was attained within 28 days.

More than 60 % degradation of the control substance, sodium benzoate, was observed within 14 days which confirmed the validity of the test. The toxic control attained 90% degradation after 28 days confirming that the fluopicolide was not toxic to sewage treatment micro-organisms.

III. Conclusion

Under the strict terms and conditions of OECD Guideline 301B fluopicolide cannot be considered to be readily biodegradable, however the potential for rapid degradation was observed. In terms of the classification and labelling requirements (EU directive for Dangerous Substances, L110A) fluopicolide may be considered as readily biodegradable as > 70 % degradation was attained within 28 days.

Assessment and conclusion by applicant:
The study is considered valid to assess the ready biodegradability of fluopicolide.

Data Point:	KCA 7.2.2.1/02
Report Author:	[redacted]
Report Year:	1996
Report Title:	Determination of the ready biodegradability of 2,6-dichlorobenzamide in the closed bottle test
Report No:	C034065
Document No:	M-234316-01-1
Guideline(s) followed in study:	EU (=EEC): 92/69/EEC C.4-E; OECD: 310 D
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The ready biodegradability of M-01 (2,6-dichlorobenzamide) was determined according to EEC Method C.4-E (1992) and OECD Guideline 301 D (1992). M-01 was dissolved in aqueous mineral salt medium at a concentration of 2 mg/L and exposed to relatively low number of unadapted micro-organisms under aerobic conditions for a period of 28 days. M-01 was referred to as BAM in the report and AE C653711 in this summary. The samples were incubated in the dark at 20 ± 1°C. The test compound was the only source of carbon and energy which was available to the micro-organisms. The test systems were inoculated at a final concentration of 2 mg dry weight/L and test vessels were prepared as follows:

- Group A consisting of culture medium without inoculum (ten vessels)
- Group B consisting of inoculated culture medium (ten vessels)
- Group C consisting standard material (sodium acetate) in inoculated culture medium at a concentration of 6.6 mg/L (ten vessels)
- Group L consisting of M-01 in inoculated culture medium at a concentration of 2.0 mg/L (ten vessels)

Oxygen consumption by the micro-organisms was determined in order to follow the biodegradation of M-01. Biodegradation was calculated as the ratio of the biochemical oxygen demand (BOD) and the theoretical oxygen demand (ThOD) where BOD is the amount of oxygen consumed by microrganisms to metabolise a test compound and ThOD is the total amount of oxygen required to oxidise a compound completely calculated from the molecular formula.

The amount of dissolved oxygen present in the test samples was measured after 0, 7, 14, 21 and 28 days. Duplicate samples were taken at each time point from each group. The pH was measured at day 0 and 28.

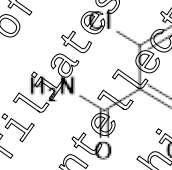
Biodegradation of M-01 reached 9 % within 28 days whereas 66 % of the reference compound sodium acetate, was degraded within 14 days, demonstrating inoculum activity was sufficient. Oxygen depletion in the inoculum remained within acceptable limits.

I. Materials and Methods

A. Materials

1. Test Item

M-01 (AE C653711, called 2,6-dichlorobenzamide, BAM in the report)



Purity: >99.5% w/w

B. Study Design

1. Experimental Conditions

The ready biodegradability of M-01 (2,6-dichlorobenzamide) was determined according to EEC Method C.4-E (1992) and OECD Guideline 301 D (1992). M-01 was dissolved in aqueous mineral salt medium at a concentration of 2 mg/L and exposed to relatively low number of unadapted micro-organisms under aerobic conditions for a period of 28 days. M-01 was referred to as BAM in the report and AE C653711 in this summary. The samples were incubated in the dark at 20 ± 1°C. The test compound was the only source of carbon and energy which was available to the micro-organisms. The test systems were inoculated at a final concentration of 2 mg dry weight/L and test vessels were prepared as follows:

- Group A consisting of culture medium without inoculum (ten vessels)
- Group B consisting of inoculated culture medium (ten vessels)
- Group C consisting standard material (sodium acetate) in inoculated culture medium at a concentration of 6.6 mg/L (ten vessels)
- Group L consisting of M-01 in inoculated culture medium at a concentration of 2.0 mg/L (ten vessels).

2. Sampling

The amount of dissolved oxygen present in the test samples was measured after 0, 7, 14, 21 and 28 days. Duplicate samples were taken at each time point from each group. The pH was measured at day 0 and 28.

3. Analytical procedures

Oxygen consumption by the micro-organisms was determined in order to follow the biodegradation of M-01. Biodegradation was calculated as the ratio of the biochemical oxygen demand (BOD) and the theoretical oxygen demand (ThOD) where BOD is the amount of oxygen consumed by microorganisms to metabolise a test compound and ThOD is the total amount of oxygen required to oxidise a compound completely calculated from the molecular formula.

II. Results and Discussion

A. Data

The percentage biodegradation of M-01, the control sodium acetate are presented in Table 7.2.2.1- 2.

Table 7.2.2.1- 2: Biodegradation of M-01 according to dissolved oxygen depletion

Time (days)	% Biodegradation	
	M-01	Sodium acetate
0	0.0	0.0
7	2.3	55
14	11	66
21	14	54
28	9.2	53

B. Biodegradation of test substance

Biodegradation of M-01 reached 9 % within 28 days whereas 66 % of the reference compound, sodium acetate, was degraded within 14 days, demonstrating inoculum activity was sufficient. Oxygen depletion in the inoculum remained within acceptable limits.

III. Conclusion

M-01 was found to be not readily biodegradable according to OECD Test Guideline 301D.

Assessment and conclusion by applicant:

The study is considered valid to assess the ready biodegradability of M-01 (AE C653711)

CA 7.2.2.2 Aerobic mineralisation in surface water

An assessment of aerobic mineralisation in surface water is new study requirement under Regulation 1107/2009. An aerobic mineralisation study (OECD 309) with [phenyl-U-^{14}C]-fluopicolide is provided as new data not yet reviewed. Fluopicolide was found to be stable in surface water under the conditions of the test. A second study with [2,6-pyridyl-^{14}C]-labelled fluopicolide would not have added any additional information on the fate of the compound and consequently is not required.

Report reference	Author, Year	Phenyl Label	Pyridyl Label	Comment
KCA 7.2.2.2/01 M-558747-01-2	[redacted] 2016	✓	×	New data not yet reviewed.

Data Point:	KCA 7.2.2.2/01
Report Author:	[redacted]
Report Year:	2016
Report Title:	[Phenyl-UL-^{14}C]fluopicolide: Aerobic mineralization in surface water
Report No:	MEACN032
Document No:	M-558747-01-2
Guideline(s) followed in study:	OECD Test Guideline No. 309; Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The aerobic mineralisation of [phenyl-U-^{14}C]-fluopicolide was studied in one surface water system from Beaver Dam Lake, Wake Forest, North Carolina, USA under pelagic conditions in the dark at 20 ± 2 °C for 63 days. The pH and Total Organic Carbon were 7.25 and 9.5 mg/L respectively.

Two application rates were used, 10.7 µg [phenyl-U-^{14}C]-fluopicolide/L and 107.7 µg [phenyl-U-^{14}C]-fluopicolide/L of surface water for low and high concentration test systems, respectively.

The test systems consisted of 250 mL flasks each containing 100 mL of surface water equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds equilibrated for 3 days prior to treatment. The surface water in the test systems was kept in motion during the entire study period to maintain aerobicity.

Sterile test systems were prepared to determine influences on the degradation of the test substance induced by hydrolysis. Test systems (surface water, test vessels and trap attachments) were sterilized by autoclaving for 60 minutes at 121 °C.

Duplicate test systems of each test concentration were processed and analysed at 0, 7, 14, 21, 31, 42, 51, and 63 days after treatment (DAT). Sterile test systems for both concentrations were processed and analysed at day 63.

At each sampling interval the concentration of any dissolved carbon dioxide and then the remaining radioactive content of the water was determined. The water phase was transferred to a measuring cylinder and the test vessel rinsed with acetonitrile. The acetonitrile washes were then combined with the water sample. The amounts of test item and degradation products in the water extracts were determined by HPLC/radiodetection analysis.

Mean material balances were 103.8% AR for low concentration test systems (range from 101.1 to 105.9% AR) and 103.5% AR for high concentration test systems (range from 102.0 to 105.0% AR).

Formation of carbon dioxide was not significant at <0.1% AR at all sampling intervals for both biologically active and sterile test systems. Formation of volatile organic compounds (VOC) was also insignificant (<0.1% AR).

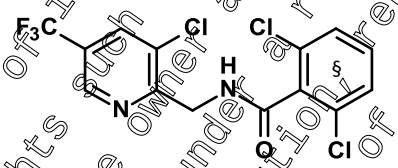
Fluopicolide was found to be stable in surface water at both concentrations and was ≥ 100.9% at all intervals. One minor degradation product was formed in the high concentration test system which reached a maximum of 1.6% AR at day 21. No major degradation products were formed.

I. Materials and Methods

A. Materials

1. Test Item

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



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

Specific activity: 59.404 mCi/mMole

Batch number: C-966A, 17097

Radiochemical purity: 99.0%

2. Water

Water collected from the Beaver Dam Lake, Wake Forest, North Carolina, USA was used in the study. A summary of the physical and chemical properties of the surface water is provided in Table 7.2.2.2- 1 below.

Table 7.2.2.2- 1: Physico-chemical characteristics of surface water

Parameter	Value
Water Identity	Beaver Dam, NC
pH	7.25
Biochemical Oxygen Demand (O_2/L)	1.9
Temperature (°C)	22.4
Dissolved Oxygen [mg/L]	7.74
Total Organic Carbon (TOC) [mg/L]	9.5
Redox Potential @ 24.0 °C, pH 7.3 [mV]	243.4
Dissolved Organic Carbon (DOC) [mg/L]	8.6

B. Study Design

1. Experimental conditions

The aerobic mineralisation of [phenyl-U-^{14}C]-fluopicolide was studied in a natural surface water under pelagic conditions in the dark at 20 ± 2 °C for 63 days.

Two application rates were used, 10.7 and 107.7 µg [phenyl-U-^{14}C]-fluopicolide/L of surface water for low and high concentration test systems, respectively.

The test systems consisted of 250-mL flasks each containing 100 mL of surface water equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds equilibrated for 3 days prior to treatment. The surface water in the test systems was kept in motion during the entire study period to maintain aerobicity.

Sterile test systems were prepared to determine influences on the degradation of the test substance induced by hydrolysis. Therefore, the respective test systems (surface water, test vessels and trap attachments) were sterilized by autoclaving for 60 minutes at 121 °C.

Positive control flasks treated with [^{14}C]-benzoic acid at a nominal dose rate of 9.3 mg/L were used as control items to verify that the test water showed a good level of biological activity as it is known to mineralize rapidly in most natural waters. Solvent control flasks were treated to show the effects of the addition of organic solvent into the test system.

2. Sampling

Duplicate test systems of each test concentration were processed and analysed at 0, 7, 14, 21, 31, 42, 51, and 63 days after treatment (DAT). Sterile test systems for both concentrations were processed and analysed at day 63.

3. Analytical procedures

At each sampling interval, an aliquot of water was initially removed, and the concentration of any dissolved carbon dioxide determined. The radioactive content of the water was then determined by LSC. Once the physical measurements had been determined the water phase was transferred to a measuring cylinder and the test vessel rinsed with acetonitrile. The acetonitrile washes were then combined with the water sample. The amounts of test item and degradation products in the water extracts were determined by HPLC/radiodetection analysis. The limit of detection for the primary chromatographic method was determined to be 1.1% AR.

Any carbon dioxide absorbed by soda lime traps was liberated and trapped in a scintillation cocktail selective for binding of carbon dioxide. The soda lime of the trap attachments was transferred into an Erlenmeyer flask, which was then attached with a dropping funnel, containing 60 mL of aqueous hydrochloric acid, and connected to a series of five trapping vessels, each filled with 15 mL of ice-cooled scintillation cocktail. The hydrochloric acid was added dropwise under continuous stirring 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.

Any organic volatiles trapped by the PU foam plug was extracted with 50 mL ethyl acetate for approximately 5 minutes in an ultrasonic bath. The radioactivity content was determined by LSC.

Degradation kinetics

The degradation kinetics determined in the report were evaluated according to the FOCUS guidance document on degradation kinetics using the software KinGUI 2. The degradation of fluopicolide was best described by the simple first order (SFO) model in both systems.

DT_{50} and DT_{90} values for the degradation of fluopicolide have been re-calculated from the reported data following the recommendations of the FOCUS work group using the software KinGUI (version 2.1). An initial comparison was performed between the SFO and FOMC fits for fluopicolide. As reported the data was best fit by the simple first order (SFO) model at both concentrations, with an $\chi 2$ error of 1.31 and 0.82%.

II. Results and Discussion

A. Data

The distribution and characterisation of radioactivity for the low and high concentration test systems following application of [phenyl-U-^{14}C]-fluopicolide are summarized in Table 7.2.2.2- 2 and Table 7.2.2.2- 3.

Table 7.2.2.2- 2: Biotransformation of fluopicolide (10.7 µg/L) including sterilised test system (% AR)

Component	Mean SD	Incubation time (DAT)								
		0	7	14	21	31	42	51	63	63 sterile
Fluopicolide	Mean	101.6	102.6	104.6	101.0	103.5	105.0	105.4	105.8	104.9
	SD	± 0.2	± 0.2	± 0.8	± 0.5	± 1.6	± 0.9	± 1.1	± 0.9	± 1.0
Water Phase	Mean	101.6	102.6	104.6	101.0	103.5	105.0	105.4	105.8	104.9
	SD	± 0.2	± 0.2	± 0.8	± 0.5	± 1.6	± 0.9	± 1.1	± 0.9	± 1.0
Carbon Dioxide	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
Volatile Organic Compounds [A]	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
Recovery	Mean	101.6	102.7	104.8	101.1	103.6	105.1	105.6	105.9	105.0
	SD	± 0.2	± 0.2	± 0.8	± 0.5	± 1.6	± 0.9	± 1.0	± 0.9	± 0.9

DAT: days after treatment
n.a.: not analysed,
SD: standard deviation,
[A] Sum of radioactivity in soda lime and polyurethane foam
All values expressed as percentage of total applied radioactivity

Table 7.2.2.2- 3: Biotransformation of fluopicolide (107.7 µg/L) including sterilised test system (% AR)

Component	Mean SD	Incubation time (DAT)								
		0	7	14	21	31	42	51	63	63 sterile
Fluopicolide	Mean	103.5	100.9	103.4	102.0	104.2	101.6	103.3	102.1	103.8
	SD	± 0.1	± 1.4	± 1.0	± 0.8	± 1.8	± 0.1	± 0.1	± 1.7	± 1.1
Minor Unknown 1	Mean	n.d.	1.0	0.6	1.6	0.7	1.4	n.d.	1.2	0.7
	SD		± 0.0	± 0.0	± 0.0	± 0.0	± 0.2		± 0.0	± 0.0
Water Phase	Mean	103.5	101.9	104.0	103.6	104.9	103.0	103.3	103.3	104.5
	SD	± 0.1	± 1.4	± 1.7	± 0.8	± 1.0	± 0.10	± 0.1	± 1.7	± 0.4
Carbon Dioxide	Mean	n.a.[B]	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
Volatile Organic Compounds [A]	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
Recovery	Mean	103.5	101.9	104.0	103.6	104.9	103.0	103.3	103.3	104.5
	SD	± 0.1	± 1.4	± 1.7	± 0.8	± 1.0	± 0.10	± 0.1	± 1.7	± 0.4

DAT: days after treatment,
n.a.: not analysed,
SD: standard deviation,
[A] Sum of radioactivity in soda lime and polyurethane foam
All values expressed as percentage of total applied radioactivity

B. Mass Balance

Mean material balances were 103.8% AR for low concentration test systems (range from 101.1 to 105.9% AR) and 103.5% AR for high concentration test systems (range from 102.0 to 105.0% AR).

C. Mineralisation

Formation of carbon dioxide remained low throughout the study at <0.1% AR at all sampling intervals for both concentrations in degradation test systems as well as in sterile test systems. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of <0.1% AR at all sampling intervals for both concentrations in degradation test systems as well as in sterile test systems.

Microbial activity of the surface water was demonstrated by extensive mineralization of benzoic acid, with 85.1% AR completely mineralized to carbon dioxide at day 14. Following analysis no control substance was present in test systems after 14 days of incubation confirming that the surface water was microbially active throughout the experiment see Table 7.2.2.2- 4.

Table 7.2.2.2- 4: Mineralisation results of reference compound [^{14}C]-sodium benzoate (9.3 µg/L)

DAT [A]	Benzoic acid			Carbon dioxide		
	Water [% AR]	Organic rinse [% AR]	Mean [% AR]	Water [% AR]	Soda lime [% AR]	Total [% AR]
0	104.2	0.2	103.5	n.a.	n.a.	n.a.
	102.4	0.2		n.a.	n.a.	n.a.
14 BIO^{-} [B]	n.d.	n.d.	n.d.	28.5	47.4	75.9
	n.d.	n.d.	n.d.	24.0	64.0	88.0
14 BIO^{+} [C]	n.d.	n.d.	n.d.	3.5	82.0	85.4
	n.d.	n.d.	n.d.	9.6	82.9	92.5

[A] DAT: Days after treatment, n.a.: not analysed, n.d.: not detected,
[B] BIO^{-}: Treated with benzoic acid in water,
[C] BIO^{+}: Treated with benzoic acid in water as well as 110 µL methanol

D. Transformation of Parent Compound

Fluopicolide was found to be stable in surface water at both concentrations and was ≥ 100.9% at all intervals. One minor degradation product was formed in the high concentration test system which reached a maximum of 1.6% AR at day 21. No major degradation products were formed.

In the sterile test systems, no major degradation products were formed.

E. Degradation Kinetics

No significant degradation of fluopicolide was observed in aerobic surface water at either test concentration over 63 days.

The reported degradation of [phenyl-U-^{14}C]-fluopicolide followed single first order (SFO) kinetics for both the low and high concentrations (see Table 7.2.2.2- 5).

Table 7.2.2.2- 5: Reported DT_{50} and DT_{90} values for fluopicolide in aerobic aquatic systems

Test water (applied concentration)	Best Fit Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]	Prob. > t*
Low concentration 10.7 µg/L	SFO	>1000	>1000	1.93	0.5
High concentration 107 µg/L	SFO	1000	>1000	0.84	0.0596

* In order to assess the fitted degradation rates as statistically acceptable, Prob. > t (i.e. the p-value) should be < 0.05

The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). A summary of the fit statistics in the standard EFSA kinetics template are shown below. Best fit kinetics are highlighted in bold. Parameter confidence was low for SFO fits due to extremely slow decline in residues. The FOMC fits were not accepted, as extrapolation beyond the experimental period is not recommended for deriving robust DT_{90} values using this model (EFSA, 2009) and the FOMC fit at the high test concentration did not generate a full set of statistics. Thus the SFO fits were accepted.

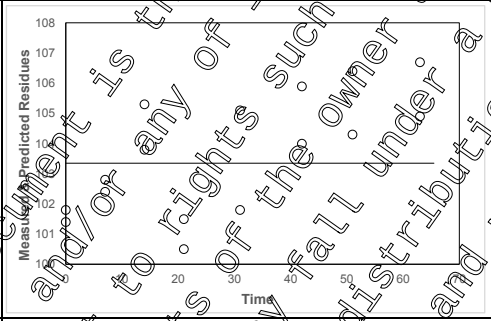
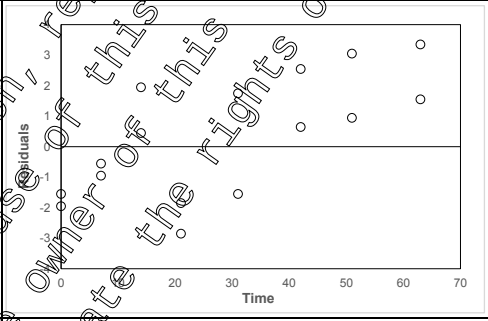
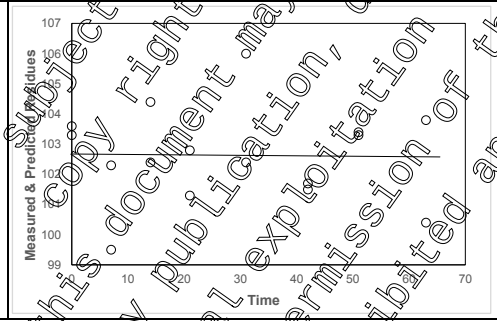
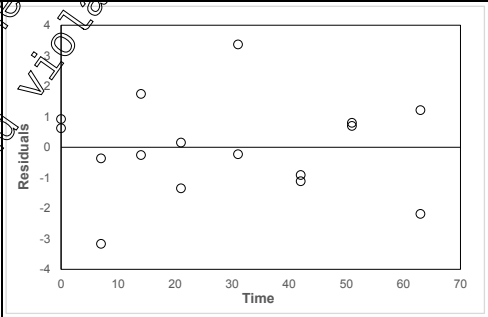
Table 7.2.2.2- 6: Re-evaluated degradation rate of fluopicolide in surface water under aerobic conditions

Test	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Low concentration, 10.7 µg/L M-558747-01-2	**SFO**	**103.4**	**k 0.00E+00**	**1.31**	**0.5**	**-0.000471**	**0.00E+00**	**>10000**	**>10000**
	FOMC	103.4	α 0.00E+00 β 0.3912	1.38	n.r. n.r.	-0.006491 0.3912	0.006 0.391	>10000	>10000
High concentration, 107.7 µg/L M-558747-01-2	**SFO**	**102.7**	**k 1.44E-05**	**0.82**	**0.471**	**-0.0003617**	**0.00E+00**	**>10000**	**>10000**
	FOMC	103.4	α 0.000134 β 2.24E-28	Inf	n.r. n.r.	NA NA	NA NA	>10000	>10000

Best fit model highlighted in bold

A graphical representation of the final kinetic fit is shown below.

Table 7.2.2.2- 7: Degradation of fluopicolide in surface water under aerobic conditions (best-fit DT_{50} values)

Test Model Reference	Modelled vs observed	Residuals
Low concentration SFO M-558747-01-2		
High concentration SFO M-558747-01-2		

III. Conclusion

Fluopicolide was not degraded in both the low and high concentration tests. The DT_{50} values of fluopicolide in the tested surface water under aerobic conditions were >1000 days for both the low and high concentrations.

Formation of volatiles such as carbon dioxide was minimal and reached a maximum of 0.2 % AR indicating very little potential for it to be a major degradation pathway of fluopicolide and its degradation products. No major degradation products were formed.

Fluopicolide is not expected to be degraded by mineralization in surface water under aerobic conditions.

Assessment and conclusion by applicant:

The study is considered appropriate to assess the aerobic mineralisation of fluopicolide in surface water bodies.

CA 7.2.2.3 Water/sediment study

The degradation and fate of fluopicolide in aerobic water sediment systems has been investigated in Study KCA 7.2.2.3/01, which was evaluated during the previous EU review and is still considered as reliable to assess the behaviour in water sediment systems.

For procedural reasons the previously submitted kinetic evaluation report also has to be included under Point KCA 7.2.2.3 in the current dossier (KCA 7.2.2.3/02). This report is fully superseded by the latest kinetic evaluation report (KCA 7.2.2.3/03).

Report reference	Author, Year	Phenyl Label	Pyridyl Label	Comment
KCA 7.2.2.3/01 M-241425-01-1	[redacted] 2003	✓	✓	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable.
KCA 7.2.2.3/02 M-234725-01-1	[redacted] 2003	-	-	Submitted and reviewed for first approval of fluopicolide, 2005. Considered valid and acceptable for the initial submission. Superseded by M-685681-01-1
KCA 7.2.2.3/03 M-685681-01-1	[redacted] 2020	-	-	New data not yet reviewed.

Data Point:	KCA 7.2.2.3/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Degradation of [2,6-14C-pyridinyll] and [U-14C-benzoyl]-AE C638206 in two contrasting sediment-water systems under laboratory aerobic conditions at 20° C
Report No:	B004510
Document No:	M-241425-01-1
Guideline(s) followed in study:	EU (=EEC): Annex II, Section 7, Point 7.2.1.3.2; PMRA: T-1-255; USEPA (=EPA): Section N, 162-4
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The fate of fluopicolide was studied in two different sediment and water systems under aerobic conditions at 20 °C. A fine texture sediment (loamy sand/silty sand) and course texture sediment (sand) both from the United Kingdom. To elucidate the complete metabolic pathway, two radiolabelled forms of fluopicolide were used: [Phenyl-U-^{14}C] label and [2,6-pyridyl-^{14}C]-label.

The test system was designed to provide a laboratory representation of a "worst case" scenario resulting from direct overspray or run-off of the test substance into a stationary body of water and then subjected to aerobic conditions. Cylindrical metabolism flasks of 7.2 cm inner diameter were filled with a layer of sediment (*ca.* 70 to 75 ml) and overlying water (*ca.* 225 to 250 ml) at either a ratio of 1:4 or 1:6.

[Phenyl-U-^{14}C]-fluopicolide or [2,6-pyridyl-^{14}C]-fluopicolide was applied to the surface of the water overlying sediment at a target rate of 163 µg of a.i. per flask, which was equivalent to a field application rate of 400 g/ha.

Humidified air was passed through each treated flask continuously to maintain aerobic conditions. Effluent gas was then passed through traps containing ethylene glycol and either ethanolamine or aqueous sodium hydroxide for the collection of volatile organic components and carbon dioxide, respectively.

For each radiolabel, non-sterile samples were taken for analysis after 0, 3, 7, 15, 28, 85, 135, 182, 273 and 365 days of incubation. Duplicate sample were taken at day 0, at other timepoints single samples were analysed. Additionally, single sterile flasks of each sediment/water type and radiolabel were removed from the incubation system after 29, 85 and 135 days.

At each sampling interval the water phase was decanted from the sediment and particulates at the water / sediment interface were removed by filtration. From day 182 to day 365, particulates in the water phase were removed by centrifugation. The sediments were extracted two to four times at ambient temperature with acetonitrile / water (4:1, v/v). After ambient extractions, sediment samples from day 135, 273 and 365 timepoints were further extracted by Soxhlet with acetonitrile for four hours.

The overall recoveries of applied radioactivity ranged from 96.7% to 108.7% during the course of the study.

In the Mill Stream water / sediment system the radioactivity initially transferred rapidly from the water phase to the underlying sediment before reaching a more steady level after approximately one to two months. In the Iron Hatch system the partitioning of radioactivity from the water phase was slower but also reached a relatively steady level after one to two months. In the Mill Stream system ca. 93% of applied radioactivity was accounted for in the water at time zero, declining to *ca*. 25% after 28 days and

ca. 14% at 365 days. In the Iron Hatch system 95% of applied radioactivity was accounted for in the water at time zero, declining to *ca*. 67% after 28 days and *ca.* 60% at 365 days. The distribution of radioactivity was very similar for both radiolabelled treatments.

The changing levels of radioactivity in the sediment reflected those for the water, with a rapid initial rise in the Mill Stream system and a steadier rise in the Iron Hatch system being followed in both sediments by the attainment of a relatively steady level. Thus for the Mill Stream system the total radioactivity in the sediment rose rapidly from *ca.* 3% at time zero to *ca.* 78% at day 85 and *ca.* 73% at day 365. In the Iron Hatch system the total activity in the sediment rose from *ca.* 3% at time zero to *ca.* 36% at day 85 and *ca.* 30% at day 365. The levels of radioactivity not extracted by ambient extraction of sediments did not exceed 13% throughout the study and soxhlet extraction of the sediment residue released less than 5% of applied radioactivity. Unextractable sediment residues were ≤10% throughout the study in both systems.

Volatilization was not a significant dissipation pathway for fluopicolide or its metabolites which was demonstrated by the low amount radioactivity (< 0.05% of applied) recovered in the ethylene glycol traps. Mineralization to carbon dioxide was observed but accounted for less than 3% of applied radioactivity by day 365

Fluopicolide dissipated from the water after application by a combination of degradation and partitioning to the sediment. At the end of the study after 365 days incubation, levels of fluopicolide remaining in the water phase were *ca.* 41% in the Iron Hatch samples, and *ca.* 9% in the Mill Stream samples. Differences in the dissipation of fluopicolide in the water of the two sediments over time were likely to be due to differences in sorption properties of the sediment (organic carbon, cation exchange capacity). More rapid dissipation of fluopicolide from the water phase was observed in the Mill Stream system which had a higher organic carbon content and cation exchange capacity.

In sediment after 135 days incubation, fluopicolide was present at *ca.* 36% in the Iron Hatch samples and *ca*. 75% in the Mill Stream samples. The residues in the Iron Hatch and Mill Stream sediments generally decreased thereafter to *ca.* 28% and 69%, respectively.

The primary route of degradation in sediment water system was assumed to be oxidation of fluopicolide followed by rapid cleavage of the double ring system of the parent molecule to simultaneously form M-02 (referred to as AE C657188) from the pyridine ring and M-01 (referred to as AE C653711) in the study report from the benzene ring.

M-01, resulting from the cleavage of [phenyl-U-^{14}C]-fluopicolide increased gradually over time to a maximum of 20.3% at day 365 in the Iron Hatch system and 9.0% at day 365 in the Mill Stream system. M-02, resulting from the cleavage of [2,6-pyridyl-^{14}C]-fluopicolide increased gradually over time to a maximum of 8.2% at day 365 in the Iron Hatch system and 1.5% at day 135 in the Mill Stream system. The levels of M-01 and M-02 detected at equivalent timepoints in sterile and non sterile samples were generally comparable indicating the cleavage of fluopicolide was not facilitated by microbial activity. Both metabolites were found predominantly in the water and with lesser amounts found in the sediment.

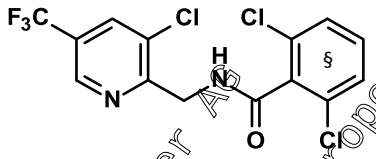
A re-evaluation of the degradation kinetics for both water sediment systems according to FOCUS guidance (2006, 2014) using the software KinGUI 2.1 resulted in SFO DT_{50} values for fluopicolide in the total system ranging from 856.3 days to 1340.0 days with a geometric mean of 1071.2 days. The dissipation of fluopicolide from the water phase was best described by the DFOP model in Iron Hatch and by the FOMC model in Mill Stream. Persistence endpoint DT_{50} values for the water phase were 228.9 and 5.6 days respectively. Pseudo-SFO DT_{50} values of 594.5 days (from the slow phase of the DFOP fit) in Iron Hatch and 57.0 days (from the FOMC DT_{90}/3.32) in Mill Stream were derived from the biphasic fits for modelling endpoints according to FOCUS recommendations.

I. Materials and Methods

A. Materials

1. Test Items

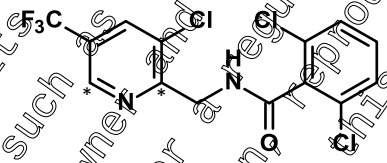
[Phenyl-U-^{14}C]-fluopicolide



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

Batch Number:	ESR- 283
Specific Activity:	5.74 MBq/mg (155 µCi/mg or 344,100 dpm/µg)
Radiochemical Purity:	96.4% (by HPLC)

[2,6-Pyridyl-^{14}C]-fluopicolide



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

Batch Number:	ESR- 282
Specific Activity:	5.99 MBq/mg (162 µCi/mg or 359,640 dpm/µg)
Radiochemical Purity:	96.5% (by HPLC)

2. Water Sediment

The sediments were classified as a sand (USDA and German BBA classification) and a loamy sand/silty sand (USDA/German BBA classification) and were collected from the Institute of Freshwater Ecology, Dorset, United Kingdom with their respective overlying water. The properties of the sediment water systems are summarised in Table 7.2.2.3-1

Table 7.2.2.3- 1 Physico-chemical characteristics of the water sediment systems

Sediment Characteristic	Units	EFS -98	EFS-99
Origin	State, Country	Dorset, UK	Dorset, UK
Location		Iron Hatch	Mill Stream
Particle Size Analysis, USDA:			
Total Sand	(0.05 - 2.00 mm)	95%	81%
Silt	(0.002 - 0.05 mm)	2%	16%
Clay	(< 0.002 mm)	3%	3%
Textural Class	USDA	Sand	Sand
Particle Size Analysis, ADAS:			
Total Sand	(0.063 – 2.0 mm)	93%	79%
Silt	(0.002 – 0.063 mm)	4%	18%
Clay	(<0.002 mm)	3%	3%
Textural Class	ADAS	Sand	Loamy Sand
Particle Size Analysis, BBA:			
Total Sand	(0.063 – 2.0 mm)	93%	79%
Silt	(0.002 – 0.063 mm)	4%	18%
Clay	(<0.002 mm)	3%	3%
Textural Class	BBA	Sand	Loamy Sand
pH	Water (1:1)	7.2	6.6
	0.01M CaCl2	6.5	6.6
Organic Carbon	%	0.5	5.3
Organic Matter	%	0.9	9.1
Cation Exchange Capacity	meq/100g	5.0	16.5
Water Holding Capacity	% at 0 bar	28.5	93.6
	% at 1/10 bar	5.7	44.3
	% at 1/3 bar	3.5	28.6
	% at15 bar	1.9	15.9
Disturbed Bulk Density	g/cc	1.52	0.77
Microbial Biomass Initial	µg microbial C / g soil	29.1	92.5
Day 135	µg microbial C / g soil	25.6	20.4
Final	µg microbial C / g soil	43.4	21.6
Water Characteristic	**Units**	**EFW-12**	**EFW-13**
At Time of Collection			
pH		8.20	8.20
Dissolved oxygen concentration	%	113.3	117.7
Water temperature	°C	10.1	10.5
Post-Collection Analysis			
pH		8.4	8.3
Conductivity	mS/cm	0.64	0.60
Hardness	mg $CaCO_3$/L	263	265
Alkalinity	mg $CaCO_3$/L	214	215
Total suspended solids	mg/L	2	6
Nitrate/nitrite	mg/L	4.9	4.5

B. STUDY DESIGN

1. Experimental conditions

The behaviour of [phenyl-U-^{14}C]- and [2,6-pyridyl-^{14}C]-fluopicolide were investigated in two contrasting sediment water systems ('Iron Hatch' and 'Mill Stream') over a period of 365 days.

The test system was designed to provide a laboratory representation of a "worst case" scenario resulting from direct overspray or run-off of the test substance into a stationary body of water and then subjected to aerobic conditions. Cylindrical metabolism flasks of 7.2 cm inner diameter were filled with a layer of sediment (*ca.* 70 to 75 ml) and overlying water (*ca.* 225 to 250 ml) at a ratio of 1:4 (Iron Hatch) and 1:6 (Mill Stream).

Prior to treatment, sixteen of the sediment/water systems to be used for later time-points, were transferred to a different testing facility and connected to a humidified air supply. After equilibrating for approximately two weeks, the flasks were treated in the same manner as the samples at the original testing facility.

[Phenyl-U-^{14}C]-fluopicolide or [2,6-pyridyl-^{14}C]-fluopicolide was applied to the surface of the water overlying sediment at a target rate of 163 µg of a.i. per flask, which was equivalent to a field application rate of 400 g/ha.

Humidified air was passed through each treated flask continuously to maintain aerobic conditions. Effluent gas was then passed through traps containing ethylene glycol and either ethanolamine or aqueous sodium hydroxide for the collection of volatile organic components and carbon dioxide, respectively. Oxidation/reduction potential (E_h) of water and sediment phases, dissolved oxygen concentration in water, and pH of the water were measured prior to treatment and at each timepoint during the study.

2. Sampling

For each radiolabel, non-sterile samples were taken for analysis after 0, 3, 7, 15, 28, 85, 135, 182, 273 and 365 days of incubation. Duplicate sample were taken at day 0, at other timepoints single samples were analysed. Additionally, single sterile flasks of each sediment/water type and radiolabel were removed from the incubation system after 29, 85 and 135 days. Untreated sterile controls were monitored for microbial activity at the same timepoints.

3. Analytical procedures

The water phase was decanted from the sediment and particulates at the water / sediment interface were removed by filtration. From day 182 to day 365, particulates in the water phase were removed by centrifugation. The sediments were extracted two to four times at ambient temperature with acetonitrile / water (4:1, v/v). After ambient extractions, sediment samples from day 135, 273 and 365 timepoints were further extracted by Soxhlet with acetonitrile for four hours. After the completion of extractions, the remaining residue was combusted to quantify non-extractable residue (NER).

Radioactivity in the water phase, extracted from the sediment phase and in the volatile traps was quantified by LSC.

Extracts from the water and sediment were analyzed against authentic reference standards by reverse phase HPLC. Aqueous samples were analysed directly. Sediment extracts from days 3 to 29 were concentrated by rotary evaporation (< 40 °C) prior to HPLC analysis. All other sediment extracts were analysed directly. Soxhlet extracts, all of which contained < 5%, were not analysed.

4. Degradation kinetics

The degradation kinetics determined in the report were conducted prior to the issuing of the FOCUS guidance document on degradation kinetics and are no longer considered valid. DT_{50} and DT_{90} values for the degradation of fluopicolide has been re-calculated from the reported data according to FOCUS guidance (2006, 2014) using the software KinGUI 2.1. As the degradation was investigated using two radiolabel positions of fluopicolide and similar behaviour was observed for each, these radiolabels have been considered as true replicates, and included together in a single optimisation. Full details, including the approach to derived modelling endpoints, are provided in Document CA 7.2.2.3/03 (M-685681-01-1). A brief summary of the approach to derive persistence endpoints is provided below.

Analysis was conducted at level I of the FOCUS assessment schemes, using a single-compartment approach to derive (i) degradation endpoints for the total system or (ii) dissipation endpoints for the individual water and sediment compartments. Dissipation endpoints are derived from maximum residue onwards. However, after removal of all data points prior to the maximum residue in sediment (DAT 182) only three data points remained for each system, which was insufficient to provide a robust fit. Kinetic analysis was therefore conducted for the total system and water compartment only, and no fits were included for the sediment compartment.

To derive endpoints for the degradation of fluopicolide in the total system, an initial comparison was performed for each system between the SFO and FOMC fits. In both cases the FOMC model provided no significant improvement on the SFO fit, and the SFO fit was therefore accepted.

To derive endpoints for the dissipation of fluopicolide from water, an initial comparison was performed for each system between the SFO and FOMC fits. In both systems, the FOMC model provided a better fit to the residues, and the DFOP and HS models were therefore also fitted. For the Iron Hatch system, DFOP provided the best fit to the residues, with the lowest χ^2 err% value, while for the Mill Stream system, the best fit and lowest χ^2 err% value were provided by the FOMC model.

II. Results and Discussion

A. Data

The distribution and recovery of radioactivity in each water sediment system is presented in Table 7.2.2.3- 2 to Table 7.2.2.3- 5.

Table 7.2.2.3- 2 Recovery and distribution of radioactivity following treatment of Iron Hatch system with [phenyl-U-^{14}C]-labelled fluopicolide (% AR)

Component	DAT													
	Non sterile											Sterile		
	0	0	3	7	15	28	85	135	182	273	365	29	85	135
Water phase	94.6	93.0	89.0	83.6	72.5	67.6	60.0	56.9	56.4	53.7	60.6	70.1	64.8	65.4
Sediment ambient ext	3.2	4.5	8.4	14.8	23.7	29.2	35.2	36.9	36.5	33.4	29.7	27.2	34.2	33.7
Sediment soxhlet ext	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	4.8	2.8	n.a	n.a	n.a
Sediment unextractables	0.1	0.2	0.3	0.8	1.9	2.2	2.5	3.8	6.6	4.3	4.9	0.7	0.9	1.0
$^{14}CO_2$	n.a	n.a	n.d	n.d	n.d	0.1	0.5	0.9	1.1	2.7	2.8	n.d	n.d	n.d
Organic volatiles	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Recovery	98.0	97.6	97.7	99.2	98.2	99.1	98.1	98.6	100.7	99.0	100.8	98.1	99.8	100.1

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

Table 7.2.2.3- 3 Recovery and distribution of radioactivity following treatment of Iron Hatch system with [2,6-pyridyl-^{14}C] labelled fluopicolide (% AR)

Component	DAT													
	Non sterile											Sterile		
	0	0	3	7	15	28	85	135	182	273	365	29	85	135
Water phase	94.6	94.5	89.0	82.2	75.5	66.6	59.7	57.1	57.0	61.1	58.9	68.5	64.8	66.9
Sediment ambient ext	4.2	3.0	8.6	16.4	20.8	29.5	36.9	37.1	42.4	32.3	30.7	30.0	32.6	30.0
Sediment soxhlet ext	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	2.5	2.4	n.a	n.a	n.a
Sediment unextractables	0.2	0.1	0.3	0.8	1.7	2.0	2.1	4.1	7.7	4.3	5.8	1.1	2.0	2.9
$^{14}CO_2$	n.a	n.a	n.d	n.d	n.d	n.d	n.d	0.1	0.3	0.7	1.9	n.d	n.d	n.d
Organic volatiles	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Recovery	99.0	97.6	97.9	97.5	98.0	98.1	98.8	98.4	107.4	100.8	99.4	99.6	99.3	99.8

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

Table 7.2.2.3- 4 Recovery and distribution of radioactivity following treatment of Mill stream system with [phenyl-U-^{14}C]-labelled fluopicolide (% AR)

Component	DAT													
	Non sterile											Sterile		
	0	0	3	7	15	28	85	135	182	273	365	29	85	135
Water phase	93.6	92.9	66.1	39.8	35.9	26.3	16.3	12.6	12.0	13.7	15.3	34.1	19.6	18.5
Sediment ambient ext	2.9	3.6	31.8	54.7	60.2	70.0	78.9	78.9	78.8	77.3	74.1	64.9	78.5	76.4
Sediment soxhlet ext	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	4.1	3.3	n.a	n.a	n.a
Sediment unextractables	0.8	0.7	1.3	2.8	3.9	3.3	4.5	7.1	7.9	3.8	6.1	1.6	2.4	3.2
$^{14}CO_2$	n.a	n.a	n.d	n.d	0.1	0.1	0.3	0.4	0.1	0.8	1.3	n.d	n.d	n.d
Organic volatiles	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Recovery	97.3	97.2	99.2	97.3	100.0	100.7	100.0	99.0	98.9	99.6	100.1	100.7	100.5	98.1

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

Table 7.2.2.3- 5 Recovery and distribution of radioactivity following treatment of Mill Stream system with [2,6-pyridyl-^{14}C] labelled fluopicolide (% AR)

Component	DAT													
	Non sterile											Sterile		
	0	0	3	7	15	28	85	135	182	273	365	29	85	135
Water phase	91.7	93.3	61.4	36.6	32.9	22.4	16.1	11.7	12.2	13.1	12.9	33.5	19.9	18.2
Sediment ambient ext	5.1	4.1	34..8	57.8	60.4	72.7	77.1	76.2	77.7	72.3	71.6	64.3	77.7	77.2
Sediment soxhlet ext	n.a	n.a	n.a	n.a	n.a	n.a	n.a	3.3	n.a	4.2	2.6	n.a	n.a	n.a
Sediment unextractables	1.2	0.8	1.4	2.9	3.8	3.4	5.6	5.4	10.3	8.6	9.7	1.7	2.5	3.1
$^{14}CO_2$	n.a	n.a	n.d	n.d	n.d	n.d	0.1	0.1	0.6	0.7	1.2	n.d	n.d	n.d
Organic volatiles	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Recovery	97.9	98.2	97.6	97.3	97.1	98.5	98.8	96.7	100.9	98.9	97.9	99.5	100.1	98.6

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

The results from the characterisation of the radioactivity in water and ambient sediment extracts are presented in Table 7.2.2.3- 6 to Table 7.2.2.3- 9

Table 7.2.2.3- 6 Characterisation of radioactivity following treatment of Iron Hatch system with [phenyl-U-^{14}C]-labelled fluopicolide (% AR)

Component	Phase	DAT													
		Non sterile											Sterile		
		0	0	3	7	15	28	85	135	182	273	365	29	85	135
Total	Water	94.6	93.0	89.0	83.6	72.5	67.6	60.0	56.9	56.4	53.7	60.6	70.1	64.8	65.4
	Sediment	3.2	4.5	8.4	14.8	23.7	29.2	35.2	36.9	36.5	33.3[1]	29.7[1]	27.2	34.2	33.7
Fluopicolide	Water	93.6	92.4	87.9	82.1	69.9	64.0	57.5	52.4	48.0	41.3	40.9	68.4	60.3	59.0
	Sediment	n.a	n.a	8.2	14.0	23.6	28.7	34.6	36.2	35.3	31.7	27.6	26.0	33.6	32.0
M-01 (AE C653711)	Water	1.0	0.6	1.2	1.3	1.4	1.5	2.5	3.9	7.3	12.4	18.2	1.7	4.5	5.7
	Sediment	n.a	n.a	0.1	0.2	0.1	0.2	0.3	n.d	1.2	1.7	2.1	0.2	0.6	n.d
Unk 1 (20.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 2 (22.5)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 3 (30.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 7 (34.5)	Water	n.d	n.d	n.d	n.d	1.2	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 8 (34.6)	Water	n.d	n.d	n.d	n.d	n.d	2.1	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.a: not applicable, n.d: not detected, Figures in parenthesis = retention time (minutes),
[1] Soxhlet extracts not analysed as <5%

Table 7.2.2.3- 7 Characterisation of radioactivity following treatment of Iron Hatch system with [2,6-pyridyl-^{14}C] labelled fluopicolide (% AR)

Component	Phase	DAT												
		Non sterile											Sterile	
		0	0	3	7	15	28	85	135	182	273	365	29	85	135
Total	Water	94.6	94.5	89.0	80.2	75.5	66.6	59.7	57.1	57.0	61.1	58.9	68.5	64.8	66.9
	Sediment	4.2	3.0	8.6	16.4	20.8	29.5	36.9	37.1	42.4	32.3[1]	30.7[1]	30.0	32.6	30.0[2]
Fluopicolide	Water	94.5	93.8	88.5	78.7	74.1	63.8	57.2	52.9	47.7	48.6	41.6	67.8	63.6	68.1
	Sediment	n.a	n.a	8.6	16.2	20.7	28.7	36.0	32.7	40.6	30.6	28.2	29.1	32.3	32.4
M-02 (AE C657188)	Water	n.d	n.d	n.d	0.8	0.8	1.2	1.4	3.3	5.4	5.6	7.4	0.7	1.2	1.8
	Sediment	n.a	n.a	n.d	0.1	n.d	0.1	n.d	n.d	0.6	0.6	0.8	n.d	0.3	n.d
Unk 1 (20.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2.7	4.7	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 2 (22.5)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2.4	4.3	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 3 (30.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.6	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 7 (34.5)	Water	n.d	n.d	n.d	n.d	n.d	1.6	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 8 (34.6)	Water	n.d	n.d	n.d	n.d	n.d	n.d	1.8	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.a: not applicable, n.d: not detected, Figures in parenthesis = retention time (minutes)
[1] Soxhlet extracts not analysed as <5%, [2] Report states figure is 64.8%, correct figure is 30.0%

Table 7.2.2.3- 8 Characterisation of radioactivity following treatment of Mill Stream system with [phenyl-U-^{14}C]-labelled fluopicolide (% AR)

Component	Phase	DAT													
		Non sterile											Sterile		
		0	0	3	7	15	28	85	135	182	273	365	29	85	135
Total	Water	93.6	92.9	66.1	39.8	35.9	26.3	16.3	12.6	12.0	13.7	15.3	34.1	19.6	18.5
	Sediment	2.9	3.6	31.8	54.7	60.1	70.0	78.9	78.9	78.8	77.3[1]	74.1[1]	64.9	78.5	76.4
Fluopicolide	Water	92.7	92.0	65.4	38.6	34.2	24.7	13.6	10.4	9.4	9.6	8.0	32.7	17.0	16.4
	Sediment	n.a	N a	31.4	52.9	59.0	68.5	76.2	73.1	74.8	73.4	68.8	63.3	75.8	76.9
M-01 (AE C653711)	Water	1.0	0.8	0.7	0.9	0.8	0.9	1.6	1.5	2.0	2.9	3.1	1.4	1.9	2.9
	Sediment	n.a	n.a	0.4	0.4	0.6	0.8	n.d	1.9	1.5	2.5	3.9	0.6	0.8	1.0
Unk 1 (20.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 2 (22.5)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 3 (28.4)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 4 (30.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 5 (32.4)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.1	n.d	n.d	n.d
Unk 6 (33.5)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.1	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	0.5	n.d	n.d	0.4	n.d	n.d	n.d
Unk 7 (34.5)	Water	n.d	n.d	n.d	n.d	0.9	0.7	n.d	n.d	0.2	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	0.5	n.d	n.d	1.0	n.d	0.6	n.d	n.d	n.d	n.d
Unk 8 (34.6)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	1.0	n.d	n.d	n.d	n.d	n.d

n.a: not applicable, n.d: not detected, Figures in parenthesis = retention time (minutes) ,
[1] Soxhlet extracts not analysed as <5%

Table 7.2.2.3- 9 Characterisation of radioactivity following treatment of Mill Stream system with [2,6-pyridyl-^{14}C] labelled fluopicolide (% AR)

Component	Phase	DAT													
		Non sterile											Sterile		
		0	0	3	7	15	28	85	135	182	273	365	29	85	135
Total	Water	91.7	93.3	61.4	36.6	32.9	22.4	16.1	11.7	12.2	13.1	12.9	33.5	19.9	18.2
	Sediment	5.1	4.1	34.8	57.8	60.4	72.7	77.1	76.2[1]	77.7	72.3[1]	71.6[1]	64.3	77.2	77.3
Fluopicolide	Water	90.8	93.1	60.9	35.8	31.8	20.1	14.3	10.1	10.4	10.1	10.1	33.3	19.3	17.0
	Sediment	n.a	n.a	34.8	56.2	59.3	72.0	73.8	74.1	73.9	70.8	70.0	63.5	73.4	75.5
M-02 (AE C657188)	Water	n.d	n.d	0.5	0.5	0.6	0.7	0.8	0.8	0.5	0.7	0.6	0.1	0.7	1.3
	Sediment	n.a	n.a	n.d	0.2	n.d	0.2	n.d	0.8	n.d	n.d	n.d	0.2	n.d	n.d
Unk 1 (20.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	0.3	1.4	1.4	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 2 (22.5)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 3 (28.4)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	0.5	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 4 (30.0)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	0.2	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unk 5 (32.4)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.4	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	0.1	n.d	n.d	n.d	n.d	n.d	n.d	1.6	n.d	n.d	n.d
Unk 6 (33.5)	Water	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.0	n.d	n.d	n.d	n.d
Unk 7 (34.5)	Water	n.d	n.d	n.d	n.d	n.d	0.3	0.2	n.d	n.d	0.3	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	n.d	n.d	n.d	1.2	0.9	n.d	0.5	n.d	n.d	n.d	n.d
Unk 8 (34.6)	Water	n.d	n.d	n.d	n.d	0.4	n.d	n.d	n.d	0.4	n.d	n.d	n.d	n.d	n.d
	Sediment	n.a	n.a	n.d	0.2	n.d	n.d	n.d	n.d	1.4	n.d	n.d	n.d	n.d	n.d

n.a: not applicable, n.d: not detected, figures in parenthesis = retention time (minutes),
[1] Soxhlet extracts not analysed as <5%

B. Mass Balance

The overall recoveries of applied radioactivity ranged from 96.7% to 108.7% during the course of the study.

C. Extractable and Non-Extractable Residues

In the Mill Stream water / sediment system the radioactivity initially transferred rapidly from the water phase to the underlying sediment before reaching a more steady level after approximately one to two months. In the Iron Hatch system the partitioning of radioactivity from the water phase was slower but also reached a relatively steady level after one to two months. In the Mill Stream system ca. 93% of applied radioactivity was accounted for in the water at time zero, declining to *ca*. 25% after 28 days and *ca.* 14% at 365 days. In the Iron Hatch system 95% of applied radioactivity was accounted for in the water at time zero, declining to *ca*. 67% after 28 days and *ca.* 60% at 365 days. The distribution of radioactivity was very similar for both radiolabelled treatments.

The changing levels of radioactivity in the sediment reflected those for the water, with a rapid initial rise in the Mill Stream system and a steadier rise in the Iron Hatch system being followed in both sediments by the attainment of a relatively steady level. Thus for the Mill Stream system the total radioactivity in the sediment rose rapidly from *ca.* 3% at time zero to *ca.* 78% at day 85 and *ca.* 73% at day 365. In the Iron Hatch system the total activity in the sediment rose from *ca.* 3% at time zero to *ca.* 36% at day 85 and *ca.* 30% at day 365. The levels of radioactivity not extracted by ambient extraction of sediments did not exceed 13% throughout the study and soxhlet extraction of the sediment residue released less than 5% of applied radioactivity. Unextractable sediment residues were ≤10% throughout the study in both systems.

D. Volatile Radioactivity

Volatilization was not a significant dissipation pathway for fluopicolide or its metabolites, which was demonstrated by the low amount radioactivity (< 0.05% of applied) recovered in the ethylene glycol traps. Mineralization to carbon dioxide was observed but accounted for less than 3% of applied radioactivity by day 365.

E. Degradation of Parent Compound

Fluopicolide dissipated from the water after application by a combination of degradation and partitioning to the sediment. At the end of the study after 365 days incubation, levels of fluopicolide remaining in the water phase were *ca.* 41% in the Iron Hatch samples and *ca.* 9% in the Mill Stream samples. Differences in the dissipation of fluopicolide in the water of the two sediments over time were likely to be due to differences in sorption properties of the sediment (organic carbon, cation exchange capacity). More rapid dissipation of fluopicolide from the water phase was observed in the Mill Stream system which had a higher organic carbon content and cation exchange capacity. In sediment after 135 days incubation, fluopicolide was present at *ca.* 36% in the Iron Hatch samples and *ca*. 75% in the Mill Stream samples. The residues in the Iron Hatch and Mill Stream sediments generally decreased thereafter to *ca.* 28% and 69%, respectively.

Fluopicolide showed similar degradation in the water and sediment of sterile samples, indicating that fluopicolide degradation was not enhanced by microbial activity.

The primary route of degradation in sediment water system was assumed to be oxidation of fluopicolide followed by rapid cleavage of the double ring system of the parent molecule to simultaneously form M-02 from the pyridine ring and M-01 in the study report from the benzene ring.

M-01, resulting from the cleavage of [phenyl-U-^{14}C]-fluopicolide increased gradually over time to a maximum of 20.3% at day 365 in the Iron Hatch system and 9.0% at day 365 in the Mill Stream system. M-02, resulting from the cleavage of [2,6-pyridyl-^{14}C]-fluopicolide increased gradually over time to a maximum of 8.2% at day 365 in the Iron Hatch system and 1.5% at day 135 in the Mill Stream system. The levels of M-01 and M-02 detected at equivalent timepoints in sterile and non sterile samples were generally comparable indicating the cleavage of fluopicolide was not facilitated by microbial activity. Both metabolites were found predominantly in the water and with lesser amounts found in the sediment.

The following table summarizes the maximum levels of these metabolites observed in non sterile total sediment-water systems, water and sediment fractions.

Table 7.2.2.3- 10 Maximum occurrences of metabolites in non-sterile samples (% AR)

Compound	Iron Hatch Sediment-Water System			Mill Stream Sediment-Water System		
	Total	Water	Sediment	Total	Water	Sediment
M-01	20.3	18.2	2.1	9.0	5.1	3.9
M-02	8.2	7.4	0.8	1.6	0.8	0.8

As a consequence of the slow degradation of fluopicolide in the Mill Stream system the amounts of metabolites observed were low. In the Iron Hatch system the levels of both M-02 and M-01 were higher, with no decline in either metabolite observed by the end of the study. However, as both metabolites are formed simultaneously as a result of cleavage of the double ring system of the parent molecule and the rate of degradation of fluopicolide determined for each radiolabelled experiment showed good agreement in both sediment water systems, it is clear that degradation of M-02 did occur as the levels of this metabolite are significantly lower that the levels of M-01.

Up to eight other unidentified minor metabolites were observed occasionally, but none exceeded 5.0%. Four metabolites (listed as Unknowns 5 to 8 in the following tables) were observed in as transient metabolites in both the pyridyl and phenyl radiolabelled experiments. A further four metabolites were observed only in the pyridyl radiolabelled systems (listed as Unknowns 1 to 4 in the following tables). No metabolites other than M-01 were detected arising from the phenyl ring. Breakdown of this ring slowly leads to the formation of carbon dioxide and sediment bound residues with no intermediate products accumulating.

F. Degradation Kinetics

Fluopicolide degraded slowly in the Iron Hatch and Mill Stream sediment-water systems under aerobic conditions. Fluopicolide dissipated quickly from the water in the Mill Stream system, while dissipation from the water in the low organic carbon sediment Iron Hatch was slower. Fluopicolide dissipated in the water by a combination of degradation and partitioning to the sediment.

The reported DT_{50} values for the dissipation of fluopicolide from the water phase and total system are shown below.

Table 7.2.2.3- 11 Reported DT_{50} and DT_{90} values for fluopicolide in aerobic water sediment systems

System	Radiolabel	Water Phase DT_{50} (days)	Fitting criteria (*B* value)	Water Sediment System DT_{50} (days)	Fitting criteria (*B* value)
Mill Stream	Phenyl	7.41	0.9471	833.64	0.9965
	Pyridyl	6.25	0.9423	874.96	0.9955
	Mean	6.81	0.9441	855.15	0.9960
Iron Hatch	Phenyl	170.24	0.9599	653.39	0.9980
	Pyridyl	181.93	0.9562	751.24	0.9982
	Mean	175.95	0.9580	699.42	0.9980

The experimental data has been re-evaluated according to the FOCUS guidance document on degradation kinetics (FOCUS, 2014) using the software KinGUI (version 2.1). Further details of the evaluation are provided in the summary for KCA 7.2.3.3/03. The resulting DT_{50} values for trigger endpoints are summarised below in Table 7.2.2.3- 12. Best fit kinetics are highlighted in bold.

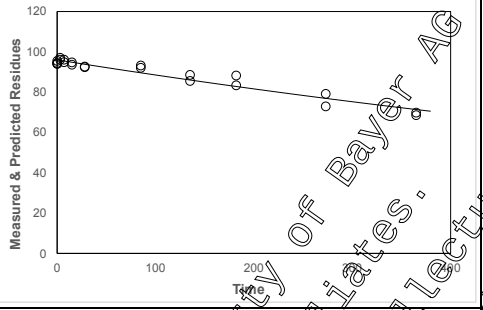
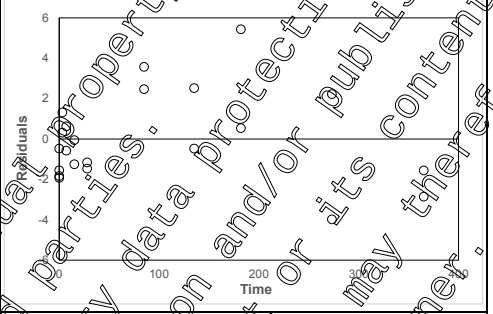
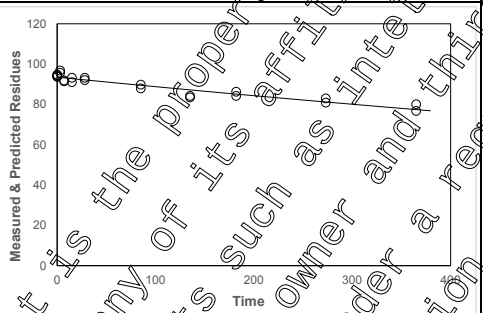
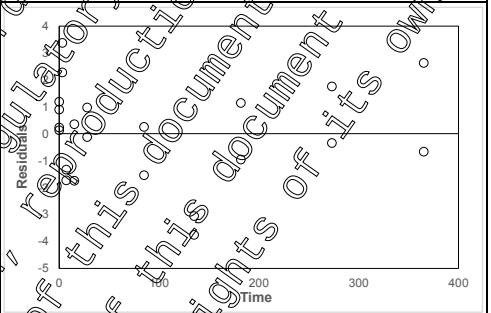
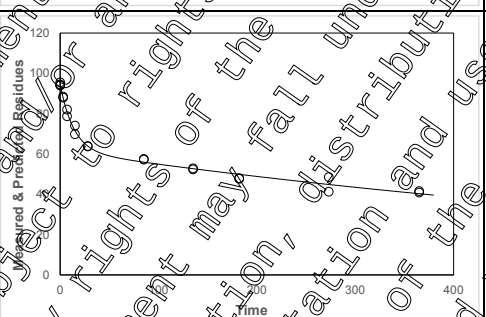
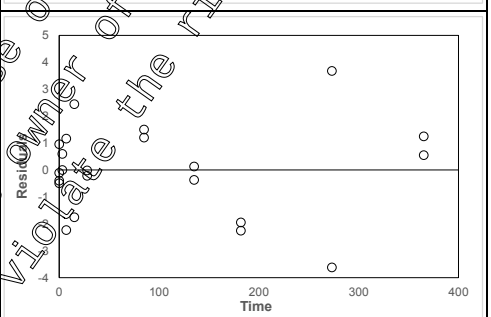
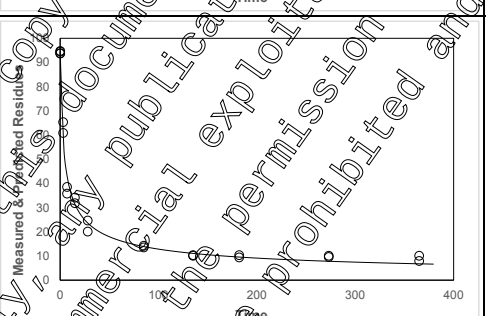
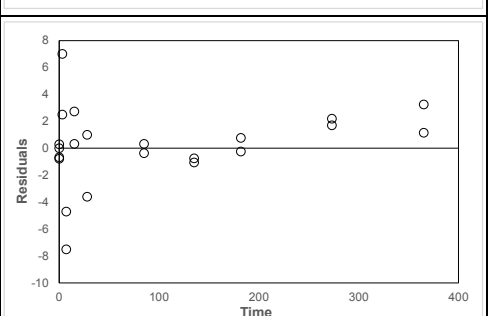
Table 7.2.2.3- 12 Re-evaluated degradation rate of fluopicolide in aerobic water sediment systems at 20 °C (DT_{50} values for trigger endpoints)

Soil	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, α, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Iron Hatch; Total system, M-241425-01-1	**SFO**	**96.01**	**k 0.0008095**	**1.56**	**3.74E-13**	**0.0007102**	**0.001**	**856.3**	**2844**
	FOMC	96.01	α 3480 β 4.30E+06	1.64	n.r. n.r.	3042 4.30E+06	3918 4.30E+06	856.3	2845
Mill Stream; Total system, M-241425-01-1	**SFO**	**93.57**	**k 0.0005174**	**1.43**	**1.08E-11**	**0.0004412**	**0.001**	**1340**	**4450**
	FOMC	94.3	α 0.1316 β 129.6	1.24	n.r. n.r.	0.02153 52.53	0.242 311.7	>10000	>10000
Iron Hatch; Water phase, M-241425-01-1	SFO	83.19	k 0.002658	10	1.21E-07	0.001973	0.003	260.8	866.3
	FOMC	94.9	α 0.1823 β 4.584	1.82	n.r. n.r.	0.1621 2.772	0.202 6.396	200.9	>10000
	DFOP	**94.58**	**k1 0.07309 k2 0.001166 g 0.347**	**1.21**	**2.52E-08 5.61E-09 n.r.**	**0.05703 0.0009342 0.3153**	**0.089 0.001 0.379**	**228.9**	**1609**
	HS	93.91	k1 0.01882 k2 0.001343 tb 21.56	1.99	2.63E-10 4.37E-10 n.r.	0.01573 0.001116 17.7	0.022 0.002 25.42	235.6	1434
Mill Stream; Water phase, M-241425-01-1	SFO	89.42	k 0.08162	27.1	2.72E-06	0.05552	0.108	8.5	28.2
	FOMC	**94.47**	**α 0.4951 β 1.826**	**7.55**	**n.r. n.r.**	**0.4228 1.126**	**0.567 2.526**	**5.6**	**189.4**
	DFOP	94.19	k1 0.2065 k2 0.004523 g 0.7306	9.35	2.09E-08 4.34E-05 n.r.	0.1617 0.002761 0.6776	0.251 0.006 0.784	5.4	219.1
	HS	94.17	k1 0.1329 k2 0.005753 tb 9.091	9.95	1.48E-41 5.25E-06 n.r.	0.1146 0.003884 7.425	0.151 0.008 10.76	5.2	199.4

Best fit model highlighted in bold

A graphical representation of the final kinetic fit is shown below.

Table 7.2.2.3- 13 Degradation of fluopicolide in aerobic water sediment systems at 20 ºC (best-fit DT_{50} value for trigger endpoints)

Soil Model Reference	Modelled vs observed	Residuals
Iron Hatch; total system SFO M-241425-01-1		
Mill Stream; total system SFO M-241425-01-1		
Iron Hatch; water DFOP M-241425-01-1		
Mill Stream; water FOMC M-241425-01-1		

III. Conclusion

Fluopicolide was stable in both sediment water systems with total system DT_{50} values of 856.3 days and 1340.0 days re-evaluated according to FOCUS kinetics. The compound dissipated quickly from the water in the Mill Stream system with DT_{50} of 5.6 days, while dissipation from the water in the low organic carbon Iron Hatch system was slower with a DT_{50} of 228.9 days. Fluopicolide dissipated in the water by a combination of degradation and partitioning to the sediment. The degradation of fluopicolide in the water and sediment of sterile samples was similar, indicating that fluopicolide degradation was not enhanced by microbial activity. The primary route of degradation in sediment water system was assumed to be oxidation of fluopicolide followed by rapid cleavage to form the metabolites M-01 (AE C653711) and M-02 (AE C657188).

M-01 was detected as a major metabolite in the water phase of one of the water sediment systems. No other major metabolites were detected.

Assessment and conclusion by applicant: The study is considered valid to assess fate and behaviour of [phenyl-U-^{14}C]-and [2,6-pyridyl-^{14}C]-fluopicolide in aerobic water sediment systems.

Data Point:	KCA 7.2.2.3/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	Kinetic evaluation of the aerobic aquatic metabolism of AE C638206 and its metabolites in water/sediment systems by inverse modelling using TOXSWA, PEST and TopFit; Code: AE C638206, AE C653711, AE C657188
Report No:	C034326
Document No:	M-234725-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For procedural reasons the previously submitted modelling report is included under Point KCA 7.2.2.3 in the current dossier. However the report has been fully superseded by a new kinetic evaluation report (KCA 7.2.2.3/03, M-685681-01-1) of the original water sediment study. Consequently, no summary of this superseded report (KCA 7.2.2.3/02, M-234725-01-1) has been included in this dossier.

Data Point:	KCA 7.2.2.3/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide (FLC) - Kinetic evaluation of aerobic aquatic metabolism in water/sediment systems
Report No:	VC/19/041G
Document No:	M-685681-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

DT_{50} values were derived for fluopicolide in the water phase, sediment phase and total water sediment system from two aerobic laboratory water/sediment systems incubated in the dark.

The evaluation was conducted to derive kinetic parameters that are suitable for use as persistence endpoints to determine whether additional studies are triggered, and modelling endpoints for use in aquatic risk assessments, according to FOCUS Kinetics guidance (FOCUS 2006, 2014). The kinetic analysis was conducted using the software tool KinGUI 2.1. The appropriate kinetic model was identified following the recommendations given by the FOCUS Kinetics Workgroup (FOCUS 2006, 2014), based on a detailed analysis including visual assessment, χ^2err statistics and a t-test to assess parameter significance.

Analysis was conducted at level I of the FOCUS assessment schemes, using a single-compartment approach to derive (i) degradation endpoints for the total system or (ii) dissipation endpoints for the individual water and sediment compartments. For simplicity, both degradation and dissipation half lives are abbreviated in the report as DT_{50}.

Fluopicolide was degraded in the test systems to the metabolites M-01 (AE C653711) and M-02 (AE C657188). These metabolites reached their maximum level at the final sampling interval. It was not possible to obtain robust fits which included these metabolites and thus the kinetic analysis is presented for fluopicolide only (i.e. analysis at level P-I).

The degradation and dissipation half-lives for fluopicolide are given in the tables below.

Degradation and dissipation in water/sediment systems: Modelling endpoints for fluopicolide, Level P-I

Fluopicolide	Distribution: Max. in sediment 76.2% after 85 d (Mill Stream)									
Water / sediment system	pH water phase	pH sed ($CaCl_2$)	t. (°C)	DT_{50} whole sys. (d)	St. (χ^2err) (%)	DT_{50} water (d)	St. (χ^2err) (%)	DT_{50} sed (d)	St. (χ^2err) (%)	Method of calculation whole sys. / water / sed
Iron Hatch, sand, Dorset, UK	8.2	6.5	20	856.3	1.56	594.5	1.21	n.e.	n.e.	SFO / DFOP slow / n.e.
Mill Stream, sand, Dorset, UK	8.2	6.6	20	1340.0	1.43	57.0	7.55	n.e.	n.e.	SFO / FOMC recalc. / n.e.
Geometric mean at 20°C				1071.2		184.1		-		

n.e. - not evaluated (insufficient data points)

Degradation and dissipation in water/sediment systems: Persistence endpoints for fluopicolide, Level P-I

Fluopicolide	Distribution: Max. in sediment 76.2% after 85 d (Mill Stream)									
Water / sediment system	pH water phase	pH sed ($CaCl_2$)	t. (°C)	DT_{50} / DT_{90} whole sys. (d)	St. (χ^2err) (%)	DT_{50} / DT_{90} water (d)	St. (χ^2err) (%)	DT_{50} / DT_{90} sed (d)	St. (χ^2err) (%)	Method of calculation whole sys. / water / sed
Iron Hatch, sand, Dorset, UK	8.2	6.5	20	856.3 / 2844.0	1.56	228.9 / 1609.0	1.21	n.e.	n.e.	SFO / DFOP / n.e.
Mill Stream, sand, Dorset, UK	8.2	6.6	20	1340.0 / 4450.0	1.43	57.6 / 189.4	7.55	n.e.	n.e.	SFO / FOMC / n.e.

n.e. - not evaluated (insufficient data points)

I. Materials and Methods

The degradation behaviour of fluopicolide has been investigated in laboratory water/sediment systems in the dark. The objective of this study was to derive dissipation or degradation half-lives for fluopicolide in the water phase, sediment phase and total water sediment system.

The evaluation was conducted to derive kinetic parameters that are suitable for use as persistence endpoints to determine whether additional studies are triggered, and modelling endpoints for use in aquatic risk assessments, according to FOCUS Kinetics guidance (FOCUS 2006, 2014). The kinetic analysis was conducted using the software tool KinGUI 2.1, implementing the IRLS (Iteratively Reweighted Least Square) error model. The identification of the appropriate kinetic model was performed following the recommendations given by the FOCUS Kinetics Workgroup (FOCUS 2006, 2014), based on a detailed analysis including visual assessment, χ2err statistics and a t-test to assess parameter significance.

Analysis was conducted at level I of the FOCUS assessment scheme, using a single-compartment approach to derive (i) degradation endpoints for the total system or (ii) dissipation endpoints for the individual water and sediment compartments. For simplicity, both degradation and dissipation half lives are abbreviated in the report as DT_{50}.

Fluopicolide was degraded in the test systems to metabolites M-01 and M-02. The residues of these metabolites increased steadily throughout the study period, with their maximum levels recorded at the final sampling interval (KCA 7.2.2.3/01). It was not possible to obtain robust fits including these metabolites, and their inclusion in optimisations resulted in worse parent fits, therefore kinetic analysis is presented in this report for fluopicolide only (i.e. analysis at level P-I).

A. Experimental data

The degradation behaviour of fluopicolide in water/sediment systems was investigated in two aerobic laboratory water/sediment test systems at 20°C in the dark, using two different radiolabelled forms of fluopicolide (KCA 7.2.2.3/01, M-241425-01-1).

Table 7.2.2.3- 14 Overview of aerobic aquatic laboratory studies with fluopicolide

Water-sediment system	Sediment texture (USDA)	Radioactive label	Duration (d)
Iron Hatch	Sand	[phenyl-U-^{14}C] [2,6-pyridyl-^{14}C]	365
Mill Stream	Sand	[phenyl-U-^{14}C] [2,6-pyridyl-^{14}C]	365

B. Data pre-processing

Residue data were checked for consistency and obvious outliers, and where outliers were removed this has been indicated clearly. Experimental data sets and data points were weighted equally in the kinetic analysis. True replicates were used individually in the optimisations, while analytical replicates were averaged prior to curve fitting. In the study degradation was investigated using two radiolabel positions, with similar behaviour observed for each, and so these radiolabels have been considered as true replicates and included together in a single optimisation.

Following the FOCUS Kinetics guidance (FOCUS 2006, 2014), all mass in the initial samples (t=0) was assumed to be the parent compound in the water phase. Initial parent residues in the water compartment were therefore set to the total recovery at t=0 multiplied by the radiochemical purity of the test solution, with residues in sediment set to 0. No samples below the Limit of Detection (LOD) or Limit of Quantification (LOQ) were reported for these test systems, therefore no further adjustments were made to the data.

The kinetic evaluation was conducted at level P-I of the FOCUS assessment scheme, using a single-compartment approach to derive (i) degradation endpoints for the total system or (ii) dissipation endpoints for the individual water and sediment compartments.

For both water/sediment systems investigated by (KCA 7.2.2.3/01), the maximum residues in sediment (averaged across both radiolabels) occurred on day 182 of the study. After removal of all data points prior to this maximum, only three data points remained for each system, which is insufficient to provide a robust, meaningful fit. Kinetic analysis was therefore conducted in this study for the total system and water compartment only, and no fits are presented for the sediment compartment.

The kinetic evaluation was performed following the recommendations of the FOCUS Kinetics workgroup (FOCUS 2006 and 2014). For each data set, the acceptability of each kinetic model to describe the experimental data was assessed using the following criteria, as described in the FOCUS guidance:

- Visual assessment of the fit up to the DT_{90}. No systematic deviations should occur before the time when measured residues reach 10% of their initial values.
- An assessment of the χ^2err. The FOCUS Kinetics guidance (2006 and 2014) recommends that for a fit to be acceptable, χ^2err should ideally be below 15%; however this should not be considered as an absolute cut-off criterion; fits with χ^2err > 15 % may be acceptable in the absence of systematic deviations, and fits with χ^2err < 15 % may be rejected due to strong systematic deviations.
- A t-test to evaluate whether rate constants are significantly different from zero.

For the derivation of persistence endpoints, FOCUS recommends using the best-fit model, while for modelling purposes the SFO model is preferred if it provides an acceptable fit.

Persistence endpoints for fluopicolide were derived following the FOCUS decision scheme. In an initial step, the residue data for the parent compound were fitted using the SFO and FOMC models, and the resulting fits were compared. If the SFO model provided a better fit overall, both visually and statistically, this fit was selected. If the FOMC model provided a better fit, however, the data were also fitted using the DFOP and HS models, and the model providing the best fit overall was selected.

Modelling endpoints for fluopicolide were derived following the FOCUS decision scheme. For modelling purposes, the preferred model is single first-order (SFO) decay. In an initial step, data for the parent compound were fitted using the SFO model, and if the resultant fit was visually and statistically acceptable, with a χ^2err that did not significantly exceed 15%, the SFO fit was accepted.

If the SFO fit was not acceptable, other models were evaluated. Where the residues remaining at the end of the study had not reached 10% of initial values, FOCUS recommends the use of the DFOP and HS models only. A pseudo-SFO half-life was then calculated from the slow phase as $\ln(2)/k_{slow}$. Where residues remaining at the end of the study did reach 10% of initial values, the FOMC model was evaluated in addition to DFOP and HS, with a pseudo-SFO half-life calculated from the best fitting of these three models as $DT_{90}/3.32$.

II. Results and Discussion

Results of the kinetic evaluation of two different water sediment studies on fluopicolide aquatic system degradation, are discussed below.

Dissipation from water (level P-I)

To derive persistence endpoints, an initial comparison was performed for each system between the SFO and FOMC fits. In both systems, the FOMC model provided a better fit to the residues, and the DFOP and HS models were therefore also fitted. For the Iron Hatch system, DFOP provided the best fit to the residues, with the lowest χ^2 err% value, while for the Mill Stream system, the best fit and lowest χ^2 err% value were provided by the FOMC model.

The SFO model provided an unacceptable fit in both systems for the derivation of modelling endpoints. For the Iron Hatch system, residues at the end of the study did not reach 10% of their initial values, therefore the DFOP and HS models were also fitted. The DFOP model provided the best overall fit to the data, with the lowest χ^2 err% value, and a pseudo-SFO DT_{50} value was derived from the DFOP fit as $\ln(2)/k_2$. For the Mill Stream system, residues at the end of the study did reach 10% of their initial values, and the FOMC, DFOP and HS models were therefore fitted. The FOMC model provided the

best fit to the data, with the lowest χ^2 err% value, and a pseudo-SFO DT_{50} value was derived from the FOMC fit as $DT_{90}/3.32$.

A summary of the fitted parameters is given in Table 7.2.2.3- 15.

Table 7.2.2.3- 15 Results for fluopicolide: summary of kinetic analysis for dissipation from water (level P-I)

Kinetic model	DT_{50}	DT_{90}	VA	χ^2err	k_1 / α	k_2 / β	t_b / g	t-test k_1 / k_2	MS
	(d)			(%)	(1/d / -)	(1/d / -)	(d / -)	(-)	
Iron Hatch									
SFO	260.8	866.3	-	10.01	0.002658	-		1.21e-07	
FOMC	200.9	>10000	+	1.824	0.18228	4.58393		-	
DFOP	228.9	1609	+	1.205	0.07309	0.001166	0.3477	2.52e-08 / 5.61e-09	M/T
HS	235.6	1434	+	1.986	0.01882	0.001343	21.86	2.63e-10 / 4.37e-10	
Mill Stream									
SFO	8.493	28.21	-	27.07	0.08162	-	-	2.72e-06	
FOMC	5.58	189.4	+	7.553	0.49505	1.82613	-	-	M/T
DFOP	5.447	219.1	-	9.349	0.2065	0.004523	0.7306	2.09e-08 / 4.34e-05	
HS	5.217	199.4	-	9.946	0.1329	0.005753	0.091	1.48e-11 / 5.25e-06	

MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

Degradation in total system (level P-I)

To derive persistence endpoints, an initial comparison was performed for each system between the SFO and FOMC fits. In both cases the FOMC model provided no significant improvement on the SFO fit, and the SFO fit was therefore accepted.

For the derivation of modelling endpoints, the SFO model provided an acceptable fit for fluopicolide in both systems.

A summary of the fitted parameters is given in Table 7.2.2.3- 16.

Table 7.2.2.3- 16 Results for fluopicolide: summary of kinetic analysis for degradation in total system (level P-I)

Kinetic model	DT_{50}	DT_{90}	VA	χ^2err	k_1 / α	k_2 / β	t_b / g	t-test k_1 / k_2	MS
	(d)			(%)	(1/d / -)	(1/d / -)	(d / -)	(-)	
Iron Hatch									
SFO	856.3	2844	-	1.563	8.10e-04	-	-	3.74e-13	M/T
FOMC	856.3	2845	+	1.641	3480	4.30e+06	-	-	
Mill Stream									
SFO	1340	4450	+	1.43	5.17e-04	-	-	1.08e-11	M/T
FOMC	>10000	>10000	+	1.235	0.13159	129.56171	-	-	

MS: Model selected (T: for trigger evaluation; M: for modelling evaluation)

Modelling endpoints

A summary of the modelling endpoints derived for fluopicolide is given in Table 7.2.2.3- 17.

Table 7.2.2.3- 17 Degradation and dissipation in water/sediment systems: modelling endpoints for fluopicolide, Level P-I

Fluopicolide	**Distribution: Max. in sediment 76.2% after 85 d (Mill Stream)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**t. (°C)**	**DT_{50} whole sys. (d)**	**St. (χ^2err) (%)**	**DT_{50} water (d)**	**St. (χ^2err) (%)**	**DT_{50} sed (d)**	**St. (χ^2err) (%)**	**Method of calculation whole sys. / water / sed**
Iron Hatch, sand, Dorset, UK	8.2	6.5	20	856.3	1.56	594.5	1.21	n.e.	n.e.	SFO / DFOP slow / n.e.
Mill Stream, sand, Dorset, UK	8.2	6.6	20	1340.0	1.43	57.0	7.55	n.e.	n.e.	SFO / FOMC recalc / n.e.
Geometric mean at 20°C				1071.2		184.1				

n.e. - not evaluated (insufficient data points)

Persistence endpoints

A summary of the persistence endpoints derived for fluopicolide is given in Table 7.2.2.3- 18.

Table 7.2.2.3- 18 Degradation and dissipation in water/sediment systems: persistence endpoints for fluopicolide, Level P-I

Fluopicolide	**Distribution: Max. in sediment 76.2% after 85 d (Mill Stream)**									
Water / sediment system	**pH water phase**	**pH sed ($CaCl_2$)**	**t. (°C)**	**DT_{50} / DT_{90} whole sys. (d)**	**St. (χ^2err) (%)**	**DT_{50} / DT_{90} water (d)**	**St. (χ^2err) (%)**	**DT_{50} / DT_{90} sed (d)**	**St. (χ^2err) (%)**	**Method of calculation whole sys. / water / sed**
Iron Hatch, sand, Dorset, UK	8.2	6.5	20	856.3 / 2844.0	1.56	228.9 / 1609.0	1.21	n.e.	n.e.	SFO / DFOP / n.e.
Mill Stream, sand, Dorset, UK	8.2	6.6	20	1340.0 / 4450.0	1.43	5.6 / 189.4	7.55	n.e.	n.e.	SFO / FOMC / n.e.

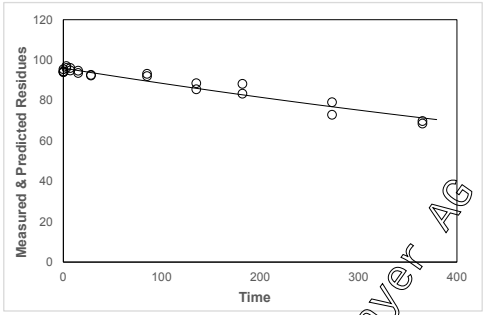
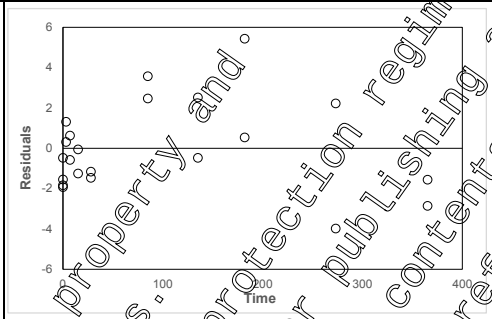
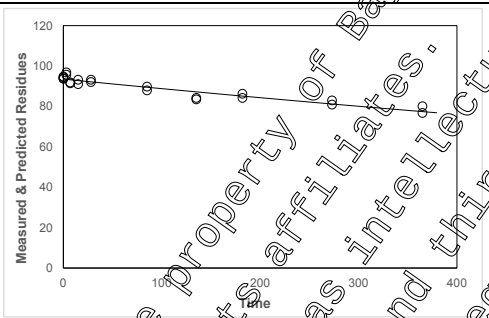
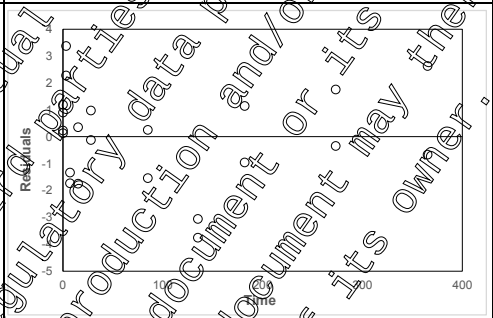
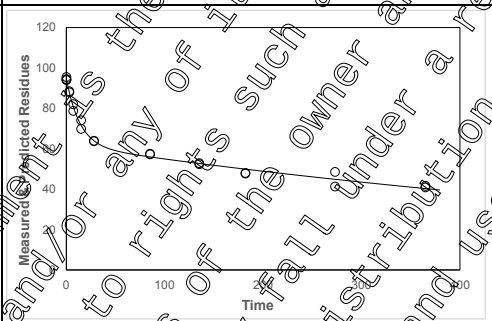
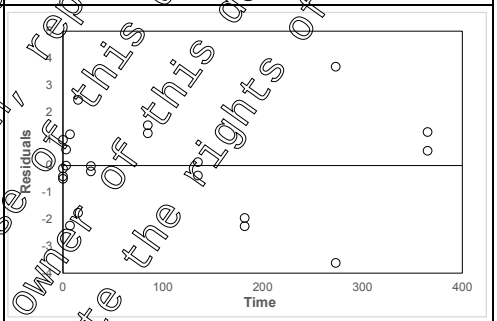
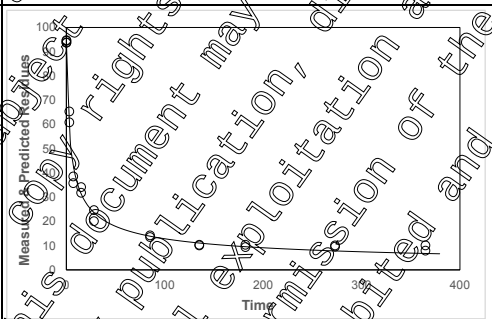
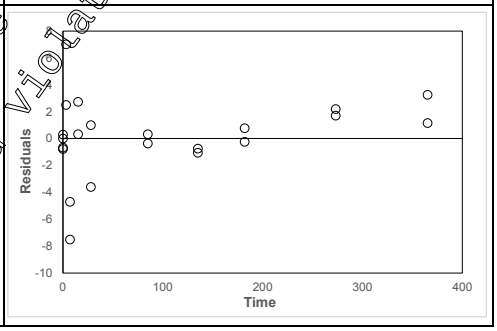
n.e. - not evaluated (insufficient data points)

The standard EFSA template can be seen in Table 7.2.2.3- 19 and graphical representations in Table 7.2.2.3- 20.

Table 7.2.2.3- 19 Standard EFSA template for kinetic fitting

Soil	Kinetic model	M_0	Parameter (k, k1, k2, g, tb, a, β)	χ^2, %-error	Prob >t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Iron Hatch; Total system	**SFO**	**96.01**	**k 0.0008095**	**1.56**	**3.74E-13**	**0.0007102**	**0.001**	**856.3**	**2844**
	FOMC	96.01	α 3480 β 4.30E+06	1.64	n.r. n.r.	3042 4.30E+06	3918 4.30E+06	856.3	2845
Mill Stream; Total system	**SFO**	**93.57**	**k 0.0005174**	**1.43**	**1.08E-11**	**0.0004412**	**0.001**	**1340**	**4450**
	FOMC	94.3	α 0.1316 β 129.6	1.24	n.r. n.r.	0.02152 -52.53	0.242 311.7	>10000	>10000
Iron Hatch; Water	SFO	83.19	k 0.002658	10	1.21E-07	0.001975	0.003	260.8	866.3
	FOMC	94.9	α 0.1823 β 4.584	1.82	n.r. n.r.	0.1621 2.772	0.202 6.396	2009	>10000
	DFOP	**94.58**	**k1 0.07309 k2 0.001166 g 0.347**	**1.21**	**2.52E-08 5.61E-09 n.r.**	**0.05703 0.0009342 0.3153**	**0.089 0.001 0.379**	**228.9**	**1609**
	HS	93.91	k1 0.01882 k2 0.001343 tb 21.56	1.99	2.63E-10 4.37E-10 n.r.	0.01373 0.001116 17.7	0.025 0.002 25.42	235.6	1434
Mill Stream; Water	SFO	89.42	k 0.08162	2.11	2.72E-06	0.05552	0.108	8.5	28.2
	FOMC	**94.47**	**α 0.4951 β 1.826**	**7.55**	**n.r. n.r.**	**0.4228 1.126**	**0.567 2.526**	**5.6**	**189.4**
	DFOP	94.19	k1 0.2065 k2 0.004523 g 0.7306	9.35	2.09E-08 4.34E-05 n.r.	0.1617 0.002761 0.6776	0.251 0.006 0.784	5.4	219.1
	HS	94.17	k1 0.1329 k2 0.005753 tb 9.091	9.95	1.48E-11 5.25E-06 n.r.	0.1146 0.003884 7.425	0.151 0.008 10.76	5.2	199.4

Table 7.2.2.3- 20 Graphical representations of the best fit model in aquatic systems

Soil Model	Modelled vs observed	Residuals
Iron Hatch; Total system SFO		
Mill Stream; Total system SFO		
Iron Hatch; Water DFOP		
Mill Stream; Water FOMC		

III. Conclusion

Data from two European water sediment systems has been used in a kinetic evaluation to derive parameters suitable for use as modelling and persistence endpoints for fluopicolide.

SFO DT$_{50}$ values for fluopicolide in the total system, suitable as both modelling and persistence endpoints, ranged from 856.3 days to 1340.0 days with a geometric mean of 1071.2 days.

The dissipation of fluopicolide from the water phase was best described by the DFOP model in Iron Hatch and by the FOMC model in Mill Stream. Persistence endpoint DT$_{50}$ values for the water phase were 228.9 and 5.6 days respectively. Pseudo-SFO DT$_{50}$ values of 5942.5 days (from the slow phase of the DFOP fit) in Iron Hatch and 57.0 days (from the FOMC DT$_{90}$/3.32) in Mill Stream were derived from the biphasic fits for modelling endpoints according to FOCUS recommendations.

<u>Assessment and conclusion by applicant:</u>
The modelling report was conducted according to FOCUS Degradation Kinetics (2006, 2014) and is considered valid to assess trigger and modelling DT$_{50}$ values for fluopicolide in water sediment systems.

CA 7.2.2.4 Irradiated water/sediment study

An irradiated water sediment study is an optional higher tier study which is not required for fluopicolide.

CA 7.2.3 Degradation in the saturated zone

Lysimeter and field leaching studies have demonstrated (Points CA 7.1.4.2 and CA 7.1.4.3) that the risk of groundwater contamination by fluopicolide is negligible. The studies have also shown that there is negligible potential for the metabolites M-03 (AE 0608000) and M-02 (AE C657188) to reach groundwater. The metabolite M-01 (AE C653711) and the pyridyl ring metabolites M-05 (AE 1344122), M-10 (AE 1344123), M-14 (AE 1388273), M-11, M-12 and M-13 have been proven to be non-relevant metabolites according to the criteria of the 'Guidance Document On The Assessment Of The Relevance Of Metabolites In Groundwater Of Substances Regulated Under Council Directive 91/414/EEC (Sanco/221/2000 – Revision 10 – Final; 25 February 2003)'.

Additional information is available from a soil study summarised under Point CA 7.1.1.2 (Anaerobic degradation). Treated soil samples were incubated under anaerobic conditions. Fluopicolide was slowly degraded under anaerobic conditions. Anaerobic metabolism of fluopicolide was the same as that observed under aerobic conditions, with cleavage of fluopicolide to form M-01 (AE C653711) and M-02 (AE C657188). No unique metabolites were formed under anaerobic conditions.

It is therefore concluded that no investigation into the fate of fluopicolide or its metabolites in the saturated zone is required.

CA 7.3 Fate and behaviour in air

Fluopicolide has a very low vapour pressure [3.03 x 10^{-7} Pa at 20°C] and Henry's Law constant [4.15 x 10^{-5} Pa m^3 mol^{-1} at 20°C] and thus would not be expected to be found in any significant concentration in the air. The theoretical rate of degradation of fluopicolide in air has been calculated according to the methods developed by Atkinson. These indicate that any material that might reach the atmosphere would be steadily degraded with the calculation indicating a half-life of 2.2 to 3.4 days.

CA 7.3.1 Route and rate of degradation in air

Data Point:	KCA 7.3.1/01
Report Author:	[redacted]
Report Year:	2004
Report Title:	Estimation of the Reaction of AE C638206 with Photochemically Produced Hydroxyl Radicals in the Atmosphere
Report No:	B004573
Document No:	M-241479-01-2
Guideline(s) followed in study:	EU (=EEC) 94/37, Annex I, Section 2.10
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The rate constant for the atmospheric reaction between photochemically produced hydroxyl radicals and fluopicolide was estimated using the computer programme AOPWIN. The estimated rate constants enabled the calculation of the atmospheric half-life of fluopicolide based upon average atmospheric concentration of hydroxyl radicals. As fluopicolide contains neither olefin nor acetylene groups, which are assumed to be necessary for reaction with ozone, no ozone reaction was estimated.

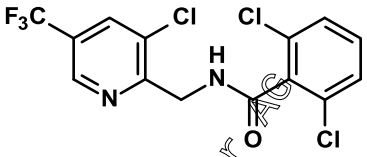
The half-life of fluopicolide in the atmosphere was calculated as being in the range of 2.2 to 3.4 days dependent upon the mean aerial OH concentration chosen for the calculation.

I. Materials and Methods

A. Materials

1. Test Item

Fluopicolide



CAS No.	239110-15-7
Molecular Formula:	$C_{14}H_8Cl_3F_3N_2O$
Molecular Weight:	383.59 g/mol
Smiles Code:	FC(F)(F)c1cnc(CNC(=O)c2c(Cl)cccc2Cl)c(Cl)c1 [as stated in Document N3] or CLc1cc(C(F)(F)F)cnc1CNC(=O)c2c(CL)cccc2CL) [as stated in the report] The codes are equivalent.

In the troposphere, there are three important photochemical transformation processes that may contribute to the degradation of a chemical. These are direct phototransformation (see Section 7.2.1.2), indirect photoreaction with hydroxyl radicals and oxidation with ozone. The rate constant for the atmospheric reaction between photochemically produced hydroxyl radicals and fluopicolide was estimated using the computer programme AOPWIN (v1.90, 200). The estimated rate constants enabled the calculation of the atmospheric half-life of fluopicolide based upon average atmospheric concentration of hydroxyl radicals. As fluopicolide contains neither olefin nor acetylene groups, which are assumed to be necessary for reaction with ozone, no ozone reaction was estimated.

The programme estimated an overall OH-radical reaction rate constant by summing the individual OH-radical reaction pathways, which could be regarded as operating independently.

II. Results and Discussion

The overall OH-radical reaction rate constant resulting from addition of OH radicals to the aromatic rings in fluopicolide was calculated as:

$$k_{OH} = 4.7570 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ sec}^{-1}$$

The resultant half life in the atmosphere was then calculated based upon two separate scenarios with differing assumptions of mean hydroxyl radical concentrations.

Scenario	Long term	Short term
OH concentration [10^6 radicals cm^{-3}]	0.5	1.5
Time frame [hours day^{-1}]	24	12
OH rate constant [cm^3 $molecule^{-1}$ sec^{-1}]	4.7570×10^{-12}	
Half life [days]	3.373	2.248

III. Conclusion

The half-life of fluopicolide in the atmosphere was calculated as being in the range of 2.2 to 3.4 days dependent upon the mean aerial OH concentration chosen for the calculation.

Assessment and conclusion by applicant:

The estimate of the half-life of fluopicolide in the upper atmosphere complies with current guidance.

CA 7.3.2 Transport via air

Being a new potential requirement, this has not previously been evaluated within the process for EU renewal.

The vapour pressure of fluopicolide is below the triggers of 10^{-4} Pa for soil and 10^{-5} Pa for plant and consequently no study on transport of the active substance has been conducted.

Fluopicolide has a very low vapour pressure [3.03 x 10^{-7} Pa at 20°C] and Henry's Law constant [4.15 x 10^{-5} Pa m^3 mol^{-1} at 20°C]. Therefore, it is unlikely that the fluopicolide enters the atmosphere through volatilization.

In the FOCUS Air Group report (FOCUS, 2008) a DT_{50} in air of 2 days has been selected as the initial screen to determine whether a pesticide has a potential for long range transport and the Atkinson calculation is considered an acceptably accurate basis for a first-tier assessment. The trigger value of 2 days is based on calculations with a 12-hour day with a hydroxyl concentration of 1.5 x 10^6 OH radicals cm^{-3}. The half-life of fluopicolide in the troposphere based on an average hydroxyl radical concentration of 1.5 x 10^6 molecule cm^{-3} during a 12-hour daylight period was calculated as 2.2 days. Thus the photochemical oxidative DT_{50} of fluopicolide just exceeds the FOCUS AIR trigger of 2 days. However, the compound does not meet the criteria for classification as a persistent organic pollutant as the compound does not bioaccumulate (fluopicolide BCF = 653 L/kg versus trigger of >5000).

CA 7.3.3 Local and global effects

Being a new potential requirement, this has not previously been evaluated within the process for EU renewal.

The potential for local effects from use of fluopicolide is considered in risk assessments performed following its use under field conditions in particular by considering factors like spray drift. The combination of exposure assessments with potential effects measured in soil and surface water do thus cover the environmental compartments of interest. In contrast and since there is no aerial application envisaged, air is not a compartment regarded to be major compartment of potential for fluopicolide occurrence following its intended use in the field.

The setting of global effects like contributions to global warming potential (GWP), ozone depleting potential (ODP), photochemical ozone creation potential (POCP) would require a high probability for the molecule assessed to evaporate and thus occur in the gas phase. This probability can be expressed by the volatility in terms of the vapour pressure (and the Henry constant). The very low potential of fluopicolide residues to occur in the atmosphere has been addressed before under CA 7.3.1 and 7.3.2.

Any accumulation in the troposphere would require high volumes of active substance applied and a significant volatility combined with persistence in the gas phase. An acidification potential (AP) would require the generation of acidifying gases like sulfur dioxide or nitrogen oxides in a free form. An eutrophication potential (EP) would require the generation of ammonia or phosphorous compounds acting as nutrients.

Assessment of criteria to be considered a POP (Persistent Organic Pollutant), PBT (Persistent, Bio-accumulative and Toxic) or vPvB (very persistent and very bio-accumulative) substance: Summary of POP, PBT and vPvB Evaluation for Fluopicolide

It is considered that fluopicolide does not meet all of the necessary screening criteria to be considered a POP (Persistent Organic Pollutant) or to be classed as a PBT (Persistent, Bio-accumulative and Toxic) compound or as a vPvB (very Persistent, very Bio-accumulative) compound.

Detailed Assessment of POP, PBT and vPvB classification

Annex II, section 7 of Regulation (EC) No 1107/2009 states that substances deemed to meet the POP, PBT or vPvB criteria cannot be approved. Also, if an active substance meets two out of three PBT criteria, it will be a 'Candidate for Substitution'. The potential for fluopicolide to meet the criteria to be considered as a POP, PBT or vPvB substance is shown below.

POP (Persistent Organic Pollutant)

A POP is defined as a chemical which is extremely stable or persistent in the environment; will bioaccumulate in organisms or the food chain; is toxic to humans or animals and has potential to be transported in the environment over long distances far from the place of release. A substance that fulfils all three of the criteria below is a POP.

Criteria for Classification of a Compound as a POP

Criterion	Definition	Fluopicolide Data	Criteria Met?
Persistence	$DT_{50\ water} \geq 2$ months	$DegT_{50}$ (water sediment, water phase) = 1841 days, n=2 (Geometric mean of pseudo SFO DT_{50} values from water sediment studies at 20 °C). $DegT_{50}$ (water sediment, water phase) = 391.1 days, n=2 (Geometric mean of pseudo SFO DT_{50} values from water sediment studies normalized to 12 °C).	Yes

Criterion	Definition	Fluopicolide Data	Criteria Met?
	$DT_{50\ soil}$ > 6 months	Laboratory studies DT_{50} (soil) = 181.6 days, n=22 (Geometric mean of SFO DT_{50} or pseudo SFO DT_{50} values from laboratory studies normalized to 20 °C). Field studies DT_{50} (soil) = 336 days, n =13 (Geometric mean of SFO DT_{50} or pseudo SFO DT_{50} values from field studies, un-normalised).	
	$DT_{50\ sediment}$ > 6 months	$DegT_{50}$ (water sediment, total system) = 1071.2 days, n=2 Geometric mean of SFO DT_{50} values from water sediment studies at 20 °C. $DegT_{50}$ (water sediment, total system) = 2276 days, n=2 Geometric mean of SFO DT_{50} values from water sediment studies normalized to 12 °C.	
Bioaccumulation	BCF or BAF > 5000 or in absence log K_{ow} > 5 or evidence that the substance presents other reasons for concern, such as high bioaccumulation in other non-target species, high toxicity or ecotoxicity.	The BCF of fluopicolide is 65 L/kg (lipid normalized bioconcentration factor).	No
Potential for Long-Range Transport (LRT)	Monitoring data showing that long range transport (LRT) may have occurred via air, water or migrating species or fate properties or modelling demonstration LRT or DT_{50} (air) > 2 days for a chemical migrating through the air.	DT_{50} (air) = 2.2 days	Yes

Fluopicolide does not fulfil all of the criteria for classification as a POP.

PBT (Persistent, Bioaccumulative, Toxic)

A PBT is defined as a chemical which is extremely stable or persistent in the environment; will bioaccumulate in organisms or the food chain and is toxic to humans or animals. A substance that fulfils all three of the criteria below is a PBT.

Criteria for Classification of a Compound as a PBT

Criterion	Definition	Fluopicolide Data	Criteria Met?
Persistence	The half-life in marine water is higher than 60 days The half-life in fresh or estuarine water is higher than 40 days	No data in marine water available. $DegT_{50}$ (water sediment, water phase) = 184.1 days, n=2 (Geometric mean of pseudo SFO DT_{50} values from water sediment studies at 20 °C). $DegT_{50}$ (water sediment, water phase) = 391.1 days, n=2 (Geometric mean of pseudo SFO DT_{50} values from water sediment studies normalized to 12 °C).	Yes
	The half-life in marine sediment is higher than 180 days The half-life in fresh or estuarine water sediment is higher than 120 days, or	No data in marine sediment or available $DegT_{50}$ (water sediment, total system) = 1071.2 days, n=2 Geometric mean of SFO DT_{50} values from water sediment studies at 20 °C. $DegT_{50}$ (water sediment, total system) = 2276 days, n=2 Geometric mean of SFO DT_{50} values from water sediment studies normalized to 12 °C.	
	The half-life in soil is higher than 120 days Assessment of persistency in the environment shall be based on available half-life data collected under appropriate conditions, which shall be described by the applicant	Laboratory studies DT_{50} (soil) = 181.6 days, n=22 (Geometric mean of SFO DT_{50} or pseudo SFO DT_{50} values from laboratory studies normalized to 20 °C). Field studies DT_{50} (soil) = 336 days, n =13 (Geometric mean of SFO DT_{50} or pseudo SFO DT_{50} values from field studies, un-normalised).	

Bioaccumulation	BCF or BAF > 5000 or in absence log K_{ow} > 5 or evidence that the substance presents other reasons for concern, such as high bioaccumulation in other non-target species, high toxicity or ecotoxicity.	The BCF of fluopicolide is 65 L/kg (lipid normalized bioconcentration factor).	No
Toxicity	The long-term NOEC for aquatic organisms is < 0.01 mg/L. The substance is classified as carcinogenic (category 1A or 1B), mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) pursuant to Regulation (EC) No 1272/2008 There is other evidence of chronic toxicity, as identified by the classifications STOT RE 1 or STOT RE 2 pursuant to Regulation (EC) No 1272/2008.	The lowest chronic aquatic toxicity relevant endpoint is 0.0160 mg/L (E_rC_{50} at 72h from the study on *Skeletonema costatum*) and therefore does not satisfy the criteria for fluopicolide being classified as T. For a full discussion on the endpoint, see KCA 8.2.6.2/07. At the 53rd meeting of the Committee for Risk Assessment (RAC-53), it was agreed that fluopicolide should be classified as a reproductive (developmental) toxicant, category 2 (H361d) in accordance with regulation (EC) No 1272/2008. However it was also agreed at RAC-53 that fluopicolide should not be classified as carcinogenic (category 1A or 1B) or mutagenic (category 1A or 1B), and that there was no evidence of chronic toxicity, which would require classifications for STOT RE 1 or STOT RE 2 pursuant to Regulation (EC) No 1272/2008.	Yes

Fluopicolide does not fulfil all of the criteria for classification as a PBT.

vPvB (very Persistent, very Bioaccumulative)

A vPvB is defined as a chemical which is extremely stable or persistent in the environment and will bioaccumulate in organisms of the food chain. A substance that fulfils both of the criteria below is a vPvB.

Criteria for Classification of a Compound as a vPvB

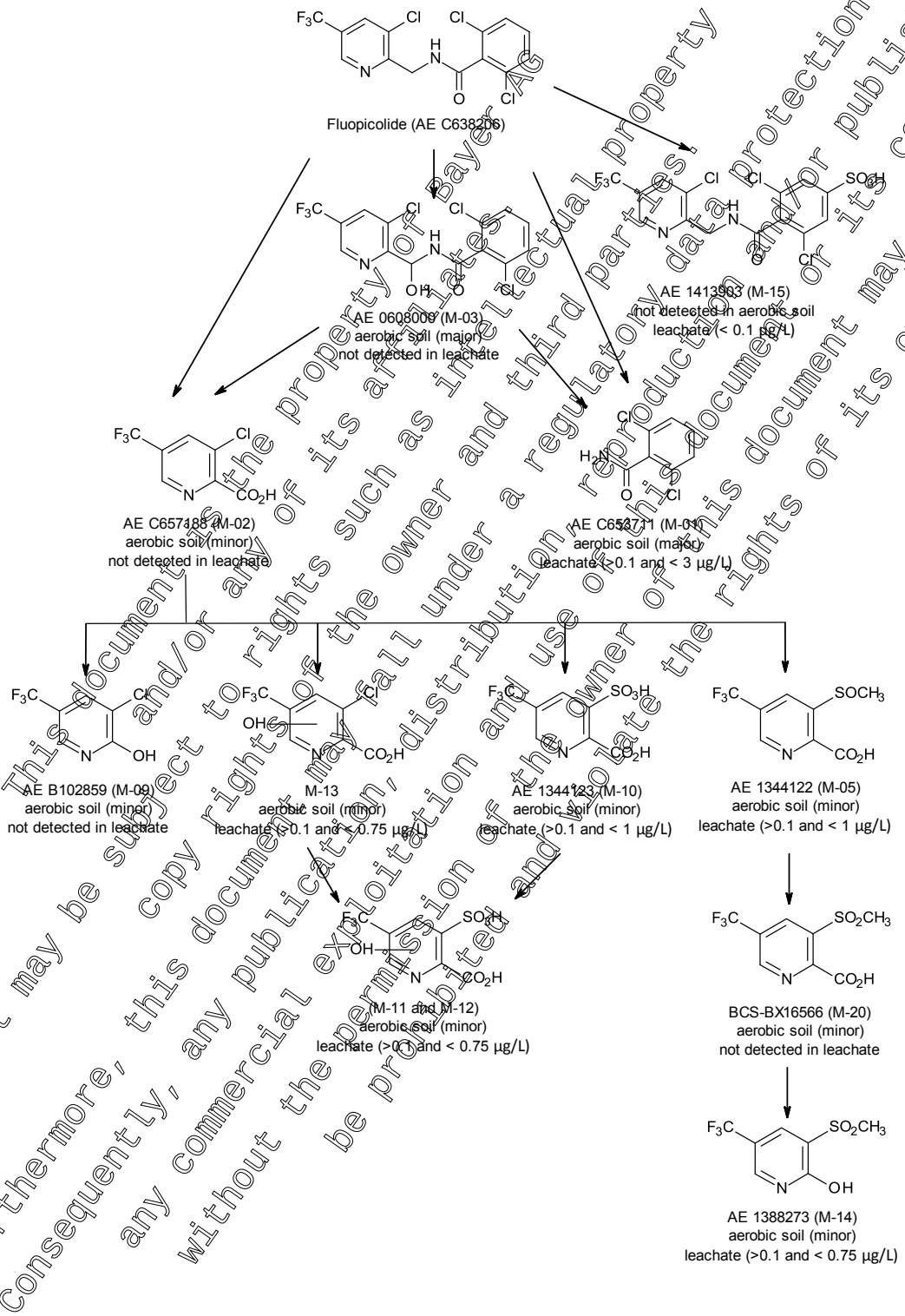
Criterion	Definition	Fluopicolide Data	Criteria Met?
Persistence	The half-life in marine, fresh or estuarine water is higher than 60 days	$DegT_{50}$ (water sediment, water phase) = 184.1 days, n=2 (Geometric mean of pseudo SFO DT_{50} values from water sediment studies at 20 °C). $DegT_{50}$ (water sediment, water phase) = 391.1 days, n=2 (Geometric mean of pseudo SFO DT_{50} values from water sediment studies normalized to 12 °C).	Yes
	The half-life in marine, fresh or estuarine sediment is higher than 180 days	$DegT_{50}$ (water sediment, total system) = 1071.2 days, n=2 Geometric mean of SFO DT_{50} values from water sediment studies at 20 °C. $DegT_{50}$ (water sediment, total system) = 2276 days, n=2 Geometric mean of SFO DT_{50} values from water sediment studies normalized to 12 °C.	
	The half-life in soil is higher than 180 days	Laboratory studies DT_{50} (soil) = 181.6 days, n=22 (Geometric mean of SFO DT_{50} or pseudo SFO DT_{50} values from laboratory studies normalized to 20 °C). Field studies DT_{50} (soil) = 336 days, n=13 (Geometric mean of SFO DT_{50} or pseudo SFO DT_{50} values from field studies, un-normalised).	
Bioaccumulation	BCF or BAF > 5000 or in absence log K_{ow} > 5 or evidence that the substance presents other reasons for concern, such as high bioaccumulation in other non-target species, high toxicity or ecotoxicity.	The BCF of fluopicolide is 65 L/kg (lipid normalized bioconcentration factor).	No

Fluopicolide does not fulfil all of the criteria for classification as a vPvB.

CA 7.4 Definition of the residue

The overall fate of fluopicolide in the environment has been extensively studied, with the metabolic pathway shown being established.

Figure 7.4- 1: Metabolic pathway for fluopicolide in the environment



Relevant Residue in Soil

The route and rate of degradation of fluopicolide has been investigated in a series of laboratory studies under both aerobic and anaerobic conditions. The potential effect of sunlight upon this degradation has also been studied. During the course of these studies, only three metabolites have been observed at amounts > 6% of applied at any time:

The maximum amount of each metabolite in soil is summarised below.

Table 7.4- 1: Soil metabolites formed from fluopicolide

Maximum observed in laboratory studies (as % of applied fluopicolide)	Metabolite	Aerobic	Anaerobic	Soil Photolysis
	M-01 (AE C653711)	58.0	2.1	8.6
	M-02 (AE C657188)	7.3	8.9	2.6
	M-03 (AE 0608000)	10.6	not detected	not detected

M-01 (AE C653711) is slowly degraded in soil, with a geometric mean field $DegT_{50}$ value of 146 days. M-02 (AE C657188) is rapidly degraded in soil, with a geometric mean laboratory $DegT_{50}$ value of 1.6 days. M-03 (AE 0608000) has been shown to have a very short half-life in most soils and was only detected at significant levels in very acidic soils (pH < 6), with a geometric mean laboratory $DegT_{50}$ value of 17.9 days for soils with a pH < 6 and 0.19 days for soils with a pH >6. The rapid chemical degradation of the molecule resulted in the formation of M-01 (AE C653711) and M-02 (AE C657188).

Relevant Residue in Water

Groundwater

Lysimeter and field leaching studies have shown that there is negligible potential for fluopicolide to appear in groundwater even under unfavourable soil and weather conditions. A number of metabolites of fluopicolide have been observed at annual average concentrations > 0.1 µg/L in groundwater at 1 m depth. The maximum annual average concentrations of each metabolite are summarised in Table 7.3-2. Full details of the FOCUS groundwater modelling are provided in Document MCP 9 FLC+PCH SC 687.5 for potato and lettuce and Document MCP 9 FLC+FXA FS 350 for winter oilseed rape.

Table 7.4- 2: Maximum annual average concentration of metabolites formed from fluopicolide observed or predicted in groundwater at 1 m depth (as µg/L)

Metabolite	Lysimeter	Field leaching	FOCUS PECgw		
			Potatoes	Lettuce	Winter OSR
M-01 (AE C653711)	NA	2.928	6.980	5.895	1.024
M-02 (AE C657188)	ND	ND	0.055	0.051	0.008
M-03 (AE 0608000)	ND	ND	0.163	0.134	0.020
M-05 (AE 1344122)	0.902	NA	0.548	0.434	0.072
M-10 (AE 1344123)	0.831	NA	1.737	1.604	0.225
M-11	0.546	NA	0.740	0.641	0.092
M-12	0.364	NA	0.493	0.427	0.061
M-13	0.137	NA	0.427	0.397	0.049
M-14 (AE 1388278)	0.194	NA	0.175	0.138	0.023
M-15 (AE 1413903)	0.095	NA	0.041	0.032	0.006

NA = Not applicable; ND = Not detected

The metabolite M-02 (AE C657188) was not detected in leachate from lysimeter and field leaching studies conducted in acidic soils nor is it predicted in FOCUS groundwater modelling calculations at concentrations greater than 0.1 µg/L. As M-02 does not exceed the regulatory end point of 0.1 µg/L it is considered to be a non-relevant metabolite in groundwater and consequently, further regulatory testing is not formally required. However, this metabolite is sufficiently structurally similar to the metabolites M-11, M-12 and M-13 to enable conclusions on their toxicological properties to be made. M-02 has been included in non-relevance testing as toxicological testing of M-11, M-12 and M-13 is not possible as they cannot be synthesized.

The metabolite M-03 (AE 0608000) was not detected in leachate from lysimeter and field leaching studies conducted in acidic soils but is predicted in FOCUS groundwater modelling at concentrations > 0.1 µg/L. It should be noted for M-03 the values are highly conservative; in soils with pH ≥ 6 no exposure of groundwater from M-03 is predicted and once refinement of PEC_{gw} calculations with aged sorption parameters for the parent are considered all PEC_{gw} values M-03 in all crops are <0.1 µg/L. Moreover, aqueous hydrolysis is the major route of degradation for the M-03 in the environment. The degradation of M-03 in water and aquatic systems at environmentally relevant pHs is extremely rapid with DT_{50} values ranging from 0.1 hour at pH 8 to 45.5 hours at pH 5. The rapid chemical degradation of the molecule resulted in the formation of M-01 (AE C653711) and M-02 (AE C657188).

The metabolite M-15 (AE 1413903) was detected in lysimeter leachate but did not exceed an annual average concentration of 0.1 µg/L. As M-15 does not exceed the regulatory end point of 0.1 µg/L it is considered to be a non-relevant metabolite in groundwater and consequently, further regulatory testing is not formally required. However, EFSA were concerned that under some circumstances M-15 may exceed this trigger value during the previous EU review. Consequently M-15 has been included in FOCUS PEC_{gw} modelling and its non-relevance established using the results of studies provided in the Confirmatory Data on Fluopicolide.

Full details of the non-relevance assessment of each metabolite listed in Table 7.4- 2 are provided in Document N4. On the basis that they do not exceed any regulatory triggers for risk assessments the metabolites M-02 (AE C657188) and M-15 (AE 1413903) are not included in the residue definition for risk assessment but full assessments are provided for both metabolites.

Surface Water and Sediment

No major metabolites have been detected in laboratory hydrolysis and aqueous photolysis studies or in an aerobic mineralisation study.

In a water sediment study, M-01 (AE C653711) was the only major metabolite detected reaching a maximum of 20.3% in the total system (sediment compartment maximum 3.9%, water compartment maximum 18.2%). M-02 (AE C657188) was also detected as a significant minor metabolite, >5% at 3 consecutive timepoints and increasing at final timepoint, reaching a maximum of 8.2% in the total system (sediment compartment maximum 0.8%, water compartment maximum 7.4%). The metabolite M-03 (AE 0608000) was not detected as an aquatic metabolite. All three metabolites have been included in FOCUS surface water and sediment modelling, as exposure from formation of a metabolite in soil, with subsequent exposure of surface water and sediment from drainage or runoff from soil, has to be considered in addition to the formation of a metabolite in aquatic systems. However, M-03 is unstable in aquatic systems making aquatic ecotoxicological testing unfeasible (see KCA 7.2.1.1/03 and KCA 8.2.6.2/11 for further details).

Table 7.4- 3: Aquatic metabolites formed from fluopicolide

Maximum observed in laboratory studies (as % of applied fluopicolide)	Metabolite	Hydrolysis	Photolysis	Water Sediment		
				Total	Water	Sediment
	M-01 (AE C653711)	4.0	4.1	20.3	18.2	3.9
	M-02 (AE C657188)	NA	not detected	8.2	7.4	0.8
	M-03 (AE 0608000)	not detected	not detected	not detected	not detected	not detected

NA = Not applicable

In summary M-01 is a major metabolite in the water phase. M-02 is as a significant minor metabolite in the water phase, >5% at 3 consecutive timepoints. No metabolites requiring risk assessment are formed in sediment (<5 %AR, maximum 3.9% AR).

Relevant Residue in Air

It is recommended that fluopicolide be defined as the relevant residue in air.

CA 7.4.1 Definition of the residue for risk assessment

Soil: Fluopicolide, M-03 (AE 0608000), M-01 (AE C653711), M-02 (AE C678188)

Ground water: Fluopicolide, M-03 (AE 0608000), M-01 (AE C653711), M-05 (AE 1344122), M-10 (AE 1344123), M-11, M-12, M-13 and M-14 (AE 1388273)

Surface water: Fluopicolide, M-03 (AE 0608000), M-01 (AE C653711), M-02 (AE C657188)

Sediment: Fluopicolide

Air: Fluopicolide

CA 7.4.2 Definition of the residue for monitoring

Soil: Fluopicolide

Ground water: Fluopicolide

Surface water: Fluopicolide

Sediment: Fluopicolide

Air: Fluopicolide