Bayer CropScience

Document Title

Summary of the fate and behaviour in the environment for

Flurtamone

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013

Document MCA

Section 7: Fate and behaviour in the environment

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

Date

14th March 2014

Author(s)

[redacted]

[redacted].

Bayer CropScience AG

M-482301-01-3

OWNERSHIP STATEMENT

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **From Bayer CropScience; or**
- **From other applicants once the period of data protection has expired.**

Version history

Date	Data points containing amendments or additions[1]	Document identifier or version number

[1] Note how the amendments or additions are represented (italics/colour etc.)

Table of Contents

Section 7 - Fate and behaviour in the environment

Introduction

Flutamone is an herbicidal active substance and was included into Annex I of Directive 91/414 in 2003 (Directive 2003/84/EC, dated 25th of September 2003, Entry into Force, 1st of January 2004.

Data on the fate and behavior of flurtamone in soil, water, sediment and air were submitted within the EU Dossier (Baseline Dossier), which resulted in the Annex I inclusion under Directive 91/414/EEC in 2003. In the Supplemental Dossier for renewal of approval of flurtamone presented here only those environmental fate studies are described in sections 7.1 to 7.5, which were not submitted within the Baseline Dossier. However, for a better understanding of the behaviour of flurtamone in soil, water and sediment, and air, short summaries including the results of all environmental fate studies are given additionally in this summary.

According to the guidance of EFSA on the "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011, 9 (2), 2092), literature for the active substance and its metabolites need to be presented, covering the last 10 years prior to the submission of this Annex I renewal dossier. In case where reliable and adequate literature is found for flurtamone and its metabolites during this literature search, summaries are integrated in the respective sections of this document.

In addition, literature older than 10 years is included for the common and ubiquitous in the environment occurring metabolite trifluoroacetic acid (TFA). However these articles were not evaluated according to the above mentioned EFSA Guidance. Summaries are presented in the respective sections in the MCA document. Ecotoxicological endpoints extracted from these articles will be used in the risk assessment for the metabolite TFA and presented in the respective sections of the MCP document.

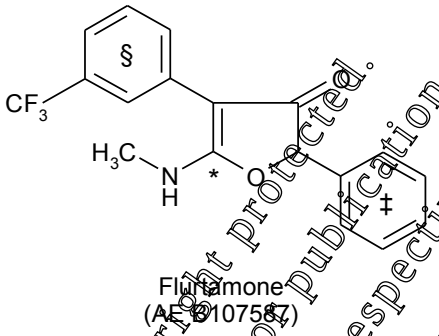
List of synonyms and codes

Flurtamone is a compound that was originally developed by the Chevron Chemical Company. It was purchased by Rhône-Poulenc Agriculture, which later merged with a division of Schering Agrochemicals to form Aventis CropScience. Aventis CropScience was purchased by Bayer to form part of Bayer CropScience, the current owner of flurtamone. As a result of this sequence, flurtamone and many of its metabolites have a number of different codes associated with them.

The original code number for flurtamone was RE 40885 and for its metabolites the codes were also RE followed by a five-digit number. In some reports there is a hyphen between the RE and the digits (eg RE-40885). When the molecule was owned by Rhône-Poulenc the compounds were given new (RPA) codes. In addition, metabolites not previously identified were detected and these were given RPA codes. When Aventis CropScience was formed the compounds were given another set (AE) of codes. The AE codes continued to be used by Bayer CropScience except where studies conducted since the molecules' acquisition have resulted in additional metabolites (for which there was no AE code existing). More recently Bayer CropScience codes (BCS) have also been used. The key codes and standardized names for flurtamone and its environmental degradates/metabolites are summarized in the table below. A full list containing structural formula, various names, short forms, codes and

occurrences of degradation products is provided in Document N3. The physical and chemical properties of the metabolites are given in section 2 of this this Supplemental Dossier.

Studies have been conducted with the radiolabelling in the three separate rings. These radiolabel positions are sufficient to define the route of degradation of flurtamone.



Flurtamone
(AE B107587)

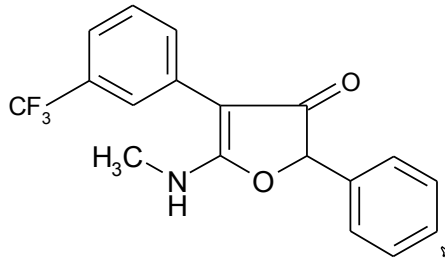
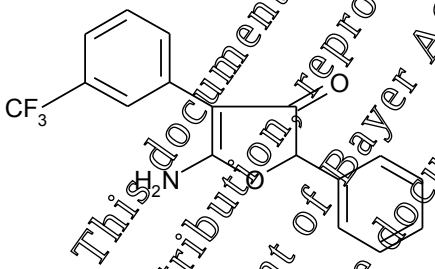
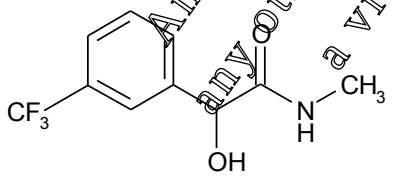
Position of radiolabel

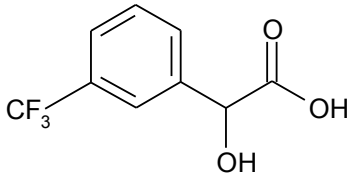
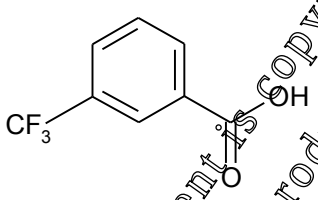
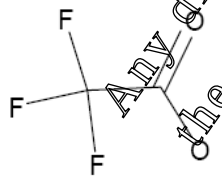
§ = uniformly in trifluoromethylphenyl ring

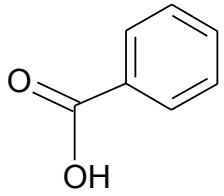
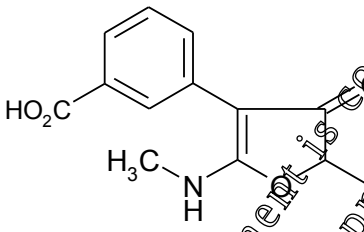
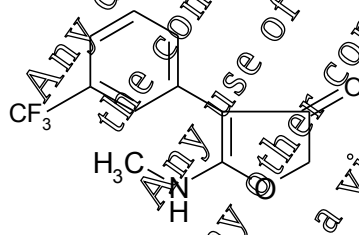
* = 5-position of the furanone ring

‡ = uniformly in phenyl ring

Table 7-1 Flurtamone and its metabolites (including Aventis and/or BCS [a], Chevron [b] and Rhone-Poulenc [c] codes)

No.	Name, Structure IUPAC name CAS name, CAS number (if known)	Molecular formula molar mass Other names / codes	Occurrence Major/Minor Compartment(s)
AS	FLURTAMONE  Name IUPAC: 5-Methylamino-2-phenyl-4-(3-trifluoromethylphenyl)-3(2H)-furanone Name CAS: 3(2H)-Furanone, 5-(methylamino)-2-phenyl-4-[3-(trifluoromethyl)phenyl]-, (±)- CAS No.: 96525-23-4	$C_{18}H_{14}F_3NO_2$ 333.3 g mol^{-1} [a] AE B107587 [a] BCS-AD26195 [b] RE 40885 [c] RPA 590515 (also 201918 and 304563) Report name: flurtamone	Active substance
M01	SM1/PM5/AM8  Name IUPAC: 5-amino-2-phenyl-4-(3-trifluoromethylphenyl)-3(2H)-furanone Name CAS: 3(2H)-Furanone, 5-amino-2-phenyl-4-[3-(trifluoromethyl)phenyl]-, (±)- CAS No.: 96525-22-3	$C_{17}H_{12}F_3NO_2$ 319.2 g mol^{-1} [a] AE B107584 [a] BCS-AC85393 [b] RE 39748 [c] RPA 202450 aka desmethyl flurtamone Report name: flurtamone-desmethyl	Minor in soil (Aerobic soil – 'trace') Cereals, Sunflower Rat, Hen
M02	SM2/PM8/AM23  Name IUPAC: 2-Hydroxy-N-methyl-2-(3-trifluoromethylphenyl)acetamide Name CAS: Benzeneacetamide, a-hydroxy-N-methyl-3-(trifluoromethyl)-CAS No.: 143236-54-8	$C_{10}H_{10}F_3NO_2$ 233.2 g mol^{-1} [a] AE 0540067 [a] BCS-AX71147 [b] RE 53285 [c] RPA 591119 aka N-methyl-3-trifluoro methyl mandelamide Report name:flurtamone-trifluoromethyl-N-methyl-mandelamide	Minor in soil/aquatic Aerobic soil – max. 4% Soil photolysis – max. 1.4% in dark controls Water / sediment total – max. 3.5% Cereals, Sunflower Rat, Goat

No.	Name, Structure IUPAC name CAS name, CAS number (if known)	Molecular formula molar mass Other names / codes	Occurrence Major/Minor Compartment(s)
M03	SM3/PM10/AM27  Name IUPAC: 2-hydroxy-2-(3-trifluoromethylphenyl)acetic acid Name CAS: Benzeneacetic acid, a-hydroxy-3-(trifluoromethyl)- CAS No.: 349-10-0	$C_9H_7F_3O_3$ 220.2 g mol^{-1} [a] AE 0592368 [a] BCS-AX85453 [b] RE 54580 [c] RPA 406510 aka 3-trifluromethyl mandelic acid Report name: flurtamone-trifluoromethyl-mandelic acid	Minor in soil : Soil photolysis – max. 0.2% irradiated, 9.3% in dark controls Cereals, Sunflower Rat, Hen, Goat
M04	SM4/PM11/AM30  Name IUPAC: 3-Trifluoromethylbenzoic acid Name CAS: Benzoic acid, 3-(trifluoromethyl)- CAS No.: 454-92-2 Sodium salt: Name IUPAC: sodium 3-(trifluoromethyl)benzoate CAS No.: 69226-41-1	$C_8H_5F_3O_2$ 190.1 g mol^{-1} [a] AE C518919 [a] BCS-AA52670 [a] BCS-CY97256 (sodium salt) [b] RE 54488 [c] RPA 025905 Common abbreviation: TFMBA Report name: TFMBA	**Major in soil** **Aerobic soil – max. 24.7%** Soil photolysis – max. 3.8% Water/sediment total – max. 4.1% Cereals, Sunflower Rat, Hen, Goat
M05	SM5/PM12/  Name IUPAC: Trifluoroacetic acid Sodium trifluoroacetate Name CAS: Trifluoroacetic acid Sodium trifluoroacetate CAS No.: 76-05-1 (acid) 2923-18-4 (sodium salt)	$C_2HF_3O_2$ 114.0 g mol^{-1} [a] AE C502988 (acid) [a] BCS-AL85845 (acid) [b] none given [c] RPA 017503 (acid) [a] AE1046319 (sodium salt) [a] BCS-AZ56567 (sodium salt) Common abbreviation: TFA (or TFAA) Report name: Trifluoroacetic acid or trifluoroacetate	**Major in soil** **Aerobic soil – max. 9.8%** **Confined rotational crops**

No.	Name, Structure IUPAC name CAS name, CAS number (if known)	Molecular formula molar mass Other names / codes	Occurrence Major/Minor Compartment(s)
M06	SM6  Name IUPAC: Benzoic acid Name CAS: Benzoic acid CAS No.: 65-85-0	$C_7H_6O_2$ 122.1 g mol^{-1} [a] BCS-AG74706 [b] none given [c] RPA435413 Report name: Benzoic acid	**Major in soil:** **Soil photolysis – max 7.2%**
M07	AQM1  Name IUPAC: 3-(2-Methylamino-4-oxo-5-phenyl-4,5-dihydrofuran-3-yl)benzoic acid Name CAS: Benzoic acid, 3-[4,5-dihydro-2-(methylamino)-4-oxo-5-phenyl-3-furanyl]- CAS No.: 148681-60-1	$C_{18}H_{15}NO_4$ 309.3 g mol^{-1} [a] AE 1083976 [a] BCS-BA29451 [b] none given [c] RPA 203597 Report name: flurtamone-carboxylic acid	**Major in Aqueous photolysis – max. 33.5%**
M08	AQM2  Name IUPAC: 5-methylamino-4-(3-trifluoromethylphenyl)-3(2H)-furanone Name CAS: 3(2H)-Furanone, 5-(methylamino)-4-[3-(trifluoromethyl)phenyl]- CAS No.: 96525-53-0	$C_{12}H_{10}F_3NO_2$ 257.2 g mol^{-1} [a] AE 2093305 [a] BCS-BT61400 [b] none given [c] RPA 591120 Report name: flurtamone-desphenyl	**Major in Aquatic** Water – max. 7.8% Sediment – max.3.6% **Total max. 10.7%**

CA 7.1 - Fate and behaviour in soil

CA 7.1.1 - Route of degradation in soil

CA 7.1.1.1 - Aerobic degradation

The original aerobic degradation study in soil ([redacted], 1993, M-158234-01-1 and [redacted] 1994, M-158348-01-1) demonstrated that flurtamone is degraded via biological processes, with 24 to 40 % mineralization after 100 days and 32 % non-extractable residues. Two metabolites were observed: M04 TFMBA (3-trifluoromethylbenzoic acid, AE C518019) which was detected as a major metabolite at 8.3 to 10.8 % and M05 TFA (trifluoracetate, BCS-AZ56567, AE C502988 in the acid form) which was observed at a maximum of 9.8%. This study used only two agricultural soils, the compound labelled in only one ring and a flawed experimental study design resulting in the possibility of significant errors and therefore was not used again. This study is discussed in a position paper Lowden, 2013 below. New studies to remedy these deficiencies are presented below (along with a pilot study not previously available).

Report:	**KCA-7.1.1.1.01; [redacted] P, 2013a**
Title:	An Assessment of the 1991 Study on the Aerobic Soil Degradation of Flurtamone
Organisation:	[redacted]
Report No.:	VC/12/006B Bayer CropScience Document **M-460121-01-1**
Publication:	unpublished
Dates of experimental work:	Not relevant
Guidelines:	**Not applicable**
Deviations:	Not relevant
GLP/GEP	**Not applicable.**

Executive Summary

This position paper reviews the old flurtamone aerobic soil degradation study of [redacted], 1993, M-158234-01-1 and Burr, [redacted], 1994, M-158348-01-1. There is also an interim report that was not audited that gave further details of the study. The soil samples were treated at a rate equivalent to 325 g ha^{-1} using flurtamone labelled in the trifluromethylphenyl ring. The incubations conditions were those required by US EPA, namely a moisture content of 1/3 bar moisture holding capacity and a temperature of 22°C. The duration of the study also conformed to US EPA recommendations with samples being taken for analysis at intervals up to a year (366 days).

The study used only two agricultural soils, the third soil being an artificial soil (later discontinued). The study design was experimental. Instead of individual flasks, each with its own set of traps, the soil samples were placed on petri dishes and these were stacked on aluminium stands. The stands were housed in vertical glass towers which were placed in a temperature-controlled room. Moistened air was passed into the towers and after passage through the tower it was drawn through ethylene glycol and potassium hydroxide traps. This design was quickly abandoned (never used again).

The unusual study set-up may have caused, or at least contributed to, a number of other defects in the study. There was significant variability between replicate samples and a loss of mass balance at later time-points. Some soil samples were frozen prior to analysis.

The behaviour of the artificial soil was very different from that of the agricultural soils and it was agreed by the EU peer review that the rate of degradation was excluded from modelling. Given the major differences in the conduct of the study from a standard study (and current guidelines) and the very different results obtained in comparison to those obtained from modern, guideline studies it was concluded that, in addition to the artificial soil already excluded from risk assessment, the clay loam and sandy loam soil should be also excluded.

Material and Methods

The original report plus addendum and the interim report were comprehensively reviewed.

Findings

The reports showed that the study, which ran for a year, was conducted under EPA conditions with two agricultural soils plus one artificial soil. The study set-up was experimental and some aspects gave the possibility of significant errors. The set-up was not used again. Some soil samples were frozen and stored for a significant length of time prior to analysis.

The results showed considerable variation between replicates and loss of material balance. The mass balances (means of replicates) were > 90% up to 42 days after treatment (with a few exceptions) but then fell below that level particularly in the agricultural soils, for which the values were 77% and 78% at study termination. A number of individual samples had recoveries that fell below 90% prior to 42 days, even as early as one day and seven days and some of these recoveries were very low (75%).

Conclusions

This study is now over 20 years old and has a number of deficiencies and some of the results are questionable. Since first review in the period 1995 – 1998, standards for conducting soil metabolism studies have been raised significantly in particular due to the new guidelines published by the EU (directive 95/36/EC) in 1995 and by OECD (OECD Test Guideline 307) published in 2002. Therefore given the new studies which have recently been completed to the new standards combined with the deficiencies of the previous study, when compared to the new standards as well as considering that the data from this study fall well outside the DT$_{90}$ range from the new studies that (conducted with both ^{14}C-phenyl and ^{14}C-TFMP-labelled flurtamone, see table below) it can be concluded that this study is not fully reliable. Thus in addition to the Speyer 2.2 soil already excluded from risk assessment, the clay loam and sandy loam soil should be also excluded.

Report:	**KCA-7.1.1.1 /02; [redacted] B.V. 1991a**
Title:	Flurtamone aerobic and anaerobic soil metabolism – Pilot Study
Organisation:	[redacted];
Report No.:	Chevron report number – None given Bayer CropScience Document M-249325-02-1
Publication:	unpublished
Dates of experimental work:	1989-1991 (not stated in report)
Guidelines:	**The study was conducted as a pilot study prior to conducting a full EPA study.**
Deviations:	Not relevant
GLP/GEP	**No**

Executive Summary

The route and rate of degradation of the herbicide, flurtamone was investigated in a US sandy loam soil. The soil was incubated in the dark, at a moisture content equivalent to 75% of field capacity (⅓ bar) under aerobic conditions at 25 ºC, after treatment with radiolabelled flurtamone. Experiments were separately performed with compound uniformly labelled in either the phenyl or trifluoromethyl phenyl rings or labelled at the 5-position of the furanone ring. Treatment rates were equivalent to very high field rates of between 6 and 7.5 kg/ha. Single samples were taken after 0, 15, 28 and 42 days of incubation. The samples were extracted and the extracts were examined by HPLC and TLC.

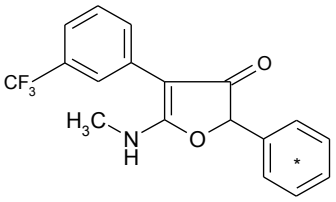
Flurtamone degraded at a rapid rate. Up to 22% of the radioactivity was detected as $^{14}CO_2$, indicating the potential for rapid mineralization of the phenyl and furanone rings of flurtamone. Mineralization of the trifluoromethylphenyl ring was somewhat slower with 6% detected as $^{14}CO_2$ after 42 days.

Two metabolites were observed: M04 TFMBA, (3-trifluoromethylbenzoictrifluoromethylbenzoic acid, AE C518919, RE 54488 in the report) which was detected as a major metabolite in excess of 20% and M02 RE 53285 (3-trifluoromethyl-N-methyl-mandelamide, AE 0540067, RE 53285 in the report) which was observed at a maximum of 4%.

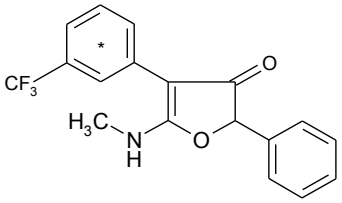
Material and Methods

The route and rate of degradation of the herbicide, flurtamone (5-methylamino-2-phenyl-4-(3-trifluoromethylphenyl)-3(2H)-furanone) was investigated in a US sandy loam soil (USDA classification) under laboratory conditions. The soil was incubated in the dark, at a moisture content equivalent to 75% of field capacity (⅓ bar) under aerobic conditions at 25 ºC. In addition, the route and rate of degradation of flurtamone incubated under anaerobic conditions was also studied. The details of the aerobic metabolism of flurtamone are given here and those pertinent to the anaerobic metabolism are summarized under point 7.1.1.2.

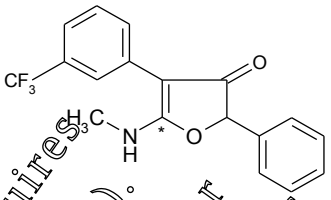
Aerobic soil experiments have been separately performed with radiolabelling in each of the three rings; uniformly labelled in either the phenyl or trifluoromethylphenyl rings or labelled at the 5-position of the furanone ring. The radiochemical purity and specific activity of each radiolabelled test item were 98.4% and 6.22 MBq/mg for [phenyl-UL-^{14}C]-flurtamone, 99.0% and 5.67 MBq/mg for [trifluoromethylphenyl-UL-^{14}C]-flurtamone and 98.8% and 5.88 MBq/mg for [furanone-5-^{14}C]-flurtamone.



[Phenyl-UL-^{14}C]-flurtamone



[Trifluoromethylphenyl-UL-^{14}C]-flurtamone



[Furanone-5-^{14}C]-flurtamone

* = position of radiolabel

The soil was collected from an agricultural field in Greenville, Mississippi and its properties are summarized in Table 7.1.1.1-1.

Table 7.1.1.1-1 Properties of the soil used in a flurtamone pilot aerobic soil study

Characteristic	Value
Origin (country)	USA
Location	Greenville, Mississippi
Particle Size Analysis: Total Sand (%) Silt (%) Clay (%)	 56 32 12
Textural Class (USDA)	Sandy Loam
pH	5
Organic* (%)	0.9
Cation Exchange Capacity Meq/100g	8.1
Moisture Content at ⅓ bar (%)	10.2
Bulk Density (g/mL)	1.43

*matter or carbon not defined.

Soil samples were incubated under static conditions in biometer flasks equipped with traps for the collection of volatile organic compounds (polyurethane bung) and CO_2 (sodium hydroxide). Aerobic conditions were maintained by connection of each flask to a low pressure oxygen supply.

Treatment. Soil samples (50 g) were weighed into biometer flasks. Three sets of 16 were prepared. Aliquots (87, 89 or 90 µL) of ethanolic solutions of the three radiolabelled forms of flurtamone were applied to soil samples. This procedure, during which treatment checks were made, gave application rates of 8 to 10 ppm, equivalent to 6 to 7.5 kg/ha.

Sampling. Single soil samples of each radiolabelled test item were taken for analysis after 0, 15, 28 and 42 days of incubation.

Sample processing. The soil samples were extracted at ambient temperature three times with methanol followed by three further extractions with 10 mM calcium sulphate solution. The volatile organics were extracted from the polyurethane bung using methanol. The extracts and post-extract soils and traps were radioassayed. Soil bound radioactivity remaining in the soil was greatest in the soil treated with [trifluoromethyl phenyl-UL-^{14}C]-flurtamone. Extracted soil residues of the final time-point from this radiolabelled experiment were further extracted with acidic or alkali solvents. The soil residue was further extracted with either acidic methanol followed by 6N hydrochloric acid, or with 0.5N sodium hydroxide followed by 20% sodium hydroxide.

Quantitative analysis. Radioactivity extracted from soil and in the volatile traps was quantified by liquid scintillation counting (LSC). Following extraction soil residues were air dried, ground to a fine powder and the radioactivity remaining unextracted quantified by combustion and LSC.

Qualitative analysis. Methanol extracts were pooled and concentrated by rotary evaporation prior to analysis with authentic reference standards by reverse phase high performance liquid chromatography (HPLC). Selected methanol extracts were analysed by normal phase thin layer chromatography (TLC) and liquid chromatography mass spectrometry (LC/MS). Selected calcium sulphate samples were analysed directly, without concentration, by reverse phase HPLC.

The HPLC system used comprised a Beckman Ultrasphere ODS-IP column connected to a UV Diode Array detector (set at 276 nm) and a Radiomatic Flo-One radiodetector (Beta model A-250) with a solid cell. The solvent system was a gradient of 1% glacial acetic acid in water and 20% acetonitrile in water. Retention times of standards were determined by UV detection.

TLC was carried out on silica gel 60 F254 plates. Extracts of selected samples were spotted on to these plates which were then developed in chloroform/acetic acid (9:1, v/v). After development and drying the plates were exposed to X-ray films, so that the radioactive areas could be located. Sample spots were co-chromatographed with unlabelled standards. The standards were visualized under UV light and their locations compared with those of the radioactive areas.

Mass spectral data were obtained on a Finnigan quadrupole mass spectrometer equipped with a Vestec 701A Thermospray LC-MS interface. The liquid chromatography conditions were as described above. The mass range covered m/z 200-650 amu. Ionization was achieved by the 'filament on' with the source block at 260°C. Under these conditions all the compounds of interest exhibited protonated molecular ions (M+1). In addition many of the species showed significant daughter ions, which served to confirm the identifications.

Findings

The day zero mass balances in the aerobic experiment ranged from 80.7 to 98.3% for the three radiolabelled studies. The distribution of radioactivity as percentage of recovered radioactivity in day zero samples are summarized in Tables 7.1.1.1-2 to 7.1.1.1-4. The mean recoveries were 88.7% for soil treated with furanone ring labelled flurtamone, 92.9% for phenyl ring labelled and 110.1% trifluoromethylphenyl ring labelled. The distribution of radioactivity was similar in the phenyl and furanone ring labelled experiments, with increasing amounts of carbon dioxide and lower amounts of extractable and unextractable soil residues with time in comparison to the trifluoromethylphenyl ring labelled experiment.

Table 7.1.1.1-2 Distribution of radioactivity following treatment of aerobic soil with [furanone-5-^{14}C]-flurtamone (as % of radioactivity)

Time (days)	% of radioactivity					
	Methanol Extract	$CaSO_4$ Extract	Total Extracted	Unextracted Soil Residues	Carbon Dioxide	Total
0	95.6	2.6	98.2	1.7	0.0	100.0
15	75.8	3.0	78.8	9.2	4.3	92.3
28	45.5	3.9	49.4	11.9	17.0	78.3
42	44.8	3.4	48.2	13.6	22.3	84.1
Overall Mean						88.7

Table 7.1.1.1-3 Distribution of radioactivity following treatment of aerobic soil with [phenyl-UL-^{14}C]-flurtamone (as % of radioactivity)

Time (days)	% of radioactivity					
	Methanol Extract	$CaSO_4$ Extract	Total Extracted	Unextracted Soil Residues	Carbon Dioxide	Total
0	96.1	1.8	97.9	2.1	0.0	100.0
15	73.8	3.2	77.0	9.9	9.0	95.9
28	62.3	1.6	63.9	5.3	13.2	82.4
42	58.8	2.6	61.4	11.6	20.2	93.2
Overall Mean						92.9

Table 7.1.1.1-4 Distribution of radioactivity following treatment of aerobic soil with [trifluoromethylphenyl-UL-^{14}C]-flurtamone (as % of radioactivity)

Time (days)	% of radioactivity					
	Methanol Extract	$CaSO_4$ Extract	Total Extracted	Unextracted Soil Residues	Carbon Dioxide	Total
0	92.4	4.2	96.6	3.3	0.0	100.0
15	89.2	10.9	100.1	9.2	1.9	111.2
28	83.3	11.3	94.6	14.1	3.6	112.3
42	69.1	13.3	82.4	28.1	6.2	116.7
Overall Mean						110.1

Extractable radioactivity was quantitative in the time zero soil extracts and decreased with time, with a corresponding increase in the levels of unextractable soil residues and carbon dioxide detected. In the phenyl and furanone ring labelled experiments the amount of carbon dioxide detected reached 20 and 22% of applied radioactivity by the end of the incubation period, while in the trifluoromethylphenyl ring labelled experiment only 6% of the initial radioactivity was mineralised to carbon dioxide. Virtually no volatile organic products were detected with any of the radiolabelled material throughout the study.

In the phenyl and furanone ring labelled experiments with 50 to 60% of applied radioactivity remained extractable after 42 days, compared with 80% in the trifluoromethyl phenyl ring. Methanol extracts removed the majority of the radioactivity in soil in all three experiments. Calcium chloride extraction removed a maximum of 3 to 4% in the phenyl and furanone ring labelled experiments and up to 13% in the trifluoromethylphenyl ring labelled experiment. The levels of unextractable radioactivity were also similar in the phenyl and furanone labelled experiments (11.6% and 13.6%) and higher in the trifluoromethylphenyl ring labelled experiment (28.1%) by 42 days.

The acidic and alkali treatments of post-extract soil from the 42 day samples of the trifluoromethylphenyl ring labelled experiment showed that an additional 10% could be extracted with acidic conditions and an additional 15% with alkali conditions. The acid and base extracts were not analysed further.

Flurtamone was the principal radiolabelled component detected in all three experiments.

In the trifluoromethylphenyl ring labelled experiment levels of parent accounted for 87% of applied radioactivity at Day 0 and declined to 33% of applied radioactivity at termination of the study at 42 days. In addition to parent material, two metabolites M04 TFMBA (AE C518919) and M02 3-trifluoromethyl-N-methyl-mandelamide (AE 0540067) were identified by radio-HPLC and LC/MS. Confirmation by radio-TLC was also obtained. M04 TFMBA was detected at a maximum of 20% in the trifluoromethylphenyl ring labelled experiment. The position of the radiolabel in the other two

experiments meant that this metabolite would not have contained a carbon-14 atom and consequently it was not observed using radiochemical detection methods. M02 3-trifluoromethy-N-methyl-lmandelamide was observed as a minor metabolite (maximum 4%) in the trifluoromethylphenyl ring labelled experiment and was also observed in the furanone ring labelled experiment. It was not observed in the phenyl ring labelled experiment due to the position of the carbon-14 atom.

The results from the characterisation of the radioactivity in methanol extracts of soil from the trifluoromethylphenyl ring labelled experiment are presented in Table 7.1.1-5. The calcium sulphate extract of the final time-point from this experiment, which contained 13% of the initial radioactivity, was analysed and found to contain M04 TFMBA. Approximately 50% of the radioactivity in the aqueous extract was identified as this metabolite. In addition, concentration of the methanol extracts, noticeable in the final time-point, led to distillation of the M04 TFMBA into cold traps on rotary evaporating. This information was used to adapt the extraction and concentration procedures used for later studies and for the anaerobic portion of the study.

Table 7.1.1.1-5 Characterisation of radioactivity following treatment of aerobic soil with trifluoromethylphenyl-UL-^{14}C- flurtamone (as % of radioactivity)

Time (days)	% of radioactivity as			
	Methanol Extract	Flurtamone	M04 2-Trifluoromethyl-3-trifluoro–N-methyl-benzoic acid	M02 mandelamide
0	92.4	88	2	2
15	89.2	86	12	1
28	83.3	62	17	1
42	69	33	30	4

In the phenyl ring labelled experiment parent was the only significant component detected in methanol extracts of the final time-point, while in the furanone ring experiment a small amount of N-methyl-3-trifluoromethylmandelamide were observed in addition to parent.

Flurtamone degraded at a rapid rate in soil with a reported DT_{50} value of 28 days.

Conclusion:

Flurtamone degraded at a rapid rate in sandy loam soil incubated at 25 ºC and 75% of field capacity (⅓ bar) under aerobic conditions. Up to 22% of the radioactivity was detected as $^{14}CO_2$, indicating the potential for rapid mineralization of the phenyl and furanone rings of flurtamone. Mineralization of the trifluoromethylphenyl ring was somewhat slower with 6% detected as $^{14}CO_2$ after 42 days.

Two metabolites were observed: M04 TFMBA which was detected as a major metabolite in excess of 20% and M02 3-trifluoromethyl-N-methyl-mandelamide which was observed at a maximum of 4%.

The DT_{50} value for the degradation of flurtamone in sandy loam soil incubated at 25 ºC was 28 days, assuming first order kinetics.

Report: **KCA-7.1.1.1 /03; [redacted] M. 2012a**
Title: [Trifluoromethylphenyl-UL-^{14}C]-Flurtamone: Aerobic Metabolism/Degradation in Four European Soils.
Organisation: [redacted]
Report No.: EnSa-12-0469
Bayer CropScience Document **M-442039-01-1**
Publication: unpublished
Dates of experimental: 9th November 2011 to 29th June 2012

work:

Guidelines:	**OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The biotransformation of [trifluoromethylphenyl-UL-^{14}C]-flurtamone was studied in four European soils. Due to the fast degradation and the high mineralization of the compound, the study was terminated after 87 days of incubation. Flurtamone was applied at a nominal rate of 1000 µg/kg soil (dry weight), corresponding to a field rate equivalent of 375 g/ha.

The test system consisted of Erlenmeyer flasks equipped with traps for the collection of CO_2 and volatile organic compounds. Samples were analyzed after 0, 1, 3, 7, 15, 22, 36, 59 and 87 days of incubation. At each sampling date, the soil samples were extracted three times at ambient temperature and once by hot (microwave) extraction. Combined organic soil extracts were concentrated and analyzed by TLC to quantify the test item as well as its transformation products. Representative extracts were additionally analyzed using a second chromatographic method (HPLC).

The test conditions outlined in the study protocol were maintained throughout the study. Mean material balances were 98.6, 98.0, 98.7, and 97.7% of the applied radioactivity. Extractable ^{14}C-residues decreased from 96.8, 95.2, 94.9, and 95.3% of AR at DAT-0 to 13.1, 11.7, 13.1, and 11.8% at the study end (DAT-87). The amounts of the test item in the extracts declined from 96.3, 95.0, 94.3, and 94.8% of AR at DAT-0 to 4.5, 3.3, 3.2 and 4.0% of AR at the end of the study. Both flurtamone enantiomers showed similar degradation behaviour.

The half-life of flurtamone was calculated by the best fit kinetics according to FOCUS (for trigger evaluation) as 13.2, 12.8, 10.7 and 9.7 days (single first order, SFO).

Besides the test item, two major transformation products were detected in the extracts. M04 TFMBA accounted for up to 12.1, 15.5, 9.8 and 24.7% of applied radioactivity (AR). The amounts of trifluoroacetate (M05 TFA) reached up to 4.6, 5.5, 4.9 and 4.9% of AR at the end of the study. Furthermore, three minor degradation products reaching up to 3.1% of AR were characterized according to their separation distances in TLC. The sum of the non-characterized minor transformation products did not exceed 2.5% of AR.

The NER increased from 0.8, 1.3, 1.1 and 1.2% of AR at DAT-0 to maximum values of 36.0, 34.8, 41.8 and 36.6% of AR and declined already slightly to 33.6, 32.9, 37.2 and 33.9% of AR towards the end of the study.

A further characterization (fractionation into humin, humic acids and fulvic acids) was shown for all four soils for samples taken at 59 days after treatment. The maximum amounts of $^{14}CO_2$ were 51.5, 55.1, 51.1 and 52.0% of AR at study termination. Volatile organic compounds were not formed in the course of the study.

The test item was rapidly degraded. The high amount of formed carbon dioxide as the final product indicates a complete mineralization of flurtamone in soil.

Materials and Methods

Test Material:

[Trifluoromethylphenyl-UL-^{14}C]-flurtamone, radiochemical purity > 99%, Batch no.: KML 9073

Test Design:

The metabolism of flurtamone in soil under aerobic conditions was investigated in four agricultural soils at 20°C. The soil characteristics are listed in the following page.

The soils were collected from agricultural areas of Germany and were taken fresh from the field. A few days before starting the study, the soil was sieved to a particle size of ≤ 2 mm. Subsequently the soil moisture was determined by drying aliquots of the soils at 105°C. Ultrapure water was added to adjust each soil aliquot to 55% of the maximum water holding capacity. The weights of all test vessels were recorded and the samples were pre-equilibrated at about 20 °C in the dark for three days.

The incubation systems were static systems and consisted of Erlenmeyer flasks (300 mL) with 100 g soil (dry weight equivalent) for each sampling interval. The flasks were closed with trap attachments, which were easily permeable for oxygen. The traps contained soda lime for adsorption of CO_2 and a polyurethane foam plug for adsorption of volatile organic compounds.

An application solution with a concentration of approximately 100 mg/mL was prepared. 1 mL of this was applied drop-wise, by use of a micropipette, to each pre-equilibrated soil sample. Dose checks were taken during the application procedure. Metabolite identification samples were also prepared. These were in case of the necessity for additional amounts of metabolites for HPLC-MS/MS analysis. These samples were treated at an exaggerated rate (but, in the event, were not required).

Water loss due to evaporation from the soil was determined by weighing the sampled flasks without the traps on each processing day. If necessary, the evaporated portions were replaced.

Table 7.1.1.1-6 Properties of the soils used in a guideline flurtamone aerobic soil study

Parameter	Result/Value			
Soil	Laacher Hof AXXa	Dollendorf II	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh
Geographic Location[1]	Monheim am Rhein	Blankenheim	Monheim am Rhein	Burscheid
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudoll	Fine-loamy, nixed, active, frigid Typic Eutrudept	Loamy, mixed, mesic Typic Argudalf	Loamy, mixed, mesic Typic Argudalf
Map Reference	N 51° 04.65' E 06° 53.52'	N 50° 22.90' E 06° 43.00'	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'
Textural Class (USDA)	Loamy sand	Loam	Sandy Loam	Silt loam
Sand (%)	78	39	57	19
Silt (%)	16	36	28	64
Clay (%)	6	25	15	17
pH in $CaCl_2$ (1:2)	6.2	7.4	5.3	6.5
pH in water (1:1)	6.5	7.5	5.5	6.7
pH in water (saturated paste)	6.6	7.4	5.5	6.8
pH in KCl (1N)	6.0	7.1	4.7	6.1
Organic Matter (%)	3.1	8.9	3.3	2.8
Organic Carbon (%)	1.8	5.2	1.9	1.6
Cation Exchange Capacity (meq/100g)	9.4	22.3	9.9	12.2
Water Holding Capacity at pF 2.5 (%)	10.9	27.4	16.9	21.0
Maximum Water Holding Capacity (%)	43.8	79.3	60.2	51.8
Bulk Density (disturbed, g/cm^3)	1.22	1.01	1.13	1.12
Soil Biomass at:				
0 days	756	3088	213	668
36 days	363	1896	268	349
121 days	470	2640	334	477

[1] in North Rhine-Westphalia, Germany.

Samples (in duplicate) were taken at 0, 1, 3, 7, 15, 22, 36, 59 and 87 days after treatment. The corresponding trap attachments were collected to determine the amount of $^{14}CO_2$ and organic volatiles. At the respective sampling dates, the soil samples from each flask were extracted completely. The extracts were analyzed by LSC and TLC within three days. HPLC analysis was performed within a maximum of four days. After analysis, the extracts were stored cold. The trap attachments containing soda lime and PU foam were processed within about three weeks. Bound residues were analyzed by combustion and LSC within four weeks after sampling. A further characterization of bound residues was performed within about five months.

Soil biomass measurements were conducted using the substrate-induced initial respiratory response (SIR) method.

For soil extraction the entire soil amount of each test vessel was transferred in a centrifuge beaker and extracted using a mechanical shaker. The extraction procedure comprised an extraction with 100 mL acetonitrile/water 80/20 (v/v) followed by two extractions with 80 mL acetonitrile/water 80/20 (v/v)

both at ambient temperature. These extracts were combined and radioassayed. . The final extraction used 80 mL acetonitrile/water 50/50 (v/v) with microwave heating to 70°C. This was then radioassayed.

The residual radioactivity (bound residues) in freeze-dried, homogenized soil was determined by combustion of three aliquots (approx. 1 g) of each sample followed by LSC. The bound residue in soil (DAT-59 samples) was characterized and fractionated into humin, humic acid and fulvic acid by addition of sodium hydroxide and subsequent precipitation of the supernatant with hydrochloric acid.

Volatile organic compounds possibly contained in the foam plugs were extracted by shaking with ethyl acetate. Aliquots of the extracts were submitted to LSC measurement. Chromatographic analyses of the PU foam extracts were not performed, because they contained < 0.1% of the AR in all test systems.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in hydrochloric acid. The liberated CO_2 was absorbed by a special absorption/ scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Prior to chromatographic analysis, the cold and microwave organic extracts were combined. Aliquots were concentrated and radioassayed to allow the determination of recovery. Aliquots of the concentrates were analyzed by TLC and the concentrated extracts sampled at DAT-0, 7, 22, 36, 59 and 87 were additionally analyzed by HPLC.

For TLC analysis aliquots of the concentrates of the extracts were spotted on silica gel plates (Si60, F254, 20 cm x 20 cm Merck) using an automatic applicator. The plates were developed with ethyl acetate/2-Propanol/water (75/20/5, v/v/v) in a plate chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products in the extracts was calculated based on the distribution of the TLC-zones and the amount of radioactivity in the extracts.

The assignment of the TLC peaks to the test item and the transformation products M04 TFMBA and M05 TFA was done by comparing their separation distances with the separation distances of radiolabelled flurtamone, M04 TFMBA and M05 TFA. The radiolabelled test item and the radiolabelled reference items were applied in separate lanes onto each TLC plate. All minor transformation products were characterized according to their separation distances.

One HPLC method was used to confirm the qualitative and quantitative TLC results. The system comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm; 5 µm column connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. A second HPLC method was used to isolate the flurtamone fraction from the application solution and from representative concentrated soil extracts. This used the same column as the first method but a gradient of ultrapure water against acetonitrile. An additional method, a chiral HPLC method, was used to analyze the ratio of the flurtamone enantiomers. This used a Chiralcel OD, 250 x 4.6 mm; 10 µm column and an isocratic mobile phase of heptane/ethanol (90:10, v/v).

The electro-spray ionization MS spectra (ESI) were obtained with a LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The HPLC instrument used for chromatography was an Agilent HP1100 with a Nucleodur C18 Gravity, 3 µm, 250 x 2 mm (MN) column. The mobile

phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

Findings

The DAT-0 extraction efficiencies were 96.9, 95.2, 94.9, and 95.3% of applied radioactivity (AR). The test item was stable under the conditions of extraction and accounted for 96.3, 95.0, 94.3, and 94.8% of AR in the combined organic soil extracts at DAT-0. These results demonstrate that the extraction method was well suitable to extract the compound from the soil matrix. The recoveries of radioactivity after the concentration step were exemplarily determined and were > 90% for all samples.

A good selectivity and reproducibility demonstrated the suitability for separation and quantification of the TLC method. The TLC limit of quantification (LOQ) for a single peak in the combined organic extracts was < 1% of radioactivity applied to the plate (0.2% of AR). The HPLC recovery-checks gave mean recoveries that ranged from 96.7 to 102.2% for the four soils, which showed that no radioactivity was lost during analysis.

Recoveries of applied radioactivity from samples were between 90% and 110% for all soils at all time-points. No time-dependent tendency was observed for the total recovery over the study period, demonstrating that no significant radioactivity dissipated from the flasks or was lost during processing.

The amount of formed $^{14}CO_2$ increased steadily during the entire study period. At the end of the study, 87 days after application, between 51.1 and 52.1% of AR was quantified as carbon dioxide. No significant amounts of volatile organic compounds were detected in the polyurethane foam of the trap attachments (values being ≤ 0.1% of AR at all sampling intervals). At the end of the incubation period the recovered radioactivity in the extracts had decreased to 11.7 - 13.1% of AR. Non-extractable ^{14}C-residues increased from 0.8, 1.3, 1.1 and 1.2% of AR at DAT-0 to maximum amounts of 36.0, 34.8, 41.8 and 36.6% of AR at DAT-36 or DAT-59 and declined already slightly to 33.6, 32.9, 37.2 and 33.9% of AR by the end of the study at DAT-87. The mean recoveries and distribution of applied radioactivity are shown in the following tables.

Table 7.1.1.1-7 Recovery and distribution of applied radioactivity in Laacher Hof AXXa soil

Fraction	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Carbon dioxide	n.a.	0.1	0.6	2.89	10.3	19.9	35.1	44.3	51.5
Organic volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	< 0.1
Total volatiles	**n.a**	**0.2**	**0.7**	**3.0**	**10.4**	**20.0**	**35.2**	**44.4**	**51.5**
Ambient extract	95.4	96.4	89.2	82.1	62.2	48.1	22.8	12.2	10.4
Microwave extract	1.6	2.0	2.5	3.7	4.3	4.3	4.3	3.1	2.7
Total extractable	**96.9**	**98.5**	**91.8**	**85.8**	**66.6**	**52.4**	**27.1**	**15.3**	**13.1**
Non-extractable	**0.8**	**3.0**	**7.0**	**11.6**	**20.7**	**28.2**	**33.6**	**36.0**	**33.6**
Total recovery	**97.7**	**101.6**	**99.5**	**100.4**	**97.6**	**100.7**	**95.9**	**95.7**	**98.3**

Table 7.1.1.1-8 Recovery and distribution of applied radioactivity in Dollendorf II soil

Fraction	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Carbon dioxide	n.a.	0.1	0.7	2.8	13.8	20.3	36.7	45.3	55.1
Organic volatiles	n.a.	< 0.1	0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a**	**0.1**	**0.7**	**2.8**	**13.9**	**20.3**	**36.7**	**45.3**	**55.1**
Ambient extract	93.3	91.0	87.0	78.9	60.8	48.2	25.4	11.1	9
Microwave extract	1.9	3.1	2.8	3.8	4.2	5.3	3.5	2.5	2.2
Total extractable	**95.2**	**94.1**	**89.8**	**82.7**	**65.0**	**53.5**	**29.3**	**13.6**	**11.7**
Non-extractable	**1.3**	**4.1**	**7.5**	**11.8**	**20.6**	**27.3**	**31.5**	**34.8**	**32.9**
Total recovery	**96.6**	**98.4**	**98.0**	**97.3**	**99.5**	**101.1**	**97.5**	**93.8**	**99.7**

Table 7.1.1.1-9 Recovery and distribution of applied radioactivity in Laacher Hof Wurmwiese soil

Fraction	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Carbon dioxide	n.a.	0.2	1.0	4.0	16.2	28.3	38.5	41.3	51.1
Organic volatiles	n.a.	< 0.1	0.1	0.1	0.1	0.1	0.1	0.1	< 0.1
Total volatiles	**n.a**	**0.1**	**1.3**	**5.4**	**16.3**	**28.4**	**38.6**	**41.3**	**51.1**
Ambient extract	93.3	94.0	85.1	73.8	46.8	28.5	13.8	11.1	10.4
Microwave extract	1.6	2.1	2.6	3.6	3.5	4.9	3.3	3.3	2.8
Total extractable	**94.9**	**96.2**	**87.5**	**77.4**	**50.2**	**33.4**	**17.1**	**14.4**	**13.1**
Non-extractable	**1.1**	**3.9**	**10.2**	**17.0**	**30.2**	**40.2**	**41.8**	**40.0**	**37.2**
Total recovery	**95.9**	**100.1**	**99.0**	**99.8**	**96.8**	**101.9**	**97.5**	**95.7**	**101.4**

Table 7.1.1.1-10 Recovery and distribution of applied radioactivity in Hoefchen Am Hohenseh 4a soil

Fraction	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Carbon dioxide	n.a.	0.1	0.5	2.3	9.5	18.2	35.1	44.8	52.0
Organic volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a**	**0.2**	**0.6**	**2.4**	**9.5**	**18.3**	**35.1**	**44.8**	**52.0**
Ambient extract	93.6	93.6	87.7	78.8	59.1	46.0	22.9	11.1	9.6
Microwave extract	1.7	2.6	3.0	4.4	4.3	4.4	3.2	2.6	2.2
Total extractable	**95.3**	**96.2**	**90.7**	**83.2**	**63.4**	**50.3**	**26.1**	**13.7**	**11.8**
Non-extractable	**1.2**	**3.7**	**7.8**	**12.7**	**24.2**	**31.3**	**34.7**	**36.6**	**33.9**
Total recovery	**96.5**	**100.0**	**99.1**	**98.3**	**97.1**	**99.9**	**95.9**	**95.2**	**97.7**

n.a = not analyzed (Tables 7.1.1.1-7 to 7.1.1.1-10 inclusive)

The results of the fractionation of unextractable residues from the 59 day samples into humin, humic acid and fulvic acid are shown in the table below.

Table 7.1.1.1-11 Distribution of unextractable radioactivity in humic substance fractions (as % applied radioactivity)

Soil	Humin Fraction (% AR)	Humic Acid Fraction (%AR)	Fulvic Acid Fraction (%AR)	Total (%AR)
Laacher Hof AXXa	12.9	7.8	15.8	36.5
Dollendorf II	16.0	7.2	11.4	34.6
Laacher Hof Wurmwiese	14.7	12.4	12.5	39.5
Hoefchen Am Hohenseh	17.8	9.1	9.8	36.8

Flurtamone was rapidly degraded. Besides the test item, two major transformation products were detected in the extracts. M04 TFMBA accounted for up to 12.1, 15.5, 4.8 and 24.7% of AR and the amounts of M05 TFA reached up to 4.6, 5.5, 4.9 and 4.9% of AR at the end of the study in soils. Furthermore, three minor degradation products reaching up to 3.1% of AR were characterized according to their separation distances in TLC. The sum of the non-characterized minor transformation products did not exceed 2.5% of AR. The biotransformation of flurtamone is summarized in the following tables.

Table 7.1.1.1-12 Biotransformation of flurtamone in Laacher Hof AXXa soil under aerobic conditions

Compound	% applied radioactivity, it days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Flurtamone	96.3	98.3	83.6	70.5	44.5	33.4	10.9	5.2	4.5
M05 TFA	n.d.	n.d.	0.5	0.9	1.2	1.5	3.4	3.8	4.6
M04 TFMBA	n.d.	1.5	3.9	9.5	12.1	10.6	5.9	0.9	0.5
U1	n.d.	1.2	1.7	2.0	1.6	0.8	n.d	n.d.	
U2	n.d.	0.4	0.6	0.8	2.4	2.2	1.9	1.5	<LOD
U3	n.d.	n.d.	0.2	0.3	1.0	0.5	0.7	0.9	n.d.
Sum of minor mets.	< LOD	< LOD	1.3	1.7	1.6	1.5	2.3	2.2	0.7
Total extractable	**96.3**	**98.3**	**91.8**	**85.8**	**66.6**	**52.4**	**27.1**	**15.3**	**13.1**
Carbon dioxide	n.a.	0.1	0.6	2.9	10.3	19.9	35.1	44.3	51.5
Organic volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	< 0.1
Non-extractable	0.8	3.0	7.0	11.6	20.7	28.2	33.6	36.0	33.6
Total recovery	**97.7**	**101.6**	**99.5**	**100.4**	**97.6**	**100.7**	**95.9**	**95.7**	**98.3**

n.d = not detected & n.a = not analyzed , < LOD = Less than limit of detection

Table 7.1.1.1-13 Biotransformation of flurtamone in Dollendorf II soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Flurtamone	95.0	90.5	78.9	65.7	41.9	31.4	10.4	4.9	3.3
M05 TFA	n.d.	n.d.	< lod	0.7	1.3	1.6	3.7	4.1	5.5
M04 TFMBA	n.d.	2.7	7.8	12.4	15.5	14.1	9.7	1.1	0.4
U1	n.d.	0.5	0.9	0.9	0.7	1.0	n.d.	n.d.	n.d.
U2	< LOD	< LOD	0.5	0.8	2.1	1.2	0.5	0.5	0.8
U3	n.d.	n.d.	< LOD	< LOD	1.3	< LOD	0.5	0.5	n.d.
Sum of minor mets	< LOD	< LOD	0.9	1.6	0.8	0.6	2.3	1.1	0.7
Total extractable	**95.3**	**94.1**	**89.8**	**82.7**	**65.0**	**53.5**	**29.5**	**15.6**	**11.7**
Carbon dioxide	n.a.	0.1	0.7	2.8	13.7	20.3	36.7	45.3	55.1
Organic volatiles	n.a.	< 0.1	0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	1.3	4.1	7.5	11.8	20.6	27.3	31.5	34.8	32.9
Total recovery	**96.6**	**98.4**	**98.0**	**97.3**	**99.5**	**101.1**	**97.5**	**95.8**	**99.7**

n.d = not detected & n.a = not analyzed , < LOD = Less than limit of detection

Table 7.1.1.1-14 Biotransformation of flurtamone in Laacher Hof Wurmwiese soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Flurtamone	94.3	93.6	82.5	68.0	38.5	29.9	5.4	3.5	3.2
M05 TFA	n.d.	n.d.	0.5	1.5	1.8	2.4	4.6	4.6	4.9
M04 TFMBA	n.d.	1.6	2.8	4.8	3.9	3.9	1.1	< LOD	0.4
U1	n.d.	0.6	0.5	0.6	0.7	1.0	n.d.	n.d.	n.d.
U2	0.2	0.3	0.5	1.0	2	1.2	3.1	2.5	1.0
U3	n.d.	n.d.	< LOD	0.3	0.7	0.8	0.7	0.9	< LOD
Sum of minor mets	< LOD	n.d.	0.3	1.1	1.1	2.5	0.9	1.6	0.8
Total extractable	**94.9**	**96.3**	**87.5**	**77.4**	**50.2**	**33.4**	**17.1**	**14.4**	**13.1**
Carbon dioxide	n.a.	0.2	1.2	5.3	16.2	28.3	38.5	41.3	51.1
Organic volatiles	n.a.	< 0.1	0.1	0.1	0.1	0.1	0.1	0.1	< 0.1
Non-extractable	1.1	3.9	10.2	17.0	30.2	40.2	41.8	40.0	37.2
Total recovery	**95.9**	**100.4**	**99.0**	**99.8**	**96.8**	**101.9**	**97.5**	**95.7**	**101.4**

n.d = not detected & n.a = not analyzed , < LOD = Less than limit of detection

Table 7.1.1.1-15 Biotransformation of flurtamone in Hoefchen Am Hohensee soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:								
	0	**1**	**3**	**7**	**15**	**22**	**36**	**59**	**87**
Flurtamone	94.9	92.6	81.7	63.5	32.8	17.8	6.5	4.5	4.0
M05 TFA	n.d.	n.d.	0.4	0.8	1.1	1.8	3.7	4.3	4.9
M04 TFMBA	n.d.	1.9	5.2	13.0	21.1	24.7	12.4	1.2	0.8
U1	n.d.	1.3	1.9	2.5	2.7	1.7	0.4	n.d.	n.d.
U2	< LOD	0.3	0.7	1.2	2.1	1.6	1.1	0.6	0.6
U3	n.d.	n.d.	< LOD	0.4	0.9	0.3	0.5	0.6	n.d.
Sum of minor mets	< LOD	n.d.	0.5	1.4	1.1	1.8	0.6	1.4	0.7
Total extractable	**95.3**	**96.2**	**90.7**	**83.2**	**63.4**	**50.3**	**26.1**	**15.7**	**11.8**
Carbon dioxide	n.a.	0.1	0.5	2.3	9.5	18.2	35.1	44.8	52.0
Organic volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	,0.1	,0.1	,0.1
Non-extractable	1.2	3.7	7.8	12.7	24.2	31.3	34.7	36.6	33.9
Total recovery	**96.5**	**100.0**	**99.1**	**98.3**	**97.4**	**99.9**	**95.9**	**97.2**	**97.7**

n.d = not detected & n.a = not analyzed , < LOD = Less than limit of detection

The data for flurtamone were evaluated according to FOCUS guidelines and the best-fit kinetic model was chosen on the basis of the chi-squared confidence criterion and visual assessment. The results are summarized in the following table.

Table 7.1.1.1-16 Flurtamone DT_{50} and DT_{90} values in soils under aerobic conditions

Soil	Soil Type	Kinetic Model	DT_{50} (d)	DT_{90} (d)	Visual Assessment	Chi^2 Error (%)
Laacher Hof AXXa	Loamy sand	SFO	13.2	44.0	Good	3.5
Dollendorf II	Loam	SFO	12.8	42.4	Good	3.1
Laacher Hof Wurmwiese	Sandy loam	SFO	10.7	35.6	Good	5.0
Hoefchen Am Hohenseh	Silt loam	SFO	9.7	32.3	Good	4.4

Conclusions

Flurtamone is rapidly degraded in soil under aerobic conditions, with a high degree of mineralization. Both enantiomers show similar degradation behaviour. The major metabolite is M04 TFMBA (maximum 27% in any soil). M05 TFA is formed to a level of about 5% AR after 120 days. All other degradates are very minor.

Report: **KCA-7.1.1.1 /04; [redacted]. 2012b**
Title: [Phenyl-UL-^{14}C]-Flurtamone: Aerobic Metabolism/Degradation in Four European Soils.
Organisation: [redacted]
Report No.: EnSa-12-0417
Bayer CropScience Document **M-440226-01-1**
Publication: unpublished
Dates of experimental work: 20th May 2011 to 19th September 2011
Guidelines: **OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100**
Deviations: None
GLP/GEP **Yes**

Executive Summary

The biotransformation of [phenyl-UL-^{14}C]-flurtamone was studied in four European soils. Flurtamone was applied at a nominal rate of 1000 µg/kg soil (dry weight), corresponding to a 375 g/ha field rate equivalent. Due to the fast degradation and the high mineralization of the compound, the study was terminated after 59 days of incubation.

The test system consisted of Erlenmeyer flasks equipped with traps for the collection of CO_2 and volatile organic compounds. Samples were analyzed after 0, 1, 3, 7, 14, 22, 35 and 59 days of incubation. At each sampling date, the soil samples were extracted three times at ambient temperature and once by hot (microwave) extraction. Combined organic soil extracts were concentrated and analyzed by HPLC to quantify the test item as well as its transformation products. Representative extracts were additionally analyzed using a second chromatographic method (TLC).

The test conditions outlined in the study protocol were maintained throughout the study. Mean material balances were 98.1, 99.8, 97.9, and 98.9% of the applied radioactivity. Extractable ^{14}C-residues decreased from 101.9, 99.3, 98.8, and 99.2% of AR at DAT-0 to 7.1, 7.1, 5.9, and 4.9% at the study end (DAT-59). The amounts of the test item in the extracts declined from 101.1, 98.5, 97.6, and 97.9% of AR at DAT-0 to 4.1, 3.7, 2.4 and 2.5% of AR at the end of the study. Both flurtamone enantiomers showed similar degradation behaviour.

The half-life of flurtamone was calculated by the best fit kinetics according to FOCUS (for trigger evaluation) as 10.3, 11.3, 9.4 and 8.5 days (single first order, SFO).

Besides the test item and a high amount of carbon dioxide only minor metabolites (of which there were several) were detected. The maximum amount of a single metabolite in any soil at any sampling time-point accounted for 2% of AR.

The NER increased from 0.7, 1.1, 0.9 and 0.8% of AR at DAT-0 to maximum values of 33.6, 32.2, 37.5 and 38.4% of AR and declined already slightly to 31.0, 31.8, 33.4 and 34.9% of AR towards the end of the study.

A further characterization (fractionation into humin, humic acids and fulvic acids) was shown for all four soils for samples taken at 59 days after treatment. The maximum amounts of $^{14}CO_2$ were 57.4, 64.0, 57.7 and 58.0% of AR at study termination. Volatile organic compounds were not formed in the course of the study.

The test item was rapidly degraded. The high amount of formed carbon dioxide as the final product indicates a complete mineralization of flurtamone in soil.

Materials and Methods

Test Material:

[Phenyl-UL-^{14}C]-flurtamone, radiochemical purity > 99%, Batch no.: KML 9124

Test Design:

The metabolism of flurtamone in soil under aerobic conditions was investigated in four agricultural soils at 20°C. The soil characteristics are listed below.

Table 7.1.1.1-17 Properties of the soils used in a guideline flurtamone aerobic soil study

Parameter	Result/Value			
Soil	Laacher Hof AXXa	Dollendorf II	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh 4a
Geographic Location[1]	Monheim am Rhein	Blankenheim	Monheim am Rhein	Burscheid
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudoll	Fine-loamy, nixed, active, frigid Typic Eutrudept	Loamy, mixed, mesic Typic Argudalf	Loamy, mixed, mesic Typic Argudalf
Map Reference	N 51° 04.65' E 06° 53.52'	N 50° 22.90' E 06° 43.00'	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'
Textural Class (USDA)	Sandy loam	Clay loam	Sandy Loam	Silt loam
Sand (%)	77	29	53	23
Silt (%)	14	38	30	60
Clay (%)	9	33	17	17
pH in $CaCl_2$ (1:2)	6.1	7.4	4.7	6.1
pH in water (1:1)	6.3	7.3	4.9	6.3
pH in water (saturated paste)	6.3	7.2	5.0	6.3
pH in KCl (1N)	5.9	6.9	4.2	5.8
Organic Matter (%)	3.4	8.8	3.8	3.1
Organic Carbon (%)	2.0	5.1	2.2	1.8
Cation Exchange Capacity (meq/100g)	9.6	21.8	10.8	11.7
Water Holding Capacity at pF 2.5 (%)	10.7	23.5	18.3	23.9
Maximum Water Holding Capacity (%)	48.2	81.6	61.2	55.2
Bulk Density (disturbed, g/cm^3)	1.25	0.99	1.13	1.13
Soil Biomass at:				
0 days	1261	4970	1034	1258
35 days	399	1896	297	437
78 days	23	1631	217	378

[1] in North Rhine-Westphalia, Germany.

The soils were collected from agricultural areas of Germany and were taken fresh from the field. A few days before starting the study, the soil was sieved to a particle size of ≤ 2 mm. Subsequently the soil moisture was determined by drying aliquots of the soils at 105°C. Ultrapure water was added to adjust each soil aliquot to 55% of the maximum water holding capacity. The weights of all test vessels were recorded and the samples were pre-equilibrated at about 20 °C in the dark for three days.

The incubation systems were static systems and consisted of Erlenmeyer flasks (300 mL) with 100 g soil (dry weight equivalent) for each sampling interval. The flasks were closed with trap attachments, which were easily permeable for oxygen. The traps contained soda lime for adsorption of CO_2 and a polyurethane foam plug for adsorption of volatile organic compounds.

An application solution with a concentration of approximately 100 mg/mL was prepared. 1 mL of this was applied drop-wise, by use of a micropipette, to each pre-equilibrated soil sample. Dose checks were taken during the application procedure. Metabolite identification samples were also prepared.

These were in case of the necessity for additional amounts of metabolites for HPLC-MS/MS analysis. These samples were treated at an exaggerated rate (but, in the event, were not required).

Water loss due to evaporation from the soil was determined by weighing the sampled flasks without the traps on each processing day. If necessary, the evaporated portions were replaced.

Samples (in duplicate) were taken at 0, 1, 3, 7, 14, 22, 35 and 59 days after treatment. The corresponding trap attachments were collected to determine the amount of $^{14}CO_2$ and organic volatiles. At the respective sampling dates, the soil samples from each flask were extracted completely. The extracts were analyzed by LSC and TLC within three days. HPLC analysis was performed within a maximum of four days. After analysis, the extracts were stored cold. The trap attachments containing soda lime and PU foam were processed within about three weeks. Bound residues were analyzed by combustion and LSC within four weeks after sampling. A further characterization of bound residues was performed within about five months.

Soil biomass measurements were conducted using the substrate-induced initial respiratory response (SIR) method.

For soil extraction the entire soil amount of each test vessel was transferred in a centrifuge beaker and extracted using a mechanical shaker. The extraction procedure comprised an extraction with 100 mL acetonitrile/water 80/20 (v/v) followed by two extractions with 80 mL acetonitrile/water 80/20 (v/v) both at ambient temperature. These extracts were combined and radioassayed. . The final extraction used 80 mL acetonitrile/water 50/50 (v/v) with microwave heating to 70°C. This was then radioassayed.

The residual radioactivity (bound residues) in freeze-dried, homogenized soil was determined by combustion of three aliquots (approx. 1 g) of each sample followed by LSC. The bound residue in soil (DAT-59 samples) was characterized and fractionated into humin, humic acid and fulvic acid by addition of sodium hydroxide and subsequent precipitation of the supernatant with hydrochloric acid.

Volatile organic compounds possibly contained in the foam plugs were extracted by shaking with ethyl acetate. Aliquots of the extracts were submitted to LSC measurement. Chromatographic analyses of the PU foam extracts were not performed, because they contained ≤ 0.1% of the AR in all test systems.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in hydrochloric acid. The liberated CO_2 was absorbed by a special absorption/ scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Prior to chromatographic analysis, the cold and microwave organic extracts were combined. Aliquots were concentrated and radioassayed to allow the determination of recovery. Aliquots of the concentrates were analyzed by HPLC and the concentrated extracts sampled at DAT-0, 7, 22 and 35 were additionally analyzed by TLC.

The HPLC method to separate and quantify the test item and its metabolites used a system that comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm; 5 µm column connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. The assignment of the HPLC peak to the test item in the application solution was done by co-chromatography using the [^{12}C]-reference substance. Within routine chromatograms of the concentrated combined extracts, comparison of

retention times in different chromatograms was used for the assignment of the HPLC peaks to test item and the transformation products. The quantification of the test item and its degradation products in the extracts was calculated based on the distribution of the HPLC - zones and the amount of RA in the extracts.

The electro-spray ionization MS spectra (ESI) were obtained with a LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The HPLC instrument used for chromatography was an Agilent HP1100 with a Nucleodur C18 Gravity, 3 µm, 250 x 2 mm (MN) column. The mobile phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

For TLC analysis aliquots of the concentrates of the extracts were spotted on silica gel plates (60, F254, 20 cm x 20 cm Merck) using an automatic applicator. The plates were developed with ethyl acetate/2-Propanol/water (75/20/5, v/v/v) in a plate chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products in the extracts was calculated based on the distribution of the TLC-zones and the amount of radioactivity in the extracts.

The assignment of the TLC peaks to the test item was done by comparing their separation distances with the separation distances of radiolabelled flurtamone. The radiolabelled test item and the radiolabelled reference item were applied in separate lanes onto each TLC plate. All minor transformation products were characterized according to their separation distances.

Findings

The DAT-0 extraction efficiencies were 101.9, 99.3, 98.8, and 99.2% of applied radioactivity (AR). The test item was stable under the conditions of extraction and accounted for 101.1, 98.5, 97.6, and 97.9% of AR in the combined organic soil extracts at DAT-0. These results demonstrate that the extraction method was well suitable to extract the compound from the soil matrix. The recoveries of radioactivity after the concentration step were exemplarily determined and were > 90% for all but one of the samples.

A good selectivity and reproducibility demonstrated the suitability for separation and quantification of the HPLC method. The HPLC limit of quantification (LOQ) for a single peak in the combined organic extracts was < 1% of radioactivity applied to the plate (0.1% AR). The HPLC recovery-checks gave mean recoveries that ranged from 92.6 to 102.7% for the four soils, which showed that no radioactivity was lost during analysis.

Recoveries of applied radioactivity from samples were between 90% and 110% for all soils at all time-points. No time-dependent tendency was observed for the total recovery over the study period, demonstrating that no significant radioactivity dissipated from the flasks or was lost during processing.

The amount of formed $^{14}CO_2$ increased steadily during the entire study period. At the end of the study, 59 days after application, between 57.4 and 64.0% of AR was quantified as carbon dioxide. No significant amounts of volatile organic compounds were detected in the polyurethane foam of the trap attachments (values being ≤ 0.1% of AR at all sampling intervals). At the end of the incubation period the recovered radioactivity in the extracts had decreased to 4.9 - 7.1% of AR. Non-extractable ^{14}C-residues increased from 0.7, 1.1, 0.9 and 0.8% of AR at DAT-0 to maximum amounts of 33.6, 32.2,

37.5 and 38.4% of AR at DAT-35 and declined already slightly to 31.0, 31.8, 33.4 and 34.9% of AR by the end of the study at DAT-59.

The mean recoveries and distribution of applied radioactivity are shown in the following tables.

Table 7.1.1.1-18 Recovery and distribution of applied radioactivity in Laacher Hof AXXa soil

Fraction	% applied radioactivity at days after treatment::							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Carbon dioxide	n.a.	0.8	3.0	9.7	24.9	36.7	47.9	57.4
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**0.8**	**3.0**	**9.7**	**24.9**	**36.7**	**47.9**	**57.4**
Ambient extract	100.1	95.3	85.5	70.1	41.7	25.7	12.6	6
Microwave extract	1.8	2.4	2.6	3.0	2.7	2.6	1.6	1.5
Total extractable	**101.9**	**97.7**	**88.1**	**73.1**	**44.4**	**28.3**	**13.7**	**7.1**
Non-extractable	**0.7**	**3.2**	**9.3**	**15.9**	**26.1**	**30.3**	**33.6**	**31.0**
Total recovery	**102.6**	**101.6**	**100.4**	**98.6**	**95.4**	**95.3**	**95.2**	**95.5**

n.a not analyzed

Table 7.1.1.1-19 Recovery and distribution of applied radioactivity in Dollendorf II soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Carbon dioxide	n.a.	0.9	3.6	11.1	24.5	38.4	49.0	64.0
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**0.5**	**3.6**	**11.1**	**24.5**	**38.4**	**49.0**	**64.0**
Ambient extract	97.2	92.9	84.4	69.4	45.6	29.4	15.0	5.7
Microwave extract	2.1	2.7	2.8	2.9	2.7	2.4	1.3	1.4
Total extractable	**99.3**	**98.5**	**87.2**	**73.3**	**48.4**	**31.8**	**16.3**	**7.1**
Non-extractable	**1.1**	**4.4**	**9.5**	**15.0**	**23.0**	**28.7**	**32.2**	**31.8**
Total recovery	**100.4**	**103.9**	**100.3**	**98.5**	**95.9**	**98.9**	**97.5**	**102.9**

n.a not analyzed

Table 7.1.1.1-20 Recovery and distribution of applied radioactivity in Laacher Hof Wurmwiese soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Carbon dioxide	n.a.	1.1	3.4	10.7	24.0	39.3	48.7	57.7
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**1.1**	**3.4**	**10.7**	**24.0**	**39.3**	**48.7**	**57.7**
Ambient extract	96.9	93.2	80.9	63.7	39.5	18.8	6.8	4.3
Microwave extract	2.0	2.8	2.7	3.3	2.9	2.6	1.6	1.6
Total extractable	**98.8**	**95.9**	**83.6**	**67.0**	**42.4**	**21.4**	**8.3**	**5.9**
Non-extractable	**0.9**	**4.7**	**12.5**	**20.4**	**28.9**	**36.6**	**37.5**	**33.4**
Total recovery	**99.8**	**101.7**	**99.5**	**98.2**	**95.3**	**97.4**	**94.5**	**97.0**

n.a not analyzed

Table 7.1.1.1-21 Recovery and distribution of applied radioactivity in Hoefchen Am Hohenseh 4a soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Carbon dioxide	n.a.	0.9	3.2	11.7	28.6	43.0	49.6	58.0
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**0.9**	**3.2**	**11.7**	**28.6**	**43.0**	**49.6**	**58.0**
Ambient extract	97.0	95.3	84.5	64.9	32.8	15.4	6.3	3.1
Microwave extract	2.2	2.9	2.9	3.2	2.6	2.2	1.2	1.2
Total extractable	**99.2**	**98.2**	**87.4**	**68.1**	**35.4**	**17.6**	**7.6**	**4.9**
Non-extractable	**0.8**	**4.0**	**10.6**	**19.3**	**32.0**	**37.3**	**38.4**	**34.9**
Total recovery	**100.0**	**103.1**	**101.3**	**99.2**	**96.0**	**97.9**	**95.6**	**97.8**

n.a not analyzed

The results of the fractionation of unextractable residues from the 59 day samples into humin, humic acid and fulvic acid are shown in the table below.

Table 7.1.1.1-22 Distribution of unextractable radioactivity in humic substance fractions (as % applied radioactivity)

Soil	Humin Fraction (%AR)	Humic Acid Fraction (%AR)	Fulvic Acid Fraction (%AR)	Total (%AR)
Laacher Hof AXXa	14.0	8.0	9.1	31.1
Dollendorf II	18.0	7.5	6.2	31.7
Laacher Hof Wurmwiese	14.9	8.0	10.4	33.3
Hoefchen Am Hohenseh	18.0	7.0	9.9	34.9

Flurtamone was rapidly degraded. Besides the test item, four minor degradation products were detected in the extracts and characterized according to their retention times in HPLC. The maximum amount of a single transformation product was 2.0% of AR (soil Dollendorf II, DAT-22). In addition, several very minor metabolites were detected. Their sum did not exceed 4% of AR. The biotransformation of flurtamone is summarized in the following tables.

Table 7.1.1.1-23 Biotransformation of flurtamone in Laacher Hof AXXa soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Flurtamone	101.1	95.8	85.5	68.2	39.0	21.2	9.7	4.1
U1	n.d.	0.4	0.3	0.5	1.1	1.2	1.2	1.1
U2	< LOD	0.3	0.4	0.7	0.6	0.4	0.4	0.3
U3	0.3	0.6	0.5	0.7	0.6	0.4	0.4	0.3
U4	< LOD	0.2	0.3	0.4	0.3	0.6	0.2	0.2
Sum of minor mets	0.3	0.3	1.0	2.7	2.6	4.4	1.9	1.4
Total extractable	**101.9**	**97.7**	**88.1**	**73.1**	**44.4**	**28.3**	**13.7**	**7.1**
Carbon dioxide	n.a.	0.8	3.0	9.7	24.9	36.7	47.9	57.4
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	0.7	3.2	9.3	15.9	26.1	30.3	33.6	31.0
Total recovery	**102.6**	**101.6**	**100.4**	**98.6**	**95.4**	**95.3**	**95.2**	**95.5**

n.d. not detected n.a not analyzed

Table 7.1.1.1-24 Biotransformation of flurtamone in Dollendorf II soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Flurtamone	98.5	96.6	84.6	67.8	43.2	24.3	12.5	3.7
U1	0.2	0.3	0.4	0.6	1.1	2.0	1.1	1.5
U2	< LOD	0.2	0.4	0.4	0.3	0.4	0.2	0.2
U3	0.3	0.6	0.8	0.7	0.7	0.7	0.4	0.3
U4	< LOD	0.2	0.3	0.4	0.5	0.4	0.3	0.3
Sum of minor mets	< LOD	0.5	0.7	2.4	2.6	3.9	1.9	1.3
Total extractable	**99.3**	**98.5**	**87.2**	**72.8**	**48.4**	**31.8**	**16.3**	**7.1**
Carbon dioxide	n.a.	0.9	3.6	11.1	24.5	38.4	49.6	58.0
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	1.1	4.4	9.5	15.0	22.0	28.7	32.2	31.8
Total recovery	**100.4**	**103.9**	**100.3**	**98.5**	**95.9**	**98.9**	**97.5**	**102.9**

n.d. not detected n.a not analyzed < LOD = Less than limit of detection

Table 7.1.1.1-25 Biotransformation of flurtamone in Laacher Hof Wurmwiese soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Flurtamone	97.6	93.4	81.2	63.2	37.3	17.3	4.6	2.4
U1	n.d.	0.2	0.3	0.4	0.7	0.9	0.8	0.8
U2	n.d.	0.4	0.5	0.7	0.4	0.2	0.3	0.3
U3	0.4	0.6	0.5	0.6	0.6	0.4	0.2	0.3
U4	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.2
Sum of minor mets	0.6	1.2	1.0	1.7	3.0	2.3	2.2	1.9
Total extractable	**98.8**	**95.9**	**83.6**	**67.0**	**42.4**	**21.4**	**8.3**	**5.9**
Carbon dioxide	n.a.	2.1	3.4	10.7	24.0	39.3	48.7	57.7
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	0.9	4.7	13.1	20.4	28.9	36.6	37.5	33.4
Total recovery	**99.8**	**101.7**	**99.5**	**98.2**	**95.3**	**97.4**	**94.5**	**97.0**

n.d. not detected n.a not analyzed

Table 7.1.1.1-26 Biotransformation of flurtamone in Hoefchen Am Hohensee soil under aerobic conditions

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**14**	**22**	**35**	**59**
Flurtamone	97.9	95.7	84.8	63.4	30.1	14.8	4.7	2.5
U1	n.d.	0.6	0.6	0.6	1.4	0.7	1.0	0.9
U2	n.d.	0.3	0.3	0.3	0.3	0.1	0.1	0.1
U3	0.4	0.6	0.7	0.8	0.6	0.4	0.2	0.2
U4	0.3	0.2	0.4	0.4	0.4	0.3	0.1	0.1
Sum of minor mets	0.6	0.8	1.0	2.6	2.7	1.3	1.4	1.1
Total extractable	**99.2**	**98.2**	**87.4**	**68.1**	**35.4**	**17.6**	**7.6**	**4.9**
Carbon dioxide	n.a.	0.9	3.2	11.7	28.6	43.0	49.6	58.0
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	0.8	4.0	10.6	19.3	32.0	37.3	38.4	34.9
Total recovery	**100.0**	**103.1**	**101.3**	**99.2**	**96.0**	**97.9**	**95.6**	**97.8**

n.d. not detected n.a not analyzed

The data for flurtamone were evaluated according to FOCUS guidelines and the best-fit kinetic model was chosen on the basis of the chi-squared confidence criterion and visual assessment. The results are summarized in the following table.

Table 7.1.1.1-27 Flurtamone DT_{50} and DT_{90} values in soils under aerobic conditions

Soil	Soil Type	Kinetic Model	DT_{50} (d)	DT_{90} (d)	Visual Assessment	Chi^2 Error (%)
Laacher Hof AXXa	Loamy sand	SFO	10.3	34.2	Good	2.8
Dollendorf II	Loam	SFO	11.3	37.5	Good	1.7
Laacher Hof Wurmwiese	Sandy loam	SFO	9.4	31.3	Good	3.1
Hoefchen Am Hohenseh	Silt loam	SFO	8.5	28.4	Good	4.7

Conclusions

Flurtamone is rapidly degraded in soil under aerobic conditions, with a high degree of mineralization. All degradates are minor, most of them very minor. This reflects the rapidity of the degradation of the phenyl ring.

CA 7.1.1.2 - Anaerobic degradation

In an anaerobic study (Simmonds and Burn C.M., 1999, M-183875-01-1) conducted to the old EU guideline, in which treatment was made to an already anaerobic system, no significant degradation of flurtamone was observed. A new study, designed to meet current guidelines, was conducted and is presented below, along with a pilot study not previously available.

Report: **KCA 7.1.1.2/03; [redacted] B.V. 1991a**
Title: Flurtamone aerobic and anaerobic soil metabolism – Pilot Study
Organisation: [redacted]
Report No.: Chevron Chemical; Report No. – not given
Bayer CropScience Document M-249325-02-1
Publication: unpublished
Dates of experimental work: 1989-1991 (not stated in report)
Guidelines: **The study was conducted as a pilot study prior to conducting a full EPA study.**
Deviations: Not applicable
GLP/GEP **No**

Executive Summary

The route and rate of degradation of [^{14}C]-flurtamone, uniformly labelled in the trifluoromethylphenyl ring was investigated under anaerobic conditions in a US sandy loam soil (USDA classification). Each sample was treated with an aliquot (97 µL) of an ethanolic solution of radiolabelled flurtamone. The application rate was not stated. Treated soil samples were incubated in the dark under aerobic conditions for 30 days, at a moisture content equivalent to 75% of field capacity (⅓ bar) at 25 ºC. After 30 days air was displaced from the sealed flasks by the introduction of nitrogen. All flasks were incubated in the dark at 25 °C and anaerobic conditions were maintained by connection of each flask

to a low pressure nitrogen supply. The flasks were incubated under anaerobic conditions for a further 42 days (92 days in total).

The soil samples were extracted. The extracts were concentrated and examined by HPLC. Only parent and the metabolite M04 TFMBA were detected and it was concluded that no unique metabolites were formed under anaerobic conditions.

Material and Methods

The experimental details of the samples pertinent to the anaerobic portion of the study are summarized here while the remainder of the study is described under 7.1.1.1/01.

The route and rate of degradation [^{14}C]-flurtamone, uniformly labelled in the trifluoromethylphenyl ring was investigated under anaerobic conditions in a US sandy loam soil (USDA classification). The radiochemical purity and specific activity of the test item were 99.0% and 6.04 MBq/mg.

Treated soil samples were incubated in the dark under aerobic conditions for 30 days, at a moisture content equivalent to 75% of field capacity (⅓ bar) at 25 ºC. Soil samples were incubated under static conditions in biometer flasks equipped with traps for the collection of volatile organic compounds (polyurethane bung) and CO2 (sodium hydroxide). After 30 days air was displaced from the sealed flasks by the introduction of nitrogen. All flasks were incubated in the dark at 25 °C and anaerobic conditions were maintained by connection of each flask to a low pressure nitrogen supply. The flasks were incubated under anaerobic conditions for a further 42 days (92 days in total).

Treatment. Each sample was treated with an aliquot (97 µL) of an ethanolic solution of radiolabelled flurtamone. The application rate was not stated.

Sampling. Samples of soil were taken for analysis after 30 and 42 days anaerobic incubation. Samples from the 30 day time point were not reported.

Sample processing. The soil samples were extracted at ambient temperature three times with methanol followed by three further extractions with 10 mM calcium sulphate solution. Prior to adding methanol to the soil samples, 1M sodium hydrogen sulphate and water were added to improve extraction of the metabolite m04, detected under aerobic conditions. Extracts and post-extract soil residues were radioassayed.

Methanol extracts were pooled and concentrated by rotary evaporation prior to analysis with authentic reference standards by reverse phase high performance liquid chromatography (HPLC). The pH of methanol extracts was adjusted to pH 6 to 7 by the addition of concentrated sodium acetate prior to concentrating to prevent distillation of any TFMBA.

Quantitative analysis. Radioactivity extracted from soil and in the volatile traps was quantified by liquid scintillation counting (LSC) of aliquots. For determination of non-extractable residue, the soil residue remaining after completion of extractions was combusted and the trapped combustion gases were assayed by LSC.

Qualitative analysis. The HPLC system used comprised a Beckman Ultrasphere ODS-1P column connected to a UV Diode Array detector (set at 276 nm) and a Radiomatic Flo-One radiodetector (Beta model A-250) with a solid cell. The solvent system was a gradient of 1% glacial acetic acid in water and 20% acetonitrile in water. Retention times of standards were determined by UV detection.

Findings

In an experiment conducted with [^{14}C]-flurtamone, labelled in the trifluoromethylphenyl ring, only parent and the metabolite M04 TFMBA (AE C518919, RE 54488 in the report) were detected in soil incubated under anaerobic conditions for 42 days after 30 days of aerobic incubation.

Conclusion

No unique metabolites were formed under anaerobic conditions. Flurtamone and its aerobic soil metabolite, M04 TFMBA, are stable under anaerobic conditions.

Report:	**KCA-7.1.1.2 /04; [redacted]. 2012a**
Title:	[Trifluoromethylphenyl-UL-^{14}C]-Flurtamone: Anaerobic Degradation/Metabolism in One European Soil.
Organisation:	[redacted]
Report No.:	MEF-11/791 Bayer CropScience Document M-440634-01-1
Publication:	unpublished
Dates of experimental work:	9th November 2011 to 29th June 2012
Guidelines:	**OECD 307**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The route and rate of degradation of the herbicide flurtamone was investigated in one European soil under flooded anaerobic conditions following an aerobic incubation phase. The test item was applied to soil at a nominal rate of 100 µg/100 g soil (dry matter). Assuming a homogeneous distribution in 2.5 cm topsoil layer, this rate is equivalent to 375 g/ha field rate.

The test systems consisted of 300 mL glass Erlenmeyer flasks, each containing 100 g of soil (dry weight equivalent). During the aerobic study phase, air-permeable traps were attached for the collection of CO_2 and volatile organics (static test systems). At start of the anaerobic study phase, the traps for volatile components were replaced by sealable two-valve glass stoppers connected with plastic gas sampling bags.

Following application of [trifluoromethylphenyl-UL-^{14}C] labelled test item to soil the samples were incubated under aerobic conditions in the dark at about 20 °C and 55% of maximum water holding capacity. After 14 days of incubation the soil samples were flooded with oxygen-depleted, de-ionized water (ca. 3 cm layer above soil level) and set under an atmosphere of nitrogen. The water-logged samples were maintained under anaerobic conditions at approximately 20 °C in the dark for 120 days.

Duplicate test systems were analyzed after 0 and 14 days of aerobic incubation. Further samples were taken directly after water logging (day 14) and 17, 21, 29, 35, 48, 77, 104 and 134 days after treatment (DAT), corresponding to 0, 3, 7, 15, 21, 34, 63, 90 and 120 days after soil flooding (DASF). Soil and water layers were separated by decanting to allow for separate analysis of the phases with the water being analyzed directly. Afterwards the soil was extracted three times at ambient temperature followed by one microwave extraction step at about 70 °C.

Aliquots of the extracts were combined for HPLC-radiodetection profiling of the components. Flurtamone residues were radioassayed by reversed phase HPLC in water layers without prior concentration. The soil extracts were concentrated using a vacuum concentrator to about ¼ of their volumes for chromatographic profiling. Normal phase TLC was employed as confirmatory method for sample profiling. Characterization and identification of flurtamone residues and its transformation products M04 TFMBA and M05 TFA were achieved by spectroscopic methods (HPLC-MS, HPLC-MS/MS) and/or HPLC and TLC co-chromatography.

During the study the total recovery of radioactivity in the individual test flasks ranged from 92.8 to 98.2% of the AR. The complete material balances found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated. In the aerobic incubation phase, non-extractable radioactivity (NER) in soil increased from 1.3 to 25.1% of the AR (mean values). NER then varied between 23.4 and 26.5% of the AR until the end of the anaerobic (flooded) incubation period (mean values). During the aerobic phase, the maximum amount of $^{14}CO_2$ was 14.2% of the AR. Formation of other volatile radioactivity was insignificant (< 0.1% of the AR) in the aerobic and anaerobic incubation phase.

Within the aerobic phase of the study, the amount of the test item flurtamone in the entire test systems decreased rapidly from 93.9% to 25.4% of the AR (mean values). During the following anaerobic incubation period (i.e. flooded state) a slight decrease was observed. At the end of the study flurtamone accounted for 23.4% of the AR.

The experimental data of the anaerobic degradation of flurtamone could be well described by a first order multi-compartment model (FOMC, best fit). The anaerobic half-life of flurtamone was > 1000 days, associated with a Chi² error of 3.3%. The amounts of the transformation product M04 TFMBA in the entire system increased from 0.7% of the AR at DAT-0 to 23.2% of the AR during the aerobic incubation period and further to 32.3% of the AR towards study termination (mean values). The amounts of M05 TFA increased up to 1.6% of AR during the aerobic incubation period and remained at this low level during the anaerobic incubation period until study termination. With the TLC confirmation method similar amounts of M05 TFA were detected.

The total unidentified radioactivity in the entire systems reached values not higher than 6.1% of the AR. Maximum levels of individual unidentified minor transformation products in the entire system were not higher than 3.4% of the AR.

Materials and Methods

Test Material:

[Trifluoromethylphenyl-UL-^{14}C]-flurtamone, radiochemical purity > 98%, Batch no.: KML 9281

Test Design:

The metabolism of flurtamone in soil under anaerobic conditions (following a period of aerobicity) was investigated in one agricultural soil at 20°C during a period of anaerobic conditions. The soil characteristics are listed below. The soil was not extreme in its physico-chemical characteristics and the plant protection product history is known for the last 5 years. The test soil was freshly collected from the field by sampling the upper horizon 0-20 cm. The soil was passed through a 2 mm sieve and mixed thoroughly for optimal batch homogeneity. Soil moisture was determined by drying of aliquots at 105 °C and calculation of the mass difference.

Table 7.1.1.2-1 Properties of the soil used in a guideline flurtamone anaerobic soil study

Parameter	Value/result
Soil	Hoefchen am Hohenseh
Geographic Location[1]	Burscheid
Map Reference	N 51° 04.01' E 07° 06.33'
Taxonomic classification	Loamy, mixed, mesic Typic Argudalf
Textural Class (USDA)	Silt loam
Sand (%) Silt (%) Clay (%)	21 63 16
pH in $CaCl_2$ (1:2) pH in water (1:1) pH in KCl (1N)	6.1 6.7 5.8
Organic Matter (%) Organic Carbon (%)	3.4 2.0
Cation Exchange Capacity (meq/100g)	11.1
Water Holding Capacity at pF 2.5 (%)	20.9
Maximum water holding capacity (%)	54.8
Bulk Density (disturbed, g/cm³)	1.09
Soil Biomass at DAT-0: (microbial C/kg soil)	1449
Soil Biomass at DAT-14: (microbial C/kg soil)	908
Anaerobic plate counts (CFU/g soil) at DAT-134	$1.2 - 3.3 \times 10^4$

[1]: in North Rhine-Westphalia, Germany.

For study conduct, soil moisture was adjusted to 55% of maximum water holding capacity, by adding de-ionized water. Characterization of the soil microbial viability was achieved by (a) determination of soil microbial biomass during the aerobic incubation phase, and (b) by determination of anaerobic bacteria during the anaerobic incubation phase (plate count assay).

Biomass measurements were conducted at the beginning (DAT-0) and at the end (DAT-14) of the aerobic incubation period for untreated test systems and test systems treated only with the application solvent. Determinations of anaerobic bacteria were performed for an untreated test system and a test system treated with the application solvent at the end of the anaerobic incubation phase (DAT-134. corresponding to study end: DASF-120).

Ultrapure de-ionized water was used for flooding the soil of the test systems in the anaerobic incubation phase. To deplete dissolved oxygen, the water was de-oxygenated using nitrogen gas for 4 days before use.

For preparation of the test systems, about 120 g of sieved soil (equivalent to 100 g dry weight) were weighed into each Erlenmeyer flask. Moisture adjustment to about 55% maximum water holding capacity was achieved by adding 11.4 g of de-ionized water per flask. For preincubation, all flasks were closed with the trap attachments. After pre-incubation, all flasks were treated with test item.

At 14 days after test item application (DAT-14 = DASF-0), the trap attachments of all remaining test flasks were removed and stored for later analysis. The soil of each flask was flooded with about 150 mL of oxygen depleted de-ionized water leading to a water layer of approx. 3 cm above soil level. The flasks were then manually disturbed, equipped with sealable double-valve glass stoppers and

flushed with argon for 1 minute. The flasks were then connected to airtight plastic gas sampling bags, which had been flushed with nitrogen gas before. The valves were set to connect flask headspace and gas sampling bag, but closing the system from the outer atmosphere. Such setup allowed for pressure-less closed-flask incubation. To ensure maintenance of fully oxygen-free conditions, the test systems were placed in a nitrogen-flooded box within the incubation chamber.

During the aerobic phase each individual test flask was fitted with a separate trap attachment, in which soda lime and a polyurethane (PU) foam plug were contained as trapping media for carbon dioxide and organic volatile compounds, respectively. The assembly was permeable to atmospheric oxygen. Two separate layers of soda lime were used, allowing for the collection of $^{14}CO_2$ emanating from the flasks without interference from atmospheric CO_2. The soda lime pellets included an indicator dye, warning by colour change in case of CO_2 saturation of the pellets. During the anaerobic phase the test systems were closed with gas sampling bags which allowed for analysis of the gaseous headspace formed upon anaerobic incubation.

The test systems were treated with 364 µL of evenly distributed application solution per flask. Treatment was made as small droplets applied directly onto the soil surface using a micropipette. Finally, the test systems were weighed, fitted with the volatiles trap attachments, and placed back into the incubation chamber. Biomass and anaerobic bacteria determination test systems were either left untreated or dosed with pure application solvent. For the determination of the actual study application rate and the application homogeneity, aliquots were dosed into 10 mL volumetric flasks before, during and at the end of application.

The test systems were placed in a dark, temperature controlled incubation chamber set to 20°C (±2°C) and 55 ± 5% of the maximum water holding capacity (MWHC) target test conditions. Temperature readings were recorded by an electronic data logger system. For the aerobic phase, aerobic conditions were maintained by passive diffusion of atmospheric oxygen through the volatiles trap attachments. For the anaerobic phase anaerobic conditions were maintained by the nitrogen gas atmosphere in the closed test flasks. In addition, before closing the test systems on DAT-14 (= DASF-0), the flooded soil in the closed test flasks was flushed vigorously with argon gas for 1 minute. To ensure absence of any interfering oxygen, the entire test systems were placed into a continuously nitrogen-flooded box in the incubation chamber.

For the aerobic incubation phase, test systems were processed at two sampling time points, DAT-0 and DAT-14. For the anaerobic incubation phase samples were collected at 9 dates, namely DAT-14, 17, 21, 29, 35, 48, 77, 104 and 134, respectively, corresponding to 0, 3, 7, 15, 21, 34, 63, 90 and 120 days after soil flooding (DASF). At each sampling interval, duplicate samples were processed and analyzed. The respective flasks were removed from the incubation chamber and processed.

For aerobic phase samples, flask and volatile traps were separated. Prior to opening an incubated test vessels for processing of soil, volatile compounds possibly still present in the head space of the vessel were purged into the trap attachments. Afterwards, the trap attachments were removed and the soil of each vessel was extracted completely. Soil was extracted immediately. No storage stability experiments were therefore conducted for storage of non-extracted soil.

After collection of the respective test systems from the incubation box, they were connected to a volatiles combustion oven unit. Volatiles present in the headspace of the test system and gas sampling bag were slowly purged using a stream of nitrogen over a soda lime trap for absorption of $^{14}CO_2$, through the catalytic oven for oxidative combustion of organic volatiles (e.g. methane), and finally

through three liquid scintillation flasks filled with alkaline LSC cocktail, for absorption of $^{14}CO_2$ from combustion exhaust. Afterwards, the scintillation cocktails were directly analyzed by LSC. Next, the test flasks were opened, and oxygen content of the water layer, redox potential of water layer and of soil layer, and pH of the water layer were immediately determined by electrode measurements. The water layer was separated from the soil layer by careful decanting. For removal of suspended particles, the decanted water layers were centrifuged. After determination of the volume, the now clear supernatants were subjected to LSC and, after an additional centrifugation step, to HPLC-radiodetection analysis without concentration. The centrifugation pellets from the first centrifugation step were added to the soil phases by later re-use of the centrifuge flasks for the extraction of the respective soil layers.

The entire amount of soil per incubation flask was transferred into a screw-cap centrifuge beaker using a first portion of acetonitrile/water (4:1, v/v). Two ambient extractions were done by cycles of vigorously shaking for about 30 minutes on a mechanical shaker at ambient temperature followed by centrifugation. The supernatants were decanted and made up to a standard volume with extraction solvent. After microwave extraction with acetonitrile/water (1:1, v/v) and centrifugation, the supernatants of the microwave extracts were decanted into a graduated cylinder which was made up to a standard volume with extraction solvent. Volume and radioactivity content were determined separately by LSC for each of the two extracts.

Proportional aliquots of the extracts from each sample were combined and concentrated by use of a vacuum concentrator. The volume of each concentrate was determined and aliquots thereof were analyzed by LSC to determine the concentration recovery. The extracted soil samples were lyophilized and homogenized by grinding them to powder in a mill prior to combustion analysis. Sub-samples of the final time-point soils were subjected to a fractionation procedure for humic soil substances.

The PU foam plugs intended to trap organic volatiles were extracted with 50 mL ethyl acetate each, under sonication for 30 min. Aliquots of the extracts were radioassayed by LSC. The radioactivity (i.e. $^{14}CO_2$) absorbed to the soda lime was liberated with aqueous HCl and purged into liquid scintillation cocktails with nitrogen. For this purpose aqueous HCl was added drop-wise to the 100-mL Erlenmeyer flask containing the soda lime, and liberated $^{14}CO_2$ was carried by a stream of nitrogen for about 30 minutes whilst stirring. The $^{14}CO_2$ was absorbed in a series of three vials each filled with ice-cooled scintillation cocktail which were afterwards subjected to LSC.

Reversed-phase HPLC served as the profiling method for the quantification of the test item and its transformation products in both, water layer and soil extracts. In addition, HPLC chromatography was used for the verification of the main transformation product and for the determination of the radiochemical purity of the test item in the application solution.

The system comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm; 5 µm column connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. The quantification of the test item and its degradation products in the extracts was calculated based on the distribution of the HPLC - zones and the amount of RA in the extracts. Column recovery checks were conducted.

TLC was used within this study as confirmatory method and for transformation product verification purposes. Aliquots of the solutions were spotted onto silica gel (Merck Si60) plates as bands using an automatic TLC applicator. These were developed in ethyl acetate/2-propanol/water (75/20/5, v/v/v) .

Reference items were spotted onto the plates (200 mm x 200 mm, thickness 0.20 mm) overlapping with the radioactive bands. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer and quantified with the software package AIDA (Version 4.14, Raytest). Non-radiolabelled reference items on the plates were visualized in the UV-cabinet at a wavelength of 254 nm. The ROIs were selected manually according to the positions of the spots or bands in the chromatogram.

The electro-spray ionization MS spectra (ESI) were obtained with a Q-Exactive mass spectrometer (Thermo, San Jose, CA, USA). The HPLC instrument used for chromatography was an Agilent HP1100 with a Nucleodur C18 Gravity, 3 µm, 250 x 2 mm (MN) column. The mobile phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

Findings

The mean study incubation temperature was 19.7 °C (max: 20.4 °C; min: 18.9 °C). Oxygen content in the water layer decreased during the study from 6.7 and 5.3 mg/L at DASF-0 to 0.6 and 0.8 mg/L at DASF-120 demonstrating the shift to anaerobic conditions. Redox potential measurements indicated reducing conditions in the soil layer and in the water layer from DASF-21 of the study onwards. The pH values increased from DASF-0 from values around pH 7.2 and 7.4 until study end to values around pH 7.9. The values for each of these parameters at times during the study are shown in the table overleaf.

Microbial biomass of the test soil was 1402 mg microbial C/kg for untreated soil and 1419 mg microbial C/kg for soil treated with the application solvent. At the end of the aerobic incubation phase (DAT-14), biomass was 909 mg microbial C/kg for untreated soil and 908 mg microbial C/kg for soil treated with the application solvent. These biomass values indicate a good viability of the soil. Anaerobic bacteria plate count assays performed at DASF-120 showed the presence of at least about 12000 colony-forming units per gram of soil dry weight for untreated soil or soil treated with application solvent. This confirms the establishment of an anaerobic microflora in the test systems.

Table 7.1.1.2-2 Redox potential, oxygen content and pH of test system

DAT	DASF	Sample	Water Layer					Soil		Buffer
			O_2		Redox E_{obs}	Redox E_H	pH	Redox Eobs	Redox EH	Redox Eobs
			(%)	(mg/L)	(mV)	(mV)		(mV)	(mV)	(mV)
0	n/a	1	n/a					n/a		n/a
		2								
14	n/a	1	n/a					n/a		n/a
		2								
14	0	1	62	5.3	170	376	7.4	190	396	224
		2	53	4.7	170	376	7.2	185	391	
17	3	1	41	3.6	181	386	7.1	215	420	224
		2	48	4.1	173	378	6.9	205	410	
21	7	1	29	2.6	156	362	7.2	202	408	224
		2	27	2.4	167	373	7.2	161	367	
29	18	1	30	2.6	183	389	7.3	151	155	224
		2	26	2.4	169	375	7.3	139	167	
35	21	1	26	2.4	92	153	7.3	-123	79	224
		2	26	2.3	-109	97	7.3	-106	45	
49	34	1	20	1.8	-150	56	7.4	-186	21	224
		2	19	1.7	-140	62	7.4	-196	10	
77	63	1	9	0.8	-140	63	7.7	-179	24	227
		2	9	0.8	-129	74	7.8	-174	29	
104	90	1	16	1.4	-67	135	7.7	-156	46	228
		2	8	0.7	-132	70	7.7	-164	38	
134	120	1	7	0.6	-111	80	7.9	-182	8	240
		2	8	0.8	-126	64	7.9	-186	4	
		min	7	0.6	-150	56	6.9	-196	4	224
		max	62	5.3	183	389	7.9	215	420	240
		mean	26	2.2	112	215	7.4	-36	167	227

The extraction efficiency from DAT-0 samples was 99.6% of the AR. The stability of the test item during processing was verified by a mean purity of 99.2% in the combined soil extracts at DAT-0. These results indicated that the extraction method was very suitable for the extraction of the applied [^{14}C]-labelled flurtamone from the soil matrix. All combined soil extracts were concentrated prior to chromatographic profiling. The concentration recovery was determined for all samples and varied between 92.7 and 119.9% (mean 103.4%). No signs of artifact formation were observed upon processing and sample analysis.

The HPLC system used provided good separation and quantification of test item and its degradation products. The HPLC mean recoveries for a representative water layer and soil extract were 100.2 and 101.4%, respectively. In addition, the radiochemical purity of the test item in the application solution was verified by HPLC. The LOD was 0.64% of the AR, the LOQ 1.91% of the AR. The LOD for the normal phase TLC used as the confirmatory method was 0.30% of the AR, the LOQ 0.91% of the AR.

The achieved application rate was equivalent to about 95.8% of the intended value corresponding to the maximum field application rate. The recovery and distribution of radioactivity is summarized in the table below. During the aerobic phase, the maximum amount of $^{14}CO_2$ was 11.2% of the AR. Formation of other volatile radioactivity was insignificant (< 0.1% of the AR) in the aerobic and

anaerobic incubation phases. The total extractable radioactivity decreased from 94.6% of the AR at DAT-0 to 36.8% of the AR at DAT-134. The radioactivity in the water layer increased from 12.5% AR to 23.2% AR at DAT-77 (DASF-63) and then decreased slightly to 22.6% AR at the end of the study. Total recoveries ranged from 93.6% AR to 97.2% AR.

Table 7.1.1.2-3 Recovery and distribution of applied radioactivity (mean values) in Hoefchen Am Hohensee soil in an anaerobic soil study (aerobic phase followed by anaerobic phase)

Fraction/Phase	% applied radioactivity(mean values) at these days after treatment/soil flooding										
	0	**14**	**14/0**	**17/3**	**21/7**	**29/15**	**35/21**	**48/34**	**77/63**	**104/90**	**134/120**
Carbon dioxide - Aerobic	n.a	11.2	10.6	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Organic volatile - Aerobic	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles - Aerobic	**n.a**	**11.2**	**10.6**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**
Carbon dioxide - Anaerobic	n.a	n.a	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Organic volatile - Anaerobic	n.a	n.a	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles - Anaerobic	**n.a**	**n.a**	**n.a**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
Carbon dioxide – Total	n.a	11.2	10.6	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Organic volatile - Total	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles - Both	**n.a**	**11.2**	**10.6**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**	**10.9**
Water layer - Anaerobic	n.a	n.a	12.5	19.8	19.5	19.8	20.9	21.1	23.2	22.8	22.6
Ambient extract - Both	91.8	42.7	34.7	30.2	37.9	36.4	36.6	33.6	33.7	36.3	34.2
Microwave extract - Both	2.8	4.6	3.4	3.1	3.4	3.2	2.9	2.8	2.8	3.0	2.7
Total extractable - Both	94.6	57.3	48.1	42.4	41.3	38.5	39.6	36.4	36.5	38.3	36.8
Total extractable + water	**94.6**	**57.3**	**60.6**	**62.1**	**60.8**	**58.4**	**30.6**	**57.5**	**59.8**	**61.1**	**59.4**
Non-extractable	**1.3**	**25.1**	**23.7**	**23.4**	**24.9**	**25.1**	**24.7**	**26.5**	**25.8**	**25.2**	**26.3**
Total recovery	**95.9**	**93.6**	**94.8**	**96.4**	**96.5**	**94.4**	**96.1**	**95.0**	**96.5**	**97.2**	**95.6**

n.a not analyzed

The numerical results of the analytical investigations for the entire system concerning the degradation of the test item and formation and decline of transformation products are summarized in the table below. Under unidentified radioactivity, all very minor non-characterized peaks plus diffuse radioactivity were summed.

Table 7.1.1.2-4 Biotransformation of flurtamone in Hoefchen Am Hohensee soil under aerobic then anaerobic conditions

Compound	% applied radioactivity(mean values) at these days after treatment/soil flooding:										
	0	**14**	**14/0**	**17/3**	**21/7**	**29/15**	**35/21**	**48/34**	**77/63**	**104/90**	**134/120**
Flurtamone	93.9	25.4	28.3	27.2	27.6	24.1	26.6	24.2	22.7	24.7	23.4
M04 TFMBA	0.7	23.2	24.8	26.7	26.7	27.6	27.6	25.9	28.1	27.7	32.3
M05 TFA	n.d.	1.6	0.8	1.2	1.0	0.9	< LOD	0.8	1.5	1.0	< LOD
Unid./Diffuse radioactivity	n.d.	5.8	4.4	6.1	3.1	2.8	3.2	5.0	4.2	4.7	< LOD
Total extractable residues	94.6	56.0	58.2	61.2	58.5	55.3	57.8	55.9	56.5	58.0	57.7
Carbon dioxide	n.a.	11.2	10.6	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues	1.3	25.1	23.7	23.4	24.9	25.1	24.7	26.5	25.8	25.2	26.3
Total recovery	**95.9**	**93.6**	**94.8**	**96.4**	**96.5**	**94.4**	**96.1**	**95.0**	**96.5**	**97.2**	**95.6**

n.a not analyzed n.d. not detected , < LOD = less than limit of detection

The chemical identity of the radiolabelled test item was confirmed by 1H-NMR spectroscopy, HPLC-MS, HPLC-MS/MS (ESI positive) analysis including accurate mass determination within the application solution. The radiochemical purity was verified in the application solution by HPLC-radiodetection. In the water phases and soil extracts, the test item was identified by TLC co-chromatography using the non-labelled reference item. Within the aerobic phase of the study, the amount of the test item flurtamone in the entire test systems decreased rapidly from 93.9% to 25.4% of the AR (mean values). During the following anaerobic incubation period (i.e. flooded state) a slight decrease was observed. At the end of the study flurtamone accounted for 23.4% of the AR.

M04 TFMBA was identified by spectroscopic methods (HPLC-MS, HPLC-MS/MS). For verification, the isolated radioactive zone was also used for allocating the peak by HPLC co-chromatography. The identity of M04 TFMBA in the water phases and soil extracts was furthermore confirmed by TLC co-chromatography using the non-labelled reference item. The amounts of the transformation product M04 TFMBA in the entire system increased from 0.7% of the AR at DAT-0 to 23.2% of the AR during the aerobic incubation period and further to 32.3% of the AR towards study termination (mean values).

M05 TFA was identified by HPLC co-chromatography in a water layer sampled at DAT-29. In TLC, M05 TFA was identified by comparing its Rf value with the Rf value of the radiolabelled reference item ^{14}C-M05 TFA. The amounts of M05 TFA increased up to 1.6% of AR during the aerobic incubation period and remained at this low level during the anaerobic incubation period until study termination. With the TLC confirmation method similar amounts of M05 TFA were detected.

The total unidentified radioactivity in the entire systems reached values not higher than 6.1% of the AR. Maximum levels of individual unidentified minor transformation products in the entire system were not higher than 3.4% of the AR.

In the aerobic incubation phase, NER in soil increased from 1.9% to 25.1% of the AR (mean values). NER remained then on a similar level until the end of the anaerobic (flooded) incubation period when they accounted for 25.3% of AR. Chemical characterization of the NER was performed by organic matter fractionation after disintegration under excessive alkaline conditions. Partitioning of NER (25.5 and 25.1% of the AR) into humic acid, fulvic acid, and humin like fractions was observed. 28.3-30.0% of the NER was attributed to the humic acid fraction, 29.2-29.4% of the NER to the fulvic acid fraction and 44.1-45.7% of the NER to the humin substance fraction.

The calculated DT_{50} value of Flurtamone in the entire system for the anaerobic phase was > 1000 days. The kinetic "First Order Multi Compartment" model (FOMC) was the most suitable as indicated by visual assessment and the lowest Chi^2 Error % value. The following table summarizes the results of all DT_{50} and DT_{90} calculations.

Table 7.1.1.2-5 Flurtamone degradation kinetics in Hoefchen am Hohenseh soil under anaerobic conditions

Kinetic Model	Visual Assessment	Chi^2 error (%)	DT_{50} (d)	DT_{90} (d)
SFO	Good	4.1	488	> 1000
FOMC	**Good**	**3.3**	**> 1000**	**> 1000**
DFOP	Good	3.3	> 1000	> 1000

Conclusions

In soil under anaerobic conditions flurtamone is essentially stable. Its main aerobic soil metabolites M04 TFMBA and M05 TFA are also stable under the same conditions. As a result no new metabolites (i.e. additional to those identified in aerobic soil studies) are formed.

CA 7.1.1.3 - Soil photolysis

In the original soil photolysis study ([redacted], 1993, M-162193-01-1) the degradation rate of flurtamone was slightly enhanced in the presence of light. M04 TFMBA and M05 TFA, known soil metabolites, were detected in minor quantities at maximum occurrence of 3.8% and 1.4%, respectively. No unique photodegradation products were detected. These results confirmed those of an earlier study ([redacted], 1990, M-276959-01-1) that did not to conform to current guidelines but also showed that the rate of degradation was increased by light (in this case natural sunlight) but without the formation of unique photodegradates. The study of Lawrence and Kesterson was conducted with the compound labelled in one ring only (the TFMP ring) so a new study was carried out with the label in the phenyl ring. This is presented below.

Report:	**KCA-7.1.1.3 /01 [redacted] 2012c**
Title:	[Phenyl-UL-^{14}C]-flurtamone: Phototransformation on Soil.
Organisation:	[redacted]
Report No.:	EnSa-12-0650 Bayer CropScience Document M-443626-01-1
Publication:	unpublished
Dates of experimental work:	5th March 2012 to 2nd May 2012
Guidelines:	**OECD Draft Guideline: Phototransformation of Chemicals on Soil Surfaces, US EPA OCSPP Test Guideline No. 835.2410, DRAFT SANCO 11802/2010/rev 1**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The biotransformation of [phenyl-UL-^{14}C]-flurtamone was studied on a European silt loam soil at 20±1°C and a soil moisture of about 55% of the maximum water holding capacity. Flurtamone was applied at a nominal rate corresponding to a field use rate of 250 g flurtamone/ha.

The test system consisted of glass vessels filled with 3 g soil (dry weight) with a surface of 10.2 cm^2. The vessels were connected to traps for the collection of CO_2 and organic volatiles. The samples were continuously exposed to artificial irradiation (xenon lamp with < 290 nm cut-off filter, 643 W m^{-2}). In addition, dark controls were set up. Samples were taken in duplicate after 0, 0.25, 1, 2, 3, 5 and 6 days of incubation. At each sampling date, the soil samples were extracted three times at ambient temperature and once by hot (microwave) extraction. Combined organic soil extracts were concentrated and analyzed by HPLC to quantify the test item as well as its transformation products. Identification was by use of HPLC-MS and HPLC-MS/MS, as well as by HPLC co-chromatography with certified reference compounds.

The mean material mass balances in the irradiated and dark samples were 99.2% (RSD: 2.5%) and 99.7% (RSD: 2.1%) of the applied radioactivity (AR), respectively. For irradiated test systems, the extractable radioactivity decreased from 103.5% AR at DAT-0 to 61.1% AR towards the end of the

incubation period (DAT-6). Non-extractable residues (NER) accounted for 0.2% AR at DAT-0 and increased up to 18.5% AR until DAT-6. For dark test systems, the extractable radioactivity decreased from 103.5% AR at DAT-0 to 89.5% AR until DAT-6. NER accounted for 0.2% AR at DAT-0 and increased up to 7.7% AR towards the end of the study.

In the irradiated test systems, the amount of flurtamone decreased from an average of 100.2% AR at DAT-0 to 38.6% AR towards the end of the study (DAT-6). One major (> 5% AR at consecutive time-points) transformation product was detected and identified as M06 benzoic acid. It reached a maximum amount of 7.2% AR at DAT-5. In addition, up to 12 minor transformation products were characterized according to their retention times. Each individual one accounted for ≤ 3.2% AR. $^{14}CO_2$ formation increased up to 17.6% AR towards the end of the study. Organic volatile formation was negligible (≤ 0.1% AR).

In the extracts of the dark test systems, flurtamone decreased from an average of 100.2% AR at DAT-0 to 86.2% AR towards the end of the incubation period (DAT-6). In addition, up to 13 minor transformation products were characterized according to their retention times. Each individual one accounted for ≤ 1.1% AR. $^{14}CO_2$ formation increased up to 3.2% AR towards the end of the study. Organic volatile formation was negligible (≤ 0.1% AR).

The experimental DT_{50} values of flurtamone in the irradiated and dark samples were 4.1 and 28.5 days, respectively, according to single first order kinetics. The comparatively long half-life for flurtamone in dark samples may be due to the application technique and a higher concentration on soil. Based on the experimental DT_{50} value of 4.1 days for irradiated samples, the DT_{50} of flurtamone under environmental conditions is calculated to be e.g. 22.7 solar summer days at London, Great Britain, or 18.1 solar summer days at Athens, Greece.

Phototransformation on soil can contribute to the degradation of flurtamone under outdoor conditions. Besides carbon dioxide, M06 benzoic acid was found as a major product in the irradiated samples but not in the dark controls where it was rapidly degraded.

Materials and Methods

Test Material:

[Phenyl-UL-^{14}C]-flurtamone, radiochemical purity > 99%, Batch no.: KML 9146

Test Design:

The degradation of flurtamone on an irradiated soil surface was investigated in an agricultural soil, not treated with any pesticide for five years, at 20°C. The soil characteristics are listed overleaf. The test soil was air dried until sieving was possible, broken up and then successively sieved to ≤ 10, 5, 3.35 and 2 mm. The sieved soil was filled in a plastic bag and stored at 4-8°C until further use. The test soil was successively sieved to a particle size < 2 mm prior to use. Microbial biomass was determined with untreated soil sampled prior to the incubation period. The microbial biomass determinations show that the soil used in this study was viable.

The test systems consisted of quartz glass vessels (36 mm inner diameter, 35 mm height, inner surface area 10.2 cm2) each containing 3 g of soil (dry weight), which provided about 3 mm soil depth. A glass neck with ground joint was attached to the side of the wall. There, the flask was connected a solid trap attachment, which comprised a small glass tube of 90 mm length and 12 mm inner diameter, in which volatile compounds were bound to soda lime and polyurethane foam. The quartz glass cover

(thickness 3 mm) was sealed with metallic clips and the ground glass joint was covered with a rubber septum. Two treated test systems were analyzed prior to the start of incubation. At each subsequent sampling interval, duplicate treated test systems were analyzed for both irradiated and dark test systems (duplicate irradiated and dark test systems were reserved as spares).

The test systems containing 3.9 g of moist soil adjusted to 55% of the maximum water holding capacity were treated with 50 µL of the application solution using a pipette. The solution was applied evenly as drops across the surface of the soil. All vessels were left unsealed for 15 min to facilitate the evaporation of methanol. Then, the DAT-0 samples were immediately processed. The samples to be exposed to irradiation as well as the dark controls were closed with a quartz-glass cover, weighed and fitted with trap attachments.

Table 7.1.1.3-1 Properties of the soil used in a guideline flurtamone soil photolysis study

Parameter	Value/result
Soil	Hoefchen am Hohenloh 4a
Geographic Location[1]	Burscheid
Map Reference	N 51° 04.00' E 07° 06.30'
Taxonomic classification	Loamy, mixed, mesic Typic Argudalf
Textural Class (USDA)	Silt loam
Sand (%) Silt (%) Clay (%)	22 50 19
pH in CaCl2 (1:2) pH in water (1:1) pH in water (saturated paste) pH in KCl (1N)	6.3 6.8 6.6 6.0
Organic Matter (%) Organic Carbon (%)	2.9 1.7
Cation Exchange Capacity (meq/100g)	10.9
Water Holding Capacity at pF 2.5 (%)	19.3
Bulk Density (disturbed, g/cm³)	1.13
Soil Biomass: (microbial C/kg soil)	666.1

[1] in North Rhine-Westphalia, Germany.

The photolysis vessels were placed in a Suntest unit (Heraeus) containing a xenon lamp simulating natural sunlight. The light emission was filtered with a 290 nm cut-off UV-filter, which eliminated all wavelengths < 290 nm. The temperature inside the Suntest unit was maintained by a cooling plate connected to a refrigerated circulating chiller. The temperature was monitored by a data logger and the intensity of the xenon lamp was monitored at the beginning and the end of the overall test period using an irradiance monitor. The dark test systems were maintained in the dark at a constant temperature in a walk-in climatic chamber. The temperature of the dark test systems was recorded with a thermistor thermocouple connected to a data logger.

The radiation intensity and exposure time under experimental conditions can be related to natural solar radiation at e.g. Athens, Greece, representing extraordinary conditions in Europe.

Duplicates of both irradiated and dark soil samples were processed after 0.25, 1, 2, 3, 5 and 6 days of incubation. The soil samples were extracted on the day of sampling, except for the samples collected at DAT-3. These samples were deep-frozen together with the first extraction solvent and worked up 2 days afterwards. For soil extraction the entire soil amount of each test vessel was transferred in a centrifuge beaker and extracted using a mechanical shaker. The extraction procedure comprised an extraction with 100 mL acetonitrile/water 80/20 (v/v) followed by two extractions with 80 mL acetonitrile/water 80/20 (v/v) both at ambient temperature. These extracts were combined and radioassayed. . The final extraction used 80 mL acetonitrile/water 50/50 (v/v) with microwave heating to 70°C. This was then radioassayed.

All extracts were analyzed by LSC and the first chromatographic method (HPLC) at least within three days after sampling and then stored in a freezer. Samples for CO_2 were stored at ambient conditions after sampling and analyzed within 42 days. The PU-foams were extracted and analyzed within 41 days after sampling. The extracted soil samples were stored deep-frozen before they were freeze-dried and combusted within 19 days.

Volatile organic compounds possibly contained in the foam plugs were extracted by shaking with ethyl acetate. Aliquots of the extracts were submitted to LSC measurement. Chromatographic analyses of the PU foam extracts were not performed, because they contained < 0.1% of the AR in all test systems.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in hydrochloric acid. The liberated CO_2 was absorbed by a special absorption/ scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Prior to chromatographic analysis, the cold organic extracts and the microwave extract were combined and concentrated using a vacuum concentrator. The concentrate was diluted with acetonitrile/water (80/20, v/v), sonicated, centrifuged and weighed. Aliquots of the concentrates were analyzed by HPLC and the concentrated water layers sampled at DAT-0 and DAT-6 were additionally analyzed by TLC (irradiated and dark samples). For all sampling intervals, aliquots of the concentrates were analyzed by LSC to determine the recoveries of radioactivity after concentration.

The HPLC method to separate and quantify the test item and its metabolites used a system that comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm; 5 µm column connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. The assignment of the HPLC peak to the test item in the application solution was done by co-chromatography using the [^{12}C]-reference substance. Within routine chromatograms of the concentrated combined extracts, comparison of retention times in different chromatograms was used for the assignment of the HPLC peaks to test item and the transformation products. The quantification of the test item and its degradation products in the extracts was calculated based on the distribution of the HPLC - zones and the amount of RA in the extracts.

The electro-spray ionization MS spectra (ESI) were obtained with a Q Exactive mass spectrometer (Thermo, San Jose, CA, U.S.A.). The HPLC instrument used for chromatography was an Agilent HP1100 with Nucleodur C18 Gravity, 3 or 5 µm, 250 x 2 mm (MN) columns. The mobile phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

For TLC analysis aliquots of the concentrates of the extracts were spotted on silica gel plates (Si60, F254, 20 cm x 20 cm Merck) using an automatic applicator. The plates were developed with ethyl acetate/2-Propanol/water (75/20/5, v/v/v) in a plate chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products in the extracts was calculated based on the distribution of the TLC-zones and the amount of radioactivity in the extracts. Regions of the non-labelled reference items were detected by observation of UV 254 nm background fluorescence inhibition.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in hydrochloric acid. The liberated CO_2 was absorbed by a special absorption/ scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Findings

The average DAT-0 extraction efficiency was 103.5% of applied radioactivity (AR). HPLC analysis of the DAT-0 extracts indicated that there was only very minor degradation of the test item during sample extraction or processing.. These results demonstrate that the extraction method was well suitable to extract the compound from the soil matrix. The recoveries of radioactivity after the concentration step were exemplarily determined and averaged 98.2% and 98.6% for irradiated and dark control samples, respectively.

A good selectivity and reproducibility demonstrated the suitability for separation and quantification of the HPLC method. The HPLC limit of quantification (LOQ) for a single peak in the combined organic extracts was < 1% of radioactivity applied to the plate (0.46% AR). The HPLC recovery-checks gave recoveries of 97.6 to 100.6% for irradiated and dark control samples, respectively.

For irradiated test systems, the average material balances ranged from 95.4 to 103.7% and for dark control test systems, the average material balances ranged from 96.6 to 103.7% AR. For irradiated test systems, the extractable radioactivity decreased from 103.5% at DAT-0 to 61.1% towards the end of the incubation period (DAT-6). Non-extractable residues (NER) accounted for 0.2% AR at DAT-0 and increased up to 18.5% AR until DAT-6. $^{14}CO_2$ formation increased up to 17.6% AR towards the end of the study. Organic volatile formation was negligible throughout the study (≤ 0.1% AR).

For dark test systems, the extractable radioactivity decreased from 103.5% AR at DAT-0 to 89.5% AR until DAT-6. NER accounted for 0.2% AR at DAT-0 and increased up to 7.7% AR until the end of the study. $^{14}CO_2$ formation increased up to 3.2% AR towards the end of the study. Organic volatile formation was negligible throughout the study (≤ 0.1% AR).

The recovery and distribution of applied radioactivity are shown in the following tables.

Table 7.1.1.3-2 Recovery and distribution of applied radioactivity in irradiated samples

Fraction	% applied radioactivity at days after treatment:						
	0	**0.25**	**1**	**2**	**3**	**5**	**6**
Carbon dioxide	n.a.	0.9	3.4	5.4	6.6	13.0	17.6
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**0.9**	**3.4**	**5.4**	**6.6**	**13.0**	**17.6**
Ambient extract	102.6	94.7	87.9	80.1	72.6	60.2	57.1
Microwave extract	0.9	2.0	2.5	3.1	1.8	4.6	3.7
Total extractable	**103.5**	**96.7**	**90.3**	**83.2**	**80.4**	**64.8**	**61.1**
Non-extractable	**0.2**	**3.2**	**6.6**	**9.7**	**11.9**	**17.6**	**18.5**
Total recovery	**103.7**	**100.9**	**100.3**	**98.2**	**98.6**	**95.4**	**97.2**

n.a not analyzed

Table 7.1.1.3-3 Recovery and distribution of applied radioactivity in non-irradiated samples

Fraction	% applied radioactivity at days after treatment:						
	0	**0.25**	**1**	**2**	**3**	**5**	**6**
Carbon dioxide	n.a.	0.3	0.8	1.2	1.5	2.7	3.2
Organic volatiles	n.a.	< 0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**0.3**	**0.8**	**1.2**	**1.5**	**2.7**	**3.2**
Ambient extract	102.6	96.9	92.1	92.8	92.5	86.4	87.5
Microwave extract	0.9	1.4	1.4	1.1	1.0	2.8	2.0
Total extractable	**103.5**	**98.3**	**93.5**	**94.0**	**93.5**	**89.2**	**89.5**
Non-extractable	**0.2**	**1.3**	**2.3**	**4.0**	**5.4**	**5.9**	**7.7**
Total recovery	**103.7**	**99.9**	**96.6**	**99.2**	**100.4**	**97.8**	**100.4**

n.a not analyzed

In the irradiated test systems, the amount of flurtamone decreased from an average of 100.2% AR at DAT-0 to 38.6% AR towards the end of the study (DAT-6). The degradation behaviour in the individual test vessels was scattering as expected for soil photolysis. One transformation product was detected twice with 5% AR and was identified as M06 benzoic acid. It reached a maximum amount of 7.2% AR at DAT-5. In addition, up to 12 minor transformation products were characterized according to their retention times. Each individual one accounted for ≤ 3.2% AR.

In the extracts of the dark test systems, flurtamone decreased from an average of 100.2% AR at DAT-0 to 86.2% AR towards the end of the incubation period (DAT-6). In addition, up to 13 minor transformation products were characterized according to their retention times. Each individual one accounted for ≤ 1.1% AR. Obviously the benzoic acid was either not formed or was so rapidly degraded compared to its formation rate that it did not reach trigger levels.

Table 7.1.1.3-4 Degradation of flurtamone in irradiated samples

Compound	% applied radioactivity at days after treatment:						
	0	**0.25**	**1**	**2**	**3**	**5**	**6**
Flurtamone	100.2	91.3	78.7	63.8	65.4	38.2	38.6
M06 Benzoic acid	1.0	0.7	2.6	4.9	3.7	7.2	5.8
Sum of minor mets	2.3	4.7	9.0	14.5	11.2	10.4	16.7
Total extractable	**103.5**	**96.7**	**90.3**	**83.2**	**80.4**	**64.8**	**61.1**
Carbon dioxide	n.a.	0.9	3.4	6.4	6.6	13.0	17.6
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	0.2	3.2	6.6	9.7	11.9	17.6	18.5
Total recovery	**103.7**	**100.9**	**100.3**	**98.2**	**98.6**	**95.4**	**97.2**

n.a not analyzed

Table 7.1.1.3-5 Degradation of flurtamone in non-irradiated samples

Compound	% applied radioactivity at days after treatment:						
	0	**0.25**	**1**	**2**	**3**	**5**	**6**
Flurtamone	100.2	95.2	89.4	90.4	89.8	85.3	86.2
Sum of minor mets	3.3	2.9	4.0	3.4	3.6	3.9	3.2
Total extractable	**103.5**	**98.2**	**93.4**	**93.9**	**93.4**	**89.2**	**89.4**
Carbon dioxide	n.a.	0.3	0.8	1.2	1.5	2.7	3.2
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	0.2	1.3	2.3	4.0	5.4	5.9	7.7
Total recovery	**103.7**	**99.8**	**96.5**	**99.1**	**100.4**	**97.7**	**100.3**

n.a not analyzed

The data for flurtamone were evaluated according to FOCUS guidelines and the best-fit kinetic model was chosen on the basis of the chi-squared confidence criterion and visual assessment. The results are summarized in the following table.

Table 7.1.1.3-6 Flurtamone DT_{50} and DT_{90} values in soils under aerobic conditions

Irradiated/Non-irradiated	Kinetic Model	Chi Error (%)	DT_{50} (d)	DT_{90} (d)	Rate constant (d^{-1})	Net Phototransformation Rate constant1/Half-life
Irradiated	SFO	2.8	4.1	13.1	0.17042	0.14606 days-1/4.7 days
Non-irradiated	SFO	0	28.5	94.5	0.02436	

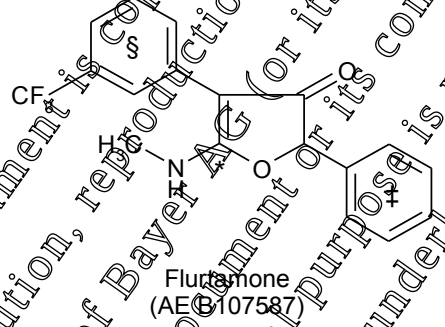
Flurtamone was degraded in dark and irradiated samples with experimental DT_{50} values of 28.5 and 4.1 days, respectively. Based on the experimental DT_{50} value of 4.1 days for irradiated samples, the DT_{50} of Flurtamone under environmental conditions is calculated to be 22.7 solar summer days at London, Great Britain, or 18.1 solar summer days at Athens, Greece. Besides carbon dioxide, benzoic acid was found as a major product in the irradiated samples but not in the dark controls where, if formed, it was rapidly degraded.

Conclusions

Phototransformation on soil can contribute to the rate degradation of flurtamone under outdoor conditions.

Overall Conclusions on the Route of degradation of flurtamone in Soil (Point 7.1.1)

The route of degradation of flurtamone has been investigated in a series of laboratory studies under aerobic, sterile and anaerobic conditions. The potential effect of sunlight upon degradation has also been studied. The laboratory studies were generally conducted with active substance uniformly labelled with ^{14}C in the trifluoromethylphenyl ring (as this has been shown out of the three rings to be the most stable) or in the unsubstituted phenyl ring. An early pilot study also used flurtamone labelled with labelled at the 5-position of the furanone ring. Flurtamone is degraded by opening of the furanone ring and splitting the trifluromethylphenyl ring from the phenyl ring. The only metabolite originating uniquely from the furanone and phenyl rings is M06 benzoic acid coming from the phenyl ring seen under soil photolysis conditions. The only two major metabolites (besides M06 benzoic acid) observed are derived from the trifluoromethylphenyl ring, other than carbon dioxide which is observed in high quantities from all three rings. The results of these studies showed that the metabolism and degradation of flurtamone is completely understood from the results of studies with the radiolabelling in the three separate rings.



Flurtamone
(AE B107587)

Position of radiolabel

§ = uniformly in trifluoromethylphenyl ring,
* = 5-position of the furanone ring,
‡ = uniformly in phenyl ring

Flurtamone was metabolized at a brisk rate in soil under aerobic conditions. The principal degradation product was carbon dioxide, which always accounted for 50 % to 55% of the applied flurtamone in modern GLP studies with labelling in the trifluoromethylphenyl ring and 57% to 64% with labelling in the unsubstituted phenyl ring. This confirms exploratory work over shorter time-periods with the labelling in the phenyl and furanone rings, indicating these rings are even more completely mineralised than the trifluoromethylphenyl ring. Levels of unextractable soil bound residues accounted for up to 42% of the applied radioactivity. The maximum levels always occurred prior to the termination of the study and had declined by the final time-point (with an increase in carbon dioxide liberated), indicating that the unextractable residues were still being mineralized. There was little degradation in sterile soils, showing that metabolism is microbially mediated.

The primary metabolic pathway in soil involved opening of the furanone ring resulting in the generation of M02 3-trifluoromethyl-N-methyl-mandelamide, which, in addition to containing the intact trifluoromethylphenyl ring retained the amide of the furanone ring. It was detected as a minor metabolite (4% AR) in soil with test item labelled in both the trifluoromethylphenyl and furanone rings, indicating that it was rapidly metabolized. More extensive metabolism led to the formation of

the major metabolite, M04 TFMBA (up to 24.7% AR), at least a portion of which is further metabolised to form M05 TFA (up to 9.8% AR) both of which were detected with flurtamone labelled in the trifluoromethylphenyl ring only. No other significant metabolites were detected in aerobic soil. M01 flurtamone-desmethyl (AE B107584) was detected as a very minor metabolite in one soil study and M03 3-trifluoromethyl-mandelic acid, also a very minor metabolite, in a soil photolysis study.

The only metabolite that has been detected arising from the phenyl ring in laboratory studies conducted with the parent is the natural product M06 benzoic acid. This was detected at up to 7.2% AR in a soil photolysis study.

Under anaerobic conditions flurtamone and M04 TFMBA are stable

The occurrence of metabolites in laboratory soil studies is summarized in the table below.

Table 7.1.1-1 Occurrence of flurtamone metabolites in laboratory soil studies

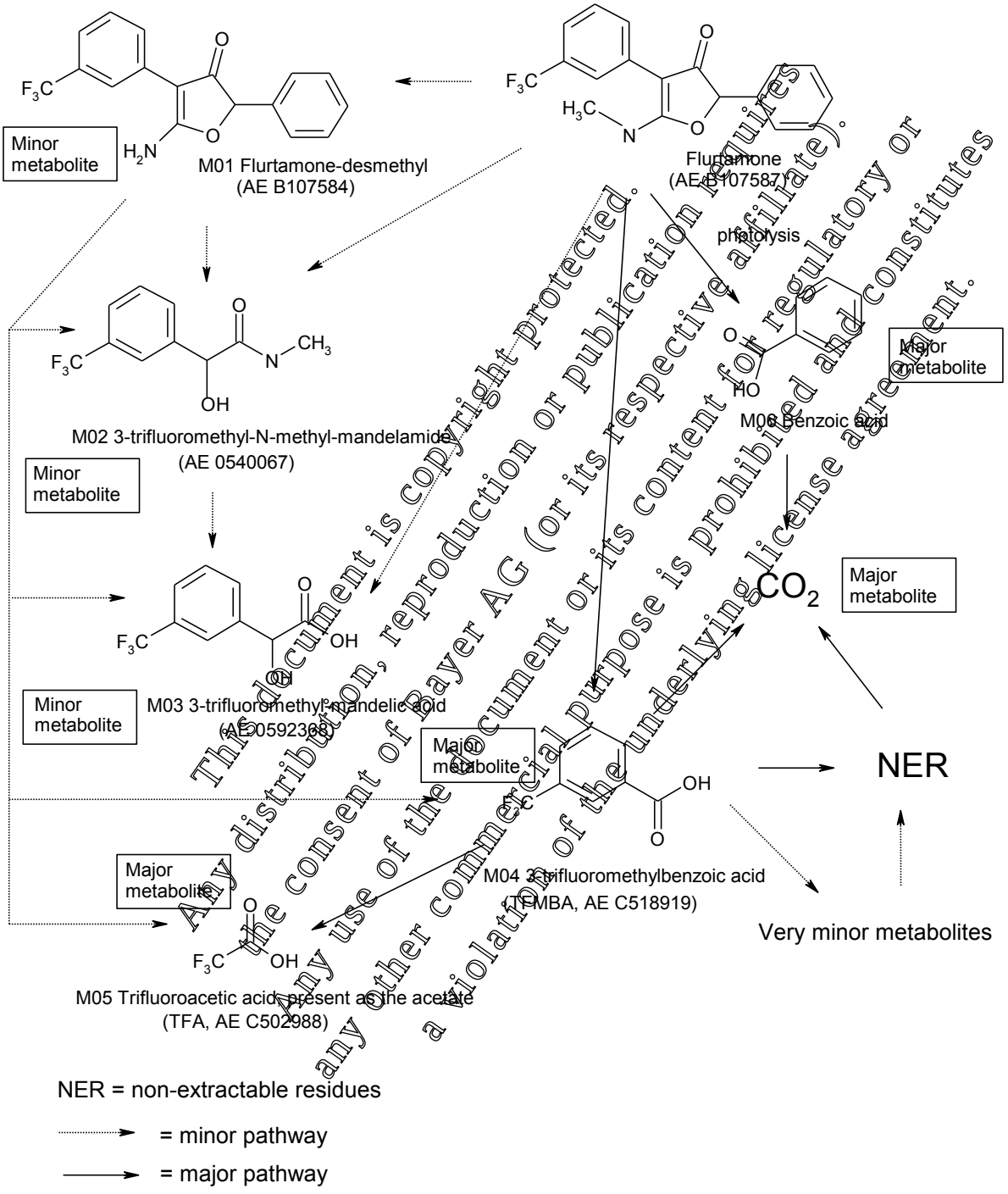
Metabolite	Current Code	Max% AR found in soil studies:		
		Aerobic	Soil Photolysis	Anaerobic
3-Trifluoromethylbenzoic acid (M04 TFMBA) Major metabolite	AE C518919	24.7	3.8	nf
Trifluoroacetate (M05 TFA) Major metabolite	BCS-AZ56567 (acid = AE C502988)	9.8	nd	nf
3-Trifluoromethyl-N-methyl-mandelamide(M02) Minor metabolite	AE 0540067*	4.0	1.4	nf
Benzoic acid (M06) Major metabolite	BCS-AG74306	nd	7.2	nd
3-Trifluromethyl-mandelic acid (M03) Minor metabolite	AE 0592368*	nd	0.3	nd
Flurtamone-desmethyl (M01) Minor metabolite	AE B107584	trace	nd	nd

* also seen in soil leaching studies at < 1% AR. nd = not detected

nf – not formed (only present in anaerobic soil because already formed under aerobic conditions)

A proposed metabolic pathway for flurtamone in soil is presented on the following page.

Figure 7.1.1-1 Proposed metabolic pathway of flurtamone in soil



CA 7.1.2 - Rate of degradation in soil

CA 7.1.2.1 - Laboratory studies

CA 7.1.2.1.1 - Aerobic degradation of the active substance

The original aerobic soil study ([redacted], 1993, M-158234-01-1 and [redacted], 1994, M-158348-01-1) conducted to EPA guidelines gave non-normalized half lives of 48.3 and 59.0 days for flurtamone in the agricultural soils, calculated according to current recommendations. In the artificial soil the report value was 211 days but this was disregarded, as being not valid by the EU review for the purposes of modelling. The normalized values from the agricultural soils are 47.8 and 41.3 days. This original study had a number of unusual aspects which may have affected the rate of degradation. These are discussed in a position paper ([redacted] 2013). With a study of dubious quality in only two agricultural soils and with compound labelled in only one ring it was necessary to conduct studies in more soils and with labels in more than one ring. These studies were commissioned and are presented below, along with the pilot study, not previously available and a study taken from literature, not previously presented.

Report:	**KCA-7.1.2.1.1 /03; [redacted] P. 2013a**
Title:	An Assessment of the 1991 Study on the Aerobic Soil Degradation of Flurtamone
Organisation:	[redacted]
Report No.:	VC/12/006B Bayer CropScience Document **M-460121-01-1**
Publication:	unpublished
Dates of experimental work:	Not relevant
Guidelines:	**Not applicable**
Deviations:	Not relevant
GLP/GEP	**Not applicable**

Executive Summary

This position paper has been fully summarized under 7.1.1.

Conclusions

This study is now 20 years old and has a number of deficiencies and some of the results are questionable. It can be concluded that this study is not fully reliable. Thus in addition to the Speyer 2.2 soil already excluded from risk assessment, the clay loam and sandy loam soil should be also excluded and only the rates of degradation derived from the modern studies conducted to current guidelines under EU conditions should be used for risk assessments

Report:	**KCA-7.1.2.1.1 /04; [redacted] B.V. 1991a**
Title:	Flurtamone aerobic and anaerobic soil metabolism - Pilot Study
Organisation:	[redacted];
Report No.:	Chevron Chemical Report No.- not given Bayer CropScience Document M-249325-02-1
Publication:	unpublished
Dates of experimental work:	1989-1991 (not stated in report)
Guidelines:	**The study was conducted as a pilot study prior to conducting a full EPA study.**
Deviations:	Not applicable
GLP/GEP	**No**

Executive Summary

This study is fully summarized under point 7.1.1.1.

Material and Methods

The details of this study are summarized under point 7.1.1.1.

The route and rate of degradation of [^{14}C]-flurtamone was investigated in one soil under aerobic conditions at 25°C and at a rate of 8 to 10 ppm, equivalent to the very high application rate, equivalent to 6 to 7.5 kg/ha.

Findings

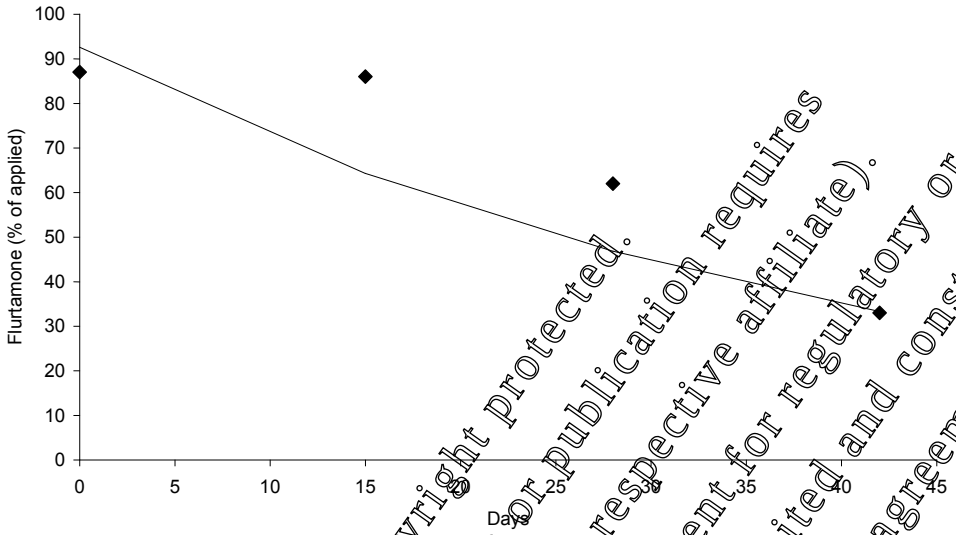
Flurtamone degraded at a rapid rate in soil with a reported DT50 value of 28 days. This is only calculated from 4 data points and is not, therefore, reliable but shows that degradation is speedy even at a very high application rate (48 to 60-fold the proposed application rate).

Table 7.1.2.1.1-1 Degradation Rate of Flurtamone under Aerobic Conditions at 25 ºC

Soil	Radiolabel	DT_{50} (days)	r^2
Sandy Loam	trifluoromethylphenyl-UL-^{14}C- flurtamone	28	0.941

The degradation of flurtamone with time is graphed below (Figure 7.1.2.1-1). The data upon which this is based have been presented previously in Table 7.1.1.1-5.

Figure 7.1.2.1.1-1 Degradation of Flurtamone in sandy loam soil at 25 °C with Time



Conclusions

Flurtamone degraded at a rapid rate in sandy loam soil incubated at 25 °C and 75% of field capacity (⅓ bar) under aerobic conditions. The DT50 value for flurtamone in sandy loam soil was 28 days, assuming first order kinetics.

Report: **KCA-7.1.2.1.1 /05; [redacted]. 2012a**
Title: [Trifluoromethylphenyl-U[^{14}C]-Flurtamone: Aerobic Metabolism/Degradation in Four European Soils.
Organisation: [redacted]
Report No.: En5a-12-0469
Bayer CropScience Document M-442039-01-1
Publication: unpublished
Dates of experimental work: 9th November 2011 to 29th June 2012
Guidelines: OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100
Deviations: None
GLP/GEP **Yes**

Executive Summary

This study has been fully summarized under 7.1.1.1.

Findings

The half-life of flurtamone was calculated by the best fit kinetics according to FOCUS (for trigger evaluation) as 13.2, 12.8, 10.7 and 9.7 days (single first order, SFO).

Conclusions

Flurtamone is rapidly degraded in soil under aerobic conditions, with a high degree of mineralization. Both enantiomers show similar degradation behaviour. The major metabolite is M04 TFMBA (maximum 24.7% in any soil). M05 TFA is formed to a level of about 5% AR after 120 days. All other degradates are very minor. The half-life of flurtamone ranged from 9.7 days to 13.2 days.

Report:	**KCA-7.1.2.1.1 /06; [redacted] M. 2012b**
Title:	[Phenyl-UL-^{14}C]-Flurtamone: Aerobic Metabolism/Degradation in Four European Soils.
Organisation:	[redacted]
Report No.:	EnSa-12-0417 Bayer CropScience Document **M-440226-01-1**
Publication:	unpublished
Dates of experimental work:	20th May 2011 to 19th September 2011
Guidelines:	**OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

This study has been fully summarized under 7.1.1.1.

Findings

The half-life of flurtamone was calculated by the best fit kinetics according to FOCUS (for trigger evaluation) as 10.3, 11.3, 9.4 and 8.5 days (single first order, SFO).

Conclusions

Flurtamone is rapidly degraded in soil under aerobic conditions, with a high degree of mineralization. All degradates are minor, most of them very minor. This reflects the rapidity of the degradation of the phenyl ring. The half-life of flurtamone ranged from 8.5 days to 11.3 days.

Report:	**KCA-7.1.2.1.1 /07; [redacted] .2013a**
Title:	Flurtamone: Kinetic Modelling Evaluation of Aerobic Soil Degradation Studies to Derive Modelling and Trigger Endpoints
Organisation:	[redacted]
Report No.:	VC/13/012A Bayer CropScience Document **M-475175-01-1**
Publication:	unpublished
Dates of experimental work:	Not applicable
Guidelines:	**Commission Regulation (EC) No 1107/2009 of 21 October 2009**
Deviations:	None
GLP/GEP	**No – but conducted to Good Modelling Practice**

Executive Summary

A kinetic evaluation of aerobic soil degradation studies with the active substance flurtamone was conducted using the computer program KinGUI2 according to FOCUS Kinetics guidance [FOCUS, 2006]. Data for flurtamone was evaluated against the FOCUS Kinetics flowcharts [FOCUS, 2006] for the determination of parent modelling and trigger endpoints.

The modelling endpoint DT_{50} values (20°C and pF2) for flurtamone gave with an overall geometric mean DT_{50} of 17.1 days. The un-normalised trigger endpoint DT_{50} values for flurtamone ranged from 9.1 to 59 days.

Material and Methods

The experimental data for the behaviour of flurtamone under laboratory conditions were taken from a number of aerobic soil degradation studies [Burr and Austin, 1993; Eckermann and Weuthen, 2012a and b]. In the Burr and Austin [1993] study, the route of degradation of flurtamone under aerobic conditions was investigated using [TFMP-U-^{14}C]-flurtamone on three soils (two agricultural soils and an artificial Speyer 2.2 soil) in the dark at 22°C and 75% at 3 bar. The artificial Speyer 2.2 soil was excluded from evaluation. In the Eckermann and Weuthen [2012a] study, the rate of degradation of [Phenyl-U-^{14}C]-flurtamone was investigated in four soils under aerobic conditions, incubated at 20°C and 55% MWHC. In the Eckermann and Weuthen [2012b] study, the rate of degradation of [TFMP-U-^{14}C]-flurtamone was investigated in four soils under aerobic conditions, incubated at 20°C and 55% MWHC.

Time zero residues for flurtamone were set to the recovered amount. For the Laacher Hof AXXa, Dollendorf II, Wurmwiese and Hoefchen am Hohenseh 4a soils the phenyl and TFMP label datasets were considered as replicates and combined.

Following the recommended procedure for determining modelling and trigger endpoints, [FOCUS, 2006], all datasets were evaluated using SFO and FOMC kinetics with free optimisation of parameters. The determination of the kinetic values followed the recommendations of FOCUS rules. These were aimed at deriving DT_{50} values for use as model and trigger inputs according to the FOCUS guidance document on degradation kinetics [FOCUS, 2006]. The kinetic evaluations were performed according to the respective decision flowchart for the determination for use in modelling and as trigger endpoints [FOCUS, 2006].

The sampling times and residue data were entered into KinGUI and optimisations carried out for SFO and FOMC kinetics. The kinetic evaluations and the statistical calculations were conducted with KinGUI (v2.0) [Meyer, 2011] using iteratively re-weighted least-square (IRLS) optimisation.

The FOCUS Kinetics modelling endpoint flowchart [FOCUS, 2006] was used to evaluate the datasets. SFO kinetics were considered visually and statistically acceptable for deriving modelling endpoints for all datasets.

Findings

The following table summarizes the DT_{50} values for flurtamone for use as modelling end-points.

Table 7.1.2.1.1-2 Normalised (20°C and pF2) DT_{50} values for flurtamone as modelling endpoints

Soil	Kinetics	DT_{50} (days)	DT_{90} (days)	Non-normalised DT_{50} (days)	DT_{50} [20°C and pF2] (days)
Ongar [[redacted], 1993 M-158234-01-1]	SFO	48.3	161	48.3	47.8
Manningtree [[redacted], 1993 M-158234-01-1]	SFO	59.0	196	59.0	44.3
Laacher Hof AXXa [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	11.7	38.8	11.7	11.7
Dollendorf II [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	12.0	39.8	12.0	12.0
Wurmwiese [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	10.1	33.4	10.1	10.1
Hoefchen am Hohenseh 4a [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	9.1	30.2	9.1	9.1
				Geomean	**17.1**

According to the trigger flowchart, SFO kinetics were determined to be the best-fit for all soils as FOMC showed no improvement over SFO.

The table below summarizes the best-fit kinetic trigger endpoint DT_{50} values derived for flurtamone.

Table 7.1.2.1.1-3 DT_{50} values for flurtamone as trigger endpoints

Soil	Kinetics	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual
Ongar [[redacted], 1993 M-158234-01-1]	SFO	48.3	161	6.9	6.84e-08	+
Manningtree [[redacted], 1993 M-158234-01-1]	SFO	59.0	196	3.0	5.07e-11	+
Laacher Hof AXXa [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	11.7	38.8	4.6	<2e-16	+
Dollendorf II [[redacted], 2012a M-442039-01-1 and b M-440226-01-1] a	SFO	12.0	39.8	2.2	<2e-16	+
Wurmwiese [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	10.1	33.4	5.1	<2e-16	+
Hoefchen am Hohenseh 4a [[redacted], 2012a M-442039-01-1 and b M-440226-01-1]	SFO	9.1	30.2	5.7	<2e-16	+

Visual assessment: + = good, o = moderate, - = poor

Conclusions

The modelling and trigger endpoint DT_{50} values derived for flurtamone can be used in exposure assessments.

Report:	**KCA-7.1.2.1.1 /08; [redacted] P. 2013b**
Title:	Flurtamone: DT_{50} Values at 10°C
Organisation:	[redacted]
Report No.:	VC/13/005A
	Bayer CropScience Document M-471674-03-1
Publication:	unpublished
Dates of experimental work:	Not relevant
Guidelines:	**Not applicable**
Deviations:	Not relevant
GLP/GEP	**Not applicable**

Executive Summary

DT_{50} values for flurtamone at 10°C have been estimated by calculation. Based on the results from aerobic soil studies conducted at, or normalized to 20°C, a range of values from 23.4 to 123.2 days has been calculated for 10°C, with a geometric mean of 44.1 days. It must be borne in mind that the values used include two from an old, discredited study (the third from which was already agreed to be excluded because it was from an artificial soil). The modern studies give consistent DT_{50} values at 20°C that range from 9.1 to 12.0 days, which would equate to 23.4 to 30.9 days at 10°C with a geometric mean of 27.4 days.

Materials and Methods

Laboratory aerobic degradation studies on six agricultural soils have been conducted. Two soils were incubated at 22°C and four soils were incubated at 20°C. In the four soils incubated at 20°C two radiolabelled versions of flurtamone were examined. The results from the two labels for each soil were consolidated and the DT_{50} for each soil determined. The values from the incubations at 22°C were normalised to 20°C (and pF 2). This resulted in six values at 20°C.

To calculate the DT50 at 10 ºC a factor was applied to the data to account for the temperature difference according to the Arrhenius equation, where typically a 10 ºC increase in temperature equates to an approximate doubling of the rate constant:

$$k = Ae^{-E/RT}$$

where:

k = the rate constant

A = constant (for small temperature changes). Known as the frequency factor.

e= 2.71828 (mathematical number)

E= activation energy for the reaction

R = the gas constant

T = temperature in º Kelvin

EFSA has recommended a Q10 factor of 2.58 for use in FOCUS models and it is therefore appropriate to use this value when calculating 10 ºC DT50 values from 20ºC data.

Findings

The results are tabulated below.

Table 7.1.2.1.1-4 Normalised DT_{50} values at 20°C and calculated DT_{50} values at 10°C for Flurtamone in soil

Soil	Normalized DT_{50} at 20°C (days)	Calculated DT_{50} at 10°C (days)
Ongar [redacted, 1993 M-158234-01-1]	47.8	123.2
Manningtree [redacted, 1993 M-158234-01-1]	41.3	106.5
Laacher Hof AXXa [redacted 2012a M-442039-01-1 and 2012b M-440226-01-1]	11.7	30.1
Dollendorf II [redacted, 2012a M-442039-01-1 and 2012b M-440226-01-1] a	12.0	30.9
Wurmwiese [redacted, 2012a M-442039-01-1 and 2012b M-440226-01-1]	10.1	25.9
Hoefchen am Hohenseh 4a [redacted, 2012a M-442039-01-1 and 2012b M-440226-01-1]	9.1	23.4
	Average	**56.7**
	SD	**45.4**
	Geomean	**44.1**

Conclusions

The calculated DT_{50} values for flurtamone in soil under aerobic conditions at 10°C were estimated to range from 23.4 days to 123.2 days with a mean value of 56.7 days and a geometric mean value of 44.1 days.

It must be borne in mind that the values used include two from an old, discredited study (the third from which was already agreed to be excluded because it was from an artificial soil). The modern studies give consistent DT_{50} values at 20°C that range from 9.1 to 12.0 days, which would equate to 23.4 to 30.9 days at 10°C with a geometric mean of 27.4 days.

CA 7.1.2.1.2 - Aerobic degradation of metabolites, breakdown and reaction products

The results from the original soil study on the flurtamone metabolite M04 TFMBA gave laboratory DT_{50}s of 11.2 to 16.7 days (SFO, n=3, mean 13.5 days), normalised 7.3 to 10.5 days (mean 8.9 days). For M05 TFA a default value of 2 years (730 days) was assumed. In order to facilitate calculations of formation fractions new studies on M04 TFMBA were conducted at the same facility, in the same soils and at the same time as the new studies on flurtamone. Similar studies were conducted on the metabolite M05 TFA. All the new studies are presented below.

Report:	**KCA-7.1.2.1.2 /02; [redacted]. 2012**
Title:	[Phenyl-UL-^{14}C]-3-Trifluoromethylbenzoic acid: Aerobic Degradation in Four European Soils
Organisation:	[redacted]
Report No.:	EnSa-12-0589 Bayer CropScience Document **M-443478-01-1**
Publication:	unpublished
Dates of experimental work:	27th June 2011 to 15th November 2011
Guidelines:	**OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The degradation of [phenyl-UL-^{14}C]-3-trifluoromethylbenzoic acid (M04 TFMBA) was studied in four European soils. Due to the fast degradation and the high mineralization of the compound, the study was terminated after 28 days of incubation. M04 TFMBA was applied at a nominal rate of 25 µg/100 g soil dry weight (250 µg/kg soil dry weight) in the test systems, which is equivalent to a field rate of 93.75 g/ha. This represents a 50% conversion of flurtamone, applied at 250 g/ha, to M04 TFMBA (equivalent to 71.3 g/ha) with a 1.3 factor to compensate for analytical limitations.

The test system consisted of Erlenmeyer flasks equipped with traps for the collection of CO2 and volatile organic compounds. Samples were analyzed after 0, 1, 3, 7, 10, 14, 21 and 28 days of incubation. At each sampling date, the soil samples were extracted three times at ambient temperature and once with respect to the formation of non-extractable residues by hot (microwave). The amounts of radioactivity in the extracts as well as the amounts of trapped volatiles were determined by liquid scintillation counting (LSC). Aliquots of the combined organic soil extracts were concentrated and analyzed by TLC to quantify the test item as well as its transformation products. Representative extracts were additionally analyzed using a second chromatographic method (HPLC).

The test conditions outlined in the study protocol were maintained throughout the study. Mean material balances were 99.1, 99.6, 97.9, and 99.2% of the applied radioactivity. Extractable ^{14}C-residues decreased from 101.6, 99.5, 100.8, and 100.5% of AR at DAT-0 to 10.5, 11.8, 10.4, and 12.3% at the study end (DAT-28). The amounts of the test item in the extracts declined from 101.5, 99.4, 100.7, and 100.5% of AR at DAT-0 to 0.9, 1.7, 0.7 and 2.4% of AR at the end of the study.

The half-life of M04 TFMBA was calculated by the best fit kinetics according to FOCUS (for trigger evaluation) as 6.0, 7.4, 8.5 and 2.8 days (single first order, SFO) under aerobic conditions.

Besides the test item, one major transformation product was detected in the extracts. The amounts of M05 TFA reached up to 5.5, 6.3, 6.6 and 5.7% of AR at the end of the study. Furthermore, six minor degradation products reaching up to 2.4% of AR were characterized according to their separation distances in TLC.

The NER increased from 1.1, 3.0, 1.3 and 1.9% of AR at DAT-0 to maximum values of 31.0, 30.8, 31.6 and 28.1% of AR at DAT-10 or DAT-21 and declined already slightly to 27.7, 28.3, 26.7 and 27.0% of AR towards the end of the study. A further characterization (fractionation into humin, humic acids and fulvic acids) was shown for all four soils for samples taken at DAT-28.

The maximum amounts of $^{14}CO_2$ were 58.5, 56.9, 57.7 and 56.0% of AR at study termination. Volatile organic compounds were not formed in the course of the study (≤ 0.2% of AR at all sampling intervals). The test item was rapidly degraded. The high amount of formed carbon dioxide as the final product indicates a near-complete mineralization of M04 TFMBA in soil.

Materials and Methods

Test Material:

[Phenyl-UL-^{14}C]-3-Trifluoromethylbenzoic acid, radiochemical purity > 99%.
Batch no.: KML 9099.

Test Design:

The metabolism of flurtamone in soil under aerobic conditions was investigated in four agricultural soils at 20°C. The soil characteristics are listed below.

The soils were collected from agricultural areas of Germany and were taken fresh from the field. A few days before starting the study, the soil was sieved to a particle size of ≤ 2 mm. Subsequently the soil moisture was determined by drying aliquots of the soils at 105°C. Ultrapure water was added to adjust each soil aliquot to 55% of the maximum water holding capacity. The weights of all test vessels were recorded and the samples were pre-equilibrated at about 20 °C in the dark for three days.

The incubation systems were static systems and consisted of Erlenmeyer flasks (300 mL) with 100 g soil (dry weight equivalent) for each sampling interval. The flasks were closed with trap attachments, which were easily permeable for oxygen. The traps contained soda lime for adsorption of CO_2 and a polyurethane foam plug for adsorption of volatile organic compounds.

An application solution with a concentration of approximately 33 mg/mL in acetone/water (1:27, v/v) was prepared. 1 mL of this was applied drop-wise, by use of a micropipette, to each pre-equilibrated soil sample. Dose checks were taken during the application procedure.

Water loss due to evaporation from the soil was determined by weighing the sampled flasks without the traps on each processing day. If necessary, the evaporated portions were replaced.

Table 7.1.2.1.2-1 Physico-chemical characteristics of the soil used in a M04 TFMBA aerobic soil study

Parameter	Result/Value			
Soil	Laacher Hof AXXa	Dollendorf II	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh
Geographic Location[1]	Monheim am Rhein	Blankenheim	Monheim am Rhein	Burscheid
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudoll	Fine-loamy, nixed, active, frigid Typic Eutrudept	Loamy, mixed, mesic Typic Argudalf	Loamy, mixed, mesic Typic Argudalf
Map Reference	N 51° 04.65' E 06° 53.52'	N 50° 22.90' E 06° 43.00'	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'
Textural Class (USDA)	Loamy sand	Loam	Sandy Loam	Silt loam
Sand (%)	78	39	57	19
Silt (%)	16	36	28	64
Clay (%)	6	25	15	17
pH in $CaCl_2$ (1:2)	6.2	7.4	5.3	6.5
pH in water (1:1)	6.5	7.5	5.5	6.7
pH in water (saturated paste)	6.6	7.4	5.5	6.8
pH in KCl (1N)	6.0	7.1	5.1	6.1
Organic Matter (%)	3.1	8.9	3.3	2.8
Organic Carbon (%)	1.8	5.2	1.9	1.6
Cation Exchange Capacity (meq/100g)	9.4	22.3	9.9	12.2
Water Holding Capacity at pF 2.5 (%)	10.9	27.4	16.9	21.0
Maximum Water Holding Capacity (%)	43.8	79.3	60.2	51.8
Bulk Density (disturbed, g/cm^3)	1.22	1.01	1.13	1.12
Soil Biomass at:				
0 days	741	3276	624	660
28 days	415	1621	312	418

[1] in North Rhine-Westphalia, Germany.

Samples (in duplicate) were taken at 0, 1, 3, 7, 10, 14, 21 and 28 days after treatment. The corresponding trap attachments were collected to determine the amount of $^{14}CO_2$ and organic volatiles. At the respective sampling dates, the soil samples from each flask were extracted completely. The extracts were analyzed by LSC and TLC within one day. HPLC analysis was performed within a maximum of two days. After analysis, the extracts were stored cold. DAT-0 and DAT-1 samples were re-analyzed by TLC after 17 and 9 days, respectively, due to a confirmation of the concentration recovery and a too short exposure time of the TLC plates. The trap attachments containing soda lime and PU foam were processed within about two weeks. Bound residues were analyzed by combustion and LSC within two weeks after sampling. A further characterization of bound residues was performed within about a month.

Soil biomass measurements were conducted using the substrate-induced initial respiratory response (SIR) method.

For soil extraction the entire soil amount of each test vessel was transferred in a centrifuge beaker and extracted using a mechanical shaker. The extraction procedure comprised an extraction with 100 mL

acetonitrile/water 80/20 (v/v) followed by two extractions with 80 mL acetonitrile/water 80/20 (v/v) both at ambient temperature. These extracts were combined and radioassayed. . The final extraction used 80 mL acetonitrile/water 50/50 (v/v) with microwave heating to 70°C. This was then radioassayed.

The residual radioactivity (bound residues) in freeze-dried, homogenized soil was determined by combustion of three aliquots (approx. 1 g) of each sample followed by LSC. The bound residue in soil (DAT-59 samples) was characterized and fractionated into humin, humic acid and fulvic acid by addition of sodium hydroxide and subsequent precipitation of the supernatant with hydrochloric acid.

Volatile organic compounds possibly contained in the foam plugs were extracted by shaking with ethyl acetate. Aliquots of the extracts were submitted to LSC measurement. Chromatographic analyses of the PU foam extracts were not performed, because they contained ≤ 0.1% of the AR in all test systems.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in hydrochloric acid. The liberated CO_2 was absorbed by a special absorption/ scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Prior to chromatographic analysis, the cold and microwave organic extracts were combined. Aliquots were concentrated and radioassayed to allow the determination of recovery. Aliquots of the concentrates were analyzed by TLC and the concentrated extracts sampled at DAT-0, 7, 14 and 28 days were additionally analyzed by HPLC.

For TLC analysis aliquots of the concentrates of the extracts were spotted on silica gel plates (Si60, F254, 20 cm x 20 cm Merck) using an automatic applicator. The plates were developed with ethyl acetate/2-Propanol/water (75/20/5, v/v/v) in a plate chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products in the extracts was calculated based on the distribution of the TLC-zones and the amount of radioactivity in the extracts.

The assignment of the TLC peaks to the test item was done by comparing their separation distances with the separation distances of radiolabelled flurtamone. The radiolabelled test item and the radiolabelled reference item were applied in separate lanes onto each TLC plate. All minor transformation products were characterized according to their separation distances.

The HPLC method used a system that comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm; 5 µm column connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. The assignment of the HPLC peak to the test item in the application solution was done by co-chromatography using the [^{12}C]-reference substance. Within routine chromatograms of the concentrated combined extracts, comparison of retention times in different chromatograms was used for the assignment of the HPLC peaks to test item and the transformation products. The quantification of the test item and its degradation products in the extracts was calculated based on the distribution of the HPLC - zones and the amount of RA in the extracts.

The electro-spray ionization MS spectra (ESI) were obtained with a LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The HPLC instrument used for chromatography was an Agilent HP1100 with a Nucleodur C18 Gravity, 3 µm, 250 x 2 mm (MN) column. The mobile

phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

Findings

The DAT-0 extraction efficiencies were 101.6, 99.5, 100.8, and 100.5% of applied radioactivity (AR). The test item was stable under the conditions of extraction and accounted for 101.5, 99.4, 100.7, and 100.5% of AR in the combined organic soil extracts at DAT-0. These results demonstrate that the extraction method was well suitable to extract the compound from the soil matrix. The recoveries of radioactivity after the concentration step were exemplarily determined and were > 90% for all samples examined.

A good selectivity and reproducibility demonstrated the suitability for separation and quantification of the TLC method. The TLC limit of quantification (LOQ) for a single peak in the combined organic extracts was < 1% of radioactivity applied to the plate (0.3% of AR). The HPLC recovery-checks gave mean recoveries that ranged from 99.0 to 101.0% for the four soils, which showed that no radioactivity was lost during analysis.

Recoveries of applied radioactivity from samples were between 90% and 110% for all soils at all time-points. No time-dependent tendency was observed for the total recovery over the study period, demonstrating that no significant radioactivity dissipated from the flasks or was lost during processing.

The amount of formed $^{14}CO_2$ increased steadily during the entire study period. At the end of the study, 28 days after application, between 56.0 and 58.5% of AR was quantified as carbon dioxide. No significant amounts of volatile organic compounds were detected in the polyurethane foam of the trap attachments (values being ≤ 0.1% of AR at all sampling intervals). At the end of the incubation period the recovered radioactivity in the extracts had decreased to 10.4 – 12.3% of AR. Non-extractable ^{14}C-residues increased from 1.1, 3.0, 7.3 and 11.9% of AR at DAT-0 to maximum amounts of 31.0, 30.8, 31.6 and 28.1% of AR at DAT-10 or DAT-21 and declined already slightly to 27.7, 28.3, 26.7 and 27.0% of AR until the end of the study at DAT-28.

The mean recoveries and distribution of applied radioactivity are shown in the following tables.

Table 7.1.2.1.2-2 Recovery and distribution of applied radioactivity in Laacher Hof AXXa soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
Carbon dioxide	n.a.	1.7	8.0	22.6	31.7	42.5	54.2	58.5
Organic volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**1.8**	**8.1**	**22.8**	**31.8**	**42.6**	**54.2**	**58.6**
Ambient extract	99.0	93.6	77.7	53.5	38.6	24.7	10.4	9.1
Microwave extract	2.6	2.9	2.9	3.2	2.8	2.9	1.8	1.4
Total extractable	**101.6**	**96.6**	**80.6**	**56.7**	**41.4**	**27.6**	**12.1**	**10.5**
Non-extractable	**1.1**	**4.6**	**13.1**	**20.6**	**24.8**	**27.6**	**31.0**	**27.7**
Total recovery	**102.6**	**103.0**	**101.7**	**100.1**	**98.0**	**97.8**	**97.4**	**96.8**

n.a not analyzed

Table 7.1.2.1.2-3 Recovery and distribution of applied radioactivity in Dollendorf II soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
Carbon dioxide	n.a.	0.7	4.6	14.8	23.8	37.7	52.7	56.9
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**0.8**	**4.6**	**14.8**	**23.8**	**37.8**	**52.8**	**56.9**
Ambient extract	94.2	91.8	83.2	63.3	48.5	29.6	12.4	10.0
Microwave extract	5.3	5.3	5.0	4.9	4.2	3.5	2.1	1.7
Total extractable	**99.5**	**97.1**	**88.2**	**68.2**	**52.8**	**33.1**	**14.4**	**11.8**
Non-extractable	**3.0**	**5.4**	**9.4**	**16.8**	**21.2**	**26.1**	**30.8**	**28.3**
Total recovery	**102.5**	**103.2**	**102.2**	**99.2**	**97.6**	**97.0**	**97.9**	**96.9**

n.a not analyzed

Table 7.1.2.1.2-4 Recovery and distribution of applied radioactivity in Laachet Hof Wurmwiese soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
Carbon dioxide	n.a.	4.7	18.7	38.8	47.6	53.1	56.0	57.7
Organic volatiles	n.a.	0.1	0.2	0.2	0.1	0.1	0.1	0.1
Total volatiles	**n.a.**	**4.8**	**18.9**	**38.9**	**47.7**	**53.2**	**56.1**	**57.7**
Ambient extract	98.0	83.5	54.2	23.5	14.4	11.0	9.7	9.2
Microwave extract	2.8	3.5	4.1	2.3	1.9	3.0	1.4	1.2
Total extractable	**100.8**	**87.0**	**58.3**	**25.8**	**16.3**	**14.0**	**11.1**	**10.4**
Non-extractable	**1.3**	**8.0**	**21.8**	**31.3**	**31.6**	**30.6**	**30.4**	**26.7**
Total recovery	**102.1**	**100.5**	**99.0**	**96.0**	**95.7**	**97.9**	**97.6**	**94.8**

n.a not analyzed

Table 7.1.2.1.2-5 Recovery and distribution of applied radioactivity in Hoefchen Am Hohenseh 4a soil

Fraction	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
Carbon dioxide	n.a.	0.7	4.1	13.3	21.5	32.9	50.6	56.0
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	0.1	0.1	0.1	0.1
Total volatiles	**n.a.**	**0.8**	**4.2**	**13.3**	**21.7**	**33.1**	**50.7**	**56.1**
Ambient extract	96.6	94.0	80.4	68.2	56.0	39.5	15.8	10.8
Microwave extract	3.9	4.0	4.0	3.7	3.0	3.6	2.3	1.5
Total extractable	**100.5**	**98.0**	**89.4**	**71.9**	**58.0**	**43.1**	**18.1**	**12.3**
Non-extractable	**1.9**	**4.0**	**7.5**	**14.1**	**18.5**	**21.8**	**28.1**	**27.0**
Total recovery	**102.3**	**102.7**	**101.1**	**99.3**	**98.2**	**98.0**	**96.9**	**95.4**

n.a not analyzed

The results of the fractionation of unextractable residues from the 28 day samples into humin, humic acid and fulvic acid are shown in the table below.

Table 7.1.2.1.2-6 Distribution of unextractable radioactivity in humic substance fractions (as % applied radioactivity)

Soil	Humin Fraction (% AR)	Humic Acid Fraction (%AR)	Fulvic Acid Fraction (%AR)	Total (%AR)
Laacher Hof AXXa	13.8	7.9	6.2	27.8
Dollendorf II	16.4	8.3	4.5	29.2
Laacher Hof Wurmwiese	13.4	5.9	8.0	27.3
Hoefchen Am Hohenseh	15.7	4.9	7.0	27.6

Trifluoromethylbenzoic acid was rapidly degraded. Besides the test item, one major transformation product was detected in the extracts. The amounts of M05 TFA reached up to 5.5, 6.3, 6.6 and 5.7% of AR at the end of the study in soils Laacher Hof AXXa, Dollendorf II, Laacher Hof Wurmwiese and Hoefchen Am Hohenseh 4a, respectively. Furthermore, six minor degradation products reaching up to 2.4% of AR (soil Laacher Hof AXXa and Laacher Hof Wurmwiese, DAT-10) were characterized according to their separation distances in TLC. The biotransformation of M04 TFMBA is summarized in the following tables.

Table 7.1.2.1.2-7 Biotransformation of M04 TFMBA in Laacher Hof AXXa soil

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
M04 TFMBA	101.5	95.5	78.8	51.8	34.9	17.9	2.3	0.9
M05 TFA	n.d.	< LOD	0.6	2.2	2.7	3.9	5.2	5.5
U1	n.d.	n.d.	n.d.	0.7	n.d.	0.9	1.3	1.1
U2	n.d.	n.d.	n.d.	< LOD	n.d.	0.5	0.6	0.5
U3	n.d.	0.6	0.7	0.7	2.4	2.2	1.0	1.7
U4	n.d.	n.d.	< LOD	0.5	n.d.	0.6	0.6	< LOD
U5	n.d.	n.d.	n.d.	0.7	n.d.	< LOD	0.4	0.3
U6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.
Total extractable	**101.6**	**96.6**	**80.6**	**56.8**	**41.4**	**27.6**	**12.2**	**10.5**
Carbon dioxide	n.a.	1.7	8.0	22.6	31.7	42.5	54.2	58.5
Organic volatiles	n.a.	0.1	0.1	0.1	0.1	0.1	< 0.1	0.1
Non-extractable	1.1	4.6	13.1	20.6	24.8	27.6	31.0	27.7
Total recovery	**102.6**	**103.0**	**101.7**	**100.1**	**98.0**	**97.8**	**97.4**	**96.8**

n.d. not detected n.a not analyzed < LOD = less than limit of detection

Table 7.1.2.1.2-8 Biotransformation of M04 TFMBA in Dollendorf II soil

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
M04 TFMBA	99.4	96.1	87.0	64.4	48.0	23.8	4.4	1.7
M05 TFA	n.d.	< LOD	0.5	1.9	1.8	4.1	5.6	6.3
U1	n.d.	n.d.	n.d.	0.3	n.d.	1.0	1.3	1.7
U2	n.d.	n.d.	n.d.	< LOD	n.d.	0.6	0.8	1.0
U3	n.d.	0.5	< LOD	0.7	1.6	1.8	0.8	1.1
U4	n.d.	n.d.	< LOD	< LOD	n.d.	0.4	0.7	0.4
U5	n.d.	n.d.	n.d.	< LOD	n.d.	n.d.	0.3	< LOD
U6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	n.d.
Total extractable	**99.5**	**97.1**	**88.2**	**68.2**	**52.7**	**33.1**	**14.4**	**11.8**
Carbon dioxide	n.a.	0.7	4.6	14.8	28.8	37.7	52.7	56.9
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable	3.0	5.4	9.4	16.3	21.2	26.1	30.8	28.3
Total recovery	**102.5**	**103.2**	**102.2**	**99.2**	**97.6**	**97.0**	**97.9**	**96.9**

n.d. not detected n.a not analyzed < LOD = less than limit of detection

Table 7.1.2.1.2-9 Biotransformation of M04 TFMBA in Laacher Hof Wurmwiese soil

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
M04 TFMBA	100.1	93.1	52.0	16.8	5	2.0	1.3	0.7
M05 TFA	n.d.	0.7	2.7	4.9	4.9	5.1	6.1	6.6
U1	n.d.	n.d.	n.d.	0.6	0.5	0.7	0.9	0.4
U2	n.d.	n.d.	n.d.	< LOD	< LOD	< LOD	< LOD	< LOD
U3	n.d.	1.4	1.1	1.9	2.4	2.4	0.8	1.2
U4	n.d.	0.3	0.6	0.8	1.3	1.0	0.8	0.5
U5	n.d.	n.d.	0.8	< LOD	n.d.	0.7	< LOD	< LOD
U6	n.d.	n.d.	n.d.	0.4	n.d.	n.d.	< LOD	n.d.
Total extractable	**100.8**	**87.0**	**58.3**	**25.8**	**16.4**	**14.0**	**11.1**	**10.4**
Carbon dioxide	n.a.	4.7	18	38.8	47.6	53.1	56.0	57.7
Organic volatiles	n.a.	0.1	0.1	0.2	0.1	0.1	0.1	0.1
Non-extractable	1.3	8.6	21.8	31.3	31.6	30.6	30.4	26.7
Total recovery	**102.1**	**100.5**	**99.0**	**96.0**	**95.7**	**97.9**	**97.6**	**84.8**

n.d. not detected n.a not analyzed < LOD = less than limit of detection

Table 7.1.2.1.2-10 Biotransformation of M04 TFMBA in Hoefchen Am Hohenseh 4a soil

Compound	% applied radioactivity at days after treatment:							
	0	**1**	**3**	**7**	**10**	**14**	**21**	**28**
M04 TFMBA	100.5	97.5	88.4	69.2	53.2	35.0	8.6	2.4
M05 TFA	n.d.	< LOD	0.4	1.4	2.3	3.5	4.9	5.7
U1	n.d.	n.d.	n.d.	0.4	0.5	0.3	1.7	1.5
U2	n.d.	n.d.	n.d.	< LOD	< LOD	0.6	0.8	0.8
U3	n.d.	< LOD	< LOD	0.3	1.0	1.4	0.9	1.0
U4	n.d.	n.d.	< LOD	< LOD	< LOD	0.5	0.6	0.4
U5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< LOD	< LOD
U6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	n.d.
Total extractable	**100.5**	**98.0**	**89.4**	**71.9**	**58.0**	**43.1**	**18.1**	**12.3**
Carbon dioxide	n.a.	0.7	4.1	13.2	20.7	32.9	50.6	56.0
Organic volatiles	n.a.	< 0.1	< 0.1	0.1	0.1	0.1	0.1	0.1
Non-extractable	1.9	4.0	7.5	14.1	18.5	21.5	25.0	27.0
Total recovery	**102.3**	**102.7**	**101.1**	**99.3**	**98.2**	**98.0**	**96.9**	**95.4**

n.d. not detected n.a not analyzed < LOD = less than limit of detection

The data for the parent compound M04 TFMBA were evaluated according to FOCUS. The best fit kinetic model was chosen based on the chi^2 confidence criterion and visual assessment. The results are summarized in the table below.

Table 7.1.2.1.2-11 M04 TFMBA DT_{50} and DT_{90} values in soils under aerobic conditions

Soil	Soil Type	Kinetic Model	DT_{50} (d)	DT_{90} (d)	Visual Assessment	Chi^2 Error (%)
Laacher Hof AXXa	Loamy sand	SFO	6.0	19.9	Good	6.4
Dollendorf II	Loam	SFO	7.4	24.8	Good	9.9
Laacher Hof Wurmwiese	Sandy loam	SFO	2.8	9.3	Good	4.9
Hoefchen Am Hohenseh	Silt loam	SFO	8.5	28.3	Good	9.1

Conclusions

Trifluoromethylbenzoic acid is rapidly degraded in soil under aerobic conditions, with a high degree of mineralization. The DT_{50} was calculated to be between 2.8 and 8.5 days. M05 TFA was detected as major transformation product. Formation of significant amounts of CO_2 and NER indicates a near-complete mineralization of M04 TFMBA and a quite usual participation in the natural carbon cycle of soil. There was no pH dependency evident. There is no potential for persistence and accumulation in aerobic soil.

Report:	**KCA-7.1.2.1.2 /03; [redacted] N. 2012a**
Title:	[1-^{14}C]-Trifluoroacetate: Aerobic Degradation in Four European Soils
Organisation:	[redacted]
Report No.:	EnSa-12-0393 Bayer CropScience Document **M-439283-01-1**
Publication:	unpublished
Dates of experimental work:	18th February 2011 to 6th September 2011
Guidelines:	**OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The degradation of [1-^{14}C]-trifluoroacetate (BCS-AZ56562) was studied in four European soils. The test system consisted of Erlenmeyer flasks equipped with traps for the collection of CO_2 and volatile organic compounds. Samples were treated at 20 µg/100 g, equivalent to a field rate of 75 g/ha, and analyzed after 0, 3, 7, 14, 28, 43, 59, 92 and 120 days of incubation. At each sampling date the soil samples were extracted three times at ambient temperature and once with respect to the formation of non-extractable residues by hot (microwave) extraction. The amounts of radioactivity in the extracts as well as the amounts of trapped volatiles were determined by liqtid scintillation counting (LSC). Aliquots of the combined organic extracts were concentrated and analyzed and quantified by TLC. The identification of the test item in the application solution was achieved by HPLC-MS and HPLC-MS/MS. The test conditions outlined in the study protocol were maintained throughout the study. Mean material balances accounted for 100.4, 100.5, 100.0 and 101.2% of the applied radioactivity (AR) for the four soils used.

The test item [1-^{14}C]-trifluoroacetate was not degraded under laboratory conditions during an incubation time of 120 days. Significant amounts of volatiles and non-extractable residues were not formed in the course of the study. The half-life of [1-^{14}C]-trifluoroacetate was calculated by the best fit kinetics according to FOCUS (single first order, SFO, for trigger evaluation) as >1000 days under aerobic conditions in all four tested soils.

Materials and Methods

Test Material:

[1-^{14}C]-Trifluoroacetate, radiochemical purity > 98%, Batch no.: KML 9072.

Test Design:

The metabolism of [1-^{14}C]-trifluoroacetate in soil under aerobic conditions was investigated in four agricultural soils at 20°C. The soil characteristics are listed below.

Table 7.1.2.1.2-12 Properties of soils used in a trifluoracetate aerobic soil study.

Parameter	Result/Value			
Soil	Laacher Hof AXXa	Dollendorf II	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh
Geographic Location[1]	Monheim am Rhein	Blankenheim	Monheim am Rhein	Burscheid
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudoll	Fine-loamy, nixed, active, frigid Typic Eutrudept	Loamy, mixed, mesic Typic Argudalf	Loamy, mixed, mesic Typic Argudalf
Map Reference	N 51° 04.65' E 06° 53.52'	N 50° 22.90' E 06° 43.00'	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'
Textural Class (USDA)	Sandy loam	Clay loam	Sandy Loam	Silt loam
Sand (%)	77	29	57	25
Silt (%)	14	40	26	60
Clay (%)	9	31	17	15
pH in $CaCl_2$ (1:2)	6.2	7.4	5.1	6.4
pH in water (1:1)	6.5	7.5	5.4	6.7
pH in water (saturated paste)	6.3	7.4	5.2	6.5
pH in KCl (1N)	6.0	7.1	4.7	6.1
Organic Matter (%)	2.8	9.5	3.3	4.1
Organic Carbon (%)	1.6	5.5	1.9	2.4
Cation Exchange Capacity (meq/100g)	8.7	21.2	10.0	13.6
Water Holding Capacity at pF 2.5 (%)	12.2	21.9	18.2	26.3
Maximum Water Holding Capacity (%)	46.9	84.9	57.6	62.0
Bulk Density (disturbed, g/cm^3)	1.26	0.97	1.13	1.08
Soil Biomass at:				
0 days	536	2930	423	833
59days	589	3344	459	844
120 days	248	1412	424	387

[1] in North Rhine-Westphalia, Germany.

The soils were collected from agricultural areas of Germany and were taken fresh from the field. A few days before starting the study, the soil was sieved to a particle size of ≤ 2 mm. Subsequently the soil moisture was determined by drying aliquots of the soils at 105°C. Ultrapure water was added to adjust each soil aliquot to 55% of the maximum water holding capacity. The weights of all test vessels were recorded and the samples were pre-equilibrated at about 20 °C in the dark over a weekend.

The incubation systems were static systems and consisted of Erlenmeyer flasks (300 mL) with 100 g soil (dry weight) for each sampling interval. The flasks were closed with trap attachments, which were easily permeable for oxygen. The traps contained soda lime for absorption of CO_2 and a polyurethane foam plug for adsorption of volatile organic compounds.

An application solution, with a concentration of approximately 20 µg/mL in water, was prepared. 1 mL of this was applied drop-wise, by use of a micropipette, to each pre-equilibrated soil sample, giving a treatment rate of 20 µg/ equivalent to a field rate of 75 g/ha. Dose checks were taken during the application procedure.

Water loss due to evaporation from the soil was determined by weighing the sampled flasks without the traps on each processing day. If necessary, the evaporated portions were replaced. Determination of the soil microbial viability (microbial biomass) was performed at the start, in the middle (DAT-59) and at the end (DAT-120) of the study.

Samples (in duplicate) were taken at 0, 3, 7, 14, 28, 43, 59, 92 and 120 days after treatment. The corresponding trap attachments were collected to determine the amount of $^{14}CO_2$ and organic volatiles. At the respective sampling dates, the soil samples from each flask were extracted completely. The extracts were analyzed by LSC and TLC within one day. TLC analysis was normally performed within a day but always within a maximum of three days. HPLC analysis was performed on 120 day samples within two days of extraction. After analysis, the extracts were stored cold. The trap attachments containing soda lime and PU foam were processed within about two weeks. Bound residues were analyzed by combustion and LSC within three weeks after sampling.

For soil extraction the entire soil amount of each test vessel was transferred in a centrifuge beaker and extracted using a mechanical shaker. The extraction procedure comprised an extraction with 100 mL acetonitrile/water 80/20 (v/v) followed by two extractions with 80 mL acetonitrile/water 80/20 (v/v) both at ambient temperature. These extracts were combined and radioassayed. . The final extraction used 80 mL acetonitrile/water 50/50 (v/v) with microwave heating to 70 °C. This was then radioassayed.

The residual radioactivity (bound residues) in freeze-dried, homogenized soil was determined by combustion of three aliquots (approx. 1 g) of each sample followed by LSC.

Volatile organic compounds possibly contained in the foam plugs were extracted by shaking with ethyl acetate. Aliquots of the extracts were submitted to LSC measurement. Chromatographic analyses of the PU foam extracts were not performed because they contained ≤ 0.1% of the AR in all test systems.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in hydrochloric acid. The liberated CO_2 was absorbed by a special absorption/ scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Prior to chromatographic analysis, the cold and microwave organic extracts were combined. Aliquots were concentrated and radioassayed to allow the determination of recovery. Aliquots of the concentrates were analyzed by TLC and the concentrated extracts sampled at DAT-120 were additionally analyzed by HPLC.

For TLC analysis aliquots of the concentrates of the extracts were spotted on silica gel plates (Si60, F254, 20 cm x 20 cm Merck) using an automatic applicator. The plates were developed with ethyl acetate/2-Propanol/ultrapure water/ glacial acetic acid (65/24/11/1, v/v/v/v) in a plate chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products in

the extracts was calculated based on the distribution of the TLC-zones and the amount of radioactivity in the extracts.

The assignment of the TLC peaks to the test item was done by comparing their separation distances with the separation distances of radiolabelled flurtamone. The radiolabelled test item and the radiolabelled reference item were applied in separate lanes onto each TLC plate.

The HPLC method used a system that comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm, 5 µm column connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water containing 5mM ammonium formate against 1% formic acid in acetonitrile water containing 5mM ammonium formate.

The electro-spray ionization MS spectra (ESI) were obtained with a LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The HPLC instrument used for chromatography was an Agilent HP1100 with a Nucleodur C18 Gravity, 3 µm, 250 x 2 mm (MN) column. The mobile phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

Findings

The DAT-0 extraction efficiencies were 100.4, 100.5, 100.0, and 101.2% of applied radioactivity (AR). The test item was stable under the conditions of extraction and accounted for 97.7, 98.4, 98.7, and 99.2% of AR in the combined organic soil extracts at DAT-0. These results demonstrate that the extraction method was well suitable to extract the compound from the soil matrix. The recoveries of radioactivity after the concentration step were exemplarily determined for DAT-0 and DAT-120 samples and were 101% to 105% for all samples examined.

A good selectivity and reproducibility demonstrated the suitability for separation and quantification of the TLC method. The TLC limit of quantification (LOQ) was determined to be 2.1% of AR (LOD = 0.7% AR).

Recoveries of applied radioactivity from samples were between 90% and 110% for all soils at all time-points. No time-dependent tendency was observed for the total recovery over the study period, demonstrating that no significant radioactivity dissipated from the flasks or was lost during processing.

No $^{14}CO_2$ was formed during the study period. No significant amounts of volatile organic compounds were detected in the polyurethane foam of the trap attachments (values being ≤ 0.1% of AR at all sampling intervals). At the end of the incubation period the recovered radioactivity in the extracts was effectively unchanged at 96.4 to 98.6% of AR. Non-extractable ^{14}C-residues increased only marginally from 0.6, 1.3, 1.0 and 0.9% of AR at DAT-0 to0.8, 2.0, 1.3 and 1.2% of AR at the end of the study (DAT-120).

The mean recoveries and distribution of applied radioactivity are shown in the following tables.

Table 7.1.2.1.2-13 Recovery and distribution of applied radioactivity in Laacher Hof AXXa soil

Fraction	% applied radioactivity at days after treatment:								
	0	**3**	**7**	**14**	**28**	**43**	**59**	**92**	**120**
Carbon dioxide	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
Ambient extract	96.1	98.0	98.8	98.3	95.6	98.0	99.4	99.4	96.5
Microwave extract	1.36	1.7	1.7	1.9	1.9	1.9	2.1	2.0	2.1
Total extractable	**97.7**	**99.7**	**100.5**	**100.2**	**97.5**	**99.9**	**101.3**	**101.4**	**98.6**
Non-extractable	**0.6**	**0.6**	**0.8**	**0.6**	**0.7**	**0.9**	**0.8**	**0.7**	**0.8**
Total recovery	**98.2**	**100.4**	**101.3**	**101.0**	**98.2**	**100.9**	**102.1**	**102.1**	**99.4**

n.a not analyzed

Table 7.1.2.1.2-14 Recovery and distribution of applied radioactivity in Dollendorf II soil

Fraction	% applied radioactivity at days after treatment:								
	0	**3**	**7**	**14**	**28**	**43**	**59**	**92**	**120**
Carbon dioxide	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
Ambient extract	95.9	97.3	97.8	96.9	93.9	96.9	97.2	97.2	93.8
Microwave extract	2.5	2.5	2.4	2.5	2.5	2.4	2.8	3.0	2.7
Total extractable	**98.4**	**99.8**	**100.2**	**99.4**	**96.4**	**99.4**	**100.0**	**100.2**	**96.4**
Non-extractable	**1.3**	**1.2**	**1.3**	**1.4**	**1.4**	**1.7**	**1.8**	**1.7**	**2.0**
Total recovery	**99.7**	**101.0**	**101.6**	**100.8**	**97.8**	**101.1**	**101.8**	**101.9**	**98.5**

n.a not analyzed

Table 7.1.2.1.2-15 Recovery and distribution of applied radioactivity in Laacher Hof Wurmwiese soil

Fraction	% applied radioactivity at days after treatment:								
	0	**3**	**7**	**14**	**28**	**43**	**59**	**92**	**120**
Carbon dioxide	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
Ambient extract	96.8	97.8	97.7	97.0	93.8	97.5	97.1	96.9	95.3
Microwave extract	1.8	1.9	2.0	2.1	2.1	2.2	2.6	2.7	2.3
Total extractable	**98.7**	**99.7**	**99.7**	**99.2**	**95.9**	**99.6**	**99.7**	**99.7**	**97.6**
Non-extractable	**1.0**	**1.1**	**1.0**	**1.0**	**1.1**	**1.2**	**1.2**	**1.1**	**1.3**
Total recovery	**99.7**	**100.9**	**100.8**	**100.3**	**97.0**	**100.9**	**100.9**	**100.8**	**99.0**

n.a not analyzed

Table 7.1.2.1.2-16 Recovery and distribution of applied radioactivity in Hoefchen Am Hohenseh 4a soil

Fraction	% applied radioactivity at days after treatment:								
	0	**3**	**7**	**14**	**28**	**43**	**59**	**92**	**120**
Carbon dioxide	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Organic volatiles	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a.**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**	**< 0.1**
Ambient extract	96.9	98.2	98.4	97.4	94.5	98.1	99.1	99.2	95.5
Microwave extract	2.4	2.4	2.4	2.6	2.9	2.4	2.6	2.9	2.8
Total extractable	**99.2**	**100.7**	**100.7**	**100.0**	**97.0**	**100.5**	**101.7**	**102.1**	**98.3**
Non-extractable	**0.9**	**1.0**	**1.0**	**0.9**	**1.1**	**1.3**	**1.3**	**1.3**	**1.2**
Total recovery	**100.1**	**101.8**	**101.8**	**101.0**	**98.2**	**101.8**	**103.1**	**103.4**	**99.5**

n.a not analyzed

[1-^{14}C]-Trifluoroacetate was not degraded during the incubation time of 120 days in the laboratory and so the total extractable percentages in the tables above are also the concentrations of M05 TFA at each time-point in each soil. The calculated half-life of the test item was >1000 days (SFO kinetics).

Conclusions

Over the course of the study (120 days) trifluoroacetate was not degraded in soils under aerobic conditions over the course of the study (120 days).

Report: **KCA 7.1.2.1.2 /04; [redacted] N. 2012b**
Title: [1-^{14}C]Trifluoroacetate: Concentration dependent Mineralization under Aerobic Conditions
Organisation: [redacted]
Report No.: EnSa-12-03445
Bayer CropScience Document M-441101-01-1
Publication: unpublished
Dates of experimental work: 28th February 2011 to 23rd August 2011
Guidelines: **OECD 307, EU 95/36/EC, EC 1107/2009, OPPTS 835.4100**
Deviations: None
GLP/GEP **Yes**

Executive Summary

The concentration dependent mineralization rate of M05 TFA [1-^{14}C] trifluoroacetate (BCS-AZ56567, the sodium salt of AE C0502988), a metabolite that may be formed in soil from use of certain pesticidally active substances, was determined in four different soils: in a sandy loam, a clay loam, a sandy loam and a silt loam for 120 days under aerobic conditions in the dark at 20 ± 1°C and 55 ± 5% WHC_{max} (maximum water holding capacity). Trifluoroacetate (sodium salt) was applied at three different rates: 21 µg/100 g soil dry weight (equivalent to a field rate of 75 g/ha), 1.1 µg/100 g soil dry weight (equivalent to a field rate of 40 g/ha), and 0.1 µg/100 g soil dry weight (equivalent to a field rate of 0.4 g/ha), in the test system. The test system consisted of Erlenmeyer flasks equipped with traps for the collection of CO_2. Samples (traps) were analyzed after 30, 59 and 120 days of incubation. At each sampling date the amounts of trapped CO_2 were determined by liquid scintillation counting (LSC). The identification of the test item in the application solution was achieved by HPLC-MS and

HPLC-MS/MS. The test conditions outlined in the study protocol were maintained throughout the study.

No significant mineralization (≥1% of AR) could be detected in any of the samples under the used laboratory conditions during an incubation time of 120 days.

Materials and Methods

Test Material:

[1-^{14}C]-Trifluoroacetate, radiochemical purity > 98%, Batch no.: KMI 9078.

Test Design:

The metabolism of [1-^{14}C]-Trifluoroacetate in soil under aerobic conditions was investigated in four agricultural soils at 20°C. The soil characteristics are listed below.

Table 7.1.2.1.2-17 Properties of soils used in a M05 TFA concentration dependent mineralization soil study.

Parameter	Result/Value			
Soil	Laacher Hof AXXa	Dollendorf II	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh
Geographic Location[1]	Monheim am Rhein	Blankenheim	Monheim am Rhein	Burscheid
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudoll	Fine-loamy, mixed, active, frigid Typic Fauludept	Loamy, mixed, mesic Typic Argudalf	Loamy, mixed, mesic Typic Argudalf
Map Reference	N 51° 04.65' E 06° 53.52'	N 50° 22.90' E 06° 43.00'	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'
Textural Class (USDA)	Sandy loam	Clay Loam	Sandy Loam	Silt loam
Sand (%)	77	29	57	25
Silt (%)	14	40	26	60
Clay (%)	9	31	17	15
pH in $CaCl_2$ (1:2)	6.2	7.3	5.1	6.4
pH in water (1:1)	6.5	7.5	5.4	6.7
pH in water (saturated paste)	6.2	7.4	5.2	6.5
pH in KCl (1N)	6.0	7.1	4.7	6.1
Organic Matter (%)	2.8	9.5	3.3	4.1
Organic Carbon (%)	1.6	5.5	1.9	2.4
Cation Exchange Capacity (meq/100g)	8.7	21.2	10.0	13.6
Water Holding Capacity at pF 2.5 (%)	12.2	34.9	18.2	26.3
Maximum Water Holding Capacity (%)	46.9	84.9	57.6	62.0
Bulk Density (disturbed, g/cm^3)	1.26	0.97	1.13	1.08
Soil Biomass at:				
0 days	642	3145	598	1016
59days	484	2798	316	696
120 days	323	1931	173	499

[1] in North Rhine-Westphalia, Germany.

The soils were collected from agricultural areas of Germany and were taken fresh from the field. A few days before starting the study, the soil was sieved to a particle size of ≤ 2 mm. Subsequently the soil moisture was determined by drying aliquots of the soils at 105°C. Ultrapure water was added to adjust each soil aliquot to 55% of the maximum water holding capacity. The weights of all test vessels were recorded and the samples were pre-equilibrated at about 20 °C in the dark over a weekend.

The incubation systems were static systems and consisted of Erlenmeyer flasks (300 mL) with 100 g soil (dry weight) for each sampling interval. The flasks were closed with trap attachments, which were easily permeable for oxygen. The traps contained soda lime for adsorption of CO_2 and a polyurethane foam plug for adsorption of volatile organic compounds.

Three application solutions with concentrations of approximately 20 µg/mL, 1 µg/mL and 0.1 µg/mL in water were prepared. 1 mL of this was applied drop-wise, by use of a micropipette, to each pre-equilibrated soil sample. This resulted in treatment rates of: 21 µg/100 g soil dry weight (equivalent to a field rate of 75 g/ha), 1.1 µg/100 g soil dry weight (equivalent to a field rate of 40 g/ha), and 0.1 µg/100 g soil dry weight (equivalent to a field rate of 0.4 g/ha). Dose checks were taken during the application procedure.

Water loss due to evaporation from the soil was determined by weighing the sampled flasks without the traps on each processing day. If necessary, the evaporated portions were replaced. Determination of the soil microbial viability (microbial biomass) was performed at the start, in the middle (DAT-59) and at the end (DAT-120) of the study.

The trap attachments from samples (in duplicate from each concentration treatment) were taken at 30, 59 and 120 days after treatment. These trap attachments containing soda lime and PU foam were processed within eight days.

For determination of $^{14}CO_2$, the soda lime contained in the trap attachments was dissolved in 60 mL 18% hydrochloric acid. The liberated CO_2 was absorbed by a special absorption / scintillation cocktail and radioactivity was measured by liquid scintillation counting.

Findings

No significant amounts of radioactivity could be detected in the processed soda lime traps.

Conclusions

No significant mineralization (≥1% of AR) could be detected in any of the samples under the used laboratory conditions during an incubation time of 120 days.

Report:	**KCA-7.1.2.1.2 /05; [redacted] P. 2013c**
Title:	An Assessment of the Environmental Impact of the Photodegradate of Flurtamone: Benzoic Acid
Organisation:	[redacted]
Report No.:	VC/12/006A Bayer CropScience Document **M-453572-02-1**
Publication:	unpublished
Dates of experimental work:	Not applicable
Guidelines:	**EC 1107/2009, OPPTS 835.4100**
Deviations:	None
GLP/GEP	**No** (position paper)

Executive Summary

A new soil photolysis study was recently conducted on flurtamone radiolabelled in the unsubstituted phenyl ring, to allow complete understanding of flurtamone degradation on soil surfaces in sunlight. In this study M06 benzoic acid was identified as a degradate and was found at > 5% of applied radioactivity at consecutive time-points. It was, therefore, a new significant metabolite. It is likely that it was a very minor metabolite in the aerobic soil study conducted on [phenyl-UL-^{14}C]-flurtamone and so its identification was not required in that study. In the soil photolysis study the concentration of benzoic acid increased (to 7.2% AR) up to the penultimate time-point and then decreased at the final time-point (to 5.8% AR).

A review of the readily available data on benzoic acid showed that it is a naturally occurring substance that is known to be readily biodegradable under both aerobic and anaerobic conditions. It is rapidly degraded in soil under aerobic and anaerobic conditions and in groundwater. When formed from flurtamone under aerobic conditions it will degrade (by mineralization). If anaerobic conditions occur after it has been formed it will still degrade by mineralization. Benzoic acid has low toxicity to vertebrates and aquatic organisms and is not therefore of ecotoxicological concern.

Materials and Methods

The review report for benzoic acid stated it is a substance naturally occurring in soil where it can be readily biodegraded. In addition the report shows that rapid and almost complete mineralization occurs in lake water samples and in sewage samples. Sodium benzoate is the reference substance required by the OECD aerobic mineralization in surface water guideline.

An assessment produced under the auspices of the World Health Authority (WHO) also reported that standardized tests on ready or inherent biodegradation showed benzoic acid to be readily biodegraded. Easy degradation of benzoic acid was also observed in different non-standardized experiments using sewage sludge as inoculum. It was found to be degraded by adapted anaerobic sewage sludge at 86-93% after 14 days by aerobic activated sludge (adapted) at 95% after 5-20 days and by unadapted aerobic activated sludge at 61-69% after 2-3 days with a preceding lag time of 2-20 h. The use of a synthetic sewage inoculated with laboratory bacterial cultures led to complete degradation of benzoic acid after 14 days under anaerobic conditions.

Rapid mineralization has been shown to occur in groundwater and subsurface soil samples. In groundwater, a half-life of 41 h has been found for benzoic acid under aerobic condition. Half-lives of

7.3 h and 18.2 h, respectively, have been observed for aerobic and anaerobic degradation of benzoic acid metabolized to $^{14}CO_2$ in subsurface soils.

From its physical/chemical properties, benzoic acid emitted to water and soil is not expected to volatilize to the atmosphere or to adsorb to sediment or soil particles. From the results of numerous removal experiments, the main elimination pathway for benzoic acid should be biotic mineralization.

The WHO assessment concluded that the available data indicate that benzoic acid has only a low toxicity potential in the terrestrial environment. This is confirmed by the ecotoxicological data on it. Benzoic acid is considered of low toxicity to vertebrates and aquatic organisms. The oral and dermal toxicity in rats resulted in a LD_{50} > 2000 mg/kg bw and dermal toxicity in rabbits was LD_{50} > 5000 mg/kg bw. Acute studies on fish (*Oncorhynchus mykiss*), aquatic invertebrates (*Daphnia magna*) and algae (*Pseudokirchneriella subcapitata*) revealed NOEC's of 120 mg/L for fish, 56 mg/L for *Daphnia* and 7.5 mg/L (E_bC_{50} = 33 mg/L) for algae. A risk to these organisms can therefore be excluded.

Conclusions

Benzoic acid produced by photodegradation of flurtamone is not a compound of concern for the environment.

Report: **KCA-7.1.2.1.2 /06; [redacted] 2013b**
Title: Flurtamone: Kinetic Modelling Evaluation of Aerobic Soil Degradation Studies to Derive Metabolite Modelling Endpoints
Organisation: [redacted]
Report No.: VC/13/912B
Bayer CropScience Document [M-475181-01-1]
Publication: unpublished
Dates of experimental work: Not applicable
Guidelines: **Commission Regulation (EC) No 1107/2009 of 21 October 2009**
Deviations: None
GLP/GEP **No – but conducted to Good Modelling Practice**

Executive Summary

A kinetic evaluation of aerobic soil degradation studies performed in the laboratory has been conducted according to FOCUS Kinetics guidance [FOCUS, 2006]. The degradation studies were performed with the active substance flurtamone or with separately dosed metabolites in order to derive DT_{50} values and formation fractions for the metabolites M04 TFMBA and M05 TFA.

Data for flurtamone and its metabolites was evaluated against the FOCUS Kinetics flowcharts [FOCUS, 2006] using the computer program KinGUI2 for the determination of metabolite modelling endpoints.

The resulting geometric mean DT_{50} values (20°C and pF2) and average formation fractions are summarized in the table below. .

Table 7.1.2.1.2-18 Normalised modelling endpoint DT_{50} values (20°C and pF2) and formation fractions for flurtamone metabolites

Compound	DT_{50} [20°C and pF2] (days)	Formation fraction (-)
M04 TFMBA	10.4	0.400
M05 TFA	1000	0.062[a] 0.791[b]

a formation directly from flurtamone b from M04 TFMBA

Materials and Methods

The experimental data for the behaviour of flurtamone metabolites under laboratory conditions have been taken from a number of aerobic soil degradation studies performed either with the parent compound [Burr and Austin, 1993; Eckermann and Weuthen, 2012] or separate dosing of metabolites to soil [Burr, 1999; Eckermann and Junge, 2012; Eckermann, 2012].

In the Burr and Austin [1993] study, the route of degradation of flurtamone under aerobic conditions was investigated using [TFMP-U-^{14}C] flurtamone on three soils (sandy loam soil, clay loam soil and artificial Speyer 2.2 soil) in the dark at 22°C and 75% 1/3 bar. The artificial Speyer 2.2 soil is excluded from evaluation. In the Eckermann and Weuthen [2012] study, the rate of degradation of [TFMP-U-^{14}C]-flurtamone was investigated in four soils under aerobic conditions, incubated at 20°C and 55% MWHC (loamy sand soil, loam soil, sandy loam soil and silt loam soil).

Due to specific activity changes during the metabolism of flurtamone to M05 TFA the tabulated data for M05 TFA (as %AR) in the original study reports [Burr and Austin, 1993; Eckermann and Weuthen, 2012] need to be multiplied by 6 prior to the kinetic modelling evaluations.

In the Burr [1999] study, the rate of degradation of [^{14}C]-M04 TFMBA was investigated in three soils (sandy loam, silty clay loam and clay loam) under aerobic conditions, incubated at 20°C and 45% MWHC. In the Eckermann and Junge [2012] study, the rate of degradation of [UL-^{14}C]-M04 TFMBA was investigated in four soils under aerobic conditions, incubated at 20°C and 55% MWHC (loamy sand soil, loam soil, sandy loam soil and silt loam soil).

In the Eckermann [2012] study, the rate of degradation of [1-^{14}C]-M05 TFA was investigated in four soils under aerobic conditions, incubated at 20°C and 55% MWHC (sandy loam soil, clay loam soil, sandy loam soil and silt loam soil). M05 TFA did not degrade under the test conditions, with the DT_{50} values determined to be >1000 days.

Time zero residues for flurtamone were set to the recovered amount. Values <LOD (ND) were set to ½ LOD for the first non-detect time-point (additional points <LOD not considered in the evaluations).

The determination of the kinetic values followed the recommendations of FOCUS rules. These were aimed at deriving DT_{50} values for use as model inputs according to the FOCUS guidance document on degradation kinetics [FOCUS, 2006]. The kinetic evaluations were performed according to the respective decision flowchart for the determination for use in modelling endpoints [FOCUS, 2006].

The sampling times and residue data were entered into KinGUI and optimisations carried out for SFO kinetics. Metabolites were fitted in a stepwise procedure (sequential addition of metabolites) with a final optimisation of all parameters. The kinetic evaluations and the statistical calculations were

conducted with KinGUI (v2.0) [Meyer, 2011] using iteratively re-weighted least-square (IRLS) optimisation.

SFO kinetics were acceptable for deriving parent flurtamone modelling endpoint DT_{50} values [Hardy, 2013]. Thus SFO kinetics were used for flurtamone in the parent/metabolite evaluations.

The flurtamone degradation data were entered into KinGUI. Simple first order (SFO) kinetics were applied to all datasets in a stepwise procedure according to the flowchart for the determination of modelling endpoints. Where formation fractions optimised to 1 during the evaluation they were fixed to 1, the optimisation repeated and that dataset used for endpoint determination. The M04 TFMBA degradation data were entered into. Simple first order (SFO) kinetics were applied to all datasets according to the flowchart for the determination of modelling endpoints.

Findings

The normalized DT_{50} values for use as modelling endpoints are shown in table on the following page.

For M05 TFA no robust DT_{50} values could be derived and a default DT_{50} of 1000 days (assumed to be at 20°C and pF2) was selected as the modelling endpoint. The parent/metabolite evaluations gave an average formation fraction of 0.063 direct from flurtamone and 0.791 from M04 TFMBA.

Table 7.1.2.1.2-19: Normalized (20°C and pF2) DT_{50} values for M04 TFMBA as modelling endpoints

Soil/study	DT_{50} (days)	DT_{90} (days)	ffm (-)	Chi^2 (%)	t-test (-)	Visual	DT_{50} 20°C/pF2 (days)
Boarded Barns Farm (91/25), Ongar, Essex, UK – ffm fixed to 1	12.1	40.0	0.473	28.5	7.04E-04	o	8.4
Manningtree Farm(91/26) Manningtree Essex, UK – ffm fixed to 1	63.0	209	0.249	21.2	2.06E-04	o	63
Laacher Hof AXXa Monheim am Rhein, Germany	7.8	25.9	0.411	11.5	2.37E-14	+	7.8
Dollendorf II, Blankenheim, Germany	8.0	26.5	0.571	8.1	2.00E-16	+	8.0
Laacher Hof Wurmwiese, Monheim am Rhein, Germany – ffm fixed to 1	4.6	15.2	0.217	12	1.55E-13	+	4.6
Hoefchen am Hohenseh 4a, Burscheid, Germany	13.4	44.5	0.483	23.3	1.16E-08	+	13.4
Manningtree Farm(98/16) Manningtree, Essex, UK [1]	9.5	31.4	-	6.4	3.01E-09	+	9.5
Flint Hall (98/22), Royston, Herts., UK[1]	13.6	45.0	-	10.0	2.22E-08	+	13.6
Boarded Barns Farm (98/24), Ongar, Essex, UK[1]	10.6	35.0	-	9.4	1.24E-08	+	10.6
Laacher Hof AXXa, Monheim am Rhein, Germany[1]	6.0	19.6	-	6.4	9.36E-13	+	6.0
Dollendorf II, Blankenheim, Germany[1]	7.4	24.6	-	9.9	4.74E-10	+	7.4
Laacher Hof Wurmwiese, Monheim am Rhein, Germany[1]	2.8	9.3	-	4.9	4.35E-15	+	2.8
Hoefchen am Hohenseh 4a Burscheid, Germany[1]	8.5	28.3		9.1	5.79E-10	+	8.5
Geometric mean*							**10.4***
Average			**0.400**				

[1] M04 TFMBA applied studies Visual assessment: + = good, o = moderate, - = poor

* Geometric mean of Laacher Hof AXXa (6.8 days), Dollendorf II (7.7 days), Wurmwiese (3.6 days) and Hoefchen (10.7 days) soils calculated first

Conclusions

Following guidance established by FOCUS [2006], DT_{50} values and formation fractions were derived for flurtamone metabolites M04 TFMBA and M05 TFA for use as modelling endpoints in exposure assessments.

CA 7.1.2.1.3 - Anaerobic degradation of the active substance

In an anaerobic study ([redacted]., 1999, M-183875-01-1) conducted to the old EU guideline, in which treatment was made to an already anaerobic system, no significant degradation of flurtamone was observed. A new study, designed to meet current guidelines, was conducted and is presented below, along with a pilot study not previously available.

Report:	**KCA-7.1.2.1.3 /02; [redacted] B.V. 1991a**
Title:	Flurtamone aerobic and anaerobic soil metabolism – Pilot Study
Organisation:	[redacted]
Report No.:	Chevron Chemical; Report No. – not given Bayer CropScience Document M-249325-02-1
Publication:	unpublished
Dates of experimental work:	1989-1991 (not stated in report)
Guidelines:	**The study was conducted as a pilot study prior to conducting a full EPA study.**
Deviations:	Not applicable
GLP/GEP	**No**

Executive Summary

This study has been fully summarized under 7.1.1.

Conclusion:

Flurtamone is stable under anaerobic conditions.

Report:	**KCA-7.1.2.1.3/03; [redacted] M. 2012**
Title:	[Trifluoromethylphenyl-UL-^{14}C]-Flurtamone: Anaerobic Degradation/Metabolism in One European Soil.
Organisation:	[redacted]
Report No.:	MEF-11/791 Bayer CropScience Document M-440634-01-1
Publication:	unpublished
Dates of experimental work:	9th November 2011 to 29th June 2012
Guidelines:	**OECD 307**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

This study has been fully summarized under 7.1.1.2.

Conclusions

In soil under anaerobic conditions flurtamone is essentially stable.

CA 7.1.2.1.4 - Anaerobic degradation of metabolites, breakdown and reaction products

In an anaerobic study ([redacted]., 1999, M-183875-01-1) conducted to the old EU guideline, in which treatment was made to an already anaerobic system, no significant degradation of flurtamone was observed. Therefore no significant metabolites were formed, the behaviour of which could be examined under anaerobic conditions. A new study, designed to meet current guidelines, was conducted and is presented below, along with a pilot study not previously available.

Report:	**KCA-7.1.2.1.4 /01; [redacted] B.V. 1991a**
Title:	Flurtamone aerobic and anaerobic soil metabolism - Pilot Study
Organisation:	[redacted]
Report No.:	Chevron Chemical; Report No: not given Bayer CropScience Document M-249325-02-1
Publication:	unpublished
Dates of experimental work:	1989-1991 (not stated in report)
Guidelines:	**The study was conducted as a pilot study prior to conducting a full EPA study.**
Deviations:	Not applicable
GLP/GEP	**No**

Executive Summary

This study has been fully summarized under 7.1.1.2.

Conclusion:

The soil metabolite of flurtamone, M04 TFMBA, is stable in soil under anaerobic conditions.

Report:	**KCA-7.1.2.1.4 /02; [redacted] M. 2012**
Title:	[Trifluoromethylphenyl-UL-^{14}C]-Flurtamone: Anaerobic Degradation/Metabolism in One European Soil.
Organisation:	[redacted]
Report No.:	MEF-11/791 Bayer CropScience Document M-440634-01-1
Publication:	unpublished
Dates of experimental work:	9th November 2011 to 29th June 2012
Guidelines:	**OECD 307**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

This study has been fully summarized under 7.1.1.2.

Conclusions

The main aerobic soil metabolites of flurtamone, M04 TFMBA and M05 TFA, are essentially stable under anaerobic conditions.

Report:	**KCA-7.1.2.1.4 /03; [redacted] P. 2013c**
Title:	An Assessment of the Environmental Impact of the Photodegradate of Flurtamone: Benzoic Acid
Organisation:	[redacted]
Report No.:	VC/12/006A Bayer CropScience Document **M-453572-02-1**
Publication:	unpublished
Dates of experimental work:	Not applicable
Guidelines:	**EC 1107/2009, OPPTS 835.4100**
Deviations:	None
GLP/GEP	**No** (position paper)

Executive Summary

This position paper is comprehensively summarized under 7.1.2.1.2/04.

Findings

The available data show that M06 benzoic acid will readily degrade under anaerobic as well as aerobic conditions. They also show that benzoic acid has low toxicity to vertebrates and aquatic organisms and is not therefore of ecotoxicological concern.

Conclusions

Benzoic acid produced by photodegradation of flurtamone is not a compound of concern for the environment.

CA 7.1.2.2 - Field studies

CA 7.1.2.2.1 - Soil dissipation studies

A study under this section that was previously submitted was a terrestrial field soil dissipation study in Europe ([redacted], 1996, M-158558-01-1). Flurtamone and M04 TFMBA were completely dissipated at all four of the test sites in Europe during the course of the field study. For flurtamone the mean DT_{50} as reported was 58 days (range 46 to 65 days) and the mean DT_{90} was 191 days (range 152 to 216 days). These values were obtained by the by the methodology available at the time, however the currently recommended kinetic analysis has been conducted on these studies. No flurtamone or M04 TFMBA could be detected after 10 months at any of the sites.

A new kinetic evaluation of the data has been conducted and is summarized below.

Report:	**KCA-7.1.2.2.1 /03; [redacted] 2013c**
Title:	Kinetic Modelling Analysis of Flurtamone from Field Soil Residue Studies Conducted in Europe
Organisation:	[redacted]
Report No.:	VC/13/012E Bayer CropScience Document M-475190-01-1
Publication:	unpublished
Dates of experimental work:	Not applicable
Guidelines:	**Commission Regulation (EC) No 1107/2009 of 21 October 2009**
Deviations:	None
GLP/GEP	**No – but conducted to Good Modelling Practice**

Executive Summary

The aim of this evaluation was to conduct a kinetic analysis of four datasets from trial locations reported in one European field soil dissipation study performed with the active substance flurtamone. The kinetic analysis derived un-normalised DT_{50} values for flurtamone, for use as modelling and trigger endpoints.

The un-normalised DT_{50} values determined under field conditions are considered appropriate for use in calculations of PEC_{Soil} and as trigger endpoints and are summarized below.

Table 7.1.2.2.1-1 Un-normalised modelling endpoint DT_{50} values (SFO) for PEC_{Soil} calculations

Trial	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual assessment
Bologna, Italy	91.6	304	16.5	4.93E-06	+
Mereville, France	37.4	124	28.1	0.000248	o
Goch, Germany	27.2	90.5	14.7	2.61E-05	+
Manningtree, UK	63.8	212	21.3	1.69E-05	+

Visual assessment: + = good, o = moderate, - = poor

Table 7.1.2.2.1-2 Un-normalised trigger endpoint DT_{50} values

Trial	Best-fit kinetic	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual assessment
Bologna, Italy	SFO	91.6	304	16.5	k 4.93E-06	+
Mereville, France	DFOP	15.3	238	13.5	k_1 0.062751 k_2 0.000160	+
Goch, Germany	DFOP	20.0	155	4.3	k_1 0.0501 k_2 0.0294	+
Manningtree, UK	DFOP	33.6	247	7.8	k_1 0.19331 k_2 2.35E-05	+

Visual assessment: + = good, o = moderate, - = poor

Materials and Methods

A terrestrial field soil dissipation study with flurtamone has been conducted at four trial locations across Europe (France, Germany, Italy and UK) [[redacted], 1996 M-158558-01-1]. Applications were made to bare soil at a nominal application rate of 325 g ha^{-1} pre-emergence of a winter wheat crop. Soil samples were collected at regular intervals for up to 436 days and analyzed for flurtamone (and M04 TFMBA). Where residue values were < LOQ (9 g/ha) a value of ½ LOQ of 4.5 g/ha was used for the first sampling time point and not used thereafter.

All datasets were evaluated (using free optimisation of parameters) for flurtamone. DT_{50} and DT_{90} values were determined for the degradation of the test item flurtamone. The determination of the kinetic values followed the recommendations of FOCUS rules and was aimed at deriving DT_{50} values for use as modelling and trigger input according to the FOCUS guidance document on degradation kinetics [FOCUS, 2006]. The kinetic evaluations and the statistical calculations were conducted with KinGUI (v2.0) [Meyer, 2011] using iteratively re-weighted least-squares (IRLS) optimisation.

The model fits were evaluated using a chi-square (χ^2) error statistic and visual inspection of residual plots. The kinetic analyses and optimisations were carried out using the replicate data, however for the χ^2 analysis the predicted concentration is compared to the mean measured value. An evaluation of the visual fit is a key assessment in FOCUS Kinetics. In this evaluation, a three-point scale has been used: good (+), moderate (o) and poor (-).

The flurtamone residue data were entered in to KinGUI and optimised using SFO kinetics according to the parent flowcharts for modelling and trigger endpoints. SFO kinetics were acceptable for the determination of modelling endpoint DT_{50} values.

The flurtamone residue data were entered in to KinGUI and optimised using FOMC kinetics according to the flowchart for parent trigger endpoints. FOMC showed improvement over SFO kinetics for all trials except Bologna. Thus, SFO was accepted as the best-fit kinetic for Bologna, with the remaining three trials requiring further evaluation with DFOP kinetics.

The flurtamone residue data for Mereville, Goch and Manningtree were entered in to KinGUI and optimised using DFOP kinetics according to the flowchart for parent trigger endpoints.

Findings

SFO kinetics for flurtamone were acceptable for modelling endpoint determination and the results are summarized in Table 7.1.2.2.1-1.

SFO kinetics were acceptable for trigger endpoint determination for the Bologna trial, with DFOP being the best-fit kinetic for Mereville, Goch and Manningtree and the results are summarized in Table 7.1.2.2.1-2

Conclusions

Kinetic modelling analysis of data from the flurtamone field soil dissipation studies showed acceptable model fits for flurtamone. The un-normalised DT_{50} values calculated can be used in PEC_{Soil} calculations and as trigger endpoints.

CA 7.1.2.2.2 - Soil accumulation studies

No studies required under this point

Overall Conclusions on the Rate of degradation of flurtamone (and its metabolites) in Soil (Point 7.1.2)

The rate of degradation of flurtamone and its two major soil metabolites has been extensively studied under laboratory conditions. An old study conducted under US conditions (and with significant issues concerning experimental set-up and study conduct) in two agricultural soils gave normalized DT_{50} values of 47.8 and 41.3 days. Recent studies conducted with flurtamone labelled in each of two rings gave DT_{50} values ranging from 9.1 days to 12.0 days. The geometric mean of all six values is 17.1 days (and 10.6 days if only the modern studies are considered).

Kinetic data on the degradation of M04 TFMBA are derivable from the old (previously submitted) study on flurtamone as well as the more recent studies on flurtamone and as the old (previously submitted) study on M04 TFMBA as well as the recent study on M04 TFMBA. As a result, some thirteen values are available. Twelve of these are in the range 2.8 days to 13.6 days. The only value to fall significantly outside this range comes from the old study on flurtamone and is 63.0 days. The corresponding normalized values are also 2.8 days to 13.6 days and the outlier is 62.3 days. The geometric mean (of all thirteen values is 10.4 days.

For M05 TFA no robust DT_{50} values could be derived and a default DT_{50} of 1000 days (assumed to be at 20°C and pF2) has been selected as modelling endpoint.

The data from the previously submitted field study gave DT_{50} values of 27.2 days to 91.6 days, with the latter figure being used for PEC_{Soil} calculations.

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

In the previously submitted adsorption/desorption study ([redacted] 1990 (revised 1992, M-163688-01-1)) the K_{OC} of flurtamone ranged from 88 to 543 mL/g (n = 5, mean 329 mL/g, 1/n = 0.978). That study deviated from the current guidelines in several respects so a new study completely consistent with the current guidelines was commissioned.

Report:	**KCA-7.1.3.1.1 /02; [redacted] 2011**
Title:	[trifluoromethylphenyl-UL-^{14}C]-flurtamone: Adsorption/Desorption in Five Different Soils
Organisation:	[redacted]
Report No.:	AS-154 Bayer CropScience Document M-401272-02-1
Publication:	Published
Dates of experimental work:	19th October 2010 to 18th January 2011
Guidelines:	**OECD 306, EU 95/36/EC, OPPTS 835.1220, PMRA.**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The adsorption/desorption characteristics of [trifluoromethylphenyl-UL-^{14}C]-flurtamone were studied in five soils of differing characteristics: Wurmwiese, Hoefchen am Hohenseh, Laacher Hof AXXa, Dollendorf II and Hanscheider Hof. The adsorption phase of the study (Definitive Test) was carried out using pre-equilibrated air-dried soil with [trifluoromethylphenyl-UL-^{14}C]-flurtamone at concentrations of nominal 1, 0.3, 0.1, 0.03, and 0.01 mg/L in the dark at 20 °C ± 2 °C for 24 hours. The equilibration solution used was 0.01 M aqueous $CaCl_2$ solution except the soil Dollendorf II, where a 0.01 M aqueous $CaCl_2$ solution with 50 ppm biocide ($HgCl_2$) was used.

The following soil to solution ratios were applied to the soils: Wurmwiese, Hoefchen am Hohenseh and Laacher Hof AXXa ratio of 1:10 and Dollendorf II and Hanscheider Hof 1:20. The 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, except for the highest concentration, where three desorption cycles were performed. The aqueous supernatant after adsorption and desorption was separated by centrifugation and the [trifluoromethylphenyl-UL-^{14}C]-flurtamone residues in the supernatant were analyzed by liquid scintillation counting (LSC). The adsorption parameters were calculated using the Freundlich adsorption isotherm. Samples without soil were used as control in preliminary test and did not show adsorption to the vessels or degradation.

For the soils Wurmwiese, Hoefchen am Hohenseh, Laacher Hof AXXa and Hanscheider Hof the parental mass balance after 96 h showed that 90.4-93.4% of applied [trifluoromethylphenyl-UL-^{14}C]-flurtamone could be recovered. The balance of soil Dollendorf II after 72 h was below 90%. Therefore the test was repeated with $HgCl_2$ as biocide and the parental mass balance after 96 h was 92.4%. The mass balance of the soils was determined by LSC of the supernatants after adsorption and desorption and by combustion of the remaining soils. The overall material balance for all concentrations for individual specimens was in the range of 97.5-107.0%, 95.9-105.2%, 94.3-105.7%, 97.1-108.2%, and 97.1-105.8% of the applied radioactivity in soils Wurmwiese, Hoefchen am

Hohenseh, Dollendorf II, Laacher Hof AXXa, Hanscheider Hof, respectively. In the definitive adsorption test 29.5-43.7%, 37.5-52.8%, 34.0-50.7%, 32.6-49.0%, and 28.6-45.3% of the applied test material was adsorbed in soils Wurmwiese, Hoefchen am Hohenseh, Dollendorf II, Laacher Hof AXXa, Hanscheider Hof, respectively.

The calculated adsorption constants $KF_{(ads)}$ of the Freundlich isotherms for the five test soils ranged from 4.5 mL/g to 10.6 mL/g and the normalized $KF_{OC\ (ads)}$ values ranged from 225.1 to 287.8 mL/g (mean 257.1 mL/g). The Freundlich exponents, 1/n, were in the range of 0.876 to 0.88 (mean 0.88), indicating that the concentration of the test item did affect the adsorption behaviour.

At the end of one adsorption and one desorption phase, 43.3-62.6%, 35.2-50.8%, 40.4-59.5%, 39.7-60.5%, and 37.8-63.4% of the initially adsorbed amount were desorbed in soils Wurmwiese, Hoefchen am Hohenseh, Dollendorf II, Laacher Hof AXXa and Hanscheider Hof, respectively.

The mean desorption $KF_{(des)}$ values ranged from 4.5 – 11.2 mL/g and the normalized $KF_{OC\ (des)}$ values ranged from 236.7 to 298.5 mL/g, and were 1.00-1.15 times higher than those obtained for adsorption phase.

The following table summarizes the key results from the study:

Table 7.1.3.1.1-3 Flurtamone sorption characteristics in five different soils

Soil	Adsorption				Desorption			
	K_F (mL/g)	1/n	R^2	K_{FOC} (mL/g)	K_F (mL/g)	1/n	R^2	K_{FOC} (mL/g)
Wurmwiese	4.47	0.884	0.9980	253.9	4.48	0.842	0.9903	254.3
Hofchen am Hohenseh	6.11	0.881	0.9985	255.1	7.1	0.866	0.9957	293.8
Dollendorf II	10.62	0.878	0.9997	225.1	11.17	0.864	0.9976	236.7
Laacher AXXa	5.30	0.870	0.9966	287.8	5.49	0.841	0.9872	298.5
Hanscheider Hof	8.21	0.859	0.9985	264.8	8.38	0.814	0.9924	270.3
Mean	6.95	0.876	0.9982	257.3	7.36	0.845	0.9927	270.7

Flurtamone can be classified as being of low mobility in soil according to the Briggs classification.

Materials and Methods

Test Material:

[Trifluoromethylphenyl-UL-^{14}C]-flurtamone, radiochemical purity > 98%,
Batch no.: KATH 64601.

Test Design:

The objectives of this study were adsorption/desorption measurements of [trifluoromethylphenyl-UL-^{14}C]-flurtamone on five different soils for the determination of K_F and K_{FOC} values. The soil characteristics are listed overleaf.

All soils were air-dried and sieved (2 mm).

Table 7.1.3.1.1-4 Properties of soils used in a flurtamone adsorption/desorption study

Parameter	Soil				
Soil	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh	Dollendorf II	Laacher Hof AXXa	Hanscheider Hof
Geographic Location[1]	Monheim am Rhein	Burscheid	Blankenheim	Monheim am Rhein	Burscheid
Map Reference	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'	N 50° 22.90' E 06° 43.00'	N 51° 04.85' E 06° 55.52'	N 51° 04.48' E 07° 06.36'
Textural Class (USDA)	Loam	Silt loam	Clay Loam	Sandy loam	Loam
Sand (%)	51	27	31	73	35
Silt (%)	28	54	38	18	50
Clay (%)	21	19	31	9	15
pH in $CaCl_2$ (1:2)	5.5	6.8	7.4	6.7	6.8
pH in water (1:1)	5.3	6.6	7.3	6.2	5.6
Organic Matter (%)	3.1	7.9	7.9	3.1	5.3
Organic Carbon (%)	1.8	4.6	4.6	1.8	3.1
Cation Exchange Capacity (meq/100g)	10.8	13.9	21.9	9.0	10.0

[1] in North Rhine-Westphalia, Germany.

Preliminary tests were conducted in order to confirm the stability of the test item in calcium chloride solution and to determine appropriate soil/solution ratios and equilibration periods. The solubility and stability of test item in 0.01 M $CaCl_2$ was checked using the highest proposed concentration for the definitive study experiments (1.0 mg/L). Two test vessels were filled with 49.95 mL of calcium chloride solution. Afterwards 50 µL of the application solution were added. The vessels were capped and placed on a horizontal shaker. After shaking periods of 24, 48, 72 and 96 hour intervals, aliquots from each solution were analyzed by LSC and HPLC.

The study guideline (OECD) requires that ratios of soil to solution should be such that 20-80% of the applied test item is adsorbed to the soil after equilibration. The appropriate amount of soil for each soil was tested by weighing 5, 2 and 1 g aliquots into the centrifuge tubes and adding 19.98 mL of calcium chloride solution. Afterwards the test vessels were pre-equilibrated for at least 16 hours. Prior to the application the suspended particles were centrifuged for about 5 minutes at 1000 rpm. Afterwards 20 µL of the application solution was applied to the test vessels. The test was conducted at a concentration of 1.00 mg/L. The centrifuge tubes were capped and shaken for 24 hours. The tubes were centrifuged and the radioactivity in the supernatants was determined by LSC. In addition, the pH was measured in one replicate at each ratio for all soils. The amount of soil to be used for subsequent tests was decided for each soil based on the outcome of the test.

The determination of the equilibration time was important in order to decide on the most appropriate shaking time for the adsorption/desorption measurements. The test was conducted using soil/solution ratios of 1:10 (soils: Wurmwiese, Hoefchen am Hohenseh and Laacher Hof AXXa) and 1:20 (soils: Dollendorf II and Hanscheider Hof). The nominal concentration in this test was 1.0 mg/L. For each soil five test systems were equilibrated by shaking with a volume of 49.95 mL of calcium chloride solution overnight (at least 16 hours). Afterwards, 50 µL of the application solution were added in order to adjust the final volume to 50 mL. The test vessels were shaken and samples were taken after 2, 4, 6, 24, 30, 48, 72, 96 and 120 hours, respectively. As a result of some instability of the test item in Dollendorf II soil the test was repeated with $HgCl_2$ present as a biocide.

At each sampling interval the mentioned test vessels were centrifuged and aliquots of 100 µL were taken from the supernatants for LSC. A mass balance was carried out from all test systems. For this the test item was extracted up to four times with 40 mL ACN for 30 min (15 min. using ultrasonic bath). The amount of radioactivity of the combined extraction solvents was determined by LSC (aliquots of 1.0 mL). The amount of the test item in the combined and concentrated extracts was investigated by means of HPLC. Radioactivity remaining in the soil was quantified after combustion in a sample oxidiser.

For the definitive experiments the parameters determined in the preliminary tests were used. The adsorption measurements were carried out in duplicate with five different concentrations of the test item. Soil/solution ratios of 1:10 (soils Wurmwiese, Hoefchen and Hohensch and Laachen Hof AXXa) and 1:20 (soil Dollendorf II and Hanscheider Hof) and equilibration times (24 hours) established for each soil in the preliminary tests were used for the definitive test. The batches were equilibrated (16 h). Following the determined shaking period, the test vessels were centrifuged and the supernatant was completely decanted. The volumes were measured gravimetrically (density of the solution was set equivalent to 1 g/mL) and recorded, and two aliquots of 1 mL from all soils were taken for LSC. The pH was measured in all supernatants (single measurements).

For all soils serial desorption cycles (including 3 desorptions) were performed on the 1.00 mg/L concentration. Single point desorption was performed on the 0.30 mg/L, 0.10 mg/L, 0.03 mg/L and 0.01 mg/L concentrations. The volume of solution removed was replaced by an equal volume of calcium chloride solution. The test vessels were shaken for the same period as for adsorption (3 x 24 hours for the first, second, and third desorption) and handled as described in the previous section. The pH was measured in the specimens with the 1.00 mg/L concentration. At the end of the desorption cycles all soil residues were mixed with approximately 0.4 g cellulose/g soil, air-dried, homogenised and completely combusted. Mass balance was established on all specimens from the definitive tests. No chromatographic analysis was carried out since the stability of the test item was already proved in the preliminary tests.

High performance liquid chromatography was carried out on a system that comprised a Kinetex C18 100 A; 50 x 4.6 mm; 2.6 µm column (with a Phenomenex C18 2,6 µm100 A ;50 x 4.6 mm; 2.6 µm pre-column) connected to a radiodetector fitted with a 500 µL scintillation cell and to a UV detector set at 210 nm. The mobile phase was a gradient of water containing 0.2% phosphoric acid against acetonitrile. The retention time of flurtamone was approximately 9 minutes. Three radio-HPLC runs without injection of radioactive compounds were conducted. The whole radioactive signal of the runs was marked (cpm was chosen as signal unit). The background was calculated as the cpm-mean value (e.g. 8.3 cpm) of these HPLC-runs. For all following HPLC-runs the two fold of the determined background (e.g.17 cpm) was subtracted from each run. All signals higher than 50 cpm and with a minimum area of 100 area units were integrated.

The equilibrium partitioning in adsorption and desorption measured for five concentration dilution series per soil was used to calculate Freundlich isotherms for adsorption and desorption.

Findings

Preliminary tests.

The preliminary tests showed that the adsorption rate varied from 22.6% to 79.8% of the applied radioactivity after a shaking period of 24 hours. Based on the adsorption rates in this test soil/solution

ratios of 1:10 were used for the soils Wurmwiese, Hoefchen am Hohenseh and Laacher Hof AXXa and 1:20 for the soils Dollendorf II and Hanscheider Hof for all tests.

With exception of soil Dollendorf II the chromatographic analysis of the clear centrifuged supernatants and the soil extracts, taken after 24, 48, 72, 96 and 120 hours shaking period, showed that under test conditions the test item was stable in all soils. Soil Dollendorf II showed a degradation behaviour. Already after 72 h of shaking the parental mass balance was determined to be 89.9%. Due to the instability of the test item the test was repeated with $HgCl_2$ as biocide.

A mass balance was carried out from all test systems. For this the test item was extracted up to three times with 40 mL acetonitrile for 30 min (15 min. using ultrasonic bath). The parental mass balances were ≥ 90.4 % for the soils Wurmwiese, Hoefchen am Hohensch, Laacher Hof AXXa and Hanscheider Hof after 96 h equilibration time calculated as recovery of the test item in supernatants and soil extracts of the test vessels. For the Dollendorf II soil the repeat test with biocide added gave a mass balance of 92.4% at 96 hours. The tests showed that equilibrium was established after 24 hours of shaking for all soils.

Definitive tests

The adsorption behaviour of [trifluoromethylphenyl-UL-^{14}C] Flurtamone 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).

For these experiments the applied radioactivity (AR) was defined as the total amount of radioactivity contained in the dosed 20 µL aliquots of the respective application solutions for each concentration. The radioactive material balance in the test soils was calculated as sum of the radioactivity detected within the decanted supernatant solutions after the adsorption and desorption steps and the radioactivity found in the air-dried and combusted soil residues. The total radioactivity recovery with respect to the individual vessel ranged from 94.3 % to 108.2 % of the applied radioactivity. The complete material balance observed for all test systems therefore demonstrated that no significant amount of radioactivity dissipated from the test vessels or was lost upon processing.

The adsorption behaviour was accurately described by the Freundlich equation for all test soils, reflected in correlation coefficients of fit of calculated adsorption isotherms to the respective measured data close to one. The constant of the adsorption isotherm according to Freundlich was calculated by linear regression from the concentrations in soil and calcium chloride solution supernatants. The Freundlich adsorption coefficients (K_{Fads}) were normalised for the percentage of organic carbon content of the test soils to obtain Freundlich K_{FOCads} values as a general comparability basis of the test item adsorption behaviour.

Evaluations of the desorption experiments performed for all soils at five test concentrations were conducted. Desorption isotherms for desorption were calculated in analogy to the adsorption experiment. A second and a third desorption step was conducted on the 1.00 mg/L concentration. Using the data determined in the adsorption and in the first, second and third desorption cycle a serial desorption isotherm and corresponding K_{Fser} and K_{FOCser} values were calculated for each soil.

The results are summarized in the following tables.

Table 7.1.3.1.1-5 Flurtamone sorption characteristics in five different soils

Soil	Adsorption				Desorption (1st)			
	K_F (ml/g)	1/n	R^2	K_{FOC} (mL/g)	K_F (mL/g)	1/n	R^2	K_{FOC} (mL/g)
Wurmwiese	4.47	0.884	0.9980	253.9	4.48	0.842	0.9903	254.3
Hofchen am Hohenseh	6.17	0.881	0.9985	255.1	7.11	0.866	0.9957	293.8
Dollendorf II	10.62	0.878	0.9992	225.1	11.17	0.864	0.9976	236.7
Laacher AXXa	5.30	0.877	0.9966	287.8	5.49	0.841	0.9853	298.5
Hanscheider Hof	8.21	0.859	0.9985	264.8	8.38	0.814	0.9924	270.3
Mean	6.95	0.876	0.9982	257.3	7.30	0.845	0.9927	270.7

Table 7.1.3.1.1-5 Flurtamone sorption characteristics in five different soils (continued)

Soil	Serial desorption (2nd & 3rd) at highest conc.			
	K_F (mL/g)	1/n	R^2	K_{FOC} (mL/g)
Wurmwiese	4.56	1.076	0.9976	258.9
Hofchen am Hohenseh	6.09	0.891	0.9930	251.8
Dollendorf II	12.06	1.076	0.9850	255.4
Laacher AXXa	8.22	1.509	0.9150	446.5
Hanscheider Hof	12.32	1.393	0.9235	397.4
Mean	8.65	1.189	0.9612	322.0

Conclusions

The adsorption coefficients K_{Fads} of [trifluoromethylphenyl-UL-^{14}C]-flurtamone in five test soils were determined to range from 4.4692 mL/g to 10.6234 mL/g based on the Freundlich equation. The corresponding organic carbon normalised adsorption coefficients K_{FOCads} ranged from 225.1 mL/g to 287.8 mL/g (mean 257.3 mL/g). The Freundlich exponents (1/n) were in the range of 0.8594 to 0.8837, indicating that the concentration of the test item affected the adsorption behaviour only slightly. The desorption coefficients K_{FOCdes} of [trifluoromethylphenyl-UL-^{14}C]-flurtamone were found to be in the same range as the respective adsorption coefficients (236.7 mL/g – 298.5 mL/g). The mean desorption $K_{F(des)}$ and the normalized $K_{FOC(des)}$ values were 1.00 - 1.15 times higher than those obtained for adsorption phase.

Based on the soil sorption parameters measured in this study and classification of soil mobility potential according to Briggs, flurtamone with a Koc value of 257 can be classified as of low mobility in soil. This value is the value that has been used for risk assessments (see overall conclusions for 7.1.3).

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

In the originally submitted study ([redacted], 1999, M-207972-01-1) the K_{OC} values for M04 TFMBA were 15 to 52 mL/g (n = 4, mean 32.5 mL/g, 1/n = 0.67).

There were no GLP guideline studies for M05 TFA and so one was commissioned. This is presented below along with published research from which the data were previously submitted but no summary was prepared.

Report:	**KCA-7.1.3.1.2 /01; Richey DG, Driscoll CT, Likens GE; 1997**
Title:	Soil Retention of Trifluoracetate
Organisation:	Department of Civil and Environmental Engineering, Syracuse University, New York, USA and Institute of Ecosystem Studies, Millbrook, New York, USA
Report No.:	Environ. Sci. Technol. 1997, *31*, 1723-1727 Bayer CropScience Document **M-263649-01-1**
Publication:	Environ. Sci. Technol. 1997, *31*, 1723-1727
Dates of experimental work:	Late 1997 – early 1997 (not stated, assumed)
Guidelines:	**Non-guideline study**
Deviations:	Not relevant
GLP/GEP	**No**

Executive Summary

An adsorption-desorption batch-equilibrium study with fifty-four soils (topsoil and sub-soils) was performed with M05 TFA as part of a terrestrial ecosystem project. Thirty-five soil samples were obtained from 15 terrestrial sites of the National Science Foundation Long-Term Ecological Research Program. Soil samples were air-dried and passed through a 2 mm sieve prior to analysis. They were characterized. Batch equilibrium soil sorption experiments were conducted on each of the soils. A 1:5 soil/solution ratio was used for organic soil and 1:20 for mineral soils. The range of concentrations used was: 0, 2, 4, 7, 10, 20, 30 and 40 µmol sodium trifluoroacetate.

The soil/solution suspensions were equilibrated on a reciprocating shaker for 24 hours at 25°C. The samples were then centrifuged and filtered through 0.45 µm polypropylene fibre membrane filters. The M05 TFA concentration of the filtrate was measured using ion chromatography. The possible pH dependence of M05 TFA adsorption was examined using 20 samples of each soil. After the initial equilibration period, acid or base was added to adjust the pH to 2 or 10, which was then followed by an additional period of equilibration.

Thirty four of the soils tested showed sorption of M05 TFA. At all sites where M05 TFA was evaluated for organic and mineral soils, the organic horizon exhibited greater adsorption. Soils with high organic content were found to retain the highest concentrations of M05 TFA, adsorbing between 20 and 60% of added M05 TFA. The reported soil properties and sorption contents for nine soils with OC <5%, considered to be most representative of agricultural soils, are shown in Table 7.1.3.1.2-1. The K_{OC} values are derived from the reported Freundlich constants and the organic carbon contents (organic matter/1.724).

Table 7.1.3.1.2-1 Soil properties and sorption coefficients of the batch-equilibrium study with M05 TFA in 9 soils with OC <5%

Name (texture)	OC (%)	Clay (%)	pH (-)	CEC (meq/100 g)	Kf (mL/g)	K_{OC} (mL/g)
AB Horizon (mesic)	3.6	9	4.2	1.4	0.68	18.9
E Horizon	1.7	2	3.1	4	0.3	17.8
Bs1 Horizon	4.6	6	3.3	11.6	0.54	11.8
Bs2 Horizon	4.1	7	3.8	7.3	0.5	12.2
Wetland	2.3	1	4.1	3.7	0.60	25.9
E Horizon	0.8	2	3.2	1	0.34	41.9
Bs1 Horizon	4.7	6	4.0	2.3	0.42	8.9
Bs2 Horizon	0.3	3	4.7	0.05	0.17	56.6
Mineral Horizon	3.5	11	4.7	10.4	0.32	9.0

Mean K_{OC} (9 soils) = 22.9 mL/g

Material and Methods

A non-GLP adsorption-desorption batch-equilibrium study with fifty-four soils (top-soils and sub-soils) was performed with M05 TFA as part of a terrestrial ecosystem project. Thirty-five soil samples were obtained from 15 terrestrial sites of the National Science Foundation Long-Term Ecological Research Program. These sites encompassed a wide range of soil and ecological conditions. Soils were also obtained from Lake Agassa Peatlands, Minnesota, near Round Lake, Wisconsin, from two sites in the Czech Republic and three sites in Brazil. At most sites a surface and subsurface soil were collected, but for some sites only a surface soil was obtained. At three sites, the two sites in the Czech Republic and a New Hampshire site, samples were obtained from each of the major soil horizons.

Soil samples were air-dried and passed through a 2 mm sieve prior to analysis. They were characterized for pH, organic matter, soil texture (% clay, % silt, %sand) exchangeable cations (Ca^{+}, Mg^{2+}, K^{+}, Na^{+}, Fe^{3+}, Al^{3+}), cation exchange capacity, water-extractable anions (Cl^{-}, NO_3^{-}, SO_4^{2-}), total soil carbon and total soil nitrogen. Acid-oxalate, pyrophosphate, and citrate-dithionite extractable Fe^{3+} and Al^{3+} were also determined.

Batch equilibrium soil sorption experiments were conducted on each of the soils (and several well characterized soil minerals, such as sodium montmorillinite. A 1:5 soil/solution ratio was used for organic soil and 1:20 for mineral soils. A range of sorbate concentrations (0, 2, 4, 7, 10, 20, 30 and 40 µmol sodium trifluoroacetate) was used for each soil to develop sorption isotherms at ambient temperature. Each soil sample was weighed into a 50 mL polypropylene centrifuge tube, the adsorbate was added and the suspension was equilibrated on a reciprocating shaker for 24 hours at 25°C. The samples were then centrifuged and filtered through 0.45 µm polypropylene fibre membrane filters. The M05 TFA concentration of the filtrate was measured using ion chromatography. The adsorption to the tubes was also investigated.

Langmuir and Freundlich adsorption isotherms were plotted and fitted to evaluate the extent of retention of M05 TFA on all of the soils. Two soils with contrasting physical and chemical characteristics that had relatively high M05 TFA retention were used to examine the factors that influence the compound's adsorption. The possible pH dependence of M05 TFA adsorption was examined using 20 samples of each soil. After the initial equilibration period, acid or base was added to adjust the pH to 2 or 10, which was then followed by an additional period of equilibration.

Comparative adsorption of other inorganic anions was determined by batch adsorption experiments on fluoride, chloride, bromide, nitrate and sulfate (using a salt of each anion). Competitive sorption of M05 TFA with other anions was also measured.

Findings

No detectable adsorption of M05 TFA to the polypropylene tubes was detected.

Thirty four of the soils tested showed sorption of M05 TFA. At all sites where M05 TFA was evaluated for organic and mineral soils, the organic horizon exhibited greater adsorption. Soils with high organic content were found to retain the highest concentrations of M05 TFA, adsorbing between 20 and 60% of added M05 TFA, whilst mineral soils retained 0-15%. The lesser effect of adsorption by mineral surfaces was dependent on pH and the presence of other anions in solution. The reported soil properties and sorption contents for nine soils with OC <5%, considered to be most representative of agricultural soils, are shown in Table 7.2.4-4. The K_{OC} values are derived from the reported Freundlich constants and the organic carbon contents (organic matter/1.724).

Table 7.1.3.1.2-2 Soil properties and sorption coefficients of the batch-equilibrium study with M05 TFA in 9 soils with OC <5%

Name (texture)	OC (%)	Clay (%)	pH (-)	CEC (meq/100 g)	Kf (mL/g)	K_{OC} (mL/g)
AB Horizon (mesic)	3.6	9	4.2	1.4	0.68	18.9
E Horizon	1.7	2	3.1	4	0.3	17.8
Bs1 Horizon	4.6	6	3.3	11.6	0.54	11.8
Bs2 Horizon	4.7	7	3.8	17.3	0.53	12.9
Wetland	2.3	1	4.1	3.7	0.60	25.9
E Horizon	0.8	2	3.2	2	0.34	41.9
Bs1 Horizon	4.1	6	4.0	2.3	0.42	8.9
Bs2 Horizon	0.3	3	4.7	0.05	0.17	58.6
Mineral Horizon	3.5	11	4.7	10.4	0.32	9.0

Mean K_{OC} (9 soils) = 22.9 mL/g

Conclusion:

Trifluoroacetate (M05 TFA) exhibited a range of adsorption constants showing mobility in some of the 54 soils tested and immobility in others. The organic matter content was shown to strongly influence the adsorption. In soils representative of agricultural soils M05 TFA was shown to be potentially mobile with K_{OC} values of 8.9 to 58.6 mL/g (mean of 22.9 mL/g).

Report:	**KCA-7.1.3.1.2 /02; [redacted] 2011**
Title:	[1-^{14}C]-BCS-AZ56567: Adsorption/Desorption in Five Different Soils.
Organisation:	[redacted]
Report No.:	AS-155. Bayer CropScience Document **M-406740-01-1**
Publication:	unpublished
Dates of experimental work:	19th October 2010 to 18th January 2011
Guidelines:	**OECD 306, EU 95/36/EC, OPPTS 835.1220, PMRA.**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The adsorption/desorption characteristics of [1-^{14}C]-BCS-AZ56567 (trifluoroacetate, M05 TFA acid AE C502988 as the sodium salt) were studied in five soils of differing characteristics: Wurmwiese, Hoefchen am Hohenseh, Dollendorf II, Guadalupe, Springfield. The adsorption phase of the study (Definitive Test) was carried out using pre-equilibrated air-dried soil with [1-^{14}C] BCS-AZ56567 at nominal concentrations of 1, 0.3, 0.1, 0.03, and 0.01 mg/L in the dark at 20 °C ± 2 °C for 24 hours. The equilibration solution used was 0.01 M aqueous $CaCl_2$ solution. The soil to solution ratio was defined for all soils as 1:1. Low to virtually no adsorption rates were determined in the preliminary testing.

The aqueous supernatant after adsorption and desorption was separated by centrifugation and the [1-^{14}C] BCS-AZ56567 residues in the supernatant were analyzed by liquid scintillation counting (LSC). The adsorption parameters were calculated using the Freundlich adsorption isotherm. Samples 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 96 h showed that 90.2-94.6% of applied [1-^{14}C]-BCS-AZ56567 could be recovered. The mass balance of the soils was determined by LSC of the supernatants after adsorption and desorption and by combustion of the remaining soils. The overall material balance for all concentrations for individual specimens was in the range of 89.9-98.7%, 96.2-98.2%, 97.4-103.1%, 97.8-100.5% and 96.1-98.9% of the applied radioactivity in soils Wurmwiese, Hoefchen am Hohenseh, Dollendorf II, Guadalupe, Springfield, respectively.

Virtually no adsorption was measured. However, using this data it was not possible to calculate any reasonable Freundlich isotherm and therefore no data describing the Freundlich isotherm (K_F-value and 1/n) were determined.

Considering the measured values it can be assumed that the [1-^{14}C] BCS-AZ56567 has a high mobility in the tested soils.

Materials and Methods

Test Material:

[1-^{14}C] BCS-AZ56567 , radiochemical purity > 98%,
Batch no.: KATH 6492.

Test Design:

The objectives of this study were adsorption/desorption measurements of [1-^{14}C]-BCS-AZ56567 (trifluoroacetate (M05 TFA)) on five different soils for the determination of K_F and K_{FOC} values. The soil characteristics are listed below. All soils were air-dried and sieved (2 mm).

Table 7.1.3.1.2-3 Properties of soils used in M05 TFA adsorption/desorption study

Parameter	Soils				
Soil	Laacher Hof Wurmwiese	Hoefchen Am Hohenseh	Dollendorf II	Guadalupe CA	Springfield NE
Geographic Location	Monheim am Rhein[1]	Burscheid[1]	Blankenheim[1]	Guadalupe, California USA	Springfield Nebraska USA
Map Reference	N 51° 04.86' E 06° 55.25'	N 51° 04.01' E 07° 06.33'	N 50° 22.90' E 06° 43.00'	N 35° 01.95' W 120° 36.10'	N 96° 15.08' W 41° 03.72'
Textural Class (USDA)	Loam	Silt loam	Clay Loam	Sandy Loam	Silt Loam
Sand (%)	51	27	31	56	13
Silt (%)	28	54	38	33	61
Clay (%)	21	19	31	11	26
pH in $CaCl_2$ (1:2)	5.5	6.8	7.4	6.8	6.2
pH in water (1:1)	5.3	6.6	7.3	6.7	6.6
Organic Matter (%)	3.0	4.1	8.0	1.9	2.9
Organic Carbon (%)	1.8	2.4	4.7	1.7	1.7
Cation Exchange Capacity (meq/100g)	10.8	13.9	21.9	16.1	16.1

[1] in North Rhine-Westphalia, Germany.

Preliminary tests were conducted in order to confirm the stability of the test item in calcium chloride solution and to determine appropriate soil/solution ratios and equilibration periods. The solubility and stability of test item in 0.01 M $CaCl_2$ was checked using the highest proposed concentration for the definitive study experiments (1.0 mg/L). Two test vessels were filled with 18 mL of calcium chloride solution. Afterwards 2 mL of the application solution were added. The vessels were capped and placed on a horizontal shaker. After shaking periods of 24, 48, 72 and 96 hour intervals, aliquots from each solution were analyzed by LSC and HPLC.

The study guideline (OECD) requires that ratios of soil to solution should be such that 20-80% of the applied test item is adsorbed to the soil after equilibration. The appropriate amount of soil for each soil was tested by weighing 20, 10 and 2 g aliquots into the centrifuge tubes and adding 18 mL of calcium chloride solution. Afterwards the test vessels were pre-equilibrated for at least 16 hours. Prior to the application the suspended particles were centrifuged for about 5 minutes at 1000 rpm. Afterwards 20 µL of the application solution was applied to the test vessels. The test was conducted at a concentration of 1.00 mg/L. The centrifuge tubes were capped and shaken for 24 hours. The tubes were centrifuged and the radioactivity in the supernatants was determined by LSC. In addition, the pH was measured in one replicate at each ratio for all soils. The amount of soil to be used for subsequent tests was decided for each soil based on the outcome of the test.

The determination of the equilibration time was important in order to decide on the most appropriate shaking time for the adsorption/desorption measurements. The test was conducted using soil/solution ratios of 1:1 for all soils. The nominal concentration in this test was 1.0 mg/L. For each soil five test systems were equilibrated by shaking with a volume of 45 mL of $CaCl_2$ solution overnight (at least 16 hours). Afterwards, 5 mL of the application solution were added in order to adjust the final volume to 50 mL. The test vessels were shaken and samples were taken after 2, 4, 6, 24, 30, 48, 72, 96 and 120 hours, respectively.

At each sampling interval the mentioned test vessels were centrifuged and aliquots of 100 µL were taken from the supernatants for LSC. A mass balance was carried out from all test systems. For this the test item was extracted up to five times with 40 mL water for 30 min (15 min. using ultrasonic bath). The amount of radioactivity of the combined extraction solvents was determined by LSC (aliquots of 1.0 mL). The amount of the test item in the combined and concentrated extracts was investigated by means of HPLC. Radioactivity remaining in the soil was quantified after combustion in a sample oxidiser.

For the definitive experiments the parameters determined in the preliminary tests were used. The adsorption measurements were carried out in duplicate with five different concentrations of the test item. Soil/solution ratios of 1:1 and equilibration times (24 hours) established for each soil in the preliminary tests were used for the definitive test. The batches were equilibrated (16 h). Following the determined shaking period, the test vessels were centrifuged and the supernatant was completely decanted. The volumes were measured gravimetrically (density of the solution was set equivalent to 1 g/mL) and recorded, and two aliquots of 1 mL from all soils were taken for LSC. The pH was measured in all supernatants (single measurements).

For all soils serial desorption cycles (including 3 desorptions) were performed on all concentrations. The volume of solution removed was replaced by an equal volume of calcium chloride solution. The test vessels were shaken for the same period as for adsorption and handled as described in the previous section. The pH was measured in the specimens with the 1.00 mg/L concentration. At the end of the desorption cycles all soil residues were mixed with approximately 0.4 g cellulose/g soil, air-dried, homogenised and aliquots of 300 mg combusted. Mass balance was established on all specimens from the definitive tests. No chromatographic analysis was carried out since the stability of the test item was already proved in the preliminary tests.

High performance liquid chromatography was carried out on a system that comprised a; Sequant ZIC®-HILIC, 5 µm, 150 x 4.6 mm column connected to a radiodetector fitted with a 500 µL scintillation cell and to a conductivity detector. The mobile phase was a gradient of water containing 0.2% phosphoric acid against acetonitrile. The retention time of [1-^{14}C]-BCS-AZ56567 was approximately 9 minutes. Three radio-HPLC runs without injection of radioactive compounds were conducted. The whole radioactive signal of the runs was marked (cpm was chosen as signal unit). The background was calculated as the cpm-mean value (e.g. 8.3 cpm) of these HPLC-runs. For all following HPLC-runs the two fold of the determined background (e.g.17 cpm) was subtracted from each run. All signals higher than 50 cpm and with a minimum area of 100 area units were integrated.

The equilibrium partitioning in adsorption and desorption measured for five concentration dilution series per soil was used to calculate Freundlich isotherms for adsorption and desorption.

Findings

Preliminary tests.

The purity of the test item determined with HPLC showed that the radiochemical purity was determined to be higher than 99.0% after the 96 h test period. T he test item did not show significant adsorption to the inner surfaces of the test vessels. No breakdown of the test item in pure $CaCl_2$ solutions was detected. There was virtually no adsorption of the test item on the tested soils. Based on the very low adsorption rates in this test soil/solution ratios of 1:1 were used for all soils.

A mass balance was carried out from all test systems. The parental mass balances were ≥ 90.2 % for all soils after 96 h equilibration time calculated as recovery of the test item in supernatants and soil extracts of the test vessels. The tests showed that equilibrium was established after 24 hours of shaking for all soils.

Definitive tests

The adsorption behaviour of 1-^{14}C]-BCS-AZ56567 (trifluoroacetate (M05 TFA)) 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).

For these experiments the applied radioactivity (AR) was defined as the total amount of radioactivity contained in the dosed 2 mL aliquots of the respective application solutions for each concentration. The radioactive material balance in the test soils was calculated as sum of the radioactivity detected within the decanted supernatant solutions after the adsorption and desorption steps and the radioactivity found in the air-dried and combusted soil residues. The total radioactivity recovery with respect to the individual vessel ranged from 89.9% to 103.1% of the applied radioactivity. The complete material balance observed for all test systems therefore demonstrated that no significant amount of radioactivity dissipated from the test vessels or was lost upon processing.

Virtually no adsorption was measured. Using the data generated it was not possible to calculate any reasonable Freundlich isotherm and therefore no data describing the Freundlich isotherm (K_F-value and 1/n) were determined. Despite these results experiments were continued and desorption cycles were performed. The results were in the same range as determined for the adsorption cycle. Since no meaningful results were measured they were not reported.

Conclusions

It can be assumed from the results with [1-^{14}C]-BCS-AZ56567 that trifluoroacetate has a high mobility in soils (i.e.K_{FOC} = 0).

CA 7.1.3.2 - Aged sorption

No studies are required under this point. This is an optional experiment and only necessary if the K_{FOC} value from standard studies indicates a leaching risk.

Overall Conclusions on the Adsorption and Desorption of Flurtamone (and its metabolites) in Soil (Point 7.1.3)

A new batch equilibrium adsorption/desorption study was conducted on flurtamone, using five soils. This gave a mean K_{FOC} of 257.3 mL/g. This value is lower than that of the previously submitted study (329 mL/g) and so its use in risk assessments is conservative. The value of 257.3 along with its corresponding 1/n value of 0.876 have therefore been used in modelling.

No new studies to determine the K_{FOC} values for M04 TFMBA were necessary. In the originally submitted adsorption/desorption study the mean K_{OC} value was 32.5 mL/g, and the mean 1/n value was 0.67.

The previously submitted literature study indicated that there were some soil types to which M05 TFA was weakly adsorbed. This was not conducted to GLP or guidelines. The new batch equilibrium adsorption/desorption study showed that there was negligible adsorption to the soils used. As a result a K_{FOC} value of zero (along with a 1/n value of 1) was chosen for use in modelling.

All the values are summarized in the table below.

Table 7.1.3-1 Summary of K_{FOC} and 1/n values for flurtamone and its metabolites

Compound	K_{FOC} (mL/g)	1/n Value
Flurtamone	257.3	0.876
M04 TFMBA	32.5	0.67
M05 TFA	0	1

CA 7.1.4 - Mobility in soil

The mobility of flurtamone and its metabolites in soil have been studied using a range of techniques. The metabolite M05 TFA has been shown to be potentially quite mobile by adsorption and desorption studies. However in addition to downward movement in soil there is also upward movement. This upward movement can be via evaporation and also as a result of plant uptake. To investigate the uptake into plants a study for the determination of the Plant Uptake Factor of the major degradation product M05 TFA has been performed. In addition the confined rotational crop studies with radiolabelled material have also been examined as they contain useful data demonstrating the uptake of M05 TFA into plants.

Report: **KCA-7.1.4/01; [redacted] B. 2013**
Title: Determination of a Suitable Plant Uptake Factor (PUF) of Trifluoroacetic Acid (TFA) for use in Environmental Fate Models in the Target Crop Wheat
Organisation: [redacted]
Report No.: EnSa-13-0545
Bayer CropScience Document **M-468684-01-1**
Publication: unpublished
Dates of experimental work: Not applicable
Guidelines: EFSA PPR-panel (2013) on the use of the Plant Uptake Factor (PUF) in exposure models
Deviations: Not relevant
GLP/GEP **No**

Executive Summary

The EFSA PPR-panel (2013) has recognized in an opinion that plant uptake via roots is significant when calculating leaching exposure concentrations and has recommended the use of the Plant Uptake Factor (PUF) in exposure models, if evidence for the actual occurrence of the process is demonstrated.

Evidence for the occurrence of plant uptake of the metabolite M05 TFA has been demonstrated consistently in crop specific plant uptake studies and supportive confined rotational crop studies, indicating significant M05 TFA translocation from soil to various plant matrices. Given the evidence on the occurrence of the plant uptake, the EFSA PPR-Panel (2013) found the use of measured PUF values appropriate for parameterization of environmental leaching models. From the study results of the target crop (wheat) specific M05 TFA plant uptake study, the average M05 TFA PUF of 0.59 is justified for modelling purposes.

Material and Methods

Evidence on the occurrence of M05 TFA uptake by plants was provided by confined rotational crop studies on wheat, turnip, swiss chard, radish and lettuce using TFA-precursor as well as the two plant uptake studies ([redacted], 2013, M-456754-03-1 and [redacted], 2012, M-440106-01-1).

Confined Rotational Crop Studies

The two confined rotational crop studies indicated that the plant uptake of M05 TFA occurs. One study examined the metabolism of a M05 TFA precursor in wheat, turnip and swiss chard, the second study in wheat, radish and lettuce. Transfer from soil into plant matrices was clearly shown as significantly high residues of M05 TFA were measured in all rotations, while the residues in soil declined simultaneously. Hence, both studies confirmed the occurrence of plant uptake of M05 TFA.

Quantification of Plant Uptake

Quantification of plant uptake is calculated according to the following definitions and formulae:

The PUF is defined as the ratio of the concentration of a compound in the solution taken up by the plant (C_{uptake}) to the concentration of that compound in the soil solution ($C_{porewater}$).

$$PUF = \frac{C_{uptake}}{C_{porewater}} \text{ with } C_{uptake} = \frac{m_{uptake}}{V_{uptake}} \qquad \text{Eq. 1}$$

Leaching models (e.g. PEARL and PELMO) use the PUF to calculate the amount of a compound taken up by a plant with the transpiration stream in each time step according to:

$$m_{uptake} = C_{porewater} \times V_{uptake} \times PUF \qquad \text{Eq. 2}$$

The EFSA PPR-Panel (2013) has stated in its opinion that plant uptake via roots is of significance when calculating leaching exposure concentrations and has recommended the use of the PUF in exposure models. Due to the possible variability of the PUF between different compounds and crops, evidence on the actual occurrence of the process is to be provided when using a PUF > 0 as a higher tier in exposure modelling.

To demonstrate evidence for plant uptake and set an appropriate PUF factor for exposure modelling, EFSA PPR (2013) outlines a tiered approach:

1 Briggs' formula estimating crop independent uptake factors based on the Koc (FOCUS, 2000)

2 Plant uptake experiments with target crop (or justified substituted) of intended PPP use.

A maximum PUF of 1.0 is defined as the upper limit for simulating passive uptake of a compound.

The Briggs approach consists of a relationship between plant uptake and the Kow derived from experimental data showing the uptake and translocation into barley shoots (i.e. the transpiration stream concentration factor (TSCF) for a limited set of non-ionic compounds). As M05 TFA is strongly ionic, it does not fall in the range of validity of Briggs equation. Furthermore EFSA PPR (2013) sees high levels of uncertainty in the Kow based relationships and suggest to limit the use of Kow based approaches (Briggs' formula) to lower tier estimates and recommends plant uptake experiments for further refinement of the PUF. Consequently plant uptake experiments have been conducted.

Plant Uptake Experiments

[redacted] (2013, M-456754-03-1) investigated the actual M05 TFA-specific passive uptake via the aqueous xylem stream in a target crop specific plant uptake study designed to determine an experimental evidence based plant uptake factor (PUF) for use in higher tier environmental leaching model calculations following the recommendations EFSA PPR Panel (2013).

The study design mimics the soil pore water containing a test compound and focuses on determination of the gross uptake of this compound from the soil pore water via the root systems into the plant. Other processes influencing the concentration of a compound in soil such as degradation or adsorption to soil particles are on purpose excluded in the test system as these processes are considered separately in leaching models.

[redacted] (2013, M-456754-03-1) studied plant uptake using five independent replicates of ten wheat plants (target crop) each were maintained in the test solution of ^{14}C-labelled compound at a pH of 6.5. Two additional test systems containing water were prepared as the control. Since the plants take up water from the test solution, an exclusion of the test compound from the water taken up will lead to an increase of the concentration in the remaining solution. The concentration of test item in the solution and the volume of test solution over time are measured during the study. As a result the concentration of the test item over time is known as well as the amount of water consumed by the plant. A comparison of the concentrations in the test solution at the start and at the end of the study (reduced volume of the remaining test solution) allows an indirect estimate of the plant uptake factor of the test compound through the following calculation:

$$PUF = \frac{\ln(m_{final} / m_{wash} / m_{day0})}{\ln(V_{final} / V_{day0})} \qquad \text{Eq. 3}$$

Legend:

m_{day0} = initial mass of test compound / 800 mL test solution [µg]

m_{final} = mass of test compound in the test solution after 8 or 11 days [µg]

m_{wash} = mass of test compound in the wash solution [µg]

V_{day0} = initial volume of test solution (default 800 mL) [L]

V_{final} = volume of test solution after 8 or 11 days [L]

To further confirm the reliability of the PUF determined indirectly by calculating the concentration differences in the test solution over time additional recovery experiments were conducted. The actual radioactivity taken up by the plant after combustion of the test samples was measured and compared it to the estimated amount of M05 TFA taken up by the plant from the test solution in the PUF experiment.

Supportive studies ([redacted], 2012, M-440106-01-1) on the plant uptake of M05 TFA on the target crop wheat and additionally on corn and tomatoes were also conducted.

Findings

Confined Rotational Crop Studies

In the first confined rotational crop study addressing the metabolism in wheat, turnip and swiss chard the radioactive residues were extracted conventionally from all RACs amounting to >97% of the TRRs. From the extract, the metabolites – amongst them M05 TFA - were quantified by HPPC and TLC with a high identification rate of >92.5% of the TRR.

In the target commodity wheat, the highest plant residues were extracted from the matrices of the 2nd rotation and a decline was noted from the material of the 3rd rotation. This is explained by the formation of the M05 TFA metabolite in soil over time followed by significant plant uptake in the 2nd rotation resulting in lower availability in soil for further uptake in the third rotation. This is fully coherent with the steady decline of soil residues with the precursor metabolizing into M05 TFA which is taken up by the plant leading to a decrease in soil from 0.162 mg/kg (day 30) to 0.034 mg/kg (day 317).

Furthermore, M05 TFA was shown to be by far the major metabolite in all commodities of all rotations, ranging from **83.6% to 99.9%** suggesting that the process of plant uptake explains the declining M05 TFA soil residues rather than further metabolization.

In the second study addressing the metabolism in wheat, radish and lettuce the transfer of M05 TFA from soil into the plant matrices was clearly shown, as significantly high total radioactive residues (TRR) were measured in all rotations, while the residues in the soil declined simultaneously.

The residue in 30 day plantings was found to be highly polar in nature with only small quantities of the M05 TFA precursor or non-polar metabolites present in any sample. The residue from the later 120 and 365 day plantings comprised almost entirely polar material. The **main** single **component** of the polar residue was **M05 TFA**, accounting for **up to 80% of the TRR** in the 30 day grain sample.

The results of this study indicate that the uptake of the M05 TFA precursor by rotational crops occurs at low levels only. Clearly identified was the soil metabolite M05 TFA as the major component of the resultant crop residue and with it was confirmed the occurrence of M05 TFA plant uptake.

Plant Uptake Studies

A summary of the results of the plant uptake factor study in wheat are tabulated below.

Table 7.1.4-1: Plant Uptake Factors of Five Independent Test Systems - Wheat

Test ID	$V_{0\,h}$ [L]	$C_{0\,h}$ [µg/L]	$m_{0\,h}$ [µg]	V_{final} [L]	C_{final} [µg/L]	m_{final} [µg]	m_{wash} [µg]	PUF
Wheat (test period for 8 days)								
Test 1	0.800	75.5	60.4	0.260	100.9	26.2	6.5	0.54
Test 2	0.800	75.3	60.2	0.270	103.4	27.9	6.5	0.51
Test 3	0.800	75.4	60.3	0.420	82.2	34.5	4.1	0.69
Test 4	0.800	75.5	60.4	0.280	105.1	29.4	5.7	0.52
Test 5	0.800	76.5	61.2	0.370	86.9	32.1	4.5	0.66
mean:		75.6						**0.59**

Additional recovery experiments demonstrate that the reduced test item amount in test solution at study end could be recovered in the plants (recovery of 92.6%) and thus, it was confirmed that the results of the PUF experiments are reliable.

The significant recovery provides further evidence on the occurrence of M05 TFA plant uptake and indicates the reliability of the test method.

From the supportive studies on wheat, corn and tomatoes, the single test on the target crop wheat provided a PUF value of **0.66**, which is in the same range as the values found in the five independent test systems. In the other two varieties corn and tomatoes higher plant uptake was determined with average PUF values of **0.98** and **0.74** respectively.

Conclusions

The EFSA PPR-panel (2013) has recognized in an opinion that plant uptake via roots is significant when calculating leaching exposure concentrations and has recommended the use of the Plant Uptake Factor (PUF) in exposure models, if evidence for the actual occurrence of the process is demonstrated.

Investigations into the M05 TFA-specific passive uptake in wheat determined an experimental evidence-based Plant Uptake Factor (PUF) of **0.59 for M05 TFA** for use in higher tier environmental leaching model calculations. The translocation of M05 TFA from the test solution into the plant was further confirmed by a high recovery rate of M05 TFA of 92.6% in the combusted plant material.

Supportive experiments showed a M05 TFA PUF-factor of 0.66 for the target crop wheat, which is in the same range as the values found in the multi-replicate wheat study.

Additional evidence for the occurrence of plant uptake of M05 TFA is demonstrated in confined rotational crop studies in which increasing concentrations of M05 TFA in various crop matrices coincided with decreasing soil residues of M05 TFA and its precursors.

Evidence for the occurrence of plant uptake of M05 TFA has been demonstrated consistently in a number of studies, which according to EFSA PPR-Panel (2013) is the necessary condition to justify the use of a PUF > 0 in environmental leaching models. For model assessment of plant protection products applied in wheat a **M05 TFA PUF of 0.59** is justified from study evidence.

Report:	**7.1.4/02; [redacted], R.; 2013**
Title:	Amendment No 2 to Determination of the Plant Uptake Factor of trifluoroacetic acid (TFA) in Wheat
Organisation:	[redacted]
Report No.:	EnSa-13-0357 Bayer CropScience Document **M-456754-03-1**
Publication:	unpublished
Dates of experimental work:	Not applicable
Guidelines:	Not applicable
Deviations:	Not relevant
GLP/GEP	**Yes**

Executive Summary

The Plant Uptake Factor (PUF) of [1-^{14}C]trifluoroacetate (report name: M05 TFA) in wheat was determined in a greenhouse climatic chamber over a study duration of 8 days under controlled

temperature, humidity and light conditions (temperature: 20 °C, approx. 60 - 75% humidity and a day/night cycle of 14 h/10 h).

The initial test item concentration in the test solution was 75.6 µg/L.

Pre-grown wheat plants (BBCH code approx. 15) were exposed to the test solution (nutrient solution plus test item) for a maximum period of 8 days.

The test was performed in 5 replicates with two additional control experiments (test systems without test item). Sample aliquots were analyzed 0, 2, 5 and 8 days after treatment (DAT).

The transpiration volume of the treated plants ranged from 380 to 540 mL at study end.

An additional recovery experiment demonstrated that the reduced test item amount in test solution at study end could be recovered in the plants (recovery of 92.6 %) and thus, it was confirmed that the results of the PUF experiments are reliable.

Plant Uptake Factors were calculated from the amount of the respective test item in the test solution and the volume of test solution at study start and study end. The Plant Uptake Factor (PUF) of M05 TFA in wheat was determined as 0.59, indicating a restricted permeability of M05 TFA through the root cell walls.

Materials and methods

Test Items

[1-^{14}C]trifluoroacetate (report name: M05 TFA)

Batch Code:	KML 8494
Specific Activity:	4.08 MBq/mg
Radiochemical Purity:	> 98% (HPLC/radiodetection)

Test Plants

Wheat plants (variety: Thasos) were pre-grown up to BBCH growth stage 15 on soil in a greenhouse under controlled temperature, humidity and light conditions. These conditions were kept similar to the natural conditions of Central Europe. On the day of study start, the soil was removed from the root system by watering and washing with a gentle water shower. Afterwards the plants were transferred to the test vessels.

Study Design

1. Experimental Conditions

The hydroponic test systems for the Plant Uptake Factor (PUF) experiments consisted of brown glass bottles (volume 1000 mL), filled with 800 mL test solution and ten wheat plants/test vessel. The plants were fixed with elastomer foam and the test vessels were covered with aluminum foil to prevent evaporation of the test solution. The experiments were performed in 5 replicates with two additional control experiments (test systems without test item for determination of the water uptake (transpiration volume)).

The initial test item concentration in the test solution was 75.6 µg/L.

The application solution was prepared in water. 24 mL of the application solution were mixed with 4.5 L nutrient solution (0.01 M 2-morpholino-ethanesulfonic acid and 0.01 M $CaCl_2$ adjusted with sodium hydroxide solution to pH 6.5) to yield the test solution.

During the study, the test systems were incubated in a greenhouse climatic chamber under controlled temperature (20 °C), humidity (approx. 60 - 75%) and light conditions (at least 35 klx and a day/night cycle of 14 h/10 h).

2. Sampling

The test was performed in 5 replicates with two additional control experiments (test systems without test item). Sample aliquots were analyzed 0, 2, 5 and 8 days after treatment (DAT). The initial and final test solution volume was determined at study start (DAT-0) and study end (DAT-8).

3. Analytical Procedures

At each sampling interval aliquots of 1 mL each were taken from each test system. The initial and final test solution volume was determined at DAT-0 and DAT-8, respectively. Additionally, at study end (DAT-8) the roots of each bunch test plants and the respective test vessel were washed with 200 mL acetonitrile/water (1/1, *v/v*). Afterwards, the wheat plants of each test systems were combined, weighed and homogenized.

Test and washing solutions were characterized by liquid scintillation counting (LSC) and HPLC/radiodetection. The amount of residues in the wheat plants was determined by combustion/ liquid scintillation counting.

The recovery rate of the test item was calculated from the amount of test item theoretically taken up by the plants and the amount of test item recovered in the plants. The theoretical amount of test item taken up by the plants was calculated from the initial test item amount in test solution minus the test item amount recovered at DAT-8 in test and washing solution.

The identity of the test item was confirmed by HPLC/radiodetection.

The Plant Uptake Factors were calculated according to the following formula:

$$PUF = \frac{ln\left(\frac{m_{DAT-8} + m_{wash}}{m_{DAT-0}}\right)}{ln\left(\frac{V_{DAT-8}}{V_{DAT-0}}\right)}$$

with:

m_{DAT-0}	= initial amount of test item in test solution	[µg]
m_{DAT-8}	= amount of test item in test solution at study end (DAT-8)	[µg]
m_{wash}	= amount of test item in washing solution	[µg]
V_{DAT-0}	= initial volume of test solution	[L]
V_{DAT-8}	= volume of test solution at study end (DAT-8)	[L]

Note: indices of mass and volumes were summarised as $\mathbf{m_{final}}$ and $\mathbf{m_{0h}}$ and $\mathbf{V_{final}}$ and $\mathbf{V_{0h}}$ in [redacted], 2013, M-468684-01-1

Findings

The transpiration volume of the treated plants ranged from 380 to 540 mL at study end. The transpiration volumes of the controls (untreated test systems) ranged from 350 to 360 mL.

The test items were stable during the entire test period of 8 days. The reliability of this plant uptake experiment was confirmed as the test item amount recovered in the plants at DAT-8 was equal to 92.6% of the theoretically up taken test item amount, calculated from the initial test item amount and the test item amount in test and washing solution at DAT-8.

The concentrations of M05 TFA in the test solution increased towards end of the study (see Table 7.1.4-2), indicating that its plant uptake is low compared with the water up take of the plants. Most probably this is caused by a restricted permeability of the test compound through the root cell walls.

The mean PUF in wheat for M05 TFA was determined as 0.59.

Table 7.1.4-2: Plant Uptake Factors of M05 TFA in Wheat

Replicate	DAT-0			DAT-8				PUF
	V [L]	c [µg/L]	m [µg]	V [L]	c [µg/L]	m [µg]	m_{wash} [µg]	
1	0.80	75.5	60.4	0.26	100.9	26.2	6.5	0.54
2	0.80	75.3	60.2	0.27	103.4	27.8	6.5	0.51
3	0.80	75.4	60.3	0.42	82.2	34.5	4.1	0.69
4	0.80	75.5	60.4	0.28	105.1	29.4	5.7	0.52
5	0.80	76.5	61.2	0.37	86.9	32.1	4.5	0.66
Mean	**0.80**	**75.6**						**0.59**

Conclusion

The Plant Uptake Factors of [1-^{14}C]trifluoroacetate (report name: M05 TFA) in wheat was determined as 0.59.

The reliability of this plant uptake experiment was confirmed by an additional recovery experiment which demonstrates that the reduced test item amount in test solution at study end could be recovered in the plants (recovery of 92.6%).

The plant uptake of the test item was lower compared to the water uptake of the plants. Most probably this is caused by a restricted permeability of the test compound through the root cell walls.

Report: **7.1.4/03; [redacted], R.; 2012**
Title: Determination of the Plant Uptake Factor of TFA (trifluoroacetic acid) in Wheat, Corn and Tomatoes
Organisation: [redacted]
Report No.: LnSa-12-0581
Bayer CropScience Document M-440106-01-1
Publication: unpublished
Dates of experimental work: Not applicable
Guidelines: Not applicable
Deviations: Not relevant
GLP/GEP **Yes**

Executive Summary

The Plant Uptake Factor (PUFs) of [1-^{14}C]trifluoroacetate (report name: M05 TFA) in wheat, tomato and corn was determined in a greenhouse climatic chamber over a study duration of 8 days (wheat and tomatoes) or 11 days (corn) under controlled temperature, humidity and light conditions (temperature: 20 °C, approx. 75% humidity and a day/night cycle of 14 h/10 h).

The initial test item concentration in the test solution was 767.8 µg/L for wheat, 711.8 µg/L for tomatoes and 769.1 µg/L for corn.

Pre-grown wheat and tomato plants were exposed to the test solution (nutrient solution plus test item) for the whole study duration.

For wheat one test system with ten wheat plants was used, whereas for tomatoes and corn two and three test systems with single plants were used, respectively.

Sample aliquots were analyzed 0, 0.1 to 0.2 (equal to 2 to 4 hours), 1, 4, 8 and 11 (only corn) days after treatment (DAT).

Plant Uptake Factors were calculated from the amount of the respective test item in the test solution and the volume of test solution each at study start and study end. The Plant Uptake Factors of M05 TFA were determined as 0.66 in wheat, 0.74 in tomato and 0.98 in corn.

The results indicate that the plant uptake in wheat and tomato was lower than the water uptake, probably due to a restricted permeability of the test item through the root cell walls. Plant uptake in corn was not restricted.

Materials and methods

Test Items

[1-^{14}C]trifluoroacetate (report name: M05 TFA)

Batch Code:	KML 9072 (used for wheat and corn) KML 9241 (used for tomatoes)
Specific Activity:	3.48 MBq/mg
Radiochemical Purity:	≥ 98% (HPLC/radiodetection).

Test Plants

Wheat plants (variety: Thasos), tomatoes and corn were pre-grown on an artificial substrate (Vermiculite) in a greenhouse under controlled temperature, humidity and light conditions. These conditions were kept similar to the natural conditions of Central Europe. On the day of study start, the Vermiculite was removed from the root system by watering and washing with a gentle water shower. Afterwards the plants were transferred to the test vessels.

Study design

1. Experimental Conditions

The hydroponic test systems for the plant uptake factor (PUF) experiments consisted of brown glass bottles (volume 1000 mL), filled with 800 mL test solution and either ten wheat plants/test vessel or one corn or tomato plant/test vessel. The plants were fixed with elastomer foam and the test vessels were covered with aluminum foil to prevent evaporation of the test solution. One test system was prepared for wheat, two test systems for tomatoes and three for corn.

The initial test item concentration in the test solution was 767.8 µg/L for wheat, 711.8 µg/L for tomatoes and 769.1 µg/L for corn.

A definite volume of the application solution was applied to 800 mL of nutrient solution (pH 6) to yield the test solution.

During the study, the test systems were incubated in a greenhouse climatic chamber under controlled temperature (20 °C), humidity (approx. 75%) and light conditions (at least 35 klx and a day/night cycle of 14 h/10 h).

2. Sampling

The test was performed with a single replicate for wheat, in duplicates for tomatoes and in triplicates for corn. Sample aliquots were analyzed 0, 0.1 to 0.2 (equal to 2 to 4 hours), 1, 4, 8 and 11 (only corn) days after treatment (DAT). The test solution volume was determined in parallel.

3. Analytical Procedures

At each sampling interval aliquots of 0.1 mL each were taken from each test system and the test solution volume was determined. Additionally, at study end (DAT-8) the roots of the test plants were washed with 50 mL water.

The radioactivity amount in test and washing solutions was determined by liquid scintillation counting (LSC).

The Plant Uptake Factors were calculated according to the following formula:

$$PUF = \frac{ln\left(\frac{m_{DAT-x} + m_{wash}}{m_{DAT-0}}\right)}{ln\left(\frac{V_{DAT-x}}{V_{DAT-0}}\right)}$$

with:

m_{DAT-0}	= initial amount of test item in test solution	[µg]
m_{DAT-x}	= amount of test item in test solution at study end (DAT-8 or DAT-11)	[µg]
m_{wash}	= amount of test item in washing solution	[µg]
V_{DAT-0}	= initial volume of test solution	[L]
V_{DAT-x}	= volume of test solution at study end (DAT-8 or DAT-11)	[L]

Findings

The transpiration volume of the treated plants at study end was 250 mL for wheat (DAT-8) and ranged from 215 to 265 mL for tomatoes (DAT-8, mean 240 mL) and from 128 to 160 mL for corn (DAT-11, mean 140 mL).

Overall, the concentration of M05 TFA in the test solutions from wheat and tomato experiments increased towards study end (see Table 7.1.4-3 and Table 7.1.4-4, indicating a restricted permeability of the test item through the root cell walls. The concentration of M05 TFA in the test solutions from corn experiments was nearly stable during the whole study period (see Table 7.1.4-5).

The mean PUFs for M05 TFA were determined as 0.66 in wheat, 0.74 in tomato and 0.98 in corn.

Table 7.1.4-3: Plant Uptake Factors of M05 TFA in Wheat

Replicate	DAT-0			DAT-8				PUF
	V [L]	c [µg/L]	m [µg]	V [L]	c [µg/L]	m [µg]	m_{wash} [µg]	
1	0.800	767.8	614.3	0.550	854.1	469.8	9.1	0.66
Mean	**0.80**	**767.8**						**0.66**

Table 7.1.4-4: Plant Uptake Factors of M05 TFA in Tomato

Replicate	DAT-0			DAT-8				PUF
	V [L]	c [µg/L]	m [µg]	V [L]	c [µg/L]	m [µg]	m_{wash} [µg]	
1	0.800	700.9	560.7	0.535	806.5	431.5	6.0	0.62
2	0.800	722.7	578.2	0.585	742.2	434.2	7.3	0.86
Mean	**0.80**	**711.8**						**0.74**

Table 7.1.4-5: Plant Uptake Factors of M05 TFA in Corn

Replicate	DAT-0			DAT-11				PUF
	V [L]	c [µg/L]	m [µg]	V [L]	c [µg/L]	m [µg]	m_{wash} [µg]	
1	0.800	762.0	609.6	0.640	750.2	480.1	3.6	1.04
2	0.800	772.6	618.0	0.672	758.1	509.5	4.4	1.06
3	0.800	772.8	618.2	0.670	785.9	526.5	4.3	0.86
Mean	**0.80**	**769.1**						**0.98**

Conclusions

The Plant Uptake Factors of [1-^{14}C]trifluoroacetate (report name: M05 TFA) were determined as 0.66 in wheat, as 0.74 in tomato and as 0.98 in corn.

The results indicate that the plant uptake in wheat and tomato was lower than the water uptake, probably due to a restricted permeability of the test item through the root cell walls, whereas the plant uptake in corn was not restricted.

CA 7.1.4.1 - Column leaching studies

CA 7.1.4.1.1 - Column leaching of the active substance

In column leaching studies ([redacted], 1994, M-162913-01-1) only small amounts of applied radioactivity appeared in the leachate of all but the sand soil. Most of applied radioactivity in the leachates was flurtamone, accompanied by very low levels (<1 %) of three metabolites: M04 TFMBA (AE C518919), M02 3-trifluoromethyl-N-methyl-mandelamide (AE 0540067) and M01 3-trifluoromethylmandelic acid (AE 0592368).

CA 7.1.4.1.2 - Column leaching of metabolites, breakdown and reaction products

The results of the aged-residue column leaching study ([redacted] 1995, M-162906-02-1) showed that the majority of applied radioactivity remained in the soil columns with only about 6% appearing in leachate. Flurtamone remained as the major compound in the soil and there was virtually no flurtamone in the leachate. The radioactive components in the leachate were M02 3-trifluoromethyl-N-methyl-mandelamide (AE 0540067), M01 3-trifluoromethylmandelic acid (AE 0592368) and M04 TFMBA (AE C518919) with M04 TFMBA being the most abundant (accounting for over half of the radioactivity in the leachate). There were only traces of unidentified components in the leachate. The results of this study add weight to the notion that flurtamone will not leach significantly under real-use conditions. No new aged column leaching studies have been

conducted but a new column leaching study on the soil metabolite trifluoroacetate has been conducted and is summarized below.

Report:	**7.1.4.1.2/02; [redacted] E. 2013**
Title:	[1-^{14}C]Trifluoroacetate: Soil Column Leaching
Organisation:	[redacted]
Report No.:	EnSa-14-0050
	Bayer CropScience Document M-427737-01-1
Publication:	unpublished
Dates of experimental work:	19th July 2011 to 20th February 2012
Guidelines:	OECD Test Guideline No. 312
	US EPA OCSPP Test Guideline No. 835.1240
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The adsorption/desorption behavior of M05 TFA (used test item:[1-^{14}C]trifluoroacetate) was studied in four different soils in the dark in the laboratory at 20 ± 1 °C using two different soil column leaching experiments:

Due to its pK_a-value < 2, trifluoroacetic acid (M05 TFA) is fully deprotonated under environmental conditions resulting in trifluoroacetate. Hence, all studies were conducted using this relevant deprotonated species to elucidate the toxicological and ecotoxicological properties of this degradation product as well as its fate in the environment, plants and animals.

Table 7.1.4.1.2-1 Properties of soils used in a [1-^{14}C]trifluoroacetate soil column leaching study

Soil	Source	Texture (USDA)	pH	OC [%]
Laacher Hof AXXa	Mannheim, Germany	loamy sand	6.2	1.8
Dollendorf II	Blankenheim, Germany	loam	7.4	5.2
Hoefchen am Hohenseh e	Burscheid, Germany	silt loam	6.5	1.6
Laacher Hof Wurmwiese	Monheim, Germany	sandy loam	5.3	1.9

[1-14C]trifluoroacetate was used as test item. Additional soil columns were treated with [triazine-UL-14C]atrazine used as reference item to check the test conditions with a moderately mobile reference item. Tritiated water was added as a tracer to the application solutions of the test item and the reference item to check the hydraulic conditions during the study.

Two different test designs were used for this soil column leaching study. Test design A reflected the test item distribution in the leachate as well as in the soil column, whereas test design B delivered detailed information of the test item distribution only in the leachate by using a larger irrigation volume. Test design A was run once with the test item plus tracer and once with the reference item plus tracer (duplicate soil columns each). The soil columns were eluted under saturated conditions with 392 mL (equal to 200 mm) artificial rain over a period of approx. 48 hours at a constant flow rate. Test design B was run only with the test item plus tracer (duplicate soil columns). The soil columns were eluted under saturated conditions with 984 mL (equal to 502 mm) artificial rain over a period of approx. 120 hours at a constant flow rate.

The test and reference items were sufficiently stable throughout the study.

Material balances for the test item were between 99.4 to 103.8% of the applied radioactivity [% AR] in all soil columns using test design A and between 93.2 to 105.2% AR using test design B. Material balances for the tracer and the reference item were between 89.1 to 105.5% AR and between 96.8 to 104.3% AR, respectively, in all soil columns and both test designs.

Using test design A 62.0 to 97.0% AR of the test item were found in the leachate of the single soil columns. The maximum test item amount was found in the fourth to fifth leachate fraction of each soil column. 58.2 to 90.3% AR of the tracer were found in the leachate of the respective soil columns. The maximum tracer amount was found in the fourth to fifth leachate fraction, i.e. after elution of approximately one saturation volume, demonstrating suitable hydrodynamic properties of the soil columns. The residual amounts of test item and tracer were almost equally distributed in the corresponding soil columns.

The maximum reference item amount was found in the first segment of each soil column using test design A, but translocation of the reference item to deeper soil column segments could be also observed, demonstrating again the suitable hydrodynamic properties of the soil columns. Only minor amounts of the reference item (< 1% AR) were found in the corresponding leachates of the single soil columns, whereas 45.1 to 93.9% AR of the tracer where found there.

Using test design B the applied radioactivity of test item and tracer was completely recovered in the leachates of the respective soil columns. The soil segments of the soil columns run with test design B were not further investigated.

The soil adsorption coefficients (K_d) for the reference item calculated according to Lambert, Hamaker and McCall ranged from 5.1 to 6.3 mL/g in the investigated soils (mean: 5.6 mL/g). The respective organic carbon normalized soil adsorption coefficients (K_{OC}) were in the range of 120.4 to 337.1 mL/g (overall mean: 258.4 mL/g).

The mobility of the test item [1-^{14}C]trifluoroacetate was determined to be almost identical to the mobility of the tracer in all soil columns and in both test designs. Thus, virtually no adsorption was determined for the test item, when calculation was performed according to Ketelle and Swoboda. According to the Briggs classification system, the mobility of M05 TFA can be classified as "very mobile" in all soils.

Considering the experimental results it can be assumed that M05 TFA has a high mobility in the tested soils.

Materials and Methods

Test Material:

[1-^{14}C]trifluoroacetate	
CAS No	2923-18-4
Specific activity	3.48 MBq/mg
Radiochemical purity	> 98% HPLC with radioactivity detector

Test Design:

The soils (see Table 7.1.4.1.2- 1) were sampled fresh from the field (upper horizon of 0 to 20 cm), sieved to a particle size of ≤ 2 mm and stored refrigerated at ≤ 8 °C for 18 days before study start. The

soils were air-dried before application. The soils were taken from agricultural areas representing different geographical origins and different soil properties as required by the guidelines.

Table 7.1.4.1.2- 2: Physico-chemical properties of test soils

Parameter	Results / Units			
Soil Designation	Laacher Hof AXXa	Dollendorf II	Hoefchen am Holzweg 4a	Laacher Hof Wurmwiese
Geographic Location				
City	Monheim	Blankenheim	Burscheid	Monheim
State	North-Rhine Westphalia	North-Rhine Westphalia	North-Rhine Westphalia	North-Rhine Westphalia
Country	Germany	Germany	Germany	Germany
GPS Coordinates	N 51° 04.647 E 006° 53.517'	N 50° 22.899' E 006° 43.001'	N 51° 04.001' E 007° 06.327'	N 51° 04.857' E 006° 55.251'
Soil Taxonomic Classification (USDA)	sandy, mixed, mesic Typic Cambudoll	fine-loamy, mixed, active, frigid Typic Eutrudept	loamy, mixed, mesic Typic Argudalf	loamy, mixed, mesic Typic Argudalf
Soil Series	no information available			
Textural Class (USDA)				
Sand [%] [50 µm – 2 mm]	78	39	19	57
Silt [%] [2 µm – 50 µm]	16	36	64	28
Clay [%] [< 2 µm]	6	25	17	15
pH				
- in $CaCl_2$ (soil/$CaCl_2$ 1/2)	6.2	7.4	6.5	5.3
- in water (soil/water 1/1)	6.5	7.5	6.7	5.5
- in water (saturated paste)	6.6	7.4	6.8	5.5
- in KCl	6.0	7.1	6.1	4.9
Organic Carbon [%]	1.8	5.2	1.6	1.9
Organic Matter [%] [1]	3.1	9.0	2.8	3.3
Cation Exchange Capacity [meq/100 g]	9.4	22.3	12.2	9.9
Water Holding Capacity				
maximum [g H_2O *ad* 100 g soil DW]	43.8	79.3	51.8	60.2
at 0.1 bar (pF 2.0) [%]	13.3	38.2	26.5	20.9
Bulk Density (disturbed) [g/cm^3]	1.22	1.01	1.12	1.13

[1] calculated as: OM [%] = OC [%] · 1.724

DAT: days after treatment

DW: dry weight

GPS: global positioning system

USDA: United States Department of Agriculture

Experimental Conditions

The test systems consisted of glass columns (45 cm length and 5 cm inner diameter) filled with soil to a height of approx. 30 cm. The glass columns were connected to a reservoir containing artificial rain solution (0.01 M aqueous calcium chloride) as well as to a peristaltic pump and a fraction collector. The desired flow rate of the artificial rain was regulated on the pre-column side by a peristaltic pump. The flow of the percolate was regulated on the post-column side in the same way. This set-up allowed

controlling and maintaining a constant level of supernatant water on top of the soil and hence, saturated flow conditions as required for the calculation of adsorption coefficients according to the chromatographic theory.

For preparation of the test systems a layer of quartz wool followed by a layer of washed sea sand was placed in the lower, conical end of the glass column to later retain the soil within the column. Afterwards, each column was dry packed with the sieved, air-dried soils to a height of approx. 30 cm, while gently vibrating. 678 to 856 g of air-dried soil was used per column.

For equilibration, the soil columns were saturated with an upward flow of artificial rain (total volume 400 mL per soil column), establishing a supernatant solution of 10-20 mm above soil surface. The soil columns were allowed to soak for approx. 16 hours in a temperature-controlled walk-in climatic chamber at 20 ± 2 °C in the dark prior to application.

The amount of test item [1-14C]trifluoroacetate for the treatment of the soil columns was based on the intended single maximum field application rate of the parent, resulting in a nominal application rate of 11.0 µg test item per soil column.

All application solutions were prepared in water. The one application solution contained the test item and the tracer side by side; the other application solution contained the reference item and the tracer side by side. For the application the artificial rain solution levels were adjusted to the soil surface levels and 500 µL of the respective application solution were applied dropwise onto the soil surface of the respective soil columns

After application, a glass frit glued to an inside down glass funnel was placed onto the top of each soil column in order to avoid whirling up the soil during the leaching test and to achieve a uniform moistening of the soil surface. The glass columns were then connected to the artificial rain reservoirs as well as to the peristaltic pumps and the fraction collector. The soil was overlaid manually with approx. 20 mL of artificial rain and a saturated flow of approx. 8.2 mL/h was established using the peristaltic pumps. A supernatant of approx. 10-20 mm was maintained above the soil layer throughout the experiment.

All experiments were performed in duplicate in a temperature-controlled walk-in climatic chamber at 20.1 °C in the dark.

Sampling

The leachate was sampled in constant time intervals using a time-controlled automatic fraction collector. For test design A the leachate fractions were sampled in intervals of 6 hours (approx. 50 mL/fraction) using a total irrigation volume of 392 mL. For test design B the leachate fractions were sampled in intervals of 6 hours (approx. 50 mL/fraction) within the first 48 hours of irrigation (equal to a irrigation volume of approx. 400 mL) afterwards they were sampled in intervals of 12 hours (approx. 100 mL/fraction) until end of irrigation using a total irrigation volume of 984 mL.

After draining, the soil columns were deep-frozen and cut each into 5 segments of approx. 6 cm height for further analysis (test design A only).

Analytical Procedures

The volume and the pH value of each leachate fraction was determined.

The single soil segments were extracted four times at ambient temperature using acetonitrile/ water (1:1, v/v). After each extraction step supernatant and soil were separated by centrifugation and decantation.

The radioactivity content in leachate fractions and soil column segment extracts was determined by liquid scintillation counting. Additionally, selected leachate fractions and soil column segment extracts were analyzed by HPLC/radiodetection. Non-extractable residues were determined by combustion/ liquid scintillation counting.

The identity of the test item was elucidated by IC-MS/MS including accurate mass determination. The identity of the reference test item was elucidated by HPLC-MS/MS including accurate mass determination.

The adsorption values for leaching compounds (e.g. the test item) were calculated according to Ketelle and Swoboda.

The adsorption values for non-leaching compounds (e.g. the reference item) were calculated according to Lambert and according to Hamaker / McCall; the results of both mathematical models were averaged.

Results and Discussion

Material Balance

Material balances for the test item were between 99.4 to 103.8% of the applied radioactivity [% AR] in all soil columns using test design A and between 93.2 to 105.2% AR using test design B. Material balances for the tracer and the reference item were between 89.1 to 105.5% AR and between 96.8 to 104.3% AR, respectively, in all soil columns and both test designs.

Degradation of Test Item

The test item was sufficient stable throughout the study, as demonstrated by HPLC/radiodetection analysis of selected leachate fraction and soil column segment extracts.

Findings

Using test design A 62.0 to 97.0% AR of the test item were found in the leachate of the single soil columns. The maximum test item amount was found in the fourth to fifth leachate fraction of each soil column. 58.2 to 90.3% AR of the tracer were found in the leachate of the respective soil columns. The maximum tracer amount was found in the fourth to fifth leachate fraction, i.e. after elution of approximately one saturation volume, demonstrating suitable hydrodynamic properties of the soil columns. The residual amounts of test item and tracer were almost equally distributed in the corresponding soil columns.

The maximum reference item amount was found in the first segment of each soil column using test design A, but translocation of the reference item in deeper soil column segments could be also observed, demonstrating again the suitable hydrodynamic properties of the soil columns. Only minor amounts of the reference item (< 1% AR) were found in the corresponding leachates of the single soil columns, whereas 45.1 to 93.9% AR of the tracer where found there.

Using test design B the applied radioactivity of test item and tracer was completely recovered in the leachates of the respective soil columns. The maximum test item amount was found in leachate fraction 8 of soil Laacherhof AXXa, in leachate fraction 3 of soil Dollendorf II, in leachate fraction 9 of soil Hoefchen am Hohenseh and in leachate fraction 3 or 10 of soil Laacherhof Wurmwiese. The tracer peak was likewise found in leachate fraction 7 to 9 of soil Laacherhof AXXa, in leachate fraction 3 to 4 of soil Dollendorf II, in leachate fraction 9 of soil Hoefchen am Hohenseh and in leachate fraction 3 or 10 of soil Laacherhof Wurmwiese. The soil segments of the soil columns run with test design B were not further investigated, as the applied radioactivity of test item was completely recovered in the leachate.

The soil adsorption coefficients (K_d) for atrazine ranged from 5.1 to 6.3 mL/g in the investigated soils (mean: 5.6 mL/g). The respective organic carbon normalized soil adsorption coefficients (K_{OC}) were in the range of 120.4 to 337.1 mL/g (overall mean: 258.4 mL/g). According to the Briggs classification system, the mobility of atrazine can be classified as “intermediate” to “low”, according to this system.

The mobility of the test item [1-^{14}C]trifluoroacetate was determined to be almost identical to the mobility of the tracer in all soil columns and in both test designs. Thus, virtually no adsorption was determined for the test item. According to the Briggs classification system, the mobility of M05 TFA can be classified as "very mobile" in all soils.

Table 7.1.4.1.2-3: Adsorption coefficients of the test item and reference item in soils
(mean values of duplicate soil columns)

Soil	[1-^{14}C]trifluoroacetate		[triazine-UL-^{14}C]atrazine	
	K_d [mL/g]	K_{OC} [mL/g]	K_d [mL/g]	K_{OC} [mL/g]
Lacherhof AXXa	0.0	0.0	5.1	287.3
Dollendorf II	0.0	0.0	6.3	120.4
Hoefchen am Hohenseh [1]	0.0	0.0	5.4	337.1
Lacherhof Wurmwiese	0.0	0.0	5.6	294.9
Overall Mean	0.0	0.0	5.6	258.4

[1] only one soil column was considered

Conclusion

The mobility of [1-^{14}C]trifluoroacetate the was determined to be almost identical to the mobility of the tracer in all soil columns and in both test designs. Thus, virtually no adsorption was determined for [1-^{14}C]trifluoroacetate. According to the Briggs classification system for mobility of organic chemicals in soil, the mobility of [1-^{14}C]trifluoroacetate can be classified as "very mobile" in all soils.

Considering the experimental results it can be assumed that M05 TFA has a high mobility in the tested soils.

CA 7.1.4.2 - Lysimeter studies

The previously submitted lysimeter study ([redacted], 1996, M-158624-01-1) was reviewed and accepted. Even though the lysimeters represented particularly high risk conditions for leaching (the soil was sandier and with less silt content than defined in the guideline, there was significant earthworm activity) and the application rate was more than double that of the currently intended use rate, parent flurtamone was hardly detectable in the leachate from the two lysimeters (average annual concentration < 0.01 µg/L). Most of the radioactivity in the leachate was identified as M05 TFA. The metabolite M04 TFMBA was also detected but at concentrations < 0.1 µg/L on an annual average basis. Flurtamone does not present a leaching risk. M04 TFMBA is indicated by its K_{OC} value to be more mobile than parent compound, but it has a short DT_{50} and is unlikely to reach groundwater in significant quantities. No new lysimeter studies have been conducted.

CA 7.1.4.3 - Field leaching studies

The results from the adsorption/desorption, column leaching and lysimeter studies show that the mobility of flurtamone and its metabolites is well understood and so studies under this point are not required.

Overall Conclusions on the Mobility in soil of flurtamone (and its metabolites) (Point 7.1.4)

Previously submitted column leaching studies and a lysimeter study showed that flurtamone does not present a leaching risk. The column leaching studies indicated that M04 TFMBA was more mobile than flurtamone but, because of its short DT_{50}, it was found in concentrations of < 0.1 µg/L in the lysimeter study. A new column leaching study on M05 TFA showed that it was poorly retained, as would be expected from the results of the adsorption/desorption studies that have been conducted on it. A study on the plant uptake of M05 TFA, supported by data from confined rotational crop studies showed that this was a significant process and that a PUF of 0.59 is appropriate for use in modelling.

CA 7.2 - Fate and behaviour in water and sediment

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

CA 7.2.1.1 - Hydrolytic degradation

The previously submitted hydrolysis study on flurtamone ([redacted], 1989, M-163684-01-1) showed that it was stable at all pHs. Hydrolysis would not be a route of degradation for flurtamone in natural water bodies.

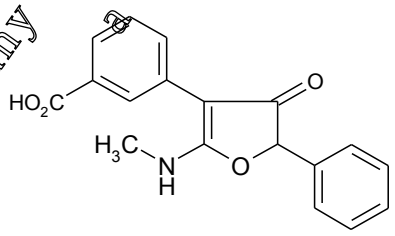
CA 7.2.1.2 - Direct photochemical degradation

Photolytic degradation of Flurtamone is rapid ([redacted], 1993, M-162273-01-1) with a photolytic half-life of 0.6 to 0.7 days of summer sunlight. One major photodegradate was formed during the study, M07 flurtamone-carboxylic acid (AE 1083976, RPA 203597 in the report) also having a short photolytic half-life (11.8 hours), as calculated below. The quantum yield of direct photolysis of flurtamone in aqueous solution ([redacted], 1993, M-162346-01-1) was found to be 3.2×10^{-2}.

Report:	**KCA-7.2.1.2/01; [redacted], 1997**
Title:	Evaluation of the aqueous photodegradation properties of RPA 203597
Organisation:	[redacted]
Report No.:	Rhone-Poulenc Document 444208 Bayer CropScience Document **M-204098-01-2**
Publication:	unpublished
Dates of experimental work:	Not relevant
Guidelines:	**Not applicable**
Deviations:	Not relevant
GLP/GEP	**Not relevant**

Executive Summary

The principal aqueous photodegradation product of flurtamone was identified as M07 flurtamone-carboxylic acid (AE 1083976, RPA 203597 in the report):



Assuming first-order photodegradation kinetics for flurtamone and M07 flurtamone-carboxylic acid, the parameters of the first-order model and the corresponding half-life were estimated using adapted statistical regression methods. The half-life of M07 AE 1083976 under the conditions of the study was estimated as 11.8 hours of irradiation. The compound will therefore be short-lived in the aqueous environment in the presence of light.

Material and Methods

The principal aqueous photodegradation product of flurtamone was identified as M07 flurtamone-carboxylic acid in the study of [redacted] (M-162275-01-1). In that study the half-life of flurtamone was estimated as 13.1 hours of irradiation. The degradation kinetics of AE 1083976 were determined on the basis of the results from the previous study.

Assuming first-order photodegradation kinetics for flurtamone and M07 AE 1083976, the parameters of the first-order model and the corresponding half-life were estimated using adapted statistical regression methods. All calculations were performed on the total measured data set.

For flurtamone:

$$C = C_0 \exp(k.t)$$

where:

C = concentration at time t

C_0 = initial concentration at time 0

k = kinetic constant

In addition to the first-order model estimate a non-linear regression method (Gustafson-Holden) was used to analyse the data for flurtamone:

$$C = C_0(1 bt)^{-a}$$

where:

C = concentration at time t

C_0 = initial concentration at time 0

a,b = non-linear model coefficients

The hypothesis [b = 0] was tested (F test) to verify whether the non-linear model provided a better adjustment of the experimental data.

For M07 AE 1083976/RPA 203597:

Assuming first-order kinetics for the photodegradation of flurtamone and M07 RPA 203597, the rate constant (k2) of the photolyte was estimated by resolving the general equation describing the variation in the concentration of the photolyte as a function of time using non-linear regression methods:

$$C1 = k_1 C_0 (\exp(k_1 t)-\exp(k_2 t))/(k_2-k_1)$$

where:

C1 = concentration of photolyte at time t

C0 = initial concentration of parent product at time 0

k1 = kinetic constant of parent (flurtamone)

k2 = kinetic constant of photolyte

Findings

The statistical analysis of the experimental data for flurtamone showed that the adjustment according to the first-order kinetics was highly significant. In contrast, the non-linear model did not provide a correct adjustment. The estimation of the initial concentration and the half-life of flurtamone used in the calculations for M07 flurtamone-carboxylic acid were, therefore:

C0 = 96.37% Half life = 13.04 hours.

The half-life of M07 flurtamone-carboxylic acid under the conditions of the study was estimated as 11.8 hours of irradiation.

Conclusions

Under the conditions of aqueous photodegradation of flurtamone used in the study, the photodegradation kinetics of flurtamone-carboxylic acid, the principal photodegradation product of flurtamone, formed directly from it, were determined. The half-life was estimated as 11.8 hours of irradiation. The compound will therefore be short-lived in the aqueous environment in the presence of light. Although it is not regarded as significant metabolite it has been considered in risk assessments, for the sake of completeness.

CA 7.2.1.3 - Indirect photochemical degradation

The calculation of the photolytic half-life of flurtamone under environmental conditions was evaluated during the Annex I inclusion ([redacted], 1993, M-102301-01-1, filed in MCA section 2). The half-lives of flurtamone in Europe for a latitude of 52° N in each calendar month ranged from 11.5 h in July to 510.8 h in December. Considering that the calculation is only valid for direct phototransformation in the top millimetres of aquatic systems and the time of year that flurtamone is applied, it is clear that photodegradation will not be an important route of dissipation of the compound in the environment.

CA 7.2.2 - Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 - "Ready biodegradability"

No ready biodegradability studies were previously submitted. A study is summarized below.

Report:	**KCA-7.2.2.1/01; [redacted] A. 2005**
Title:	Flurtamone: Biodegradation
Organisation:	[redacted]
Report No.:	BIS 2005/0079/01 Bayer CropScience DART **M-258453-01-1**
Publication:	unpublished
Dates of experimental work:	6th September 2005 to 5th October 2005
Guidelines:	**92/69/EEC Method C.4-D**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The ready biodegradability of flurtamone was assessed using the 'Manometric Respirometry Test' which is in all essential parts identical to OECD 301 F. Flurtamone batch DP639D, with a purity of

99.5%, was used for the study. A suspension of 100 mg/L in a mineral medium was inoculated with a mixed population of aquatic micro-organisms (activated sludge) and incubated under aerobic conditions in the dark at 22±2°C.

The degradation of the compounds was followed by the determination of oxygen uptake with measurements taken at 2, 6, 8, 10, 14, 16, 20, 22, 24 and 28 days of incubation. All validity criteria of the test method were met. The positive control (sodium benzoate) reached the level for ready biodegradability by 14 days. No toxicity of flurtamone to the micro-organism was observed and the difference of extremes of replicate values at the end of the test was less than 20%. The oxygen uptake of the inoculum blank was ≤ 60 mg/L. The pH of the test vessels at the end of the test was between 6.0 and 8.5.

The results showed that the oxygen demand in the flurtamone test flasks was not greater than that of the blank controls. This indicates that there was no measurable breakdown of flurtamone in those flasks. Flurtamone is not readily biodegradable.

Material and Methods

The ready biodegradability of flurtamone was assessed using the 'Manometric Respirometry Test' which is in all essential parts identical to OECD 301 F.

Flurtamone batch DP639D, with a purity of 99.5%, was used for the study. A suspension of 100 mg/L in a mineral medium was inoculated with a mixed population of aquatic micro-organisms (activated sludge) and incubated under aerobic conditions in the dark at 22±2°C. The origin of this was the aeration tank of a waste water plant treating predominantly domestic sewage (Wupper area water authority). It was collected on 6th September 2005.

Allowance was made for the endogenous activity of the inoculum the establishment of appropriate blanks that contained the inoculum but not the test item (flurtamone). Positive controls containing a reference compound (sodium benzoate) were also set up. In addition a toxicity test was run. The flasks comprising the toxicity test contained both flurtamone and the reference compound (sodium benzoate). Because of the nature of biodegradation and of the mixed bacterial populations used as innocula, the determinations are made at least in duplicate. In this study triplicate test flasks, blank control flasks and reference compound flasks were run with duplicate toxicity control flasks.

The degradation of the compounds was followed by the determination of oxygen uptake with measurements taken at 2, 6, 8, 10, 14, 16, 20, 22, 24 and 28 days of incubation. The pH of the flask contents was determined at the end of the incubation period. Because flurtamone contains nitrogen the increase in concentration of nitrite and nitrate over the 28-day period was determined and the oxygen consumed by nitrification calculated. The oxygen consumption by nitrification was subtracted from the other28 day measurements.

Findings

All validity criteria of the test method were met. The sodium benzoate reached the level for ready biodegradability by 14 days. No toxicity of flurtamone to the micro-organism was observed and the difference of extremes of replicate values at the end of the test was less than 20%. The oxygen uptake of the inoculum blank was ≤ 60 mg/L. The pH of the test vessels at the end of the test was between 6.0 and 8.5.

The biological oxygen demand values are shown in Table 7.7-1.

Table 7.2.2.1-1 Biological oxygen demand (BOD) values in a flurtamone ready biodegradability study

Sample type	Flask	BOD (mg O_2/L) at n days of incubation									
		2	6	8	10	14	16	20	22	24	28
Flurtamone	a1	10	18	20	20	24	24	27	28	28	25*
	a2	11	20	21	22	25	25	28	28	28	25*
	a3	10	18	20	21	24	25	27	28	28	24*
Blank	b1	13	21	22	22	25	27	29	29	31	32
	b2	13	21	24	24	27	28	29	31	31	34
	b3	14	24	25	27	29	29	31	31	32	34
Mean	bm	13	22	24	24	27	28	30	30	31	33
Sodium Benzoate	r1	84	147	154	158	169	172	175	176	178	179
	r2	85	144	151	157	166	168	172	173	173	176
	r3	83	144	151	157	166	168	172	175	175	176
Toxicity control	t1	84	143	151	154	164	166	169	171	172	172
	t2	80	140	147	151	161	164	168	169	169	171

* corrected for oxygen consumed by nitrogen (4 mg O_2/L)

The results show that the oxygen demand in the flurtamone test flasks was not greater than that of the blank controls. This indicates that there was no measurable breakdown of flurtamone in those flasks.

Conclusion:

Flurtamone is not readily biodegradable.

CA 7.2.2.2 - Aerobic mineralisation in surface water

This is a new study requirement.

Report: **KCA 7.2.2.2/01; [redacted]. 2013**
Title: [Trifluoromethylphenyl-UL-^{14}C]flurtamone : Aerobic Mineralization in Surface Water
Organisation: [redacted]
Report No.: Rhone-Poulenc Report 201417
Bayer CropScience Document M-467289-02-1
Publication: unpublished
Dates of experimental work: 11 September 2012 to 31 January 2013
Guidelines: OECD Test Guideline No. 309
Deviations: None
GLP/GEP **Yes**

Executive Summary

The biodegradation of [Trifluoromethylphenyl-UL-^{14}C]-flurtamone was studied in surface water under aerobic conditions ("pelagic test") in the dark for up to 71 days at 20.18 ± 0.06 °C. The test water was freshly sampled from a freshwater dam close to Wiehl near Gummersbach, Germany (Wiehltalsperre, ID: W, GPS Coordinates N 50° 56.8' E 007° 40.0') and consisted of natural water – clear water without sediment - sampled at a depth of 20 cm and 3 m away from the lakefront with pH of 7.4 and a Total Organic Carbon (TOC) content of 3 mg/L. The water was filtered before use.

The amount of test item for the treatment of the test systems was based on the intended target concentration of flurtamone of 10 µg/L (low concentration) and 100 µg/L (high concentration). The test was performed in Erlenmeyer glass flasks with baffles at the bottom each containing 100 mL surface water. The flasks were equipped with traps for the collection of carbon dioxide and volatile organic compounds. During incubation, the water was in smooth motion. Degradation of the control item benzoic acid with DT_{50} of about < 1 d confirmed the microbial activity of the test system.

Duplicate samples were analyzed after 0, 8, 14, 23, 37, 49, 59 and 71 days after treatment (DAT). At each sampling interval the water was centrifuged and decanted. The centrifugation vessels were wiped with paper tissue to take up any thin layer of microbes formed on the inner surface of the vessel. The amount of radioactivity in paper tissue and solids was determined by total combustion of the sample and liquid scintillation counting (LSC) measurement and the low amount detected radioactivity was recorded as non-extractable residue (NER).

The amounts of flurtamone and its degradation products in the water were determined by liquid scintillation counting (LSC) and by HPLC/radiodetection analysis. TLC was used as confirmatory method. Flurtamone and degradation products were identified by HPLC and TLC co-chromatography with known reference standards. The amount of volatiles was determined by LSC.

Mean material balances ranged from 99.8% to 97.6% of applied radioactivity [% AR] for both concentrations.

Flurtamone dissipated from surface water due to degradation. The mean amounts of flurtamone in the surface water decreased from 99.3% and 98.1% AR at DAT-0 to 85.0% and 84.3% AR on DAT-71 for 10 µg/L and 100 µg/L , respectively.

Degradation of flurtamone in surface water was accompanied by the formation of identified degradation product with the following maximum mean amount: M04 TFMBA (AE C518919) with 8.9% AR at DAT-59 for 10 µg/L and 3.4% AR at DAT-59 for 100 µg/L. The sum of two non-identified compounds amounted to a maximum mean value of 5.2% AR (DAT-49) at 10 µg/L and 3.6% AR (DAT-71) at 100 µg/L. No single component exceeded 3.5% AR for 10 µg/L and 2.8% AR 100 µg/L.

The maximum mean amounts of carbon dioxide were 1.1% and 0.1% AR at study end (DAT-71) for 10 µg/L (low concentration) and 100 µg/L (high concentration), respectively. The formation of volatile organic compounds was low with 0.5% and 0.1% AR at DAT-59 for 10 µg/L (low concentration) and study end (DAT-71) 100 µg/L (high concentration), respectively

The formation of non-extractable residues (NER) increased from DAT-0 towards a maximum at DAT-49 from 0.5% to 2.2% AR for 10 µg/L and from DAT-0 towards a maximum at DAT-59 from 0.5% to 2.8% AR for 100 µg/L.

When comparing sterile samples with non sterile samples on DAT-71 the sterile samples showed negligible degradation indicating degradation due to microbial activity.

The half-lives for flurtamone were between 256 and 314 days in the surface water ("pelagic test") under laboratory aerobic conditions as shown below.

Table 7.2.2.1-2 Degradation kinetics of flurtamone under pelagic conditions

System	Kinetic Model	DT_{50} (d)	DT_{90} (d)	Chi^2 Error (%)	Visual Assessment
10 µg/L (low concentration)	SFO	255.7	849.5	2.032	Moderate
100 µg/L (low concentration)	SFO	313.8	> 1000	0.898	Good

SFO = single first order

Flurtamone is degraded slowly under pelagic conditions. The metabolite M04 TFMBA is formed up to 8.9% AR.

Material and Methods

[Trifluoromethylphenyl-UL-^{14}C]-flurtamone with a radiopurity of > 99% and a specific activity of 3.59 MBq/mg was used in this study. The study was carried out using natural water • Wiehltalsperre (ID: W), GPS Coordinates N 50° 56.8' E 007° 40.0', close to Wiehl near Gummersbach, Germany: This is a fresh water dam that is used for the preparation of drinking water. The water was collected from the fore bay Nespen. The water was freshly sampled prior to the start of the study. For characterization of the natural system the following parameters were determined on-site:

- oxygen saturation of water
- temperature of water
- pH of water
- redox potential of water

The water was taken from near the lakefronts at a depth of 20 cm and 3 m away from the lakefront and filled separately in plastic containers. The water was stored for 4 days before application. During storage, the water was well aerated to maintain aerobic conditions. Within one day after sampling the waters were filtered with a 0.963 mm mesh.

At the start of the study the following parameters were measured:

- dissolved organic carbon (DOC)
- total organic carbon (TOC)
- BOD5
- total nitrogen content
- total phosphorus content

The microbial activity of the test system was confirmed by conducting the aerobic mineralization in surface water with control item benzoic acid at a concentration of 10 µg/L.

Erlenmeyer glass flasks with baffles on the bottom (volume about 250 mL) were used as incubation vessels and fitted with trap attachments (permeable for oxygen) containing soda lime for absorption of carbon dioxide and a polyurethane (PU) foam plug for adsorption of volatile organic compounds. For preparation of the test systems 100 mL of the water were added. The flasks were then fitted with trap attachments. The untreated test systems were equilibrated to study conditions by placing them in a temperature-controlled climatic shaker cabinet at 20.18 °C in the dark for 4 days prior to application. The sterile test systems were sterilized by autoclaving twice.

The quantity of 100 µL (low concentration) or 1000 µL (high concentration) of application solutions were applied drop-wise onto the water surface of the test systems to obtain the nominal test item concentrations of 1.0 µg/test system (equal to10 µg/L) or 10.0 µg/test system (equal to 100 µg/L). After application, the test vessels were fitted with trap attachments and incubated in a climatic shaker cabinet at 20.18 °C in the dark. Sterile test systems were treated in the same way. Dose checks were taken during the treatment procedure. 50 µL of application solution of the control item were applied drop-wise onto the water surface of the test systems to obtain the nominal test item concentration of 1.0 µg/test system (equal to 10.0 µg/L). For the solvent controls additionally 100 µL methanol were added to simulate the solvent content that was used in the high concentration test item application solution.

For the test item at 10 µg/L and 100 µg/L eight sampling intervals were distributed over the entire incubation period of 71 days. Duplicate samples were processed and analyzed after 0, 8, 13, 23, 37, 49, 59 and 71 days after treatment (DAT). The DAT-0 samples were processed directly after application. For the sterile samples at 10 µg/L (low) and 100 µg/L (high concentration) duplicate samples were processed and analyzed on DAT-71.

For the control item duplicate samples were processed and analyzed after 0, 3 and 7 days after treatment. The trap attachments were not processed. For the solvent control duplicate samples were processed and analyzed on DAT-7. The trap attachments were not processed.

The redox potentials, pH values and oxygen content of the water were determined at each sampling interval. Prior to opening an incubated test system for processing of the water, volatiles possibly still present in the head space of the test system were purged into the trap attachment by water-saturated air (except for DAT-0 samples). The trap attachments were removed.

For the test item (test systems non-sterile and sterile) 20 mL of the water were removed and made alkaline with 1 mL 0.5 M aqueous NaOH solution. For the control item and the solvent control 20 mL of the water were removed and 2 mL of 1% aqueous sodium azide solution were added as biocide. All samples were stored at -18 °C. These samples of test item (test systems non-sterile and sterile) were used for the determination of dissolved carbon dioxide. Aliquots of the remaining water were taken for LSC and the rest was centrifuged, decanted and the volume measured.

10 mL aliquots of the water were concentrated and taken up in acetonitrile/water (4:1, v/v). The concentrates were centrifuged and clear supernatants transferred to HPLC vials for radio-HPLC analysis.

Following centrifugation of the water the walls of the centrifugation vessels were wiped with tissues to collect the solids. These were air-dried and radioassayed by combustion/LSC. The polyurethane (PU) foam plug was extracted with 50 mL ethyl acetate to desorb possible volatile organic compounds. The radioactivity content was determined by LSC. Carbon dioxide absorbed by soda lime was liberated and trapped in a scintillation cocktail selective for binding of carbon dioxide using an air-tight assembly.

The HPLC system used for quantification and identification comprised a Purospher Star RP18e 250x4.6mm; 5µm column (with a Purospher guard column) connected to a UV detector and a radiodetector fitted with a solid cell. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. LOD of the HPLC-analysis was considered to be about 0.9%

and 0.5% of the applied radioactivity for unknown metabolites in case of 10 µg/L (low concentration) and 100 µg/L (high concentration), respectively.

Thin layer chromatography (TLC) analysis was used to confirm the qualitative and quantitative HPLC results. Samples of representative sampling points (taken at DAT-59 and at DAT-71) were spotted on silica gel plates using an automatic plate spotter. The plates were developed with ethylacetate / 2-propanol / water (75:20:5, v/v/v) in a chromatographic chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products was calculated based on the distribution of the TLC-zones and the amount of RA in the extracts. Regions of the labelled reference item were detected by the same method.

Findings

The amount of total organic carbon in the water was 3 mg/L at DAT-0 confirming that surface water without sediment was used. Degradation of the control item benzoic acid (present in the test system as benzoate) with a DT_{50} of about < 1 d confirmed the microbial activity of the test system.

The pH in the test systems remained stable . The pH in water ranged from 8.7 to 9.6 in the test system with 10 µg/L (low concentration) and from 8.1 to 9.5 in the test system with 100 µg/L (high concentration). The redox potentials measured in the waters and sediments remained at highly positive EH-values throughout the incubation period. The oxygen contents in waters were also determined and ranged from 86 % to 101 % in 10 µg/L test systems and from 90 % to 100 % in100 µg/L test systems. The clearly positive values for the redox potentials and oxygen contents indicate aerobic conditions throughout the entire incubation period.

The radioactive recoveries are summarized in Tables 7.2.2.2-2 and Table 7.2.2.2-3, below.

Table 7.2.2.2-2 Material balance of radioactivityin Wichltalsperre water containing radiolabelled flurtamone at 10 µg/L

Compartment	% applied radioactivity at DAT:								
	0	**8**	**13**	**23**	**37**	**49**	**59**	**71**	**Sterile 71**
Carbon dioxide	0.6	0.2	0.1	0.4	0.7	0.7	0.8	1.1	0.3
Volatile organics	n.a.	0.1	0.2	0.2	0.2	0.3	0.5	0.2	0.2
Total volatiles	0.6	0.3	0.4	0.7	1.0	1.0	1.3	1.3	0.5
Surface water	99.3	98.2	97.0	99.0	97.5	97.0	96.7	96.8	99.3
Non-extractable	0.5	1.4	0.9	1.0	1.6	2.2	1.8	0.8	0.7
TOTAL	100.4	99.9	98.3	100.6	100.0	100.2	99.8	98.9	100.6

Table 7.2.2.2-3 Material balance of radioactivityin Wiehltalsperre water containing radiolabelled flurtamone at 100 µg/L

Compartment	% applied radioactivity at DAT:								
	0	8	13	23	37	49	59	71	Sterile 71
Carbon dioxide	< 0.1	< 0.1	< 0.1	0.1	0.1	< 0.1	0.1	0.1	< 0.1
Volatile organics	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1
Total volatiles	< 0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Surface water	99.6	97.5	96.6	96.0	95.9	95.6	91.8	90.8	98.3
Non-extractable	0.5	1.8	1.8	1.6	2.2	2.7	2.8	1.6	0.8
TOTAL	100.1	99.3	98.4	97.6	98.2	98.4	94.6	92.5	99.2

The biodegradation is summarized in Tables 7.2.2.2-4 and Table 7.2.2.2-5 below. Flurtamone dissipated from surface water due to degradation. Degradation of flurtamone in surface water was accompanied by the formation of M04 TFMBA (AE C518919) with maxima of 8.9% AR for 10 µg/L and 3.4% AR at for 100 µg/L. The sum of two non-identified compounds amounted to a maximum mean value of 5.2% AR at 10 µg/L and 3.6% AR at 100 µg/L. No single component exceeded 3.5% AR for 10 µg/L and 2.8% AR 100 µg/L.

The maximum mean amounts of carbon dioxide were 1.1% and 0.1% AR at study end for 10 µg/L (low concentration) and 100 µg/L (high concentration), respectively. The formation of volatile organic compounds was low with 0.5% and 0.1% AR at for 10 µg/L and study end 100 µg/L, respectively

The formation of non-extractable residues (NER) increased from DAT-0 towards a maximum of 2.2% AR for 10 µg/L and 2.8% AR for 100 µg/L.

When comparing sterile samples with non sterile samples on DAT-71 the sterile samples showed negligible degradation indicating degradation due to microbial activity.

The half-lives for flurtamone were between 256 and 314 days in the surface water ("pelagic test") under laboratory aerobic conditions.

Table 7.2.2.2-4 Biodegradation of radiolabelled flurtamone at 10 µg/L in Wiehltalsperre water phase

Compartment	% applied radioactivity at DAT:								
	0	8	13	23	37	49	59	71	Sterile 71
Flurtamone	99.3	96.2	95.7	94.1	85.1	83.7	84.6	85.0	97.0
M04 TFMBA	n.d.	2.0	1.3	3.3	7.3	8.2	8.9	6.6	1.4
RT 30.3	n.d.	n.d.	n.d.	1.5	3.4	2.7	1.4	1.7	n.d.
RT 25.1	n.d.	n.d.	n.d.	n.d.	1.5	2.5	1.9	3.5	0.9
Diffuse radioactivity	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
TOTAL	99.3	98.2	97.0	99.0	97.5	97.0	96.7	96.8	99.3

Table 7.2.2.2-5 Biodegradation of radiolabelled flurtamone at 100 µg/L in Wiehltalsperre water phase

Compartment	% applied radioactivity at DAT:								
	0	**8**	**13**	**23**	**37**	**49**	**59**	**71**	**Sterile 71**
Flurtamone	98.1	95.9	93.8	92.9	90.7	89.1	85.6	84.3	94.8
M04 TFMBA	0.9	0.6	0.9	0.9	2.2	3.1	3.4	2.8	1.2
RT 30.3	0.6	0.5	0.9	1.1	1.1	1.7	n.d.	n.d.	0.6
RT 25.1	n.d.	0.5	1.0	1.1	1.8	1.7	2.8	2.5	1.1
Diffuse radioactivity	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.6
TOTAL	99.6	97.5	96.6	96.0	95.9	95.6	91.8	90.8	98.3

Conclusions

Flurtamone was degraded in surface water systems under aerobic conditions with half-lives between 256 and 314 days measured at 10 µg/L and 100 µg/L. One degradation product was detected in significant amounts and was identified as M04 TFMBA.

CA 7.2.2.3 - Water/sediment study

The original flurtamone water/sediment study ([redacted], 1995, M-203194-02-1) was treated at only 1/10th of the intended rate. A replacement water/sediment study was conducted with two contrasting systems treated at the correct rate. This study, ([redacted], 1997, M-158694-01-1) already evaluated at national levels, is presented below. Because it was conducted with flurtamone labelled only in the trifluoromethyl ring another study with the label in the phenyl ring has been performed and is also presented below.

Report:	**KCA-7.2.2.3 /02; [redacted], 1997**
Title:	[^{14}C]-Flurtamone: Degradation and Retention in two Water/Sediment Systems
Organisation:	[redacted]
Report No.:	Rhone-Poulenc Report 201413 Bayer CropScience Document M-158694-01-1
Publication:	unpublished
Dates of experimental work:	04 March 1996 to 02 October 1997
Guidelines:	**BBA Part IV, Section 5.1 (December 1990)**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The degradation of flurtamone, uniformly carbon-14 labelled in the trifluoromethylphenyl ring, applied at a rate equivalent to 375 g/ha, was studied in two contrasting water/sediment systems over a 161-day period. The aquatic incubation units were maintained in the dark at 20°C ± 2°C. Moistened carbon dioxide-free air was passed through the water in each unit and through an ethylene glycol and two 1M potassium hydroxide traps to trap liberated carbon dioxide and other evolved volatiles. Duplicate units of each sediment type (including traps) were removed for analysis at the following intervals: zero hours (immediately after application), 6, 24 and 48 hours, 7, 14, 30, 61 100, 120, 139 and 161 days after application. Single flasks were taken at 21 days. The redox potential of the sediment and water, and the oxygen content and pH of the water were measured in each unit prior to analysis.

Portions of the surface water were concentrated by a solid phase extraction method. The sediments were extracted with acidified methanol and the extracts were then concentrated. The concentrated samples were examined chromatographically. The primary method was HPLC. TLC was used as a second chromatographic technique. Liquid chromatography-mass spectrometry (LC-MS) was used to examine selected samples.

The recoveries of applied radioactivity from both systems were satisfactory. The amounts of radioactivity remaining in the water phase fell to < 10% of applied in both systems over the course of the study while the amounts associated with the sediment phases increased. At the end of the study about 70% was associated with the sediment of system 1 (31% extractable, 40% unextractable) and about 90% (50% extractable, 41% unextractable) was still associated with the sediment of system 2. The only volatile traps in which significant amounts of radioactivity were detected were the potassium

hydroxide traps (for carbon dioxide). These contained 3-6% of applied radioactivity at 100 days and 4-12% at the end of the study.

The results of the chromatographic investigations indicated that flurtamone was the most abundant component of the residue in both water and sediment phases. Bound (unextractable) residues and carbon dioxide were the major degradates formed. The degradates included M02 3-trifluoromethyl-N-methylmandelamide (AE 0540067, RE 53285 in the report), M04 TFMBA (AE C518919, RE 54488 in the report) both minor and M08 flurtamone-desphenyl (AE 2093305, RE 59120 in the report) plus three unknowns (all < 5% applied radioactivity).

Flurtamone represented less than 5% AR in the water phases of both systems by the end of the study. M08 flurtamone-desphenyl reached a maximum of 7.8% AR in the water phase of system 2 (Manningtree Stream) and a maximum of 3.6% AR in the sediment. In the total system it reached a maximum of 10.7% AR (120 days). It was not a significant metabolite in system 1 (River Roding) in which the major metabolite was carbon dioxide which reached 12.5% AR (139 days).

Using data derived from the HPLC examination of the samples the DT50 and DT90 values for the water phases and for the total systems were calculated by use of different mathematical models. These were linear regression on an Excel spreadsheet, the program of Timme & Frehse (v 2.0, Bayer AG) and the program KIM (v 1.0 Schering AG). The results indicated that flurtamone dissipation is not best described by simple first-order kinetics. Re-evaluation of the data showed that the DT50 for system 1 ranges from 9 to 11 days by first-order (FO), double first-order in parallel (DFOP) and double-first-order in sequence (DFOS) kinetics. First-order multiple compartment kinetics (FOMC) give a value of 6.7 days (exactly the same as the KIM result). The same exercise for the system 2 water results gave a range of 2 to 5 days (FO, DFOP, DFOS) and a result of 2.9 days using FOMC, extremely close to that given by the KIM program.

A metabolic pathway was proposed, based on the results of the chromatographic investigations. It was suggested that trifluoroacetic acid could be an intermediate between RE 54488 and carbon dioxide.

Material and Methods

The degradation of flurtamone applied at a rate equivalent to 375 g/ha, was studied in two contrasting water/sediment systems over a 161-day period. Radiolabelled flurtamone, batch number CSL-92-418-46-35, was used in the study. It was carbon-14 labelled in the trifluoromethylphenyl ring with a specific activity of 5.81 MBq/mg and a radiopurity of > 97%. Non-radiolabelled flurtamone, used for isotopic dilution, had the batch number IGB 838 and was 98.8% pure.

Samples of sediment and water were collected from two sites within the county of Essex in the UK. One was from the River Roding, Ongar, Essex and the other from a stream located on Aldhams Farm Manningtree, Essex. Immediately prior to sample collection the water temperature (measured just below the water surface), oxygen content (measured immediately below the water surface and at 5 cm above the sediment), pH and redox potential were measured. The water was then transferred to 25 litre plastic bottles and the sediment was collected using a spade and transferred to large plastic bags. Following collection the sediment and water were stored in the dark at approximately 4°C prior to use.

The associated water was characterised for total nitrogen, total phosphorus and total organic carbon and water hardness was determined. The pH, total nitrogen, total phosphorus, particle size distribution, organic carbon and cation exchange capacity of the sediments were measured. The microbial biomass of each sediment was determined after moisture content and water holding capacity

had been measured. The microbial biomass of each sediment was also determined post-incubation. The physico-chemical properties of the water and sediments are given in Table 7.8.3-13.

Sub-samples of sieved (2 mm) sediment were added to individual glass containers (ca. 7.5 cm diameter) to a depth of 2.5 cm and filtered (0.2 mm) water was added to a depth of 6 cm above the sediment. This level was maintained throughout the study by addition of deionised water as necessary. The degradation experiment was initiated after pre-incubation of the aquatic units for approximately 4 weeks in the dark at 20°C± 2°C to enable equilibrium with respect to oxygen content, pH, redox potential, and complete phase separation, to be established.

Table 7.2.2.3-1 Properties of sediments and associated waters used for a flurtamone water/sediment study.

Water/sediment system	**1**	**2**
	River Roding	**Manningtree Stream**
Sediment:		
Particle size distribution (BBA)		
63 µm to 2 mm (%)	45.02	52.75
63 µm to 2 µm (%)	28.84	34.23
< 2 µm (%)	26.14	13.02
Organic Carbon (%)	2.9	1.9
pH (H_20)	7.4	7.2
pH (KCl)	7.7	7.1
Total nitrogen (g/kg)	2296.0	3373.7
Total phosphorous (mg/kg)	880.9	1181.2
Cation Exchange Capacity (meq/100g)	64.3	16.8
Water:		
Total Nitrogen (mg/L)	12.9	24.0
Total Phosphorous (mg/L)	0.36	0.74
Total Organic Carbon (mg/L)	62.36	21.10
Water Hardness (mg/L as $CaCO_3$)	413.91	319.28
Initial biomass (µg/C/g)	137	390

During the equilibrium and experimental period a continuous flow of CO_2 free air was passed through the water, at a rate sufficient to allow aeration and gentle movements and at the same time avoid mixing of the two phases.

Treatment. An aliquot (190 µL) of a 174 µg/mL acetonitrile solution of [^{14}C]-flurtamone was dispensed drop-wise, on a single occasion, into the water. The aliquot contained 33 µg of flurtamone and gave a treatment rate equivalent to 372 g/ha.

The aquatic incubation units were maintained in the dark at 20°C ± 2°C. Moistened carbon dioxide-free air was passed through the water in each unit and through an ethylene glycol and two 1M potassium hydroxide traps to trap liberated carbon dioxide and other evolved volatiles.

Sampling. Duplicate units of each sediment type (including traps) were removed for analysis at the following intervals: zero hours (immediately after application), 6, 24 and 48 hours, 7, 14, 30, 61 100, 120, 139 and 161 days after application. Single flasks were taken at 21 days. The redox potential of the sediment and water, and the oxygen content and pH of the water were measured in each unit prior to analysis.

The surface water was decanted from the sediment and the volume measured. The radioactivity it contained was determined from aliquots taken for radioassay. The sediments were removed from the

incubation unit and transferred to 250 mL plastic bottles. Each unit was rinsed with methanol and the rinsings added to the sediment. The volumes of trapping solutions were recorded and aliquots of each were taken for the determination of the radioactive content.

Sample processing. Portions of the surface water were concentrated by a solid phase extraction method. Concentration of the water was by solid phase extraction using Isolute env+ spe cartridges. The column was preconditioned in acetonitrile and water before loading a known volume of sample. Elution of the sample used 3mL acetonitrile, followed by 3mL acetonitrile/water (1/1 v/v) and finally 3mL of mobile phase B (acetonitrile/water (30:70, v/v) from the HPLC gradient (see below). The eluate was evaporated to minimal volume with a stream of nitrogen and reconstituted in acetonitrile/water as above. At all processing steps recovery checks were made. The samples were then chromatographically analysed. The sediments were extracted with acidified methanol and the extracts were then concentrated. The procedures are shown below.

Sediment transferred to 250 mL plastic bottle, 7.5mL of 1M sodium hydrogen sulphate added.

Step 1	flask rinsed with 50mL methanol which was added to sediment and the whole shaken for 20 minutes on wrist action shaker.
Step 2	centrifugation (force of 2000 x g) for 5 minutes.
Step 3	supernatant decanted and step 1 repeated.
Step 4	sediment sonicated with 50mL methanol (15 minutes using a sonic probe at 70% power), then shaken for 20 minutes.
Step 5	centrifugation (force of 2000 x g) for 5 minutes.
Step 6	supernatant decanted and extracts combined in plastic bottles, volumes measured and aliquots taken for radioassay (extracts filtered as required)
Step 6	concentration by evaporation to minimal volume, using a turbovap at 35°C
Step 7	reconstitution into a small measured volume of acetonitrile/water (1:1, v/v) - recovery checks by radioassay of aliquots.

After extraction the sediment residues were air-dried and the amounts of radioactivity still remaining associated with them were determined. For the 61-day samples the distribution of the unextracted radioactivity between the humin, fulvic acid and humic acid soil fractions was determined.

In addition to the analysis of concentrated extracts for flurtamone and degradation products by high performance liquid chromatography (HPLC) and by thin layer chromatography (TLC), selected water samples and concentrated extracts were submitted for mass spectroscopic analysis (see below).

Quantitative analysis. The liquid samples (trapping solutions, water, sediment extracts) were radioassayed by the liquid scintillation counting (LSC) of aliquots. The amounts of radioactivity associated with the solid samples (post-extract sediment residues) were determined by the combustion of sub-samples followed by the LSC of trapped carbon dioxide evolved by the oxidation.

Qualitative analysis. The HPLC system used comprised a Kromasil KR100 5C8 column attached to a UV detector set at 235 nm and a radiodetector fitted with a liquid cell (ß-Ram 1B or Radiomatic A525) or one with a solid cell (Ramona). The mobile phase was a gradient of acetonitrile/water (30:70, v/v) containing 1% acetic acid and acetonitrile/water (70:30, v/v) containing 1% acetic acid. Reference standards of flurtamone and four putative metabolites were run on this system.

TLC was used as a second chromatographic technique. A glass TLC tank was lined with filter paper, approximately 100 mL of mobile phase (chloroform/acetic acid, 90:10 v/v) was added to the tank. The lid was placed on the tank and the tank left to equilibrate at room temperature. Normal phase TLC plates, Merck Silica Gel F254, 250µm, 20 cm x 20 cm were used. Samples were applied using a Camag automatic TLC Sampler, along with separate aliquots of reference standards. The plates were then placed in the TLC tank and developed for an approximate height of 16 cm, this being recorded accurately on removal of the plate. After drying, the radioactivity on the plate was quantified using an Ambis-100 Radioanalytical Imaging System. The position of the standards was viewed under UV light at a wavelength of 254 nm and their Rf positions compared to those of the [^{14}C]-sample extracts to allow metabolite identification.

Liquid chromatography-mass spectrometry (LC-MS) was used to examine selected samples. The LC system utilised the same column as the HPLC system described above. The mobile phase was a gradient of acetonitrile/water (5:95, v/v) plus 5 mL formic acid per litre against acetonitrile/water (95:5, v/v) plus 5 mL formic acid per litre. The column effluent was split approximately 20:1 such that about 0.95 mL/min went to the UV and radiodetectors and about 0.05 mL/min went to the mass spectrometer ion source. The UV detector was set at 268 nm and the radiodetector was fitted with a liquid cell. The analogue data from these detectors was captured by the mass spectrometer data system. The mass spectrometer was a Micromass Platform 3280 and the ion source was electrospray in negative ion (ESP^-) mode. The scan conditions were 100-420 amu with a 0.5 s scan cycle.

Findings

The biomass values obtained after 100 days and at the end of the study (161 days) were 222 and 131 µg/C g for system 1 (River Roding) and 375 and 296 µg/C g for system 2 (Manningtree Stream), indicating that both systems remained viable over the course of the study. The parameters measured during the incubation period (pH, redox etc) are summarized in Tables 7.8.3-14 and 7.8.3-15.

Table 7.2.2.3-2 Summary of system parameters measured in a water/sediment system (1, River Roding) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	pH	Oxygen saturation (%)	Water redox (mV)	Sediment redox (mV)
0	8.6	60	+324	-276
0.25	8.5	61	+333	-324
1	8.4	59	+288	-379
2	8.4	47	+357	-366
7	8.1	46	+398	-281
14	8.0	47	+222	-234
21*	7.9	48	+263	-415
30	7.8	53	+308	-398
61	7.8	51	+332	-398
100	7.8	54	+333	-407
120	7.3	62	+113	-388
139	8.1	48	+362	-319
161	7.4	56	+260	-66

*individual value, only one flask taken

Table 7.2.2.3-3 Summary of system parameters measured in a water/sediment system (2, Manningtree Stream) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	pH	Oxygen saturation (%)	Water redox (mV)	Sediment redox (mV)
0	8.4	62	+324	-307
0.25	8.4	69	+342	-373
1	8.6	67	+313	-235
2	8.3	59	+348	-273
7	8.4	51	+407	-257
14	8.0	36	+184	-182
21*	7.7	51	+278	-325
30	7.9	60	+291	-312
61	7.7	53	+310	-355
100	7.6	49	+329	-345
120	7.5	56	+147	-324
139	8.1	51	+420	-329
161	7.4	49	+239	-106

*individual value, only one flask taken

The recoveries of applied radioactivity from both systems were satisfactory. The recoveries from all fifty individual flasks fell between 90 and 110% except for two late time-point system 1 samples (87 and 89% and one late time-point system 2 sample (112%). The amounts of radioactivity remaining in the water phase fell to < 10% of applied in both systems over the course of the study while the amounts associated with the sediment phases increased. At the end of the study about 70% was associated with the sediment of system 1 (31% extractable, 40% unextractable) and about 90% (50% extractable, 41% unextractable) was still associated with the sediment of system 2. The only volatile traps in which significant amounts of radioactivity were detected were the potassium hydroxide traps (for carbon dioxide). These contained 3-6% of applied radioactivity at 100 days and 4-12% at the end of the study. The recovery and distribution of applied radioactivity is summarized in the tables below.

Table 7.2.2.3-4 Recovery and distribution of applied radioactivity from a water/sediment system (1, River Roding) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity in:					
	Surface Water	Sediment Extract	Un extracted	Potassium hydroxide traps	Ethylene glycol trap	Total
0	93.9	na	3.8	na	na	97.6
0.25	86.2	6.6	2.2	< 0.1	< 0.1	95.0
1	74.3	15.3	6.9	< 0.1	< 0.1	96.5
2	72.3	17.0	8.4	< 0.1	< 0.1	97.7
7	52.3	31.1	11.6	0.1	< 0.1	95.1
14	41.5	37.5	17.9	0.5	< 0.1	97.4
21*	36.7	34.9	22.5	1.2	< 0.1	95.3
30	28.0	41.0	21.4	2.7	< 0.1	93.0
61	13.9	46.4	32.0	3.2	< 0.1	95.5
100	8.5	51.8	30.1	6.2	< 0.1	96.6
120	7.9	29.2	38.7	13.2	< 0.1	89.0
139	5.3	30.2	41.1	15.5	< 0.1	92.0
161	6.8	31.3	39.6	11.8	< 0.1	89.5

na = not applicable *individual value, only one flask taken

Table 7.2.2.3-5 Recovery and distribution of applied radioactivity from a water/sediment system (2, Manningtree Stream) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity in:					
	Surface Water	Sediment Extract	Un-extracted	Potassium hydroxide traps	Ethylene glycol trap	Total
0	92.4	na	3.9	na	na	96.4
0.25	81.1	11.6	2.7	< 0.1	< 0.1	95.3
1	69.7	22.1	7.5	< 0.1	< 0.1	99.4
2	57.5	32.0	10.7	< 0.1	< 0.1	100.1
7	39.7	39.4	15.7	0.1	< 0.1	94.8
14	26.3	50.3	23.9	0.2	< 0.1	100.7
21*	22.2	67.6	16.7	0.3	< 0.1	106.8
30	16.9	69.5	11.0	0.5	< 0.1	97.9
61	6.6	75.6	14.1	< 0.1	< 0.1	101.0
100	6.8	67.5	19.5	3.5	< 0.1	97.2
120	8.0	47.4	35.1	6.0	< 0.1	96.6
139	9.3	50.9	34.9	6.4	< 0.1	100.6
161	8.9	49.9	40.7	4.3	< 0.1	103.7

na = not applicable *individual value, only one flask taken

The results of the chromatographic investigations indicated that flurtamone was the most abundant component of the residue in both water and sediment phases. Bound (unextractable) residues and carbon dioxide were the major degradates formed. The minor degradates included M02 3-trifluoro methyl-N-methylmandelamide (AE 0540067, RE 53285 in the report), M04 TFMBA (AE C518919, RE 54488 in the report) and M08 flurtamone-desphenyl (AE 2093305, RE 591120 in the report), plus three unknowns (all < 5% applied radioactivity). The amounts of flurtamone and each of its degradates detected in the water phases, sediment phases and total systems by HPLC are shown in Tables 7.8.3-18 to 7.8.3-23. All quantification was by HPLC, with TLC used to confirm the identity of parent. It was not used for as a confirmatory technique for metabolites as a number of components were shown to co-elute.

Table 7.2.2.3-6 Composition of the radioactive residue (as % applied) in the surface water of a water/sediment system (1, River Rodings) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity as:					
	Flurtamone	M04 AE C518919 (RE 54488)	M02 AE 0540067 (RE 53285)	M08 AE 2093305 (RPA 591120)	Others (total)	Total
0	93.9	nd	nd	nd	nd	93.9
0.25	86.2	nd	nd	nd	nd	86.2
1	74.3	nd	nd	nd	nd	74.3
2	72.3	nd	nd	nd	nd	72.3
7	52.3	nd	nd	nd	nd	52.3
14	39.0	1.2	1.3	nd	nd	41.5
21*	32.5	1.4	2.4	nd	0.3	36.7
30	21.3	3.1	2.7	nd	0.9	28.0
61	11.6	1.5	0.4	0.3	0.2	13.9
100	6.6	1.2	0.4	0.2	0.1	8.5
120	3.6	1.0	0.9	2.2	0.1	7.9
139	4.2	0.3	nd	0.8	0.1	5.3
161	4.6	0.7	0.8	nd	0.7	6.8

nd – not detected *individual value, only one flask taken

Table 7.2.2.3-7 Composition of the radioactive residue (as % applied) in the surface water of a water/sediment system (2, Manningtree Stream) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity as:					
	Flurtamone	M04 AE C518919 (RE 54488)	M02 AE 0540067 (RE 53285)	M08 AE 2093305 (RPA 591120)	Others (total)	Total
0	92.4	nd	nd	nd	nd	92.4
0.25	81.1	nd	nd	nd	nd	81.1
1	69.7	nd	nd	nd	nd	69.7
2	57.5	nd	nd	nd	nd	57.5
7	39.7	nd	nd	nd	nd	39.7
14	25.1	0.6	0.6	nd	nd	26.1
21*	21.1	0.5	0.6	nd	nd	22.2
30	15.6	0.8	0.6	nd	nd	16.9
61	8.3	1.0	0.2	0.2	0.1	9.6
100	4.1	0.6	1.9	nd	nd	6.8
120	0.7	0.5	0.3	6.5	0.1	8.0
139	0.1	0.8	0.5	7.8	nd	9.3
161	2.8	1.4	0.7	3.0	1.0	8.9

nd = not detected *individual value, only one flask taken

Table 7.2.2.3-8 Composition of the radioactive residue (as % applied) in the sediment extracts of a water/sediment system (1, River Roding) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity as:					
	Flurtamone	M04 AE C518919 (RE 54488)	M02 AE 0540067 (RE 53285)	M08 AE 2093305 (RPA 591120)	Others (total)	Total
0	na	na	na	na	na	na
0.25	6.6	nd	nd	nd	nd	6.6
1	14.9	0.4	nd	nd	nd	15.3
2	14.5	0.7	nd	0.3	1.5	17.0
7	24.1	1.1	0.3	0.7	4.9	31.1
14	33.9	1.2	0.2	0.3	0.3	37.5
21*	32.9	2.0	nd	nd	2.0	34.9
30	38.9	1.0	0.8	nd	0.3	41.0
61	44.7	1.0	nd	nd	0.7	46.4
100	50.0	1.3	nd	nd	0.6	51.8
120	27.4	0.7	0.3	0.3	0.6	29.2
139	29.1	0.6	0.2	nd	0.3	30.2
161	29.5	1.0	0.4	nd	0.5	31.3

na – not applicable nd = not detected *individual value, only one flask taken

Table 7.2.2.3-9 Composition of the radioactive residue (as % applied) in the sediment extracts of a water/sediment system (2, Manningtree Stream) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity as:					
	Flurtamone	M04 AE C518919 (RE 54488)	M02 AE 0540067 (RE 53285)	M08 AE 2093305 (RPA 591120)	Others (total)	Total
0	na	na	na	na	na	na
0.25	11.6	nd	nd	nd	nd	11.6
1	21.5	0.4	nd	0.1	0.1	22.1
2	31.1	0.2	nd	nd	0.7	32.0
7	38.5	0.2	0.1	nd	0.7	39.4
14	49.7	0.5	0.1	nd	0.1	50.1
21*	65.0	0.5	nd	nd	2.1	67.6
30	68.1	0.9	0.4	nd	0.1	69.5
61	74.5	1.1	nd	nd	0.1	75.6
100	63.0	nd	nd	1.6	2.9	67.5
120	41.4	0.9	0.4	3.6	1.1	47.4
139	47.4	0.6	nd	2.9	nd	50.9
161	44.0	1.4	0.4	3.4	0.6	49.9

na – not applicable nd = not detected *individual value, only one flask taken

Table 7.2.2.3-10 Composition of the radioactive residue (as % applied) in the surface water and sediment extracts of a water/sediment system (1, River Roding) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity as:					
	Flurtamone	M04 AE C518919 (RE 54488)	M02 AE 0540067 (RE 53285)	M08 AE 2093305 (RPA 591120)	Others (total)	Total
0	93.9	nd	nd	nd	nd	93.9
0.25	92.8	nd	nd	nd	nd	92.8
1	89.2	0.4	nd	nd	nd	89.6
2	86.8	0.7	nd	0.3	1.5	89.3
7	76.4	1.1	0.3	0.7	4.9	83.4
14	72.9	2.4	1.5	0.3	0.3	79.0
21*	65.4	3.4	2.4	nd	2.3	71.6
30	60.2	4.1	3.5	nd	1.2	69.0
61	56.3	2.5	0.4	0.3	0.9	60.3
100	56.6	2.5	0.4	0.2	0.7	60.3
120	31.0	1.7	1.2	2.5	0.7	37.1
139	33.3	0.9	0.2	0.8	0.4	35.5
161	34.1	1.7	1.2	nd	1.2	38.1

na – not applicable nd = not detected *individual value, only one flask taken

Table 7.2.2.3-11 Composition of the radioactive residue (as % applied) in the surface water and sediment extracts of a water/sediment system (2, Manningtree Stream) treated with [^{14}C]-flurtamone (means of duplicates).

Time-point (d)	% of applied radioactivity as:					
	Flurtamone	M04 AE C518919 (RE 54488)	M02 AE 0540067 (RE 53285)	M08 AE 2093305 (RPA 591120)	Others (total)	Total
0	92.4	nd	nd	nd	nd	92.4
0.25	92.7	nd	nd	nd	nd	92.7
1	91.2	0.4	nd	0.1	0.1	91.8
2	88.6	0.2	nd	nd	0.7	89.5
7	78.2	0.2	0.1	nd	0.7	79.1
14	74.8	1.1	0.7	nd	0.1	76.6
21*	86.1	1.0	0.6	nd	2.1	89.8
30	83.7	1.7	1.0	nd	0.1	86.4
61	82.8	2.1	0.2	0.2	0.2	85.2
100	67.1	0.6	0.4	3.6	2.6	74.3
120	42.1	1.4	0.7	10.1	1.1	56.4
139	47.5	1.4	0.5	10.7	nd	60.2
161	46.8	2.8	1.1	6.4	1.6	58.8

na – not applicable nd = not detected *individual value, only one flask taken

Flurtamone represented less than 5% AR in the water phases of both systems by the end of the study. Its identity was confirmed by the mass spectrometric results. Flurtamone was the major component present in the sediment.

The metabolite eventually identified as M08 flurtamone-desphenyl (AE 2093305, RE 591120), was originally thought to be M03 3-trifluoromethyl-mandelic acid (AE 0592368, RE 54589), on the basis of chromatographic behaviour alone. The mass spectrometric investigations indicated that it had a different structure and a reference compound was synthesized. The use of this new chromatographic marker (plus the mass spectrometric results) confirmed that the metabolite was M08 flurtamone-desphenyl (and not M03 3-trifluoromethyl-mandelic acid). M08 flurtamone-desphenyl reached a maximum of 7.8% AR in the water phase of system 2 (Manningtree Stream) and a maximum of 3.6% AR in the sediment. In the total system it reached a maximum of 10.7% AR (120 days). It was not a significant metabolite in system 1 (River Roding) in which the major metabolite was carbon dioxide, which reached 15.5% AR (139 days). It was suggested that trifluoroacetic acid could be an intermediate between M04 TFMBA and carbon dioxide (but this now seems unlikely).

Using data derived from the HPLC examination of the samples the DT50 and DT90 values for the water phases and for the total systems were calculated by use of different mathematical models. These were linear regression on an Excel spreadsheet, the program of Timme & Frehse (v 2.0, Bayer AG) and the program KIM (v 1.0 Schering AG). The results are presented below.

Table 7.2.2.3-12 Water phase and total system DT_{50} and DT_{90} values for flurtamone calculated by three mathematical models.

	System 1 (River Roding)			System 2 (Manningtree Stream)		
Phase/Model	**DT_{50} (d)**	**DT_{90} (d)**	**Goodness of Fit**	**DT_{50} (d)**	**DT_{90} (d)**	**Goodness of Fit**
Water phase by:						
Excel	34.2	113.6	0.95	22.5	74.6	0.82
Timme-Frehse	6.7	73.6	0.99	3.0	32.8	0.96
KIM	10.0	71.3	-0.999*	4.8	53.3	-0.999*
Total system by:						
Excel	107.3	356.1	0.90	158.8	527.6	0.84
Timme-Frehse	69.2	nc	0.94	160.5	nc	0.83
KIM	91.0	400.3	-0.995[#]	156.4	681.2	-0.991[¤]

KIM *3-compartment model [#]2-compartment model [¤]Power rate model

Perfect fit values would be 1.0 for the Excel and Timme-Frehse routines and -1.0 for the Kim program. The KIM goodness of fit values were taken to be the best and the KIM results were the ones that were quoted in the conclusions of the report.

The results indicate that flurtamone dissipation is not best described by simple first-order kinetics, which gives half-life values over the whole study period. Re-evaluation of the data show that the DT50 for system 1 ranges from 9 to 11 days by first-order (FO), double-first-order in parallel (DFOP) and double-first-order in sequence (DFOS) kinetics. First-order multiple compartment kinetics (FOMC) give a value of 6.7 days (exactly the same as the KIM result). The same exercise for the system 2 water results gives a range of 2 to 5 days (FO, DFOP, DFOS) and a result of 2.9 days using FOMC, extremely close to that given by the KIM program. These re-evaluations confirm that the KIM results were the most appropriate to quote.

Conclusion:

In water sediment systems flurtamone moved steadily from the water phase to the sediment such that the DT_{50} was ≤ 10 days with a DT_{90} of 53 to 71 days. The DT_{50}s for the whole systems were 91 and 156 days. Flurtamone degraded to M08 flurtamone-desphenyl (5-methylamino-4-(3-trifluoromethyl phenyl)-3(2H)-furanone, AE 2093309, RE 591120 in the report) and ultimately to carbon dioxide. Small amounts (always < 5% AR) of M02 3-trifluoromethyl-N-methyl-mandelamide (2-hydroxy-N-methyl-2-(3-trifluoromethylphenyl)-acetamide, AE 0540067, RE 53285 in the report) and M04 TFMBA (AE C518919, RE 54488 in the report) were also detected. Other degradates were present in very small quantities only.

Report:	**KCA-7.2.2.3 /03; [redacted]. 2012d**
Title:	[Phenyl-UL-^{14}C]-Flurtamone: Aerobic Aquatic Metabolism
Organisation:	[redacted]
Report No.:	EnSa-12-0590 Bayer CropScience Document **M-443489-01-1**
Publication:	unpublished
Dates of experimental work:	27th September 2011 to 11th June 2012
Guidelines:	**OECD 308, DRAFT SANCO 11802/2010/rev1 amending Regulation (EC) No 1107/2009, US EPA OCSPP Nos. 835.4300 and 835.4400.**
Deviations:	None
GLP/GEP	**Yes**

Executive Summary

The aerobic biotransformation of the herbicide flurtamone was studied in two European water/sediment systems ("Anglersee" and "Wiehltalsperre") for a maximum of 100 days at about 20.0°C in the dark. The test item [phenyl-UL-^{14}C]-flurtamone was applied to the test systems with a nominal application rate of 39.0 µg/batch (approx. 75.0 µg/L) which is equivalent to 750 g flurtamone/ha.

The test system consisted of laboratory microcosm flasks equipped with traps for the collection of CO_2 and volatile organic compounds. The water/sediment ratio used was 3/1 (v/v). During incubation, the supernatant water was in smooth motion. Duplicate samples were taken and analyzed after 0, 0.25, 1, 3, 7, 14, 30, 59 and 100 days of incubation for test system Anglersee and after 0, 0.25, 1, 3, 7, 14, 30 and 100 days of incubation for test system Wiehltalsperre.

The water layers were decanted and centrifuged. The volumes of the water layers were determined and aliquots thereof were analyzed by liquid scintillation counting (LSC) to measure the radioactivity content. From day 0.25 onwards, aliquots of the water phases were taken before to determine the dissolved amount of CO_2. The sediment samples were extracted three times at ambient temperature and once with respect to the formation of non-extractable residues by hot (microwave) extraction. The amounts of radioactivity in the extracts as well as the amounts of trapped volatiles and dissolved CO_2 were determined by liquid scintillation counting (LSC). Aliquots of the water layers and the combined organic soil extracts were concentrated and analyzed by HPLC to quantify the test item as well as its transformation products. From representative water and sediment extract samples the HPLC flurtamone fractions were collected and further analyzed by a chiral HPLC method to investigate the degradation behaviour of the single enantiomers. Representative water layers and extracts were additionally analyzed using a confirmatory chromatographic method (TLC).

The exhaustive extracted sediment phases were freeze-dried, homogenized and combusted in an oxidizer. The evolved CO2 was trapped in a scintillation cocktail and measured by LSC to determine the amounts of non-extractable residues (NERs). At the last sampling date, sediment aliquots were used for a further characterization of the non-extractable residues.

The average material balance was 102.5% AR for the "Anglersee" test systems and 102.0% AR for the "Wiehltalsperre" test systems. The radioactivity content in the water layer of the Anglersee test systems decreased from 100.1% AR at DAT-0 (approx. 1-2 min after application) to 8.5% AR at study end (DAT-100). The radioactivity content in the water layer of Wiehltalsperre test systems decreased from 96.4% AR at DAT-0 to 1.8% AR towards study termination.

The total extractable radioactivity from the sediments increased in the Anglersee water/sediment systems from 4.2% AR at DAT-0 to a maximum of 53.1% AR at DAT-14 and decreased again to 11.8% AR at study termination. Total radioactive sediment extractables in the Wiehltalsperre water/sediment systems increased from 7.2% AR at DAT-0 to 53.4% AR at DAT-7 and decreased to 37.7% AR towards study termination. The maximum amounts of non-extractable residues in the Anglersee and Wiehltalsperre test systems were 32.3% AR and 23.5% AR, respectively, at study termination. For the last sampling interval, the non-extractable residues of both water/sediment systems were further characterized by fractionation into humin, humic acids and fulvic acids.

At study termination 47.9% AR and 35.8% AR were degraded to $^{14}CO_2$ in the Anglersee and Wiehltalsperre test systems, respectively, including the dissolved amount of $^{14}CO_2$ in the water layer from DAT-0.25 onwards. Significant amounts of organic volatile compounds were not detected (≤ 0.2% AR in both test systems).

The flurtamone content in the water layer of the Anglersee water/sediment systems decreased from 98.3% AR at DAT-0 to 1.3% AR at study termination. The amount of flurtamone in the water layer of the Wiehltalsperre water/sediment systems decreased from 94.5% AR at DAT-0 to 1.7% AR at study termination. The flurtamone content in the sediment of the Anglersee test systems increased from 4.2% AR at DAT-0 to 52.1% AR at DAT-14 and declined then to 10.6% AR at study termination. The flurtamone content in the sediment of the Wiehltalsperre test systems increased from 7.2% AR at DAT-0 to 52.8% AR at DAT-7 and declined then to 36.3% AR towards study termination.

Both enantiomers of flurtamone have a similar degradation behaviour in water as well as in sediment. The amount of flurtamone in the entire Anglersee water/sediment systems declined to 11.9% AR at study termination. In the Wiehltalsperre water/sediment test systems 38.0% AR was found as unchanged test item at study end. No major metabolites were observed in the water layers, the combined sediment extracts and in the entire systems. Besides flurtamone, eight minor transformation products were detected. The maximum amount of a single minor transformation product in the entire systems was 3.1% for the Anglersee water/sediment systems (DAT-100) and 2.4% AR for the Wiehltalsperre water/sediment systems (DAT-14). Due to the low amounts of the minor transformation products, identification procedures were not performed.

The dissipation time (DT_{50}) of flurtamone from the water layer (sum of degradation and translocation processes into the sediment) was calculated to be 11.2 days for the Anglersee test systems and 7.1 days for the Wiehltalsperre test systems. The degradation half-lives (DT_{50S}) of flurtamone in the entire water/sediment systems were calculated to be 51.2 days for the Anglersee test systems and 40.9 days for the Wiehltalsperre test systems, respectively.

Materials and Methods

Test Material:

[Phenyl-UL-^{14}C]-flurtamone, radiochemical purity > 99%,   Batch no.: KML 9146.

Test Design:

The study was carried out with natural water/sediment systems from two locations:

- Anglersee (ID: A), Leverkusen, Germany: This small lake is a reclaimed gravel-pit, which is used for fishing only. The lake is entirely enclosed by a fence.
- Wiehltalsperre (ID: W), close to Wiehl near Gummersbach, Germany: This is a fresh water dam that is used for the preparation of drinking water. Water and sediment were collected from the forebay Nespen.

The waters layers and sediments were freshly sampled prior to the start of the study from the two sites. For characterization of the natural systems the following parameters were determined on site:

- oxygen saturation of water layer
- temperature of water and sediment phase
- pH of water and sediment phase
- redox potential of water and sediment phase

Water and sediment were taken from the lakefronts and filled separately in plastic containers. Sediment was obtained from the upper sediment layer. The results of the on-site measurements at the day of sampling as well as the other system characteristics, including biomass measurements made at the beginning and during the study are given in the tables below.

In addition, subsamples of both types of sediments were further characterized and the following parameters were determined:

- texture class according to USDA standards
- percentage of sand, silt and clay
- pH in water (sediment/water-ratio 1:1)
- pH in CaCl2 (0.01 M, ratio 1:2)
- cation exchange capacity
- total nitrogen content
- total phosphorus content

Table 7.2.2.3-13 Properties of waters used in a flurtamone aerobic aquatic study

Parameter	Anglersee Water	Wiehlaltsperre Sediment
Temperature (°C)	18.6	14.1
pH	6.9	6.4
Total Organic Carbon (TOC, mg/L)	3.0 / 4.0 / 7.0 / 9.0	4.0 / 4.0 / 4.0 / 2.0
Redox Potential E_h (mV)	+365	+320
Oxygen Content (mg/L)	9.45	10.16

Table 7.2.2.3-13 Properties of sediments used in a flurtamone aerobic aquatic study

Parameter	Anglersee Sediment	Wiehlaltsperre Water
Geographic Location	Leverkusen North Rhine-Westphalia Germany	Wiehl close to Gummersbach North Rhine-Westphalia Germany
Soil Classification (USDA)	Sand	Silt Loam
Sand (2000 – 50 µm, %)	98	39
Silt (< 50 – 2 µm, %)	1	52
Clay (< 2µm, %)	1	9
pH (day of sampling)	6.8	5.8
pH ($CaCl_2$)	6.4	4.4
pH (H_2O)	6.7	4.6
Organic Matter (%)	1.55 / 1.00 / 1.43 / 1.52	12.24 / 12.93 / 13.79 / 12.76
Organic Carbon (%)	0.90 / 0.58 / 0.83 / 0.88	7.14 / 7.50 / 8.00 / 7.40
Total Organic Carbon (%)	0.6	6.7
Total Nitrogen (%)	0.04	0.57
Total Phosphorus (ppm)	155	683
Cation Exchange Capacity (meq/100 g)	3.8	10.3
Redox Potential Eh (mV0	+320	+170
Temperature (°C)	18.8	13.8
Moisture (%)	29.2	211.7
Biomass (mg microbial C/kg)[2,3]	8.92 / 6.25 / 4.58 / 4.80	17.50 / 14.58 / 16.17 / 40.00

[1] day of sampling [2] start of acclimatisation, 0 / 59 / 100 days after treatment

At the day of sampling, the sediment samples were sieved through meshes down to 2 mm mesh-and the water samples were filtered with a 0.063 mm mesh size and flushed with air to maintain aerobic conditions until use. Two days after sampling the sediment weight corresponding to a height of approx. 2 cm was poured into the vessels and 320 mL (height approx. 6 cm) of the corresponding water layer were added. The water to sediment ratio was 3/1 (v/v). The dry weight of two representative samples of both types of sediment was determined.

For determination of the microbial activity, subsamples of both sediments were taken and analyzed with the SIR method at start of acclimation as well as at study start (DAT-0), in the middle of the study (DAT-59) and at the end of the study (DAT-100).

The test system consisted of a special cylindrical glass container (volume about 1000 mL, inner diameter about 10.5 cm, surface area about 86.6 cm^2). The vessels were fitted with solid trap attachments permeable for oxygen but absorbing volatile compounds formed in the test systems to soda lime (CO_2) and polyurethane foam (organic volatiles). The flasks were then fitted with trap attachments, stoppers and stirrers. For acclimatision of the test systems and for establishment of phase separation, the test systems were stored under the intended study incubation conditions for 11 days prior to application.

An application solution with a concentration of approximately 78.0 µg/mL was prepared. Biomass and TOC test systems were either left non-treated or applied with the solvent of application solution.

After the application, the test vessels (except DAT-0 vessels) were fitted with trap attachments and placed back into the temperature controlled climatic chamber.

Samples of both water/sediment systems were processed 0, 0.25, 1, 3, 7, 14, 30, 59 and 100 days after application of the test item. At each sampling interval two samples per type of water/sediment system were processed and analyzed. The corresponding trap attachments were collected to determine the amount of $^{14}CO_2$ and organic volatiles (except DAT-0, no determination of volatiles).

Prior to opening an incubated test vessel, volatile compounds possibly still present in the head space of the test system were transferred into the solid trap attachment by purging with air for 10 minutes. Then, the trap was removed from the test vessel and stored until processing. The sampled test systems were characterized by measuring the pH value and the redox potential of the water phase and the sediment layer as well as the oxygen saturation of the water phase.

A subsample (50 mL) of the undisturbed water layer was taken and 1 mL 1 M NaOH solution was added for the later determination of dissolved $^{14}CO_2$ (except on DAT-0). Afterwards, the remaining water layer of the test system was decanted in a centrifuge beaker and centrifuged for 10 min at 4200 rpm (= 5000 x *g*). The supernatant was decanted again and its volume was determined. Aliquots of the processed water were taken for LSC measurements. From DAT-0.25 onwards, 50 µL of 1 M NaOH solution was added to each LSC aliquot to prevent the volatilization of dissolved $^{14}CO_2$.

The sediment with its residual water was transferred into the same centrifuge vessel which was already used for the water layer, to combine it with the removed solids of the water layer. The extraction procedure for all intervals was 3 x 80 mL acetonitrile/water (80:20, v/v) with 30 minutes shaking at ambient temperature followed by 1 x 80 mL acetonitrile/water (50:50, v/v) in a microwave oven at 70°C for 10 minutes. After each extraction step, the suspension was centrifuged for 10 minutes at 4200 rpm (= 5000 x g) and the clear supernatant was decanted.

The three organic ambient extracts were combined; the organic microwave extract was analyzed separately for volume and radioactivity contents. The exhaustively extracted sediment was freeze-dried, weighed and homogenized in a planet mill (5 minutes). Aliquots of these homogenized, exhaustive extracted sediments were subjected to combustion/LSC to determine the amounts of non-extractable residues (NERs).

The PU foam plugs were extracted with 50 mL of ethyl acetate to desorb possible volatile organic compounds. Aliquots of 5 mL of the extracts were submitted to LSC measurement. Chromatographic analyses of the PU foam extracts were not performed, because they contained ≤ 0.2% of the AR in all test systems.

Compounds absorbed by the soda lime were liberated and trapped in an appropriate scintillation cocktail, which was later on radio-assayed by LSC. The subsamples of the water layers (50 mL) were treated likewise.

All LSC measurements were carried out without any concentration steps. For HPLC/radiodetection and TLC/radiodetection measurements, the samples were concentrated. Aliquots of the concentrates were analyzed by HPLC and the concentrated water layers sampled at DAT-0.25, DAT-30 and DAT-100 were additionally analyzed by TLC. For representative sampling intervals, aliquots of the concentrates were analyzed by LSC to determine the recoveries of radioactivity after concentration.

A further characterization of the non-extractable residues was performed using the exhaustive extracted sediments of DAT-100 (first replicates of Anglersee and Wiehltalsperre test systems, respectively). The NERs were investigated for humin, humic acids and fulvic acids.

Flurtamone occurs as a racemate in two enantiomeric structures. In order to investigate the degradation behaviour of the single enantiomers, samples were chosen in which at least a half of the flurtamone was degraded, but sufficient flurtamone was left for analyses. Representative concentrated water and organic extracts as well as the application solution were analyzed by HPLC and the fractions of flurtamone were collected. All fractions were concentrated to dryness in a stream of nitrogen and the concentrates were re-suspended in 40 µL ethanol and 160 µL heptane, respectively. Aliquots of each suspension were analyzed for radioactivity by LSC (3 x 10 µL) and by a chiral HPLC method.

All water layers and soil extracts were quantitatively analyzed by reversed phase C18 HPLC with radiodetection as first method to separate and quantify the test item and its metabolites. A system that comprised a Purospher Star RP18-e (Merck), 250 x 4.6 mm; 5 µm column (with a Purospher Star RP18-e 4 x 4 mm guard column) connected to a radioactivity detector fitted with a solid cell and to a UV detector set at 254 nm. The mobile phase was a gradient of 1% formic acid in water against 1% formic acid in acetonitrile. The quantification of the test item and its degradation products in the extracts was calculated based on the distribution of the HPLC zones and the amount of RA in the extracts.

A second HPLC method was used to isolate fractions of flurtamone from the application solution and from representative concentrated water layers and sediment extracts. This used the same column and guard column as described above but a mobile phase of a water and acetonitrile gradient. A chiral method was used for the analysis of the ratios of flurtamone enantiomers. This employed a Chiralcel OD, 250 x 4.6 mm; 10 µm analytical column and an isocratic mobile phase of heptane/ethanol (90:10, v/v).

The electro-spray ionization MS spectra (ESI) were obtained with a LTQ Orbitrap XL mass spectrometer (Thermo, San Jose, CA, U.S.A.). The HPLC instrument used for chromatography was an Agilent HP1100 with a Nucleodur C18 Gravity, 3 µm, 250 x 2 mm (MN) column. The mobile phase was a gradient of 0.1% formic acid in water against 0.1% formic acid in acetonitrile. The flow from the HPLC column was split between a UV-detector followed by a radioactivity detector (Ramona Star) and the MS spectrometer.

For TLC analysis aliquots of the concentrates of the extracts were spotted on silica gel plates (Si60, F254, 20 cm x 20 cm Merck) using an automatic applicator. The plates were developed with ethyl acetate/2-Propanol/water (75/20/5, v/v/v) in a plate chamber without solvent saturation. The distribution of radioactive zones on the plates was measured using a Bio-Imaging Analyzer (BAS 2000, Fuji Co.). Radioactive regions on the tracks were quantified using the software package AIDA (Raytest). The quantification of the test item and the degradation products in the extracts was calculated based on the distribution of the TLC-zones and the amount of radioactivity in the extracts.

Evidence that the radioactivity liberated from the soda lime was ^{14}C-carbon dioxide was obtained by use of a barium carbonate precipitation method.

Findings

Aerobic conditions were maintained throughout the study and the temperature was kept within the appropriate limits. The records of pH values, redox potentials and the amounts of dissolved oxygen at each sampling date are summarized in the tables below. The microbial activity indicated that the systems were biologically active during the entire test period.

The maximum total HPLC LODs were determined to be 0.7% AR for the water layers and 0.3% AR for the sediment extracts. Values between these LODs and LOQs (three times LOD = 2.1 and 0.9% AR, respectively) were taken into account in the tables and for calculations.

The application check showed that the application solution was homogeneous during the application procedure. The purity of the test item in the application solution was confirmed by HPLC and the stability was shown by the DAT-0 recoveries obtained for the test item in the water layer and in the combined sediment extracts. The amounts of dosed test item for the degradation samples were determined during application by the application checks.

Table 7.2.2.3-14 Redox potential, oxygen content and pH of Anglersee test system

DAT	Sample	Water Layer				Sediment			Buffer
		O_2 (mg/L)	Redox E_{obs} (mV)	Redox E_H (mV)	pH	Redox Eobs (mV)	Redox E_H (mV)	pH	Redox Eobs (mV)
0	1	8.6	144	343	8.3	88	287	7.3	231
	2	8.7	157	357	8.3	81	280	7.7	
0.25	1	8.7	146	345	8.3	102	301	7.3	231
	2	8.7	156	355	8.4	112	311	7.3	
1	1	8.7	144	342	8.1	94	292	7.0	232
	2	8.7	149	347	8.2	80	278	7.2	
3	1	7.7	141	338	7.9	-6	191	7.3	233
	2	7.9	154	351	7.9	-21	176	7.4	
7	1	8.1	147	343	7.9	-109	88	7.2	234
	2	7.7	150	346	7.9	-112	85	7.1	
14	1	8.5	155	351	8.1	-107	90	7.2	234
	2	8.4	146	342	8.2	-74	123	6.8	
30	1	8.5	152	350	8.1	47	245	6.9	232
	2	8.5	133	331	8.2	59	257	6.6	
59	1	8.8	173	371	8.3	126	324	6.9	232
	2	8.7	156	353	8.2	88	286	7.4	
100	1	9.0	161	382	9.1	79	277	8.0	233
	2	8.9	188	386	9.0	52	250	7.8	
	min	7.7	133	331	7.9	-112	85	6.6	231
	max	9.0	188	386	9.1	126	324	8.0	234
	mean	8.5	154	352	8.2	32	230	7.2	232

Table 7.2.2.3-15 Redox potential, oxygen content and pH of Wiehlaltsperre test system

DAT	Sample	Water Layer				Sediment			Buffer
		O_2 (mg/L)	Redox E_{obs} (mV)	Redox E_H (mV)	pH	Redox Eobs (mV)	Redox EH (mV)	pH	Redox Eobs (mV)
0	1	8.8	211	410	5.9	108	307	5.7	231
	2	8.7	207	406	5.7	89	288	5.7	
0.25	1	8.8	242	441	6.4	58	257	5.9	233
	2	8.7	230	429	6.4	95	294	5.9	
1	1	8.8	213	411	5.7	140	338	5.7	232
	2	8.8	199	397	5.7	144	342	5.9	
3	1	8.7	218	415	6.5	80	277	6.3	234
	2	8.5	224	421	5.8	126	323	6.4	
7	1	8.3	229	425	5.5	59	255	5.5	234
	2	8.0	194	390	5.6	47	244	6.4	
14	1	8.5	210	407	6.8	89	166	6.5	234
	2	8.4	190	387	6.9	22	218	6.6	
30	1	8.7	248	446	6.4	143	340	5.9	232
	2	8.6	221	419	6.9	113	311	6.0	
100	1	8.9	237	435	7.8	142	340	5.9	233
	2	8.8	211	409	6.3	154	352	4.9	
	min	8.0	190	387	5.5	-30	160	4.69	231
	max	8.9	248	446	7.8	154	352	6.6	234
	mean	8.6	218	415	6.3	95	291	6.0	232

The extractable radioactivity (sum of radioactivity in the water layers and in the sediment extracts) of the DAT-0 samples was determined as 104.4% AR for the Anglersee test system and 103.6% AR for the Wiehltalsperre test system, using the sediment extraction procedure previously described. These results indicate that the processing method was well suited to recover the applied test item from the sediment matrix.

Subsamples of water layers and sediment extracts were processed and subjected to the primary chromatographic method within three days after sampling. Therefore, a separate test on the storage stability was not necessary. However, representative water layers and sediment extracts were analyzed with the confirmation method after a storage period of up to 165 days and the results were comparable with the original results. This shows that flurtamone and its transformation products were stable for at least 165 days.

The HPLC method used for data evaluation gave good selectivity and reproducibility, which demonstrated the suitability for separation and quantification. The results of the primary chromatographic method and the confirmatory method (TLC) were in good agreement. The amounts of radioactivity eluted from the HPLC system (primary chromatographic method) were determined for HPLC runs performed with and without HPLC column using the first replicates of water layers and sediment extracts collected at DAT-0.25 and DAT-30 from test systems Anglersee and Wiehltalsperre. The amounts of radioactivity detected in the outflow of the runs performed with HPLC column were related to those detected in the outflows of the runs performed without HPLC column. The recoveries of radioactivity ranged from 96.3 to 102.2% for the water phases and from 97.8 to 103.6% for the combined sediment extracts.

The HPLC limit of detection (LOD) for a single peak in the water layers and combined organic extracts was < 1% of applied radioactivity, which, with the results presented in the above paragraph, demonstrate that the primary chromatographic method was well suited for the analysis of all compartments and that it fulfilled the data requirements.

The total recovery of radioactivity (mean of duplicates) of all sampling intervals of the Anglersee water/sediment systems ranged from 98.7% to 104.6% (overall mean 102.5%, RSD 1.8%) during the study course. The material balance (mean of duplicates) for the sampling intervals of the Wiehltalsperre water/sediment systems ranged from 98.9% to 104.2% (overall mean 102.0%, RSD 1.9%). The complete material balance found at all sampling intervals (mean of duplicates) demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing. The detailed results for the water layer, the sediment extracts, the NERs and the volatile fractions (soda lime and PU plugs from trap attachments) are listed in the tables below.

Table 7.2.2.3-16 Recovery and distribution of applied radioactivity from Anglersee test system

Fraction/Phase	% applied radioactivity(mean values) at these days after treatment								
	0	**0.25**	**1**	**3**	**7**	**14**	**30**	**59**	**100**
Carbon dioxide	n.a	0.3	0.4	0.8	1.3	2.3	6.4	22.9	47.9
Organic volatiles	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1
Total volatiles	**n.a**	**0.5**	**0.4**	**0.8**	**1.3**	**2.4**	**6.5**	**23.1**	**47.9**
Water layer	100.4	89.6	75.3	62.6	57.3	42.6	33.2	19.8	8.5
Ambient sediment extract	4.0	10.9	24.3	34.8	39.7	49.6	45.9	31.5	9.9
Microwave sediment extract	0.2	0.5	1.0	1.7	2.6	3.5	3.9	3.2	1.9
Total extractable from sediment	4.2	11.4	25.3	36.5	41.3	53.1	49.8	34.8	11.8
Total extractable + water layer	**104.4**	**101.0**	**100.5**	**99.2**	**98.6**	**95.4**	**83.0**	**54.6**	**20.3**
Non-extractable residues	**0.2**	**1.1**	**2.5**	**3.0**	**4.4**	**5.9**	**11.7**	**21.0**	**32.3**
Total recovery	**104.6**	**102.7**	**103.4**	**103.0**	**104.3**	**103.7**	**101.2**	**98.7**	**100.6**

n.a. – not applicable / not analyzed

Table 7.2.2.3-17 Recovery and distribution of applied radioactivity from Wiehlaltsperre test system

Fraction/Phase	% applied radioactivity(mean values) at these days after treatment							
	0	**0.25**	**1**	**3**	**7**	**14**	**30**	**100**
Carbon dioxide	n.a	0.4	0.5	0.7	0.9	2.1	34.6	35.8
Organic volatiles	n.a	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Total volatiles	**n.a**	**0.4**	**0.5**	**0.7**	**0.9**	**2.1**	**34.6**	**35.8**
Water layer	96.4	84.4	75.2	60.3	43.6	40.5	9.6	1.8
Ambient sediment extract	6.7	15.3	22.2	36.5	48.7	46.9	38.3	33.0
Microwave sediment extract	0.5	1.0	1.3	2.7	4.7	4.5	4.3	4.7
Total extractable from sediment	7.2	16.3	23.5	39.2	53.4	51.4	42.6	37.7
Total extractable + water layer	**103.6**	**100.7**	98.8	**99.5**	**97.0**	**91.9**	**52.1**	**39.5**
Non-extractable residues	**0.4**	**1.5**	**3.4**	**3.3**	**6.3**	**6.7**	**12.9**	**23.5**
Total recovery	**104.0**	**102.6**	**102.6**	**103.4**	**104.2**	**100.8**	**99.6**	**98.9**

n.a. = not applicable / not analyzed

The amount of liberated ^{14}C-carbon dioxide formed in both water/sediment systems is presented in the above tables as the sum of that determined in the soda lime of the trap attachments and in the water layers. Both water/sediment systems showed a high mineralization of flurtamone with a maximum of

47.9% AR in Anglersee and a maximum of 35.8% AR in Wiehltalsperre water/sediment systems at study termination. Significant amounts of volatile organics were not detected (≤ 0.2% of applied).

The radioactivity content in the water layer of the Anglersee test systems decreased from 100.1% AR at DAT-0 (approx. 1-2 min after application) to 8.5% at study end (DAT-100). The radioactivity content in the water layer of Wiehltalsperre test systems decreased from 96.4% AR at DAT-0 to 1.8% AR at study termination. The results of the chromatographic investigations of the supernatant water are summarized in the tables below.

After the application of the test item onto the water surface, the total extractable radioactivity from sediment increased in the Anglersee water/sediment systems from 4.2% AR at DAT-0 to a maximum of 53.1% AR at DAT-14 and decreased then to 11.8% AR towards study termination. Total radioactive sediment extractables in the Wiehltalsperre water/sediment systems increased from 7.2% AR at DAT-0 to 53.4% AR at DAT-7 and decreased then to 37.7% AR towards study termination. The chromatographic analyses of the organic sediment extracts are summarized in the tables below.

Flurtamone is eliminated from the water body via translocation into the sediment as well as via degradation. The flurtamone content in the water layer of the Anglersee water/sediment systems decreased from 98.3% AR at DAT-0 to 1.3% AR at study termination. The amount of flurtamone in the water layer of the Wiehltalsperre water/sediment systems decreased from 94.5% AR at DAT-0 to 1.7% AR at study termination. Seven minor transformation products were detected in the water layers of Anglersee and Wiehltalsperre test systems. The amounts of a single minor transformation product did not exceed 3.1% of AR.

The flurtamone content in the sediment of the Anglersee test system increased from 4.2% AR at DAT-0 to 52.1% AR at DAT-14 and declined then to 16.6% AR at study termination. The flurtamone content in the sediment of the Wiehltalsperre test system increased from 7.2% AR at DAT-0 to 52.8% AR at DAT-7 and declined then to 36.3% AR towards study termination. Besides flurtamone seven minor transformation products were detected in the sediments. The amounts of a single minor transformation product did not exceed 6.7% of AR.

The non-extractable residues for the Anglersee test system increased from 0.2% AR at DAT-0. to a maximum of 32.3% AR at study termination. For Wiehltalsperre test systems, the residues were 0.4% AR at DAT-0 and increased to a maximum of 23.5% AR at study termination. A further characterization of the non-extractable residues was conducted exemplarily for the DAT-100 samples. The distribution of the non-extractable residues in different humic substance fractions was found to be of heterogeneous nature in case of both water/sediment systems. The results are shown below:

Table 7.2.2.3-18 Distribution of non-extractable residues in two water/sediment systems

Sample	Humin (% NER)	Humic Acid (% NER)	Fulvic Acid (% NER)	Total (% NER)	Total (%AR)
Anglersee 100 d	34.7	23.5	35.6	93.7	32.0
Wiehltalsperre 100d	42.2	45.9	12.6	100.6	23.6

The biotransformation of flurtamone is summarized in the tables below.

Table 7.2.2.3-19 Biotransformation of flurtamone in Anglersee test system

Compound		% applied radioactivity as:								
	Source	0	0.25	1	3	7	14	30	59	100
Flurtamone	Water	98.3	88.8	74.7	61.3	56.6	41.5	31.6	18.4	1.3
	Sediment	4.2	11.3	24.8	36.0	40.7	52.1	48.5	33.0	10.6
	System	102.6	100.0	99.5	97.3	97.3	93.6	80.1	51.4	11.9
Sum of Minor Metabolites	Water	0.9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	4.1
	Sediment	n.d.	< LOD	< LOD	< LOD	< LOD	< LOD	0.7	< LOD	< LOD
	System	0.9	< LOD	< LOD	< LOD	< LOD	< LOD	1.4	0.9	6.1
Total Extractable Residues	Water	99.2	88.8	74.7	61.7	56.6	41.5	32.1	18.4	7.4
	Sediment	4.2	11.3	24.8	36.0	40.7	52.1	49.2	33.4	10.6
	System	103.5	100.0	99.5	97.6	97.3	93.6	81.4	52.3	17.9
Carbon dioxide		n.a.	0.5	0.4	0.8	1.3	2.3	6.5	22.8	47.9
Organic volatiles		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1
Non-extractable residues		0.2	1.1	2.5	3.0	4.4	5.9	11.7	21.0	32.3
Total recovery		**103.7**	**101.7**	**102.4**	**101.4**	**103.1**	**101.8**	**99.7**	**96.5**	**98.1**

n.a = not applicable / not analyzed < LOD = below limit of detection

Table 7.2.2.3-20 Biotransformation of flurtamone in Wiehltalsperre test system

Compound		: % applied radioactivity as							
	Source	0	0.25	1	3	7	14	30	100
Flurtamone	Water	94.5	83.2	74.3	59.7	42.9	37.9	7.8	1.7
	Sediment	7.2	16.3	23.4	38.9	52.8	50.0	41.3	36.3
	System	101.7	99.5	97.7	98.5	95.7	87.9	49.0	38.0
Sum of Minor Metabolites	Water	1.5	0.7	< LOD	< LOD	< LOD	2.0	1.0	< LOD
	Sediment	n.d.	n.d.	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	System	1.5	0.7	< LOD	< LOD	< LOD	2.4	1.4	< LOD
Total Extractable Residues	Water	96.0	83.9	74.3	59.7	42.9	39.9	8.8	1.7
	Sediment	7.2	16.3	23.4	38.9	52.8	50.4	41.7	36.3
	System	103.2	100.3	97.7	98.5	95.7	90.3	50.4	38.0
Carbon dioxide		n.a.	0.4	0.5	0.7	0.9	2.1	34.6	35.8
Organic volatiles		n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Non-extractable residues		0.4	1.5	3.4	3.3	6.3	6.7	12.9	23.5
Total recovery		**105.6**	**102.2**	**101.6**	**102.5**	**102.9**	**99.2**	**97.9**	**97.4**

n.a = not applicable / not analyzed < LOD = below limit of detection

The formation of NER and a high amount of carbon dioxide indicate a quite usual participation in the natural carbon cycle and the complete mineralization of flurtamone.

Analysis of the flurtamone enantiomers after an incubation period of 14 days in the water and 30 days in the sediment showed similar degradation behaviour of both enantiomers.

The data for the test item flurtamone were evaluated according to the FOCUS guidance document on degradation kinetics using the software KinGUI. The results of the kinetic evaluations are summarized in the table below.

Table 7.2.2.3-21 Dissipation kinetics of flurtamone in water/sediment systems

System and Phase	Kinetic Model	Visual Assessment	Chi^2 error (%)	DT_{50} (d)	DT_{90} (d)
Anglersee water layer	DFOP	Good	4.2	11.2	75.9
Wiehlaltsperre water layer	DFOP	Good	6.3	7.1	34.9
Anglersee entire system	SFO	Medium	6.9	51.2	169.9
Wiehlaltsperre entire system	DFOP	Medium	6.6	40.9	> 1000

Conclusions

Flurtamone was degraded in both water/sediment systems, resulting in mineralization rates of 47.9% AR in Anglersee and 35.8% AR in Wiehltalsperre water/sediment systems at study termination. Organic volatile radioactivity was ≤ 0.2% AR in all test systems. Besides flurtamone, eight minor radioactive zones were detected in the entire systems. The maximum amount of a single minor radioactive zone was 3.1% AR for the Anglersee water/sediment systems and 2.4% AR for the Wiehltalsperre water/sediment systems.

Along with the overall metabolism of Flurtamone non-extractable residues were formed with a maximum amount of 32.3% AR at study termination in Anglersee water/sediment systems and a maximum of 23.5% AR at study termination in Wiehltalsperre water/sediment systems.

The degradation behaviour of the Flurtamone enantiomers was analyzed in water layers and sediments and shown to be similar for both enantiomers.

The dissipation time (DT_{50}) of Flurtamone from the water layer (sum of degradation and translocation processes into the sediment) was calculated to be 11.2 days for the Anglersee test systems and 7.1 days for the Wiehltalsperre test systems. The DT_{50S} of Flurtamone in the entire water/sediment systems were calculated to be 51.2 days for the Anglersee test systems and 40.9 days for the Wiehltalsperre test systems, respectively.

Report: **KCA 7.2.2.3/04; [redacted] 2013d**
Title: Flurtamone: Kinetic Modelling Evaluation of Water Sediment Degradation Study Data to Derive Total System $DegT_{50}$ Values
Organisation: [redacted]
Report No.: VC/13/012C
Bayer CropScience Document **M-475187-01-1**
Publication: unpublished
Dates of experimental work: Not applicable
Guidelines: **Commission Regulation (EC) No 1107/2009 of 21 October 2009**
Deviations: None
GLP/GEP **No – but conducted to Good Modelling Practice**

Executive Summary

A kinetic evaluation of two water sediment studies with the active substance flurtamone has been conducted using the computer program KinGUI2 according to FOCUS Kinetics guidance [FOCUS, 2006].

Data for flurtamone was evaluated against the FOCUS Kinetics flowcharts [FOCUS, 2006] for the determination of total system trigger and modelling endpoints. The modelling endpoint total system $DegT_{50}$ values for flurtamone are summarized in the table below. The geometric mean total system modelling endpoint $DegT_{50}$ value of 82.4 days can be used as $DegT_{50water}$ in $FOCUS_{sw}$ evaluations [in combination with a conservative $DegT_{50sediment}$ value of 1000 days]. The trigger endpoint total system DT_{50} values for flurtamone derived in this evaluation are also summarized in a table below.

Table 7.2.2.3-22: Modelling endpoint total system $DegT_{50}$ values for flurtamone

Sediment system	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual assessment
River Roding	103	342	8.6	k= 0.132	o
Manningtree	167	555	7.5	k= 3.36E-09	+
Anglersee	51.2	170	6.9	k= 4.81E-09	o
Wiehltalsperre	52.3	174	7.7	k= 2.30E-05	o
Geometric mean	**82.4**				

Visual assessment: + = good, o = moderate, - = poor

Table 7.2.2.3-23: Trigger endpoint total system DT_{50} values for flurtamone

Sediment system	Best-fit kinetic	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual assessment
River Roding	DFOP	90.7	404	6.1	k_1= 0.091231 k_2= 1.01E-07	+
Manningtree	SFO	167	555	7.5	k= 3.36E-09	+
Anglersee	SFO	51.2	170	6.9	k= 4.81E-09	o
Wiehltalsperre	FIS	34.4	4000	5.6	k_1= 2.50E-06 k_2= 0.337	o

Visual assessment: + = good, o = moderate, - = poor

Material and Methods

The kinetic modelling used the results from two aerobic water / sediment studies [[redacted], 1997; [redacted], 2012].

For the first study [[redacted], 1997], [TFMP-UL-^{14}C] flurtamone was applied to two test systems, River Roding and Manningtree. The application rate of flurtamone in the water phase was equivalent to 375 g/ha. The dissipation of flurtamone, the formation of non-extractable residues (NER) and metabolites, as well as the partitioning of these components between water and sediment were observed over a period of 161 days at 20°C in the dark.

For the second study [[redacted], 2012], [phenyl-UL-^{14}C] flurtamone was applied to the Anglersee and Wiehltalsperre test systems. The application rate of flurtamone to the water phase was equivalent to 750 g/ha. The dissipation of flurtamone, the formation of non-extractable residues (NER) and metabolites, as well as the partitioning of these components between water and sediment were observed over a period of 100 days at 20°C.

Measured time zero residues for flurtamone were used directly for the River Roding and Manningtree datasets or set to the recovered amount for the Anglersee and Wiehltalsperre systems. Following the recommended procedure for determining modelling and trigger endpoints, [FOCUS, 2006], all datasets were evaluated using SFO kinetics with free optimisation of parameters, along with FOMC, DFOP and HS kinetics where appropriate.

The determinations of the kinetic values followed the recommendations of FOCUS rules. These were aimed at deriving DT_{50} values for use as trigger and model input according to the FOCUS guidance document on degradation kinetics [FOCUS, 2006]. The kinetic evaluations were performed according to the respective decision flowcharts for the determination of trigger and modelling endpoints for parent. The kinetic evaluations and the statistical calculations were conducted with KinGUI (v2.0) using iteratively re-weighted least-squares (IRLS) optimisation.

The model fits were evaluated using a chi-square (χ^2) error statistic and visual inspection of residual plots. The kinetic analyses and optimisations were carried out using the replicate data, however for the χ^2 analysis the predicted concentration is compared to the mean measured value.

Findings

SFO kinetics were initially applied to all datasets, followed by FOMC. FOMC showed no improvement over SFO kinetics for the Manningtree and Anglersee datasets, thus no further evaluations were conducted and SFO was selected as best-fit. The River Roding and Wiehltalsperre datasets required evaluation with DFOP and HS kinetics.

Table 7.2.2.3-22 (above) summarizes the total system modelling endpoint $DegT_{50}$ values for flurtamone. For flurtamone, SFO kinetics was acceptable for use as modelling endpoints for all test systems.

Table 7.2.2.3-24 (above) summarizes the total system trigger endpoint DT_{50} values for flurtamone. SFO kinetics were determined to be the best-fit in deriving trigger endpoints for the Manningtree and Anglersee systems. For the River Roding and Wiehltalsperre systems, DFOP and HS were selected as the best-fit kinetic respectively.

Conclusions

The total system kinetic modelling evaluations for flurtamone showed good model fits. The geometric mean total system modelling endpoint $DegT_{50}$ value for flurtamone of 82.4 days can be used as $DegT_{50water}$ in $FOCUS_{sw}$ evaluations [in combination with a conservative $DegT_{50sediment}$ value of 1000 days].

Report:	**KCA-7.2.2.3/05; [redacted]. 2013e**
Title:	Flurtamone: Kinetic Modelling Evaluation of Water Sediment Degradation Study Data to Derive Water Phase DT_{50} Values
Organisation:	[redacted]
Report No.:	VC/13/012D Bayer CropScience Document M-475188-01-1
Publication:	unpublished
Dates of experimental work:	Not applicable
Guidelines:	**Commission Regulation (EC) No 1107/2009 of 21 October 2009**
Deviations:	None
GLP/GEP	**No – but conducted to Good Modelling Practice**

Executive Summary

A kinetic evaluation of two water sediment studies with the active substance flurtamone has been conducted using the computer program KinGUI2 according to FOCUS Kinetics guidance [FOCUS, 2006].

Data for flurtamone were evaluated against the FOCUS Kinetics flowcharts [FOCUS, 2006] for the determination of water phase trigger and modelling endpoints.

The modelling endpoint water phase DT_{50} values for flurtamone and the trigger endpoint water phase DT_{50} values for flurtamone derived in this evaluation are summarized in the tables below.

Table 7.2.2.3-24: Modelling endpoint water phase DT_{50} values for flurtamone

Sediment system	SFO DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual assessment
River Roding	21.3	70.7	6.1	k_1= 0.0402 k_2= 4.49E-05	+
Manningtree	14.7	48.9	5.6	k_1= 4.54E-06 k_2= 1.91E-07	+
Anglersee	22.0	72.9	4.6	k_1= 0.00257 k_2= 4.95E-08	+
Wiehltalsperre	9.4	31.1	6.6	k_1= 0.019 k_2= 2.12E-07	+
Geomean	**16.0**	**52.9**	Visual assessment: + = good, o = moderate, - = poor		

* SFO DT_{50} calculated as best-fit DT_{90}/3.32

Table 7.2.2.3-25: Trigger endpoint water phase DT_{50} values for flurtamone

Sediment system	Best-fit kinetic	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual assessment
River Roding	DFOP	9.2	70.7	6.1	k_1= 0.0402 k_2= 4.49E-05	+
Manningtree	DFOP	3.8	48.9	5.6	k_1= 4.54E-06 k_2= 1.91E-07	+
Anglersee	DFOP	9.4	72.9	4.6	k_1= 0.0025 k_2= 4.95E-08	+
Wiehltalsperre	DFOP	5.8	31.1	6.6	k_1= 0.019 k_2= 2.12E-07	+
Geomean	**-**	**6.6**	**52.9**			

Visual assessment: + = good, o = moderate, - poor

Material and Methods

The kinetic modelling used the results from two aerobic water / sediment studies [[redacted], 1997; [redacted], 2012].

For the first study [[redacted], 1997], [TFMP-UL-^{14}C] flurtamone was applied to two test systems, River Roding and Manningtree. The application rate of flurtamone in the water phase was equivalent to 375 g/ha. The dissipation of flurtamone, the formation of non-extractable residues (NER) and metabolites, as well as the partitioning of these components between water and sediment were observed over a period of 161 days at 20°C in the dark.

For the second study [[redacted], 2012], [phenyl-UL-^{14}C] flurtamone was applied to the Anglersee and Wiehltalsperre test systems. The application rate of flurtamone to the water phase was equivalent to 750 g/ha. The dissipation of flurtamone, the formation of non-extractable residues (NER) and metabolites, as well as the partitioning of these components between water and sediment were observed over a period of 100 days at 20°C.

Measured time zero residues for flurtamone were used directly for the River Roding and Manningtree datasets or set to the recovered amount for the Anglersee and Wiehltalsperre systems. Following the recommended procedure for determining modelling and trigger endpoints, [FOCUS, 2006], all datasets were evaluated using SFO kinetics with free optimisation of parameters, along with FOMC, DFOP and HS kinetics where appropriate.

The determinations of the kinetic values followed the recommendations of FOCUS rules. These were aimed at deriving DT_{50} values for use as trigger and model input according to the FOCUS guidance document on degradation kinetics [FOCUS, 2006]. The kinetic evaluations were performed according to the respective decision flowcharts for the determination of trigger and modelling endpoints for parent (Level P-1) [FOCUS, 2006].

The sampling times and residue data were entered into KinGUII and optimisations carried out for SFO or FOMC, DFOP and HS kinetics. The kinetic evaluations and the statistical calculations were conducted with KinGUI (v2.0) [[redacted], 2011] using iteratively re-weighted least-squares (IRLS) optimisation.

The model fits were evaluated using a chi-square (χ^2) error statistic and visual inspection of residual plots. The kinetic analyses and optimisations were carried out using the replicate data, however for the χ^2 analysis the predicted concentration is compared to the mean measured value.

Findings

SFO kinetics were initially applied to all datasets, followed by FOMC. FOMC showed improvement over SFO kinetics for all datasets, thus further evaluations were conducted with DFOP and HS to determine the best-fit.

For the water phase modelling endpoint SFO DT_{50} values for flurtamone, SFO kinetics were not considered suitable, and modelling endpoint SFO DT_{50} values were derived as the best-fit [DFOP] DT_{90}/3.32. These are summarized in Table 7.2.2.3-24 (above).

For trigger endpoint determination DFOP kinetics were determined to be the best-fit for all systems and the results are summarized in Table 7.2.2.3-25 (above).

Report: **KCA-7.2.2.3/06: [redacted]. 2013f**
Title: Flurtamone: Kinetic Modelling Evaluation of Water Sediment Degradation Study Data to Derive Sediment Phase DT_{50} Values
Organisation: [redacted]
Report No.: VC/13/012J
Publication: Bayer CropScience Document M-476011-01-1
Dates of experimental work: unpublished
Guidelines: Not applicable
Deviations: **Commission Regulation (EC) No 1107/2009 of 21 October 2009**
GLP/GEP None
No – but conducted to Good Modelling Practice

Executive Summary

A kinetic evaluation of two water/sediment studies with the active substance flurtamone has been conducted using the computer program KinGUI2 according to FOCUS Kinetics guidance [FOCUS, 2006]. Data for flurtamone were evaluated against the FOCUS Kinetics flowcharts [FOCUS, 2006].

The sediment phase DT_{50} ($DisT_{50}$) values for flurtamone derived in this evaluation are summarized in the table below.

Table 7.2.2.3-26: Sediment phase DT_{50} values for flurtamone

System	DT_{50} (days)	DT_{90} (days)	Chi^2 (%)	t-test (-)	Visual
River Roding	68.7	228	14.3	0.0177	o
Manningtree	114	378	7.9	0.000772	+
Anglersee	48.4	161	9.9	0.000392	+
Wiehltalsperre	175	581	5.0	0.0217	+
Geomean	**90.2**	**300**	Visual assessment: + = good, o = moderate, - = poor		

Material and Methods

The kinetic modelling used the results from two aerobic water/sediment studies [[redacted], 1997; [redacted], 2012].

For the first study [[redacted], 1997], [TFMP-UL-^{14}C] flurtamone was applied to two test systems, River Roding and Manningtree. The application rate of flurtamone in the water phase was equivalent to 375 g/ha. The dissipation of flurtamone, the formation of non-extractable residues (NER) and metabolites, as well as the partitioning of these components between water and sediment were observed over a period of 161 days at 20°C in the dark.

For the second study [[redacted], 2012], [phenyl-UL-^{14}C] flurtamone was applied to the Anglersee and Wiehltalsperre test systems. The application rate of flurtamone to the water phase was equivalent to 750 g/ha. The dissipation of flurtamone, the formation of non-extractable residues (NER) and metabolites, as well as the partitioning of these components between water and sediment were observed over a period of 100 days at 20°C.

The values for the maximum flurtamone sediment concentrations and those from the time at which these occurred and the intervals between were entered into the program. Following the recommended procedure for determining modelling and trigger endpoints, [FOCUS, 2006], all datasets were evaluated using SFO kinetics with free optimisation of parameters. The determinations of the kinetic values followed the recommendations of FOCUS rules. The kinetic evaluations were performed according to the respective decision flowcharts for the determination of trigger and modelling endpoints for parent (Level P1) [FOCUS, 2006].

The kinetic evaluations and the statistical calculations were conducted with KinGUI (v2.0) [[redacted], 2011] using iteratively re-weighted least squares (IRLS) optimisation. The model fits were evaluated using a chi-square (χ^2) error statistic and visual inspection of residual plots. The kinetic analyses and optimisations were carried out using the replicate data, however for the χ^2 analysis the predicted concentration is compared to the mean measured value.

Findings

SFO kinetics were applied to all datasets and the results are summarized in Table 7.2.2.3-26, above.

Conclusions

The sediment phase kinetic modelling evaluations for flurtamone showed acceptable model fits.

CA 7.2.2.4 - Irradiated water/sediment study

No study is offered under this point. The route and rate of degradation of flurtamone in water and sediment were comprehensively studied in sections CA 7.2.1.1 to CA 7.2.1.1 and CA 7.2.2.1 to CA 7.2.2.3. Therefore, the route and rate of degradation of flurtamone in irradiated water/sediment systems was not studied separately.

CA 7.2.3 - Degradation in the saturated zone

The degradation of flurtamone in the saturated zone was not studied since flurtamone is not expected to reach the saturated zone after its use according to good agricultural practices.

Overall Conclusions on the Fate and behaviour in water and sediment (of flurtamone and its metabolites) (Point 7.2)

Studies previously submitted show that flurtamone is resistant to hydrolysis, which will therefore not be a route of dissipation in the environment, but that it is susceptible to photolysis when in aqueous solution with a quantum yield of direct photolysis of 3.2×10^{-2}. This results in the formation of one major photodegradate M07 flurtamone-carboxylic acid (3-(2-Methylamino-4-oxo-5-phenyl-4,5-dihydrofuran-3-yl)benzoic acid, AE 1083976, RPA 203597 in report) also having a short photolytic half-life (11.8 hours). This photodegradate is considered in risk assessments.

Flurtamone is not readily biodegradable and degrades only slowly in pelagic waters. Flurtamone was degraded in surface water systems under aerobic conditions with half-lives between 256 and 314 days measured at 10 µg/L and 100 µg/L. One degradation product was detected in significant amounts and was identified as M04 TFMBA.

In water sediment systems flurtamone moved steadily from the water phase to the sediment. There was significant evolution of carbon dioxide. This was particularly marked in the systems treated with flurtamone labelled in the unsubstituted phenyl ring which gave mineralization rates of 47.9% AR and 35.8% AR. In all systems there was formation of non-extractable residues.

In the systems treated with flurtamone labelled in the trifluoromethylphenyl ring flurtamone was shown to degrade to M08 flurtamone-desphenyl (5-methylamino-4-(3-trifluoromethylphenyl)-3(2H)-furanone , AE 2093305, RE 591120 in report) and ultimately to carbon dioxide. M08 flurtamone-desphenyl was found at > 10% in one of the total systems and so is included in aquatic risk assessments. Small amounts (always < 5% AR) of M02 3-trifluoromethyl-N-methyl-mandelamide (AE 0540067, RE 53285 in the report) and M04 TFMBA (AE C518919, RE 54488 in the report) were also detected. Other degradates were present in very small quantities only.

In the systems treated with flurtamone labelled in the unsubstituted phenyl ring it was extensively degraded (as shown by the degrees of mineralization) but without the formation of a major degradate. Besides flurtamone, eight minor degradates were detected in the entire systems. The maximum amount of a single minor degradate was 3.1% AR.

The occurrence of metabolites/degradates in aqueous photolysis and water-sediment studies is summarized in the table below.

Table 7.2-1 Occurrence of flurtamone metabolites/degradates in laboratory aqueous photolysis and water-sediment studies

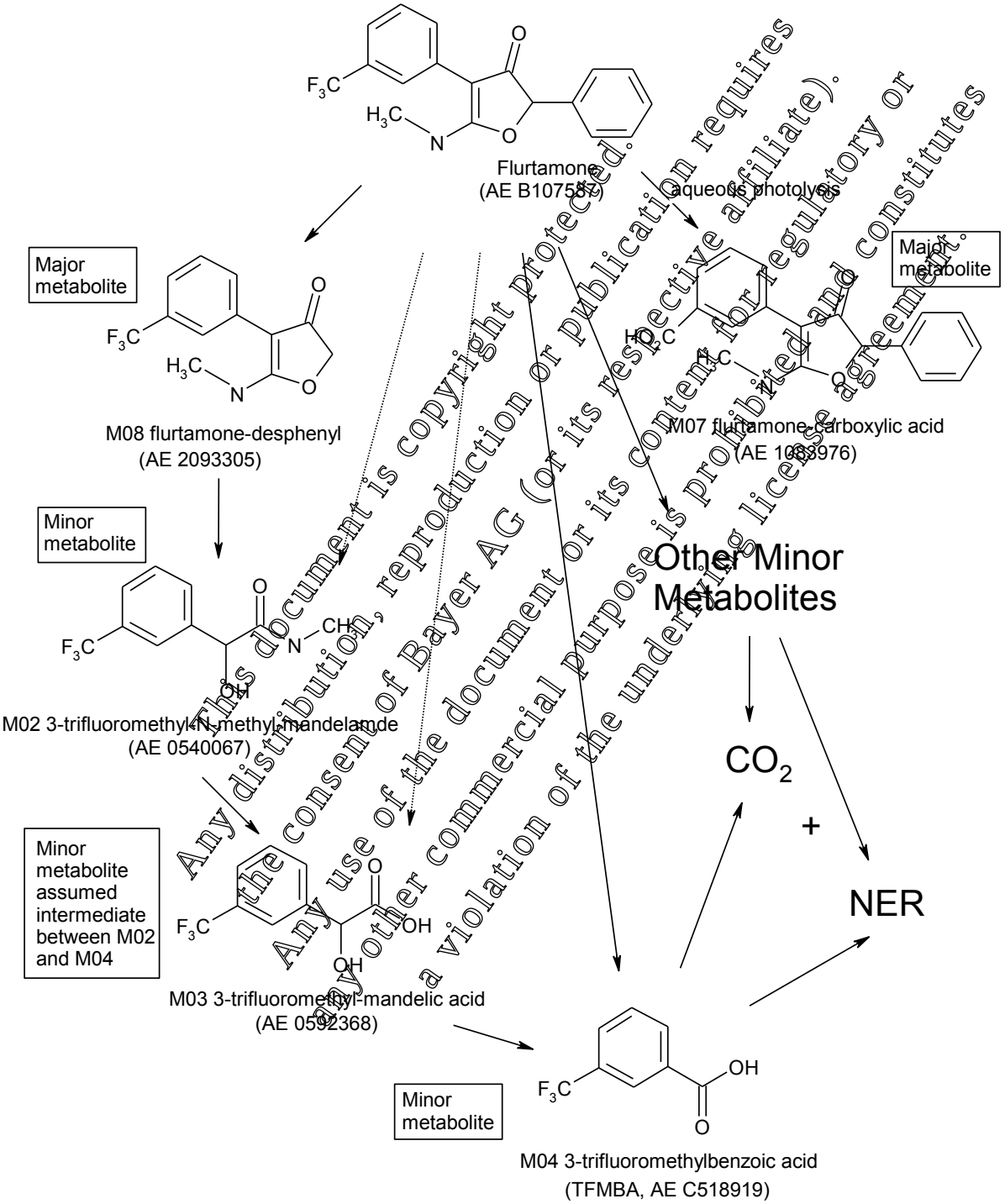
Metabolite	Current Code	Max % AR in:	
		Water Sediment Total System	Aqueous Photolysis
3-trifluoromethyl-N-methyl-mandelamide(M02) Minor metabolite	AE 0540067	3.5	nd
3-Trifluromethyl-mandelic acid (M03) Minor metabolite (assumed)	AE 0592368	nd*	nd
3-Trifluoromethylbenzoic acid (M04 TFMBA) Minor metabolite	AE C518919	4.1	nd
Flurtamone-carboxylic acid (M07) Major metabolite	AE 1083976	nd	33.7
Flurtamone-desphenyl (M08) Major metabolite	AE 2093052	10.7	nd

*not identified but assumed to be present at very low levels as an intermediate between M02 and M04

A composite metabolic/degradation pathway for flurtamone in aquatic systems is shown below.

Kinetic analyses of the water-sediment studies gave total system modelling DT_{50} values ranging from 51.2 to 167 days with a geometric mean value of 82.4 days. Water phase DT_{50} values were found to range from 9.4 to 22.0 days. Trigger DT_{50} values for the water phase gave a geometric mean value of 6.6 days and that for the sediment phase was 90.2 days.

Figure 7.2-1 Proposed metabolic/degradation pathway of flurtamone in aquatic systems



CA 7.3 - Fate and behaviour in air

CA 7.3.1 - Route and rate of degradation in air

The degradation rate of flurtamone in air using the Atkinson method ([redacted], 1994, M-162358-01-1) was evaluated during the Annex I inclusion, see also CA 7.3.2.

CA 7.3.2 - Transport via air

Data on volatility submitted for Annex I inclusion and documented in the Review Report on Flurtamone have been revised and new values determined under guideline OECD 104 have already been submitted at national level.

Flurtamone has vapour pressure values between 7.0 x 10^{-10} at 20°C and 2.0 x 10^{-9} Pa at 25° C and a Henry's law constant of 2.03 x 10^{-8} Pa m^3 mol^{-1} at 20°C ([redacted], 2006a, M-271433-01-1 and b, M-271434-01-1**,** see below). It therefore has a low potential to volatilise.

The results from studies on volatility from soil and plant surfaces ([redacted], 1995, M-219948-01-1; [redacted], 1995, M-210853-01-1) showed that there was very little volatilization of the compound (< 1% from soil and < 3% from plant surfaces) over a 24 hour period, in line with what be expected when the vapour pressure and Henry's law constant values are considered. In addition theoretical calculation of the potential for photo-oxidation ([redacted], 1994, M-162358-01-1) resulted in a half-life of 2 hours based on an OH radical concentration of 1.5 10^6 cm^{-3} on a 12 h day basis. Volatilization is not considered to be a route of dissipation of flurtamone in the soil environment. Therefore also the metabolite M07 TFA will not be found in the air from the application of flurtamone.

Report: **KCA 7.3.2./01; [redacted] G.; 2006a**
Title: Vapour Pressure of Flurtamone (AE B107587)
Organisation: [redacted]
Report No.: PA06017
Bayer CropScience M-271433-01-1
Publication: Unpublished
Dates of experimental work: 07 February 2006 to 07 March 2006
Guidelines: **92/69/EEC OECD 104**
Deviations: None
GLP/GEP **Yes**

This study is summarized in Section 2, so only an executive summary is presented in this section.

Executive Summary

The vapour pressure of flurtamone was determined by use of the gas saturation method, according to OECD 104. The tests were conducted on flurtamone batch DP539D that had a purity of 99.5%. Nitrogen was used as an inert carrier gas, passing over the test item, thereby being saturated with vapour up to the partial vapour pressure of the test item (glass columns were filled with Raschig rings coated with the test item) and transporting it into a cold trap. The columns were put into a large incubator and connected to a gas pipe and to two cold traps (serial connection). The cold traps were filled with acetonitrile and cooled down to at least -25°C to prevent evaporation. A well-defined

volume of nitrogen gas at flow rates of 1.2 and 4 L/min passed through the columns after they had reached the specified temperature. Three different test temperatures were used.

The amounts of the test item in the cold traps were determined by high performance liquid chromatography (HPLC). After quantitative determination of the test item the partial vapour pressure values were calculated using the general gas equation: pV = nRT. No flurtamone was detected in the solutions from the second traps, indicating that absorption in the first traps was complete. The linearity of the detector over the concentration range used was found to have a correlation coefficient (r) of 0.9994. The equation of the slope was y = 0.7707x – 0.0024.

The vapour pressures were calculated and are shown in Table 7.3.2-1.

Table 7.3.2-1 Vapour pressure of flurtamone at various temperatures

Temperature (°C)	Temperature (°K)	1/T	Vapour Pressure (Pa)	logP
50	323.15	3.095×10^{-3}	1.328×10^{-5}	-6.82
60	333.15	3.002×10^{-3}	2.294×10^{-6}	-5.64
70	343.15	2.914×10^{-3}	3.869×10^{-6}	-5.41

The results were used in the calculation of the vapour pressures of flurtamone at 20 and 25°C. Values of 7×10^{-10} Pa (20°C) and 2.0×10^{-9} Pa (25°C) were obtained.

Report: **KCA 7.3.2 /02; [redacted]; 2006b**
Title: Henry's Law Constant of Flurtamone (AE B107587)
Organisation: [redacted]
Report No.: AF06/032
Bayer CropScience M-271434-01-1
Publication: Unpublished
Dates of experimental work: Not applicable
Guidelines: **94/37/EEC**
Deviations: Not relevant
GLP/GEP **Not applicable**

This study is summarized in Section 2, so only an executive summary is presented in this section.

Executive Summary

The Henry's law constant for flurtamone was determined at 20°C according to the following formula:

$$K = \frac{P \times M}{Cs}$$

The Henry's law constant K was found to be 2.03×10^{-8} Pa m^3 mol^{-1}

Material and Methods

Henry's law constant was determined at 20°C according to the following formula:

$$K = \frac{P \times M}{}$$

Cs

where:

K is the Henry's law constant

P is the vapour pressure at 20°C, expressed as Pa

Cs is the water solubility, expressed as g m^{-3}

M is the molecular weight, expressed as g mol^{-1}

The relevant experimental data for flurtamone are:

- Vapour pressure at 20°C = 7 x 10^{-10} Pa
- Water solubility at 20°C = 11.5 x 10^{-3} $g.L^{-1}$ = 11.5 g m^{-3}

The calculation uses the molecular weight of flurtamone, which is 333.3 $g.mole^{-1}$

Findings

The Henry's law constant K was found to be 2.03 x 10^{-8} Pa m^3 mol^{-1}

Conclusions

The Henry's law constant for flurtamone was calculated to be 2.03 x 10^{-8} Pa m^3 mol^{-1}.

CA 7.3.3 - Local and global effects

No local or global effects have been reported for flurtamone or metabolites derived from flurtamone. The compound M05 TFA is referred to in literature as being present in the air, but this is considered to be due to being formed in the air directly from volatile refrigerants. There have been no known reported cases of M05 TFA being derived from flurtamone being present in air. Neither flurtamone or its metabolites are volatile (in the form that they will exist in the environment) so there will be no issues of transport.

Overall Conclusions on the Fate and behaviour in air (of flurtamone and its metabolites) (Point 7.3)

Based on its vapour pressure and Henry's Law constant flurtamone would not be expected to volatilize and the previously submitted volatility studies confirm that to be the case. Any flurtamone that made its way into the atmosphere would be rapidly degraded by interaction with hydroxyl radicals. In soil flurtamone forms two acid metabolites – trifluoromethylbenzoic acid and trifluoroacetic acid. Under the conditions that these are formed they will exist as salts, such as the sodium salt. These salts have low vapour pressures, that of the trifluoromethyl benzoate being 3.1 x 10^{-5} Pa and that for the trifluoroacetate being < 1 x 10^{-6} Pa. The Henry's law constants are also low with respective values of 6.4 x 10^{-8} Pa m^3/mol (at pH 7) and < 2.7 x 10^{-10} Pa m^3/mol. These metabolites will therefore not make their way into the atmosphere. This is underlined by the results from the new laboratory studies on the trifluoroacetate which showed that despite there being a constant flow of air across the samples treated with the trifluoroacetate, there was no loss, or movement to the traps, of the compound.

CA 7.4 - Definition of the residue

CA 7.4.1 - Definition of the residue for risk assessment

Soil: flurtamone, M04 TFMBA and M05 TFA

Ground water: flurtamone, M05 TFA

Surface water: flurtamone, M07 flurtamone-carboxylic acid and M08 flurtamone-desphenyl

Air: flurtamone

The soil photolysis metabolite benzoic acid has been considered as non-relevant for risk assessment as outlined in the position paper under KCP-9.1 /01: Lowden P. 2012

CA 7.4.2 - Definition of the residue for monitoring

Soil: flurtamone

Water: flurtamone

Air: flurtamone

CA 7.5 - Monitoring data

Flurtamone and its degradation products are rarely found on the list of water monitoring programs performed by water authorities throughout Europe. Results from these monitoring programs are in general not publicly available. Hence, only relevant and reliable monitoring studies found in the required literature searches of the peer reviewed open literature are presented here.

Report:	**KCA 7.5 /01; Lindqvist, B.; Hansson, J.; Jönsson, C.; Persson, K. 2007**
Title:	Presence of pesticide residues in groundwaters: monitoring in Simrishamn in 2002-2007
Source	Vatten, 63, 2, p. 159-163
Report No.:	ISBN no.: 0372-686x Bayer CropScience **M-457483-01-2**
Publication:	Published article
Dates of experimental work:	Not applicable
Guidelines:	**none**
Deviations:	Not relevant
GLP/GEP	**Not applicable -** Published study (peer-reviewed article).

Executive Summary

Simrishamn is an agricultural municipality. Around half of the municipality's land area of 393 square kilometres consists of cultivated arable land and half consists of forest and meadow land. Much of the soil in the cultivated area is sensitive to leaching and the considerable nutrient- and pesticide-intensive cultivation can lead to a risk of the local pollution of both surface and groundwater. In accordance with the drinking water regulations that came into force in 2003, the presence of pesticide residues

must be checked as part of the extended analysis of drinking water annually, depending on the amount of water being distributed by the water treatment works. However, in order to monitor the content of the untreated water, the technical department at Simrishamn municipal authority developed a programme for the inhouse-analysis of pesticide residues, which commenced in 2004 by agreement with the Environmental and Public Health Protection Authority. According to this programme, the technical department intends to check the untreated water every three years with regard to a minimum of:

1) all previously confirmed pesticide residues,

2) suspected pesticide residues known from other agricultural areas,

3) the pesticides for which the Environmental and Public Health Protection Authority has issued spreading permits in water protection areas.

The first sampling round under this programme was carried out during 2006-2007. The samples were analysed with respect to 77 different pesticides or degradation products from the pesticides. Flurtamone was not detected.

Materials and methods

Groundwater was tested for the presence of flurtamone. It was taken from 34 wells in the agricultural municipality of Simrishamn in Sweden on 10-11th January 2007. The method of analysis was by GC/LC-MS methods.

Findings

No flurtamone was detected.

Conclusions

No flurtamone had made its way into the groundwater of the agricultural municipality of Simrishamn.

Report:	**KCA 7.5 /02; Olofsson, U.; Brorström-Lundén, Kylin, H.; Haglund, P. 2013**
Title:	Comprehensive mass flow analysis of Swedish sludge contaminants
Source	Chemosphere, Volume 90, Issue Number 1
Report No.:	10.1016/j.chemosphere.2012.07.002 Bayer CropScience: **M-462150-01-1**
Publication:	Published article
Dates of experimental work:	Not applicable
Guidelines:	**none**
Deviations:	Not relevant
GLP/GEP	**Not applicable -** Published study (peer-reviewed article).

Executive Summary

A screening of metals, persistent organic pollutants, pharmaceuticals and personal care products (PPCPs), and other organic contaminants in sludge from seven Swedish sewage treatment plants (STPs) was performed in this study. This extensive screening provides information on mass flows of 282 compounds used in the Swedish society to sewage sludge. Flurtamone was not detected in sewage sludge.

Materials and methods

Dewatered digested (anaerobic) or stabilized (aerobic) sludge was collected at seven STPs (Stockholm, Gothenburg, Eslöv, Umeå, Borås, Alingsås, and Floda) spread across Sweden, in the autumn of 2004. These STPs represent large-, medium- and small-sized plants. Samples were collected during periods of normal working and weather conditions. Composite samples (n=3) from each STP were collected in dark bottles within one hour after sludge dewatering. In order to reduce the risk of microbial degradation, the sludge samples were frozen immediately after sampling and stored in freezer at -18°C until the chemical analysis. They were analyzed by a GC-MS method.

Findings

Flurtamone was not detected in samples from any of the STPs.

Conclusions

Flurtamone was not detected in the investigated sludge from seven different STPs, indicating that it is not a problem for sewage authorities.

Comments by the Notifier:

Flurtamone was not detected in samples from any of the sewage treatment plants. Thus, this study is not relevant for risk assessment.

Report:	**KCA 7.5 /03; Frank, H.; Christoph, E.H.; Holm-Hansen, O.; Bullister, J.L. (2002)**
Title:	Trifluoroacetate in Ocean Waters
Source	Environmental Science and Technology, 36, 1, p.12-15
Report No.:	not applicable Bayer CropScience **M-455778-01-1**
Publication:	Published article
Dates of experimental work:	Not applicable
Guidelines:	**none**
Deviations:	Not relevant
GLP/GEP	**Not applicable -** Published study (peer-reviewed article).

Executive Summary

Trifluoroacetate (M05 TFA) is an atmospheric pollutant which has been proved to accumulate in several environmental compartments as for instance in ocean waters. Although its environmental presence is known to arise from anthropogenic sources, the question arose whether its occurrence might be natural. M05 TFA was analytically determined in ocean water samples of different depth collected from various locations. Results indicate that M05 TFA in ocean waters is occurring naturally being homogeneously distributed in ocean waters of all ages with a concentration of about 200 ng/L.

Materials and methods

Samples were taken as tabulated below.

Sampling technique:	Niskin sampler	
Sampling frequency:	Southern Ocean	Mid-Atlantic Ocean
	Location 1: 19. and 25.01.1998	Location 4: 29. and 30.01.1998
	Location 2: 23.01.1999	
	Location 3: 26.01.1999	
Number of samples per site/soil type:	Three samples per depth	
Sampling depth (m):	10, 50, 100, 200, 500, 750, 1000, 1500, 2000	0, 1, 40, 120, 380, 1000, 4000, 4150
Transport/storage of samples:	Storage on land at 4 °C	

The processing of the samples was as follows: 3 aliquots of 10 mL were spiked with a solution of heptafluorobutyric acid in deionized water leading to an in-sample concentration 734 ng/L heptafluorobutyrate. This was followed by the addition of 2 g sodium chloride and acidification to pH 1 with 350 µL of 98 % H_2SO_4. This was then subjected to extraction with 1 mL MTBE under agitation The ethereal phases were transferred into silanised 1 mL crimp-cap vials and the acids in the ethereal extracts were derivatised to their pentafluorophenylethyl esters with 5 µL of 1-pentafluoro phenyl-diazoethane (8 vol % in MTBE), prepared from pentafluoroacetophenone.

Artificial seawater samples (pure salts in deionised water) were spiked with sodium trifluoroacetate in deionised water to give calibration concentrations of 28 to 339 ng/L M05 TFA. The final samples were examined by GC-MS with a limit of quantification of 32 ng/L and a limit of detection of 20 ng/L.

Blanks were analysed each sampling year for control. For the sampling period 1998 about 400-year-old mineral water was used as control forwarded to the sampling site. Additionally, mineral water at the University of Bayreuth, deionised water and artificial seawater were used as controls to ensure that there is no contamination with M05 TFA during sample transfer. For the sampling period of 1999, artificial seawater was forwarded to the sampling site for control.

Findings

Measured levels of M05 TFA and the calculated age of the corresponding seawater sample on basis of CFC-12 concentration are presented in Table 7.5-1 for the Mid-Atlantic and in Table 7.5-2 for the Southern ocean.

Table 7.5-1: Concentrations of M05 TFA and CFC-12 age of Mid-Atlantic seawater samples

Depth [m]	M05 TFA[a] [ng/L]	± SD[a] [ng/L]	CFC-12[b] [year]
0	190	10	< 5
SFC 2	200	8	-
40	210	12	< 5
120	205	16	< 5
380	210	6	12
1000	205	16	46
4000	195	16	> 60
4150	200	16	> 60

[a] n = 6; [b] calculated using observed CFC-12 concentration

Table 7.5-21: Concentrations of M05 TFA and CFC-12 age of Southern Ocean water samples

Depth [m]	60.6° S, 56.5°W (19. and 25.01.1998)		60.5° S, 57.5° W (23.01.1999), 60.25°S, 54.5° W (26.01.1999)	
	M05 TFA[a] [ng/L]	± SD[a] [ng/L]	M05 TFA[a] [ng/L]	± SD[a] [ng/L]
10	195	22	210	27
50	185	10	220	26
100	195	8	205	22
200	195	6	170	28
50	205	10		
750	195	12	190	21
10	195	6	165	28
50	200	12	205	24
100	200	6	205	26
200	-		190	19
500	200	10		-
750	200	22	205	18
1000	205	6	-	-
1500	220	3	-	-
2000	210	6		-

SD: standard deviation; [a] n = 6

Independent of depth and location, existing M05 TFA levels in all water samples were about 200 ng/L.

In the Mid Atlantic, subsurface waters (0 – 200 m) are rapidly ventilated over a few years and were close to equilibrium with the overlaying atmosphere revealing apparent ages less than 5 years. Waters from intermediate depth (200 – 1700 m) are ventilated primarily by subpolar-origin waters yielding in increasing ages. Water samples below 1700 m are relatively isolated, having ages larger than 60 years indicating minimal contact with the atmosphere.

Previous measurements in the Southern Ocean show a similar increase in the age of seawater with increasing depth. Down to 200 m depth the water ventilated with the atmosphere yielding in time scales of a few years whereas in deeper depth the water is isolated from the atmosphere for at least several decades.

Conclusions

Existing M05 TFA levels in ocean water samples of different depth were measured during two campaigns in 1998 and 1999 in the Mid Atlantic and Southern Ocean. Additionally, the age of the water samples in the different depth was determined. Since M05 TFA levels determined in subsurface samples and in samples of deeper depth were nearly similar with levels of about 200 ng/L, M05 TFA is likely to be a natural ionic solute in ocean water. Continuous low-level releases from geological or biological sources may have caused the present-day levels in ocean waters.

Comments by the Notifier:

This study indicates that M05 TFA in ocean waters is occurring naturally and is homogeneously distributed in ocean waters of all ages. Thus, this study will not be further considered in the risk assessment.

Report:	**KCA-7.5 /04; Scott B F; Macdonald R W; Kannan K; Fisk A; Witter A; Yamashita N; Durham L; Spencer C; Muir D C G (2005)**
Title:	Trifluoroacetate profiles in the Arctic, Atlantic, and Pacific Oceans.
Source	Environmental Science and Technology, 39, p. 6555-6560
Report No.:	not applicable Bayer CropScience **M-455832-01-1**
Publication:	Published article
Dates of experimental work:	Not applicable
Guidelines:	**none**
Deviations:	Not relevant
GLP/GEP	**Not applicable -** Published study (peer-reviewed article)

Executive Summary

A series of depth profiles was collected at 22 sites in the Arctic, North and South Atlantic and Pacific Oceans to determine spatial patterns for trifluoroacetate (M05 TFA) concentrations in the marine environment and to investigate possible natural sources of M05 TFA. Profiles were also taken over underwater vents in the North and South Pacific and the Mediterranean Sea. At the profile sites, M05 TFA values ranged from < 10 ng/L in the Pacific Ocean to greater than 150 ng/L in the Atlantic Ocean. Samples from the Canada Basin of the Arctic Ocean exhibited variable M05 TFA concentrations (60-160 ng/L) down to 700 m. Below this depth, the M05 TFA concentrations were constant (150 ng/L). Water from the Canadian Arctic had constant high M05 TFA values. Profiles from the Northern Atlantic exhibited high values at all depths but were more consistent in the Western Atlantic. The northwestern Pacific Ocean surface profile sites exhibited low M05 TFA concentrations in the top 100 m increasing to a maximum of 60 ng/L with depth. Samples from the South Pacific Ocean site had generally low values with a few depths (> 800 m) having concentrations of 50 ng/L or more. Additionally, M05 TFA concentrations from profiles over vents in the Pacific and Mediterranean Oceans were taken. The results suggest that some deep-sea vents may be natural sources of M05 TFA.

Materials and methods

Test material

Test item:	Trifluoroacetate M05 TFA

Samples were taken as tabulated below.

Sampling technique:	Niskin sampler
Sampling frequency:	sampling once per location
Number of samples per site/ocean type:	Varies from site to site: 6 – 23 samples per site, excluding duplicates (68 % of the samples had duplicates)
Sampling depth (m):	site dependent; various depth down to 5300 m
Transport/storage of samples:	Cool and dark storage during shipping; storage on land at 4 °C in the dark

Measurements

pH:	Not stated

Organic carbon:	Not applicable

Chemical analysis

Guideline/protocol:	None
Method:	GC/MSD • Derivatisation of the acid with 2,4-difluoroaniline in the presence of dicyclohexylcarbodiimide
Pre-treatment of samples:	• A 0.42 ng spike solution of labeled trichloroacetic acid was added to ca. 75% of the samples just prior to introduction of reagents to ensure complete derivatisation.
Conduction:	Liquid extracts
Reference item:	Trichloroacetic acid (TCA)
Recovery:	80–105 % (SD = 15 %) of comparative TCA
Limit of detection:	0.5 ng/L
Limit of quantification:	not stated

Findings

Validation criteria

Not applicable, monitoring study of existing M05 TFA levels.

Collection method (Niskin bottles) was validated by comparing samples collected from Lake Superior using different sample systems i.e. Niskin bottles, van Dorn bottles and PFTE-free pumps and tubing. Measured M05 TFA concentrations did not vary between the collection methods.
During seawater sampling, a laboratory blank was included in each daily sample set for control.

Recovery of reference item (trichloroacetic acid) ranges between 80-105 % with a relative standard deviation of 15%. Therefore, results were not recovery corrected.

Measured levels of M05 TFA of the corresponding seawater samples are presented in Table 1.

Table 1 Measured levels of M05 TFA in oceanic waters

Site location	Site no.	maximum monitoring depth [m]	M05 TFA [ng/L]	Difference between duplicates [%]	No. of samples
Canada Basin (Western Arctic)	1	1500	34–181	7	20[b]
Canada Basin (Western Arctic)	2	3000	61–172	18	18[b]
Nares Strait (Eastern Arctic)	3	489	120–170	7	8[b]
Nares Strait (Eastern Arctic)	4	579	120–130	5	8[b]
Nares Strait (Eastern Arctic)	5	365	8–125	20	6[b]
North Atlantic	6	1000	18–190	39	8[b]
North Atlantic	7	947	17–150	38	7[b]
North Atlantic	8	3800	120–150	24	5[b]
South Atlantic	9	3875	145–100	12	6[b]
South Atlantic	10	5300	64–155	8	8[b]
South Atlantic	11	5053	200–130	6	6[b]
South Pacific	12	3830	1–150		16
South Pacific[a]	13	2500	1–90		16
North Pacific	14	175	1–25	12	13[b]
North Pacific	15	200	1–30	8	11[b]
North Pacific	16	300	1–68	8	10[b]
North Pacific	17	300	1–80	3	9[b]
North Pacific	18	300	1–20	8	8[b]
North Pacific	19	300	2–10	10	11[b]
North Pacific[a]	20	1000–2200	3–140	not stated	not stated
North Pacific[a]	21	3968	2–230	-	23
Mediterranean Sea[a]	22	200	0.5–50	-	20

[a] vent; [b] duplicate samples

Measured M05 TFA levels ranged from 0.5 to 230 ng/L at the sampling sites (see Table 1). Levels of M05 TFA were predominantly higher in the Atlantic Ocean (>100 ng/L) than in the Pacific Ocean (< 100 ng/L) The reproducibility of concentrations between duplicates at most of the sites was < 15%, except for one location in the Eastern Acrtic and the three locations in the North Atlantic.

M05 TFA Depth profiles.

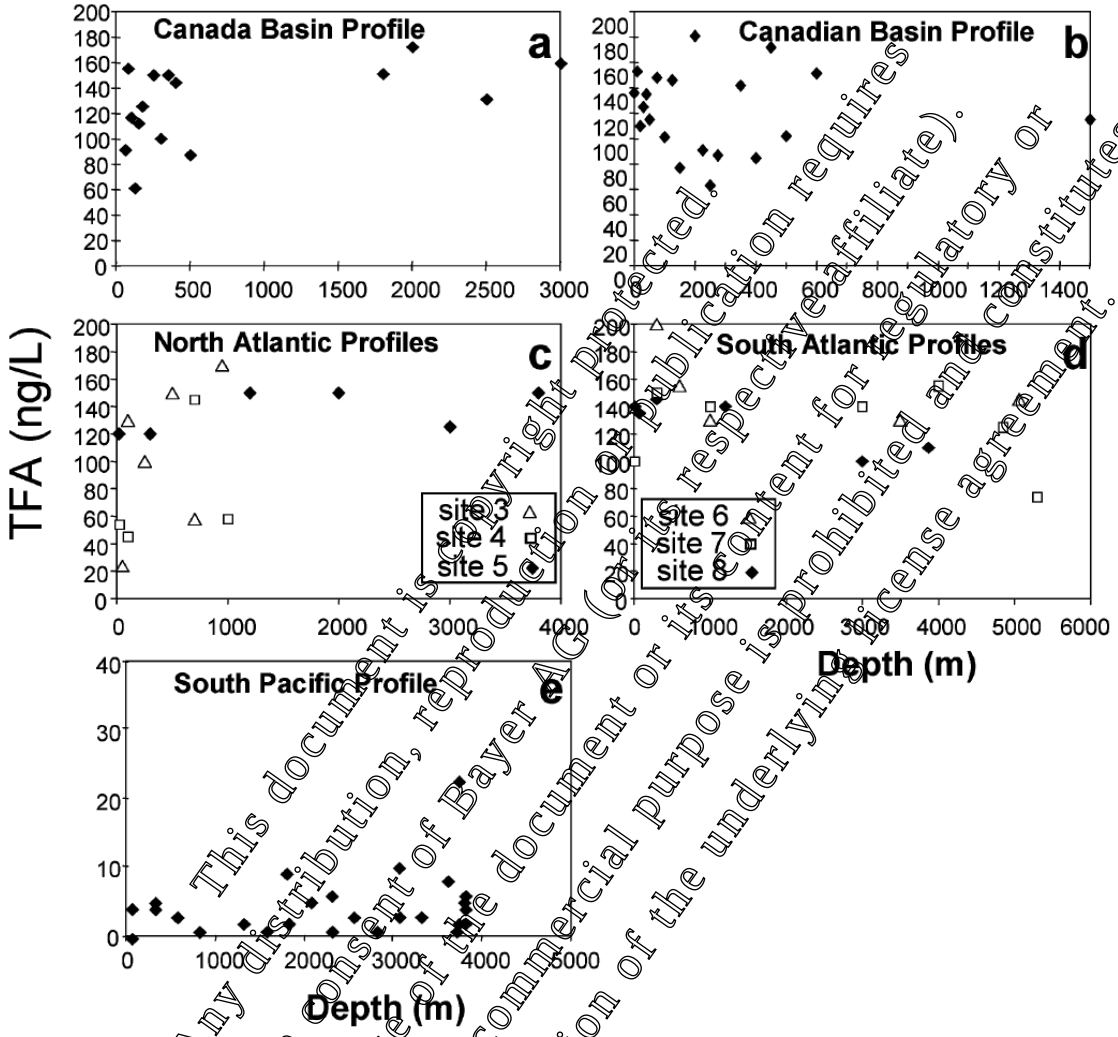


Figure 1: M05 TFA concentrations (ng/L) as function of depth (m) for (a) Canadian Basin at site 1, (b) Canadian Basin at site 2, (c) North Atlantic at sites 3–5, (d) South Atlantic at sites 6–8 and (e) South Pacific at site 12

Depth profiles of M05 TFA for the two Western Arctic sample sites (site nos. 1–2) show much variation in M05 TFA levels for the first several hundred meters. Higher concentrations of M05 TFA (about 160 ng/L) with less variation were detected for water depth from 800 to 3000 m.
Profile data from the two northern located Eastern Artic sample sites (nos. 3–4) reveal constant concentrations of M05 TFA throughout the water column at 150 ng/L with good agreement between duplicate samples (difference between duplicates < 7%). Results for the southern located Eastern Artic sample sites (no. 5) indicate high surface concentrations but significantly lower values down to depth of 250 m with increasing values similar to those observed at the two northern stations. However, differences between the duplicates for the upper 60 m were high (50 %) whereas duplicates below 60 m water depth show smaller differences (< 20%).
Two profiles (nos. 6–7) extending to depths of 1000 m were obtained at the North Atlantic Ocean

sample sites with concentrations of M05 TFA between 17 to 190 ng/L. Lowest concentrations were measured in the upper 50 m. For deeper water layers the concentration of M05 TFA was about 150 ng/L. For the third North Atlantic profile (no. 8) with a depth up to 3800 m concentrations of M05 TFA were nearly stable ranging from 120 to 150 ng/L. Overall, the three profiles from the South Atlantic Ocean sample sites exhibited consistent M05 TFA concentrations throughout the water column at 150 ng/L.

M05 TFA concentrations from the South Pacific sample site (no. 12) were generally below 20 ng/L throughout the profiles, however duplicate samples were not taken at this location.

Surface water profiles with depths of 0–300 m were obtained at six sites in the North Pacific Ocean (nos. 14–19, data shown in the supportive data to the original study). Measured concentrations of M05 TFA were > 10 ng/L in the upper 50 m, 30-60 ng/L at middle depths (150-200 m) and < 10 ng/L below 300 m

M05 TFA Vent Profiles.

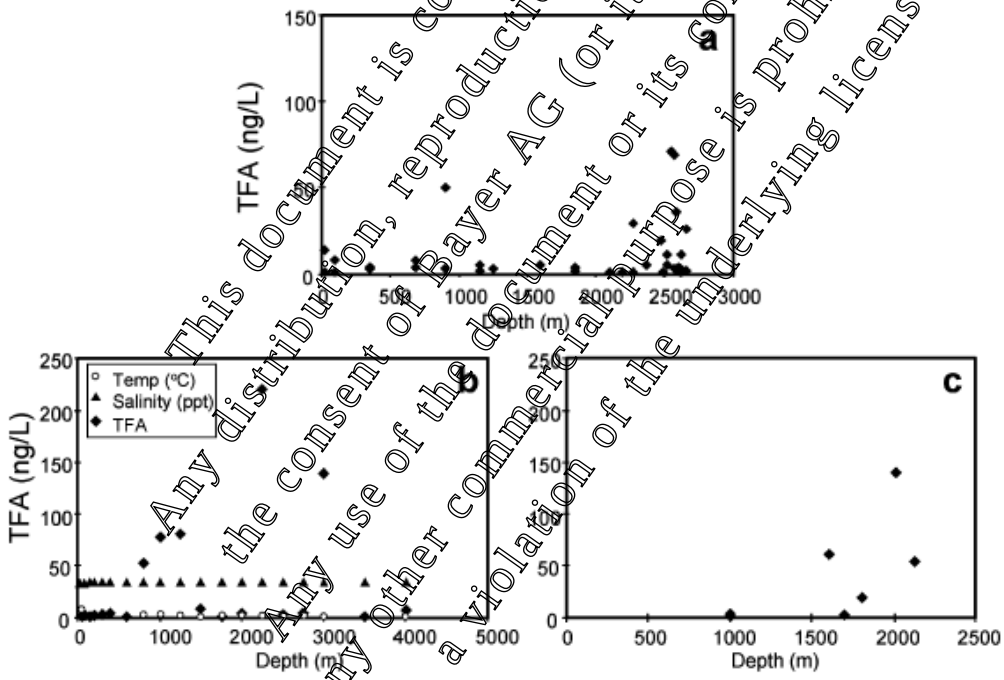


Figure 2: M05 TFA concentrations (ng/L) as function of depth (m) for sites over active vents: (a) site (13), (b) site 21 and (c) site 20

At one vent sample site (no. 13) near Easter Island in the South Pacific low M05 TFA concentration levels throughout the water column were observed. At maximum depths, higher M05 TFA values were detected (17 ng/ L). However, measured M05 TFA levels at a nearby M05 TFA depth profile (no. 9) were significantly higher with concentrations up to 150 ng/L.

At a vent area in the NE Pacific Ocean, two sampling collections (nos. 20–21) were made. One was over a deep-sea vent (4000 m, no. 21) and the other, directly over a volcanic vent (no. 20). For the deep-sea vent (no. 21), measured M05 TFA levels showed much variation over the profile. Low concentrations were observed down to a depth of 800 m and for depths around 2500 m and 3500-4000 m. Increased levels of M05 TFA were detected from 800 m to 1500 m with concentrations up to 100

ng/L. Peak concentrations of M05 TFA were measured at a depth of 2000 m and 3000 m with corresponding M05 TFA concentrations of 225 ng/L and 150 ng/L, respectively. At the other site (no. 20), directly over a volcanic vent, samples were collected at the top of the plume (1900 m), the bottom of the plume (2050 m), and within the core of the plume at 1980 to 2010 m. M05 TFA levels increased from 3 ng/L at a depth of 1000 m to 140 ng/L at the bottom of the plume(2050 m). No correlation was found between the measured M05 TFA values at the vent site with simultaneously measured salinity and temperature data.
For a depth profile taken over a vent in the Mediterranean Sea (no. 22) low concentrations of M05 TFA (often < 1 ng/L) were detected. Near the surface, levels of M05 TFA were higher (around 15 ng/L)

Conclusions

Oceanic M05 TFA depth profiles sampled over various sites reveal a high spatial heterogeneity in their horizontal and vertical distribution. Higher M05 TFA levels were observed in the Arctic Ocean and the North/South Atlantic (around 150 ng/L) whereas lower M05 TFA levels (< 100 ng/L) were measured in the Pacific Ocean. The authors concluded that this variability cannot occur without active sources or sinks. For deeper water layers, having no direct exchange with upper water layers or the atmosphere, existing M05 TFA concentrations can be only the result of natural sources.
Measurements of M05 TFA levels over active vents suggest that some deep-sea vents may be natural sources of M05 TFA.

Comments by the Notifier:

This study provides screening data on the occurance of M05 TFA in ocean waters. Measurements of M05 TFA levels over active vents suggest that some deep-sea vents may be natural sources of M05 TFA. Thus, this study will not be further considered in the risk assessment.