Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Flufenacet

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 8: Ecotoxicological studies

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

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Version history

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

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

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

Flufenacet 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.

This Supplemental Dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of flufenacet and were, therefore, not evaluated during the first EU review of this compound. In order to facilitate discrimination between new and information submitted during the first Annex I inclusion process, the old information is written in grey letters. All studies, which were already submitted by Bayer CropScience for the first Annex I inclusion, are contained in the Monograph, its Addenda and are included in the Baseline dossier provided by Bayer CropScience. These old studies are not summarized again. For all new studies detailed summaries are provided with this Supplemental Dossier. Studies which will be used in the risk assessment are marked in the tables in bold.

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 it`s 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 flufenacet 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.

Due to changes in triggers for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table 8-1). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.

Table 8- 1: Definition of the residue for risk assessment*

Compartment	Residue Definition for Risk Assessment
Soil	Flufenacet, FOE oxalate, FOE sulfonic acid, FOE methylsulfone, FOE-thiadone, FOE 5043-trifluoroethanesulfonic acid and trifluoroacetic acid
Groundwater	Same as for soil
Surface water	Same as for soil plus FOE methylsulfone
Sediment	flufenacet
Air	flufenacet

*Justification for the residue definition for risk assessment is provided in MCA Sec.7, Point CA 7.4.1 and MCA Sec. 6, Point CA 6.7.1.

In addition, a list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound flufenacet is presented in Document N3 of this dossier.

CA 8.1 Effects on birds and other terrestrial vertebrates

In addition to the parent compound flufenacet, a risk assessment (screening level only) is performed also for the metabolite trifluoroacetic acid (TFA). TFA has been identified as an environmental metabolite of different chemicals, including pesticide active substances as e.g. flufenacet. TFA has a pKa values < 2, therefore it occurs only in its deprotonated form under environmental conditions. As residues of TFA may occur in plant food items of birds and wild mammals, it was considered necessary to establish appropriate ecotoxicological endpoints to be used for risk assessment purposes. However, toxicity endpoints are only available for mammals. As birds are not expected to be more susceptible to TFA than mammals, the endpoints generated in studies performed on mammals will also be used for the bird screening assessment in the MCP documents. Nevertheless, the endpoints will only be listed in endpoint lists under CA 8.1.2 "Effects on terrestrial vertebrates other than birds".

CA 8.1.1 Effects on Birds

For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda). These studies are listed in grey in the table below.

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	acute, oral	LD_{50} 1608 1) mg as/kg bw	[redacted] (1992) M-003866-01-1
Mallard duck	acute, oral	LD_{50} > 2000 2) mg as/kg bw	[redacted] & [redacted] (1997) M-003851-01-1
Passerine bird	acute, oral	LD_{50} **434** mg as/kg bw	[redacted] 2013 M-468210-01-1 KCA 8.1.1.1/03
Bobwhite quail	*5-day dietary*	*LC_{50} > 5317 3) ppm* *LDD_{50} > 755 mg as/kg bw/d*	[redacted] *(1994)* *M-003859-0 -1*
Mallard duck	*5-day dietary*	*LC_{50} > 4970 4) ppm* *LDD_{50} > 949 mg as/kg bw/d*	[redacted] *(1993)* *M-003864-01-1*
Bobwhite quail	22-weeks feeding, reproduction	NOAEL 441 ppm NOAEL 34 mg as/kg bw/d	[redacted] (1994) M-003861-01-1
Mallard duck	21-weeks feeding, reproduction	NOAEL 88 1) ppm NOAEL **9.87** mg as/kg bw/d	[redacted] & [redacted] (1994) M-003858-01-1

Bold values: Endpoints used for TER calculation
Italics: Studies and endpoints not used in risk assessment (not required)
1) Endpoint listed in EU review report for the active substance Flufenacet (2003)
2) Highest tested dose, 2 mortalities in 1000, one in 2000 mg/kg bw group
3) Highest tested concentration, two mortalities in 2 469 ppm and one mortality in 5 317 ppm group
4) Highest tested concentration, two mortalities in 4 970 ppm group

Report:	**KCA 8.1.1./01; [redacted], D.; 2010**
Title:	Determination of residues of Flufenacet SC 500 g/L in *Poecilus cupreus* L. (Coleoptera, Carabidae) in an extended laboratory test
Document No:	M-368306-01-1
Guidelines:	None; test method according to [redacted] et al. (2000)
GLP:	Yes (certified laboratory)

Objective:

The purpose of this study was the determination of residues of Flufenacet SC 500 g/L in the carabid beetle (*Poecilus cupreus*) on the day of application and after different periods of aging under extended laboratory conditions.

Materials and Methods

A suspension concentrate of Flufenacet SC 500 g/L was tested, specified by sample description: TOX 08540-00; specification no.: 102000007779; batch ID: EFKF000636 [analysed content of active ingredient: Flufenacet: 42.1 %w/w]; density: 1.193 g/mL.

The test item was applied at a rate of 600 g as/ha (91.9 % of nominal in stock solution) on *Poecilus cupreus* beetles, the food (*Musca domestica* pupae) for the first three days and the substrate (natural soil). After spray application of the test item, the beetles were maintained in the laboratory on natural soil substrate. After defined time intervals, beetles were removed from their exposure units, deep frozen and submitted to residue analysis. Beetles maintained in separate exposure units were used as a blank control.

The samples were analysed for residues of flufenacet according to method 01160. This method describes the determination of residues of flufenacet in/on insects. Flufenacet was extracted from the sample material using a mixture of acetonitrile/water (1/1, v/v). After filtration, an aliquot of the extract was diluted with methanol/water (2/8, v/v) and mixed with an internal standard solution. The residues were quantified by reversed phase HPLC with electrospray and MS/MS-detection.

The test was performed in a controlled environment room at a temperature of 19.5 - 20.5 °C and a relative humidity of 63 – 87 %. The climatic conditions were continuously recorded with thermohygrographs. The light / dark cycle was 16 : 8 h with a light intensity of 301 - 707 Lux (measured once per week using a Luxmeter).

Results:

The samples were analysed for residues of flufenacet according to method 01160 (Analytical Method 01160 for the Determination of Flufenacet (FOE 5043) Residues in/on Insects by HPLC-MS/MS, P602094719, MR-09/089, R. [redacted], P. [redacted]).

Flufenacet was extracted as described above. The results of the analytical analysis of the control and the treated beetle samles are given in the tables below.

Flufenacet residue values in treated beetle samples

No. unit used	Sampling time	No. of Beetles	Residues Flufenacet [mg as/kg fresh weight]
37 + 38	DAA 0; 10:43 a.m.	12	**5.0**#
39 + 40	DAA 0, 19:05 p.m.	12	**0.88**
41 + 42	DAA 1, 10:40 a.m.	12	**0.25**
43 + 44	DAA 2, 10:40 a.m.	12	**0.12**
45 + 46	DAA 3, 10:45 a.m.	12	**0.33**
47 + 48	DAA 4, 10:50 a.m.	12	**0.16**
49 + 50	DAA 5, 10:50 a.m.	12	**0.14**
51 + 52	DAA 6, 10:50 a.m.	12	**0.11**
53 + 54	DAA 7, 10:40 a.m.	12	0.09
55 + 56	DAA 8, 10:50 a.m.	12	0.10
57 + 58	DAA 9, 10:45 a.m.	12	0.10
59 + 60	DAA 10, 10:45 a.m.	12	0.11
61 + 62	DAA 11,10:55 a.m.	12	0.10
63 + 64	DAA 12, 10:50 a.m.	12	0.07
69 + 70	DAA 13, 10:50 a.m.	12	0.08
71 + 72	DAA 14, 10:50 a.m.	12	0.08

LOQ = 0.10 mg/kg, LOD = 0.025 mg/kg DAA = Days after application

Values printed in **bold** are included in DT_{50} calculation (residue concentrations > LOQ)

The DT_{50} value for residue dissipation of flufenacet from the carabid beetles was calculated based on the measured residues over the sampling days 0 to 6 after application. Afterwards the measured residues fluctuated around the LOQ (0.10 mg/kg) and inclusion of the data in the calculation was not considered meaningful.

Based on single 1st order (SFO) calculation the DT_{50} for residue dissipation of flufenacet from the carabid beetles was determined at 0.15 days. However, the curve fit was poor as were the distributions of residuals. The Chi^2 error value was just above the 15% specified under FOCUS (2006).

Using best fit calculation (FOMC) gave better curve fit and distribution of the residuals. The DT_{90} for residue dissipation of Flufenacet from the carabid beetles was estimated at 0.73 days. Where an SFO DT_{50} is needed for calculation of Time-Weighted Average (TWA) residue concentrations, a usuable and conservative approximation can be calculated according to FOCUS (2006) as $DT_{50} = DT_{90} / 3.32$, i.e. DT_{50} = 0.22 days.

<u>DT_{50} Evaluation</u>

DT_{50} Evaluation early data SFO (first order)

$DT_{50\ (days)}$	0.1535
$DT_{90\ (days)}$	0.5099
Chi^2 error	15.10%
P	<0.001
Visual fit	Fair
Residual fit	poor

DT_{50} Evaluation all data from FOMC (best fit)

$DT_{50\ (days)}$	0.0645
$DT_{90\ (days)}$	0.732
$DT_{50\ (from\ DT90)\ (days)}$	0.221#
Chi² error	8.80%
P	α 0.005; β 0.11
Visual fit	Good
Residual fit	Good

Conclusions:

Based on single 1st order (SFO) calculation the DT_{50} for residue dissipation of Flufenacet from the carabid beetles was determined at 0.15 days. With best fit evaluation, a usuable and conservative approximation can be obtained as DT_{50} = 0.22 days.

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Report:	**KCA 8.1.1./02; [redacted], G.; [redacted], P.; 2012**
Title:	Determination of the residues of flufenacet in/on winter barley and winter wheat after spray application of flufenacet & diflufenican SC 600 in Germany, the Netherlands and Belgium
Document No:	M-443138-01-1
Guidelines:	EC Guidance working document 7029/VI/95 rev.5 (1997-07-22) US EPA OCSPP Guideline No. 860.1500.SUPP
GLP:	Yes (certified laboratory)

Objective:

The purpose of the study was to determine the magnitude of flufenacet residues in/on green material of winter barley and winter wheat at an early growth stage of the plants after one spraying application with Flufenacet & Diflufenican SC 600 specified by sample description: FAR 01538-00; specification no.: 102000007948-03; batch ID: EV56002670 [analysed content of active ingredients: Flufenacet: 32.7 %w/w; Diflufenican: 16.4% w/w]; density: 1.246 g/mL. The product is a suspension concentrate formulation containing 200 g/L diflufenican and 400 g/L flufenacet.

Materials and Methods

The study included four supervised residue trials conducted in northern Europe (the Netherlands, Germany and Belgium) during the 2011 season.

The actual application data are presented in the following table.

Table 1: Application summary

Trial no. Country	Sample material	Formulation	Application						
			Appl. mode	No. of appl.	Growth stage (BBCH code)	Test item rate (L/ha)	Water rate (L/ha)	a.s.	Appl. rate (kg a.s./ha)
11-2950-01 Germany	Winter barley	Flufenacet & Diflufenican SC 600	SPI	1	25	0.6	300	diflufenican	0.12
								flufenacet	0.24
11-2950-02 Netherlands	Winter barley	Flufenacet & Diflufenican SC 600	SPI	1	25	0.6	300	diflufenican	0.12
								flufenacet	0.24
11-2950-03 Germany	Winter wheat	Flufenacet & Diflufenican SC 600	SPI	1	25	0.6	300	diflufenican	0.12
								flufenacet	0.24
11-2950-04 Belgium	Winter wheat	Flufenacet & Diflufenican SC 600	SPI	1	24	0.6	200	diflufenican	0.12
								flufenacet	0.24

a.s.: Active substance SPI: Spraying
Appl.: Application

Results:
The analyses were conducted according to the following analytical method:

Table 2: Summary of analytical method criteria relevant to this study

Active substance	Analytes	Method number	Limit of quantitation [mg/kg]	Measurement principle
flufenacet	flufenacet	01300	0.01	HPLC-MS/MS

The average recoveries were within the acceptable range of 70 - 110%. RSD values were well below 20%.
The level of residues of flufenacet in the treated samples are summarised in the table below. No residues above the LOQ were found in the control samples. Results were not corrected for concurrent recoveries.

Table 3: Residue summary in/on winter barley and winter wheat

Trial No. Country	Sample material	DALT	Residues [mg/kg] a.s. flufenacet
11-2950-01 Germany	green material winter barley	0	7.5
		1	5.8
		3	1.4
		5	0.96
		13	0.27
11-2950-02 Netherlands	green material winter barley	0	9.1
		1	6.5
		3	4.1
		5	3.1
		14	0.091
11-2950-03 Germany	green material winter wheat	0	8.9
		1	6.7
		3	7.1
		5	5.1
		14	0.050
11-2950-04 Belgium	green material winter wheat	0	12
		1	9.9
		3	5.9
		5	4.8
		14	0.084

DALT = Days after last treatment a.s. = Active substance

Analyte:	Final determination as:	Residues calculated as:
flufenacet	flufenacet	flufenacet

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Report: **KCA 8.1.1/03; [redacted], F.; [redacted], C.; 2013**
Title: Statement on residue dissipation of flufenacet in treated foliage of monocotyledonous plants: kinetic evaluation
Document No: M-451178-01-1
Guidelines: -
GLP: -

This statement provides kinetic evaluations of the residues of flufenacet in green parts of monocotyledonous plants (cereals) that may represent food items for leaf-eating herbivorous birds or mammals. The residue decline data are available from regulatory plant residue studies ([redacted], G., [redacted], P. 2012, M-443138-01-1).

The single-first-order (SFO) half-lives for flufenacet derived in this evaluation are summarised in Table 1.

Table 4: Summary of DT_{50} values for flufenacet in the trials evaluated in this document calculated with SFO

Trial code	Trial description	Crop	ε [%]	DT_{50} [days]	t-test
R01	11-2950-01	winter barley	12.45	1.58	0.0059
R02	11-2950-02	winter barley	5.794	2.88	0.0013
R03	11-2950-03	winter wheat	13.63	5.20	0.0265
R04	11-2950-04	winter wheat	5.284	3.28	0.0012
		geo mean		**2.97**	

Conclusions: The DT_{50} of flufenacet residues in green plant material is 2.97 days; this value can be used for refined risk assessments addressing exposure of herbivorous and omnivorous birds and mammals.

CA 8.1.1.1 Acute oral toxicity to birds

Following a request from the US EPA, an additional oral toxicity study was performed with flufenacet techn. in passerine birds.

Report: **KCA 8.1.1.1/03;**
Title: Toxicity of Flufenacet Technical during an acute oral LD_{50} with the canary (*Serinus canaria*)
Document No: M-468210-01-1
Guidelines: OCSPP 850.2100
GLP: No

Executive summary:
An acute oral toxicity test was conducted to estimate the LD_{50} of flufenacet to canary (*Serinus canaria*).

Material and Methods:
FOE5043 (Flufenacet/AE F133402) Technical, purity: 98.83%, Batch code: AE F133402-01-19, CAS number: 142459-58-3, Specification number: 102000006978.
The acute oral avian LD_{50} study was conducted with flufenacet at single oral dose levels of 0 (blank control), 135, 236, 413, 723, and 1265 mg a.s./kg body weight. All individual birds were dosed with the appropriate amount of flufenacet to get the appropriate mg a.s./kg body weight. Treatment levels were selected based on a descending geometric progression from the highest dose of 1265 mg a.s./kg body weight, and established to determine the LD_{50} value. All birds were maintained on a basal diet throughout the study. Mortality, clinical symptoms, body weight, and feed consumption were monitored.
Birds were individually housed in commercial metal cages that each measured approximately 27 cm (L) × 33 cm (W) × 31 cm (H). The basal diet was provided *ad libitum* during acclimation and study duration with Lab Diet Advanced Protocol Small Avian Maintenance.

Results:
Body weight and feed consumption:
Body weight measurements (Day -1, Day 7 and Day 14) and changes in body weight (Day -1 to Day 7, Day 7 to 14) were not significantly different when treatment groups were compared to the control group. Bodyweight change was significantly lower than control for the 236 mg/kg bw group over the

Day -1 to 14 interval when assessed with combined sexes however no significant difference occurred for individual sexes with the exception of the 723 and 1265 mg/kg bw levels in which survival was impacted.
No significant difference from the control occurred for individual food consumption measurements (Day 1 to Day 7, Day 8 to Day 14, and Day 1 to Day 14)

Table 1. Mean canary body weights- both sexes combined

Descriptive Statistics						
Treatment Level (mg a.s./kg bw)	**Randomization (Day -1)**		**Day 7**		**Termination (Day 14)**	
	Mean ± S.D.	***n***	**Mean ± S.D.**	***n***	**Mean ± S.D.**	***n***
Control	21.7 ± 1.6	10	21.3 ± 1.8	10	23.0 ± 1.8	9
135	22.0 ± 1.7	10	21.3 ± 1.8	10	22.4 ± 1.8	10
236	21.9 ± 1.3	10	20.7 ± 1.7	9	21.8 ± 1.5	9
413	21.2 ± 1.7	10	20.0 ± 1.2	6	21.7 ± 1.1	6
723	21.4 ± 1.7	10	18.2	1	19.5	1
1265	21.5 ± 1.6	10	-	0	-	0

SD=standard deviation; n = number of surviving birds

<u>Mortality and clinical observations:</u>
No symptoms of toxicity were observed within the control group however one control bird was found dead in the water dish following observations on Day 14. The death was considered accidental and no symptoms of toxicity were noted prior to this observation.
The number of bird moralities during the study were: control (1), 135 (0), 236 (1), 413(4), 723 (9), and 1265 (10) mg ai/kg body weight. All bird mortality occurred by Day 1 with the exception of one accidental mortality in the control group which occurred on Day 14. Ataxia (loss of muscular coordination), hypo-reactivity to stimuli, and/or immobility were observed in all treatment groups with the exception of the 135 mg/kg bw level. No sub-lethal effects were observed in the control group during the study. Severity and prevalence of clinical observations were primarily dose dependent and all surviving birds recovered by Day 2 from the observed symptoms.

Conclusions:
The acute oral LD_{50} for flufenacet technical in canary was 434 mg a.s./kg body weight (95% CL = 337 to 560 mg a.s./kg body weight). The slope of the dose-response curve was 5.6 (95% CL = 2.9 to 8.3). The lowest lethal dose was 236 mg a.s./kg body weight.

CA 8.1.1.2 Short-term dietary toxicity to birds

No new studies have been conducted with flufenacet since Annex I inclusion. For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda).

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new studies have been conducted with flufenacet since Annex I inclusion. For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. Addenda). These studies are listed in grey in the table below. For details on the studies please refer to the respective section in the MCA Section 5 "Summary of the toxicological and metabolism studies for Flufenacet".

Test species	Study	Ecotoxicological endpoint			Reference
Flufenacet a.s.					
Rat	acute oral	LD_{50}	1617 ♀ **589**[1)]	mg a.s./kg bw	[redacted] (1993) M-004865-02-1 and M-004865-02-1
Rat		LD_{50}	1083	mg a.s./kg bw	[redacted] (1992) M-004864-01-1
Mouse		LD_{50}	♂ 1281 ♀ 1756 1529 [2)]	mg a.s./kg bw	[redacted] (1991) M-004850-01-1
Rat	two-generation reproduction	NOAEL	500 **37.4** [3)]	ppm mg a.s./kg bw/d	[redacted] & [redacted] (1995) M-004984-03-1
Rat	developmental	NOAEL	25	mg a.s./kg bw	[redacted] et al. (1995) M-004976-02-1
Rabbit	developmental	NOAEL	25	mg a.s./kg bw	[redacted] et al. (1995) M-004979-01-1
		NOAEL	500 **37.4** [3)]	ppm mg a.s./kg bw/d	Endpoint evaluation: [redacted] (2014) M-476600-01-1 KCA 8.1.2.2/01
TFA					
Rat	acute oral	LD_{50}	**> 2000**	mg a.s./kg bw	[redacted]-[redacted] (2013) M-444479-01-1 KCA 5.8.1/24
Rat	28 days dietary	NOAEL	16000 ♂ 1305 ♀ 1544 1329 [2)]	ppm mg a.s./kg bw/d	[redacted]-[redacted] (2005) M-259106-01-1 KCA 5.8.1/26
Rat	90 days dietary	NOAEL	1600 ♂ 98 / ♀ 123	ppm mg a.s./kg bw/d	[redacted]-[redacted] (2007) M-283994-01-1 KCA 5.8.1/27
		NOAEL	**98**	mg a.s./kg bw/d	Endpoint evaluation: [redacted] (2014) M-477154-01-1 KCA 8.1.2.2/02

Underlined bold values: Endpoints used for Tier 1 TER calculation
Bold values: Endpoints used for refined TER calculation
[1)] As difference between male and female > 25% the lower endpoint is used; endpoint from EU review report (2003)
[2)] Geometric mean of male and female
[3)] According to the Toxicology section of the EU review report (2003) as there is no mammalian reproductive endpoint listed in the Ecotoxicoloy section

CA 8.1.2.1 Acute oral toxicity to mammals

For details on the studies please refer to the respective section in the MCA Section 5 "Summary of the toxicological and metabolism studies for Flufenacet".

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

For details on the studies please refer to the respective section in the MCA Section 5 "Summary of the toxicological and metabolism studies for Flufenacet".

As part of this Supplemental Dossier for renewal of approval of flufenacet two statements are submitted discussing the long-term endpoint to be used in ecotoxicological risk assessments for mammals for the parent substance flufenacet and the metabolites TFA. Summaries of these statements are presented below.

Report:	**KCA 8.1.2.2/01; [redacted], L. (2014)**
Title:	Toxicity Endpoint for the Wild Mammal Reproductive Risk Assessment
Document No:	M-476600-01-1
Guidelines:	-
GLP:	No

In this summary, relevant studies from the Toxicology section are referenced. For details please refer to MCA Section 5 "Summary of the toxicological and metabolism studies for Flufenacet".

Objective

In the scope of the last EU review of flufenacet an official ecotoxicological endpoint addressing the reproductive and long-term risks for wild mammals has not been set. Below the relevant reproduction and developmental toxicity data available for flufenacet are summarized and an appropriate no-observed-adverse-effect-level (NOAEL) is proposed that should be used for the wild mammal risk assessment.

Assessment

Flufenacet has been tested for adverse effects on fertility and reproduction performance in a two-generation rat study. Developmental toxicity studies addressing embryotoxic and teratogenic effects of flufenacet were performed in rats and rabbits. The studies were done in accordance with the testing requirements valid at that time. An overview on the dose levels tested is given in the following table.

Reproduction study						
Species	ppm	0	20	100	500	
rat	mg/kg bw/day (premating ♂ / ♀)	0	1.4 / 1.5	7.4 / 8.2	37.4 / 41.4	
Developmental studies						
rat	mg/kg bw/day	0	5	25	125	
rabbit	mg/kg bw/day	0	5	25	125	200

An overview on the toxic effects induced by flufenacet is shown in the table below. The treatment-related findings from reproduction and developmental toxicity studies are listed in a dose-dependent way.

Dose-effect relationship in reproductive toxicity studies

	dose level ppm	dose level mg/kg bw/day ♂ / ♀	Findings
Reproduction main study	20	1.3 / 2.4	NOEL
Reproduction main study	100	7.4 / 8.2	liver weight ↑, hepatocellular hypertrophy; NOAEL
Reproduction pilot study	100	~10	NOAEL
Developmental rat		25	NOAEL
Developmental rabbit		25	NOAEL
Reproduction main study	500	**37.4** / 41.4	bw ↓ (5 – 7%); **NOAEL$_{ecotox}$**
Reproduction pilot study	400	~40	bw ↓ (<10%); pup weight ↓
Developmental rat		125	bw (↓), food consumption (↓); fetus weight ↓; delayed ossification, no. of variations ↑
Developmental rabbit		125	soft feces; fetus weight ↓; delayed ossification, no. of variations ↑
Reproduction pilot study	1600	~160	bw ↓ (12 – 23%); litter size ↓; pup weight ↓↓
Developmental rabbit		200	soft feces, bw ↓ (<5%); fetus weight ↓; delayed ossification, no. of variations ↑
Reproduction pilot study	3000	~300	bw ↓↓ (16 – 37%); litter size ↓, pup weight ↓↓, pup viability ↓

↓: decrease; (↓): slight decrease; ↓↓: strong decrease bw: body weight

The following assessment can be made from this:

- The overall reproduction performance was adversely impaired only at rather high dose-levels; the number of pups per litter was lower at ≥160 mg/kg bw/day and pup viability was decreased at 300 mg/kg bw/day. As indicated by the substantially decreased body weights, severe somatic toxicity was apparent in mother animals at these dose levels.
- Lower birth weights of pups/fetuses were found to be the most sensitive treatment-related effect with possi¬ble direct ecotoxicological relevance: At 125 mg/kg bw/day fetuses were ~3% lighter in rabbits and ~6% lighter in rats; at 200 mg/kg bw/day the rabbit fetuses were ~10% lighter. For both rodent species a clear NOAEL for lower fetus weights was established at 25 mg/kg bw/day in the develop¬mental toxicity studies.
- In the rat reproduction study no adverse effects on pup weights were detectable up to the highest dose tested. Birth weights of pups from the 500 ppm dose group were identical to that of the control group and also during the lactation period pups gained similar weight in all dose groups.
- In the main reproduction study body weight development of high dose females was slightly retarded in comparison to the control group. Beginning at week 6 of the premating period body

weights of par-ent females were ca. 4 – 7 % lower at 500 ppm. This difference was maintained throughout the gestation period but at the end of the lactation period the difference to the control group was less than 3%. No body weight effects occurred in male rats at 500 ppm.

- The marginally lower body weights in females are considered to be of no ecotoxicological relevance as viability, fertility and reproduction performance were not adversely impacted at this dose level. In addition, also the food intake was lower at 500 ppm, so that reduced palatability of the feed may have contributed to the retarded body weight gain of females.
- The morphological findings in the liver characterised by organ weight increase and hypertrophy of hepatocytes have no relevance for the wild mammal risk assessment; they are to be seen as physiological adaptation of the organ to an increased metabolic burden and not as adverse toxic effect.

Conclusion
The wild mammal long-term/reproductive risk assessment for flufenacet should be based on the ecotoxicological NOAEL obtained in the rat reproduction study: 500 ppm, equivalent to 37.4 mg/kg bw/day.

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Report:	**KCA 8.1.2.2/02; [redacted], L. (2014)**
Title:	Trifluoroacetate (TFA) Toxicity Endpoint for Terrestrial Vertebrate Risk Assessment
Document No:	M-477154-01-1
Guidelines:	-
GLP:	No

In this summary, relevant studies from the Toxicology section are referenced. For details on these studies please refer to MCA Section 5 "Summary of the toxicological and metabolism studies for Flufenacet".

Objective
Trifluoroacetate (TFA) has been identified as an environmental metabolite of different chemicals including several pesticide active ingredients. As residues of TFA may occur in plant food items of birds and wild mammals, it becomes necessary to establish an appropriate ecotoxicological endpoint that can be used for risk assessment purposes. The present paper reviews the ecotoxicologically relevant studies available for TFA and proposes suitable endpoints for the acute and long-term/reproductive risk assessment.

Assessment
For TFA a limited package of toxicological studies is available in mammalian species; no studies have been performed in birds.

Acute endpoint

TFA was found to be nontoxic following single oral administration: The limit dose for acute toxicity testing of 2000 mg/kg bw was tolerated without any signs of intoxication. Thus, for the acute risk scenario the following endpoint can be used: **LD_{50} >2000 mg/kg bw**.

Long-term / reproductive endpoint

A full rat reproduction toxicity study is not available for TFA but in a rat developmental toxicity study no specific adverse reproductive findings were obtained at the highest dose level tested (150 mg/kg bw/day). In two rat feeding studies over 28 and 90 days respectively mild effects on certain clinical chemistry and haematology parameters were seen which however were not considered to be relevant for the setting of an ecotoxicological endpoint. The only finding with possible ecotoxicological relevance was related to slight retardations of body weight development at 16000 ppm (equivalent to 1043 mg/kg bw/day) in the 90 day study. The next lower dose level (1600 ppm) is proposed as an appropriate endpoint for the long-term / reproductive risk assessment:

NOAELecotox: 1600 ppm, equivalent to 98 mg/kg bw/day.

With TFA no toxicity studies are available for bird species but under consideration of the overall favourable toxicological profile of this compound, it is not expected that birds would be more susceptible to TFA than mammals. It is proposed therefore that the mammal endpoints can be used also for screening assessments addressing risks for birds.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{ow} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation.

As the log P_{ow} of the active substance flufenacet (but not for its metabolites) is above the trigger (>3), evaluation of secondary poisoning is needed. See MCP point 10.1.1.2 for more details.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No additional studies have been conducted with flufenacet sine the last Annex I inclusion process. For details on available studies, please refer to the beforementioned annex points.

CA 8.1.5 Endocrine disrupting properties

Wild Mammals

The Flufenacet toxicology database has been updated over the past years with a number of OECD and US EPA guideline studies. Mechanistic studies submitted for evaluation during the initial evaluation of Flufenacet demonstrated that effects on thyroid hormone levels and minimal changes in thyroid gland histopathology are secondary to increased T4 clearance by the liver. Flufenacet itself does not possess endocrine disrupting properties.

Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of Flufenacet in mammals. The secondary effects of the increased clearance of T4 in the liver are covered by the apical endpoints relevant for the wild mammal risk assessment.

Birds

The population relevant effects of Flufenacet on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. No statistically significant effects on adult birds, offspring or reproductive parameters were found at 88 mg Flufenacet/kg diet in mallard ducks and 441 mg Flufenacet/kg diet in bobwhite quails. Reduced hatching success and delayed body weight development of hatchlings were the most prominent effects observed in both species.

No tests are currently available for birds to determine whether such findings are indeed caused by endocrine disruption or are a result of a secondary mechanism. However, since no direct endocrine disrupting potential was found in mammals it is questionable if these effects were indeed primarily triggered by an endocrine mode of action.

As there have been established levels at which reproduction was not affected in two avian species, it is concluded that based on an appropriate risk assessment there are no population relevant adverse effects of Flufenacet.

No further testing for endocrine disrupting properties is warranted.

CA 8.2 Effects on aquatic organisms

For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda). These studies are listed in grey in the table below.

Test species	Test system	Test duration	Endpoint [mg a.s./L]	Reference
Flufenacet				
Oncorhynchus mykiss (Rainbow trout)	acute, static-renewal	96 h	LC_{50} 5.84 (mm)	[redacted] & [redacted] (1995) M-002379-01-1
Oncorhynchus mykiss (Rainbow trout)	ELS, flow-through	97 d	NOEC 0.334 (mm)	[redacted] (1995) M-002357-01-1
Lepomis macrochirus (Bluegill sunfish)	acute, static-renewal	96 h	**LC_{50}** **2.13** (mm)	[redacted] (1995) M-002378-01-1
Pimephales promelas (Fathead minnow)	FFLC, flow-through	279 d	**NOEC** **0.138** (mm)	[redacted] & [redacted] (2002) M-082934-01-1 KCA 8.2.2.2/01
Lepomis macrochirus (Bluegill sunfish)	bioconcentration	28 d (+14 d)	BCF 71.4 (mm) $BCF_{recalc.}$ 14.3 (mm)	[redacted] (1994) M-003803-01-1 [redacted] & [redacted] (1994) M-003804-01-1

Test species	Test system	Test duration	Endpoint [mg a.s./L]	Reference
Daphnia magna (Water flea)	acute, static	48 h	**$E C_{50}$** **30.9** (mm)	[redacted] (1994) M-003805-01-1
Daphnia magna (Water flea)	chronic, static-renewal	21 d	**NOEC** **3.26** (mm)	[redacted] & [redacted] (1994) M-003795-01-1
Chironomus riparius	chronic, static, spiked water	28 d	**NOEC** **5.0** (nom)	[redacted] (2010) M-372857-01-1 KCA 8.2.5.3/01
Hyalella azteca	acute, static	96 h	$L C_{50}$ 2.45 (mm)	[redacted] (1995) M-002374-01-1
Pseudokirchneriella subcapitata [1] (Green algae)	chronic, static	96 h	96h-$E_r C_{50}$ 0.0031 (im) 96h-$E_b C_{50}$ 0.00182 120h-$E_r C_{50}$ 0.00452 120h-$E_b C_{50}$ 0.00245	[redacted] (1995) M-002348-02-1 recalculated: [redacted] (1998) M-002348-02-1
Pseudokirchneriella subcapitata [1] (Green algae)	Static 96 h using pre-exposed cells	96 h	$E_r C_{50}$ 0.00699 (nom)	[redacted] (1997) M-002343-01-1
Pseudokirchneriella subcapitata [1] (Green algae)	chronic, static	72 h	72h-$E_r C_{50}$ 0.138 (mm) 72h-$E_b C_{50}$ 0.00669 (mm)	[redacted] (2010) M-363891-03-1 KCA 8.2.6.1/09
Pseudokirchneriella subcapitata [1) 3)] (Green algae)	chronic, static	72 - 96 h	**$E_r C_{50}$** **0.0144**	Geometric mean of the three endpoints above
Pseudokirchneriella subcapitata [1] (Green algae)	flow-though	35 d	Recovery after short term peak exposure up to 0.0216 mg/L	[redacted] (2013) M-451657-01-1 KCA 8.2.6.1/11
Desmodesmus subspicatus (Green algae)	chronic, static	72 h	$E_r C_{50}$ 0.675 (nom)	[redacted] (2011) M-415813-01-1 KCA 8.2.6.1/16
Chlamydomonas terricola (Green algae)	chronic, static	9 d	$E_r C_{50}$ 0.657 (nom)	[redacted] (2011) M-418627-01-1 KCA 8.2.6.2/06
Chlorella vulgaris (Green algae)	chronic, static	72 h	$E_r C_{50}$ 11.1 (nom)	[redacted] (2011) M-416169-01-1 KCA 8.2.6.2/05
Anabaena flos-aquae (Blue-green algae)	chronic, static	5 d	$E C_{50}$ 32.5 (mm)	[redacted] & [redacted] (1993) M-002423-01-1
Synechococcus leopoldiensis (Blue algae)	chronic, static	72 h	$E_r C_{50}$ >10 (nom)	[redacted] (2011) M-415814-01-1 KCA 8.2.6.2/04
Navicula pelliculosa (Diatom)	chronic, static	5 d	$E C_{50}$ 2.07 (im)	[redacted] & [redacted] (1995) M-002355-01-1

Test species	Test system	Test duration	Endpoint [mg a.s./L]	Reference
Lemna gibba (Duckweed)	chronic, static	14 d	14d-EC_{50} 0.00243 (nom) 7d-E_rC_{50} 0.0318 (nom)	[redacted] & [redacted] (1993) M-002418-02-1 recalculated: [redacted] (1998) M-086479-01-1 KCA 8.2.7/03
Lemna gibba (Duckweed)	chronic, static	7 d	$E_rC_{50, \text{frond no}}$ 0.016 **$E_rC_{50, \text{frond area}}$ 0.0139**	[redacted] (2013) M-451198-01-1 KCA 8.2.7/11
Lemna gibba (Duckweed)	-	-	Justification to use the new E_rC_{50} ([redacted] 2013) for risk assessment purposes	[redacted] (2014) M-478762-01-1 KCA 8.2.7/13
Lemna gibba (Duckweed)	Peak exposure: one or two 24 h-peaks; total test duration 14 d	14 d	No inhibition >50% up to 0.126 mg a.s./L peak E_rC_{50} >0.126 mg/L	[redacted] (2013) M-452567-01-1 KCA 8.2.7/12
Myriophyllum spicatum	chronic, static	14 d	shoot length yield E_yC_{50} 0.0262 (mm)	[redacted] et al. (2011) M-408819-01-1 KCA 8.2.7/09
Aquatic community (incl. macrophytes & periphyton)	indoor microcosm; Flufenacet WG 60	84 d	**NOEC** **0.012** (nom) **EAC** **0.024** (nom) DT_{50} = 18.8 d	[redacted] & [redacted] (1999) M-023412-01-1 & [redacted] (2009) M-329959-01-1 KCA 8.2.8/03
African clawed frog *Xenopus laevis*	acute	48 h	**LC_{50}** **>10**	[redacted], C. S.; [redacted], T. M.; [redacted], S., (2013) M-471899-01-1 KCA 8.2.8/04
Flufenacet - Saltwater organisms *				
Cyprinodon variegatus (Sheepshead Minnow)	acute, static-renewal	96 h	LC_{50} 3.31 (mm)	[redacted] & [redacted] (1994) M-002422-01-1 KCA 8.2.1/05
Cyprinodon variegatus (Sheepshead Minnow)	ELS	35 d	**NOEC** **0.049** (mm)	[redacted], [redacted] & [redacted] (2013) M-464909-01-1 KCA 8.2.2.1/02
Mysidopsis bahia	acute, flow-through	96 h	**LC_{50}** **5.6**	[redacted], M.B. et al. (2013) M-452205-01-1 KCA 8.2.4.2/03
Crassostrea virginica	acute, flow-through	96 h	EC_{50} 12.6 (mm)	[redacted] & [redacted] (1993) M-002427-01-1 KCA 8.2.8/01

Test species	Test system	Test duration	Endpoint [mg a.s./L]	Reference
Mysidopsis bahia	chronic, flow-through	28 d	**NOEC 0.221**	[redacted], M.B. et al. (2013) M-452207-01-1 KCA 8.2.5.2/01
Skeletonema costatum	chronic, static	5 d	5d-EC_{50} 0.00559 (mm) **4d-E_rC_{50} 0.00949 (mm)**	[redacted] (1995) M-002353-02-1 recalculated: [redacted] (1998) M-086470-01-1 KCA 8.2.6.2/07
FOE oxalate				
Pseudokirchneriella subcapitata [1)] (Green algae)	chronic, static	72 h	**E_rC_{50} > 100 (nom)** E_bC_{50} > 100 (nom)	[redacted] (2009) M-358823-01-1 KCA 8.2.6.1/08
Lemna gibba (Duckweed)	chronic, static	7 d	**E_rC_{50} > 100** (nom)	[redacted] (2009) M-359515-02-1 KCA 8.2.7/05
FOE sulfonic acid				
Oncorhynchus mykiss (Rainbow trout)	acute, static	96 h	**LC_{50} > 86.1** (nom)	[redacted] (1995) M-004932-01-1
Daphnia magna (Water flea)	acute, static	48 h	**EC_{50} > 87.3** (nom)	[redacted] (1995) M-004930-01-1
Desmodesmus subspicatus [2)] (Green algae)	chronic, static	72 h	**E_rC_{50} > 86.7** (nom)	[redacted] (1995) M-004931-01-1
Lemna gibba (Duckweed)	chronic, static	14 d	**EC_{50} > 75.9** (mm)	[redacted] (1995) M-004929-01-1
FOE methylsulfide				
Pseudokirchneriella subcapitata [1)] (Green algae)	chronic, static	72 h	**E_rC_{50} 83.8** (nom)	[redacted] (1998) M-002341-01-1
Lemna gibba (Duckweed)	chronic, static	7 d	$E_rC_{50, frond no.}$ 125 **E_rC_{50} frond area 106** (nom)	[redacted] (2010) M-393709-01-1 KCA 8.2.7/07
FOE methylsulfone				
Pseudokirchneriella subcapitata [1)] (Green algae)	chronic, static	72 h	**E_rC_{50} >10.0** (nom)	[redacted] (2010) M-364591-01-1 KCA 8.2.6.1/10
Lemna gibba (Duckweed)	chronic, static	7 d	**$E_rC_{50, frond no.}$ >100** (nom) $E_rC_{50 frond area}$ >100	[redacted] (2010) M-369703-01-1 KCA 8.2.7/06

Test species	Test system	Test duration	Endpoint [mg a.s./L]	Reference
TFA				
Brachydanio rerio (Zebra fish)	acute, static	96 h	**LC_{50} > 1200**	[redacted] et al., (1992) M-247889-01-1 KCA 8.2.1/10
Brachydanio rerio (Zebra fish)	ELS	144 h	LC_{50} 3000 EC_{50} 700 NOEC 3000 (heart rate) **NOEC 300 (hatching time)**	[redacted] et al. 2013 M-462660-01-1 KCA 8.2.2.1/01
Daphnia magna (Water flea)	acute, static	48 h	**EC_{50} > 1200**	[redacted] et al. (1992) M-247890-01-1 KCA 8.2.4.1/04
Pseudokirchneriella subcapitata (Green alga)	chronic (growth inhibition test), static	72 h	**E_rC_{50} 160** E_bC_{50} 4.8	[redacted] et al. (1992) M-247820-01-1 KCA 8.2.6.1/12
Selenastrum capricornutum	chronic, static	72 h	NOEC >1.2	[redacted] & [redacted] (1993) M-247818-02-1 KCA 8.2.6.1/07
Anabaena	chronic, static	125h	E_rC_{50} >2400	[redacted] (1996) M-247822-01-1 KCA 8.2.6.1/14
Navicula		96h	E_rC_{50} >2400	
Skeletonema costatum		96h	E_rC_{50} >2400	
Chlorella vulgaris		72h	E_rC_{50} >1200	
Chlamidomonas reinhardii		72 h	E_rC_{50} >120	
Dunaliella tertiolecta		72 h	E_rC_{50} >124	
Euglena gracilis		192 h	E_rC_{50} >112	
Phaedactylum tricornutum		72 h	E_rC_{50} >117	
Microcysstis aeruginosa		144 h	E_rC_{50} >117	
Scenedesmus subspicatus	chronic, static	72 h	E_rC_{50} >120	[redacted] et al. (1995) M-247825-01-1 KCA 8.2.6.1/13
Lemna gibba (Duck weed)	chronic, static	7 d	$EC_{50, \text{frond increase}}$ 1100	[redacted] et al. (1993) M-247900-01-1 KCA 8.2.7/04
Lemna gibba (Duck weed)	chronic, static	7 d	$EC_{50, \text{wet mass}}$ **618.3**	[redacted] & [redacted] (2004) M-455787-01-1 KCA 8.2.7/14
Myriophyllum sibiricum	chronic, static	14 d	$EC_{50, \text{wet mass}}$ 357.0	
Myriophyllum spicatum	chronic, static	14 d	$EC_{50, \text{wet mass}}$ **312.9***	
FOE 5043-trifluoroethane sulfonic acid				
Pseudokirchneriella subcapitata (Green algae)	chronic, static	72 h	E_rC_{50} **> 100 (nom)**	[redacted] (2012) M-444217-01-1 KCA 8.2.6.1/15
Lemna gibba (Duckweed)	chronic, static	7 d	**$E_rC_{50 \text{ frond area}}$ > 10**	[redacted] (2013) M-445884-01-1 KCA 8.2.7/10
FOE-Thiadone				
Oncorhynchus mykiss (Rainbow trout)	acute, static	96 h	**LC_{50} 9.1** (mm)	[redacted] & [redacted] (1998) M-005388-01-1

Test species	Test system	Test duration	Endpoint [mg a.s./L]	Reference
				KCA 8.2.1/06
Lepomis macrochirus (Bluegill sunfish)	acute, static	96 h	LC_{50} 18.6 (mm)	[redacted] & [redacted] (1999) M-016583-01-1 KCA 8.2.1/08
Daphnia magna (Water flea)	acute, static	48 h	**EC_{50} 31.7** (mm)	[redacted] & [redacted] (1998) M-005390-01-1 KCA 8.2.4.1/03
Pseudokirchneriella subcapitata [1)] (Green algae)	chronic, static	96 h	**72h-E_bC_{50} 4.1** 72h-E_rC_{50} 15.0 (mm)	[redacted] & [redacted] (1999) M-009214-01-1 KCA 8.2.6.1/06
Lemna gibba (Duckweed)	chronic, static	7 d	$E_rC_{50\ frond\ no}$ 20.8 **$E_rC_{50\ frond\ area}$ 18.3** (mm)	[redacted] (2010) M-393718-01-3 KCA 8.2.7/08
FOE-Thiadone - Saltwater organisms *				
Cyprinodon variegatus (Sheepshead Minnow)	acute, static	96 h	LC_{50}: **15.3** (mm)	[redacted] & [redacted] (1999) M-009684-01-1 KCA 8.2.1/07
Mysidopsis bahia	acute, flow-through	96 h	LC_{50}: > **15.1** (mm)	[redacted] & [redacted] (1998) M-005110-01-1 KCA 8.2.4.2/02
Crassostrea virginica	acute, flow-through	96 h	EC_{50}: 22.0 (mm)	[redacted] & [redacted] (1998) M-005108-01-1 KCA 8.2.8/02

[1)] *Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*
[2)] *Desmodesmus subspicatus*, formerly known as *Scenedesmus subspicatus*
[3)] *geometric mean of two or three studies – see rightmost column*
* wet mass considered to be the most relevant endpoint
mm = mean measured; nom = nominal; im = initially measured
Bold values: Endpoints considered relevant for risk assessment

Selection of algae endpoints for risk assessment

Processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) is more suitable to measure effects in algae. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for yield or biomass based endpoints. Following current state of science, the test guidelines OECD TG 201, the EU-Method C3, the EC regulation for Classification and Labeling (EC regulation 1272/2008), the PPR Opinion (EFSA Journal 461, 1-44; 2007) and also the EFSA Aquatic Guidance Document (2013, not yet formally noted by SCFCAH), list growth rate as the relevant endpoint of the algae inhibition test. The previous Guidance Document on Aquatic Toxicology (SANCO/3268/2001 rev. 4) still states that "As there is no clear evidence available to indicate which is the most relevant endpoint for the field situation the lower figure should be used in the risk assessment". As this statement is clearly superseded by recent scientific and regulatory developments toxicity-exposure-ratios in this assessment were based on the E_rC_{50}, when available.

Three studies with the same algal species (*Pseudokirchneriella subcapitata*, the most susceptible freshwater alga) are available. According to the EFSA Opinion Paper on additional species testing (EFSA 2005[1]) endpoints of these studies should be combined and the geometric mean be used in the risk assessment. Two studies are clearly suitable for this combination, [redacted] (1995) and [redacted] (2010). A third study ([redacted] 1997) deviated in terms of design, as it used pre-exposed algal cells to demonstrate that exposure does not limit the potential for recovery (i.e. flufenacet is algistatic and not algicidal). However, as the study also generated a low end point and the geometric mean based on all three studies is lower than the one based on the two standard studies, the former approach was chosen as the more conservative one.

Selection of Lemna endpoints for risk assessment (see also Statement from [redacted] P, 2014, M-478762-01-1, KCA 8.2.7/13)

So far the EU-agreed endpoint for aquatic plants is based on a 14-day Lemna study form 1993 ([redacted] & [redacted]; M-002418-02-1). This study was done according to the FIFRA Guideline 123-2 and the endpoint was based on frond counts solely. In 1998, [redacted] (M-086479-01-1) recalculated a 7-day E_rC_{50} based on frond count out of this study with 31.8 µg/L. This endpoint was rarely considered by authorities. However, this study by [redacted] & [redacted] (1993; M-002418-02-1) is considered to be not valid according to current guidelines (OECD 221, 2006) as a second endpoint like frond dry weight or frond area has not been determined.

To address this data requirement with a fully valid study a new 7-day Lemna study ([redacted] 2013; M-451198-01-1) was performed. In this study two parameters, frond number and frond area, were assessed as required by the currently valid OECD 221 guideline. The determined endpoint relevant for risk assessment – the 7-day ErC50 based on growth rates of frond area– was by more than a factor of 2 lower than the one recalculated by [redacted] (1998) out of the 14-day study. In addition the OECD guideline 221 states that growth related endpoints should be used for risk assessment purposes to allow comparison of sensitivity of different species. As in addition the no observed effect concentrations (NOECs) from both studies reveal that the test organisms were of equal sensitivity (0.44 and 0.658 µg/L from the old and new study, respectively) it is considered justified to the new fully valid and according to current state of the science performed 7-day Lemna-study supersedes the old 14-day Lemna study where the endpoint is based solely on the frond counts. Consequently the risk assessment will be performed using the new 7-day E_rC_{50} of 13.9 µg a.s./L based on growth rate.

CA 8.2.1 Acute toxicity to fish

Report:	**KCA 8.2.1/05; [redacted], G.G., [redacted], L.M.; 1994**
Title:	Acute toxicity of FOE 5043 to the Sheepshead minnow (*Cyprinodon variegatus*) under static renewal conditions.
Document No.:	M-002422-01-1
Guidelines:	FIFRA 72-3 (a) Saltwater Fish Acute Toxicity Study
GLP:	Yes (certified laboratory)

[1] Question N° EFSA-Q-2005-042 - The EFSA Journal (2005) 301, 1-45

Objectives:
The objective of the study was to determine the acute toxicity of FOE 5043 to the sheepshead minnow *(Cyprinodon variegatus)* in a 96-hour exposure period under static conditions.

Materials and Methods:
FOE 5043), purity: 96.8%, Batch No.: FL036.
The test temperature during the 96-hour exposure ranged from 20.4 to 22.9°C as measured hourly by the data logger. Dissolved oxygen concentrations ranged from 4.0 to 7.0 mg/L representing 47 to 83 percent saturation, respectively, at 21°C. The depressed (<60 percent saturation) dissolved oxygen levels were observed on Day 2 in the old test solutions of 0.60 and 2.34 mg/L dose levels. Therefore, gentle aeration was added to all test chambers after they were renewed with fresh test solution. A small air stone, connected to an oil-free compressed air supply by silicone tubing, was submerged in each aquaria. The gentle aeration did not affect the concentration of the test compound after 48 hours since the measured concentrations were well above 70 percent of the Day 0 measured concentrations. The pH values ranged from 7.5 to 7.9, and the salinity was 12‰ (parts per thousand) throughout the test.

The FOE 5043 exposures were conducted under static conditions. Five concentrations of the test material, a dilution water control, and a solvent control were used for the test. One replicate of twenty fish each was used at each test concentration. Nominal test concentrations were 0.63, 1.25, 2.5, 5 and 10 mg/L, solvent control and control. Five fish were exposed and there was one replicate per test level. Sheepshead minnows were randomly distributed, by twos, to each test chamber until twenty fish were distributed to each. Daily observations were made for mortality and sublethal effects. Dead fish were removed daily. Fish were not fed during the test. Fish from the control and solvent control chambers were weighed and measured at test termination to determine the biomass loading factor.

Findings:
The mean measured concentrations during the test period ranged from 79 to 106 percent of the nominal concentration. The mean measured concentrations were 0.6, 1.18, 2.34, 4.65 and 9.62 mg a.s./L.

Test substance	FOE 5043
Test object	Sheepshead minnow
Exposure	96 hour, Static
LC_{50} mg a.s./L	3.31 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	2.34 mg a.s./L
Highest Test Concentration Without Toxic Effect (NOEC)	1.18 mg a.s./L

Observations:
At 96 hours there was 100% mortality at 9.62 mg/L, 75% at 4.65 mg/L, 25% at 2.34 mg/L and 0% in all other levels. There were no mortalities in the 1.18 mg/L, 0.60 mg/L, control and solvent control levels. Based on the mortality data the 96-hour LC50 was 3.31 mg/L with a 95% confidence interval of 2.73 to 4.02 mg/L. The slope of the 96-hour LC_{50} toxicity curve was +5.34 as determined by the probit method. The 24, 48, and 72-hour LC_{50} values were calculated to be 9.62 mg/L, 6.47 mg/L, and 4.75 mg/L, respectively.

At 96 hours 100% of the fish showed adverse sublethal effects in the 4.65 and 2.34 mg/L test levels. The highest test level had no survivors. The control, solvent control, 1.18, and 0.60 mg/L test levels exhibited no sublethal effects.
The no-observed-effect-concentration (NOEC) was 1.18 mg/L based upon the lack of mortality and sublethal effects at this concentration.

Conclusions:
Based on mortality and the mean measured concentrations, the FOE 5043 96-hour LC_{50} was 3.31 mg a.s./L as determined by the Probit method.

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Report:	**KCA 8.2.1/06; [redacted], L. M.; [redacted], C. V.; 1998**
Title:	Acute toxicity of thiadone to the rainbow trout *(Oncorhynchus mykiss)* under static conditions
Document No.:	M-005388-01-1
Guidelines:	FIFRA Guideline 72-1
GLP:	Yes (certified laboratory)

Objectives:
The objective of the study was to evaluate the acute toxicity of thiadone to Rainbow trout *(Oncorhynchus mykiss)* during a 96-hour exposure period under static conditions.

Materials and Methods:
Thiadone (a metabolite of FOE 5043), purity: 99.4%, Reference No.: M-90-10-76. CAS number 84352-75-0.
The test temperature during the 96-hour exposure ranged from 12.0 to 13.0 as measured hourly by the datalogger. Dissolved oxygen (DO) concentrations ranged from 6.5 to 10.0 mg/L representing 60 and 93 percent saturation, respectively, at 12°C. The pH values ranged from 7.0 to 7.8. The mean conductivity was 113 µmhos/cm. The mean hardness and alkalinity were 52 mg/L as CaCO3 and 44 mg/L as CaCO3, respectively.

A 96-hour static range find study was conducted to determine the concentrations for the definitive study. The range find test concentrations were control, solvent control, 50, 5 and 0.5 mg/L. Five fish were placed in each 20 liter stainless steel aquaria. Each aquarium contained approximately 15.3 liters of test solution. The range find test had one replicate per test level. There was no mortality in the control, solvent control, 0.5 and 5 mg/l test levels.
There was 100% mortality at 50 mg/L after two hours of exposure to Thiadone. No sublethal effects were noted in any surviving fish.
Based upon the range find data and historical toxicity data, the definitive study test levels were control, solvent control, 2.5, 5, 10, 20 and 40 mg/L.
Water samples were collected from each replicate of the control, solvent control and all test chambers on Day 0 and Day 4. The solutions were analyzed on Day 0 (new solutions) and Day 4 (old solutions) to measure actual exposure concentrations.

Findings:
The mean measured concentration of thiadone during the test period was 2.4, 5.0, 10.3, 20.3 and 41.7 mg a.s./L.
Rainbow trout biomass loading was 0.6 g fish tissue per liter of test solution.

Test substance	Thiadone (a metabolite of FOE 5043)
Test object	*Rainbow trout*
Exposure	96 hour, Static
LC_{50} mg a.s./L	9.1 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	10.3 mg a.s./L
Highest Test Concentration Without Toxic Effect (NOEC)	5.0 mg a.s./L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	7.2 mg a.s./L

Observations:
The compound was stable in the test system. No undissolved test substance was observed in the test chambers.
Toxic symptoms at the LOEC level observed included mortality, labored respiration and darkened coloration. There was no mortality in the control, solvent control, 2.4 and 5.0 mg a.s./L test levels. There was 65% mortality at 10.3 mg a.s./L, 100% mortality at 20.3 mg a.s./L and 41.7 mg a.s./L test levels. At 41.7 mg a.s./L fish were all dead within 2 hours of test initiation. The 24-hour LC_{50} was 10.8 mg a.s./L (95% CI = 10.3 to 20.3 mg a.s./L) and the 48-, 72- and 96-hour LC_{50}s were 9.1 mg a.s./L (95% CI = 5.0 - 10.3 mg a.s./L).
Two fish jumped out of the aquaria during the exposure period. The chambers were covered with clear, plexiglass but the fish jumped through some small gaps between the chamber and the plexiglass. On Day 2, one fish jumped out of replicate A, solvent control. On Day 3, one fish jumped out of replicate A, control. These fish were observed to be swimming in the waterbath and survived the duration of the test. For statistical purposes, the control and solvent control test levels were considered to have 19 fish instead of 20; the escaped fish were not considered mortalities.

Conclusions:
Thiadone is moderately toxic to rainbow trout. Based upon mortality the lowest-observed effect-concentration (LOEC) was 10.3 mg a.s./L, and the no-observed effect-concentration (NOEC) was 5.0 mg a.s./L. The 96 hour LC_{50} was 9.1 mg a.s./L (95% confidence interval = 5.0 to 10.3 mg a.s./L).

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Report:	**KCA 8.2.1/07; [redacted], L. M.; [redacted], C. V.; 1999**
Title:	Acute toxicity of thiadone to the sheepshead minnow (*Cyprinodon variegatus*) under static conditions.
Document No.:	M-009684-01-1
Guidelines:	FIFRA Guideline 72-3 (a)
GLP:	yes (certified laboratory)

Objectives:
The objective of the study was to determine the acute toxicity of thiadone to the sheepshead minnow *(Cyprinodon variegatus)* in a 96-hour exposure period under static conditions

Materials and Methods:
Thiadone (a metabolite of FOE 5043), purity: 99.4%, Reference No.: M-90-10-76. Batch No.: K778. The test temperature during the 96-hour exposure ranged from 21.5 to 22.9°C with a mean of 22.2°C as measured hourly by the data logger. Dissolved oxygen concentrations ranged from 5.2 to 6.3 mg/L representing 80 to 96 percent saturation, respectively, at 22°C. The pH values ranged from 6.9 to 7.8, and the salinity was 15‰ (parts per thousand) throughout the test. Light intensity ranged from 50 to 70 foot-candles (mean = 604 lux).

The thiadone exposures were conducted under static conditions. Five concentrations of the test material, a dilution water control, and a solvent control were used for the test. One replicate of twenty fish each was used at each test concentration. Nominal test concentrations were 50, 5 and 0.5 mg/L, solvent control and control. Five fish were exposed and there was one replicate per test level. Sheepshead minnows were randomly distributed, by twos, to each test chamber until twenty fish were distributed to each. There was one replicate at each test concentration. The fish were added to the test solutions within forty minutes of test solution preparation on Day 0. Daily observations were made for mortality and sublethal effects. Dead fish were removed daily. Fish were not fed during the test. Fish from the control and solvent control chambers were weighed and measured at test termination to determine the biomass loading factor.

Findings:
The mean measured concentrations during the test period ranged from 97 to 104 percent of the nominal concentration. The mean measured concentrations were 2.48, 5.20, 9.97, 20.5 and 38.8 mg a.s./L.

Test substance	Thiadone (metabolite of FOE 5043)
Test object	Sheepshead minnow
Exposure	96 hour, Static
LC_{50} mg a.s./L	15.3 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	9.97 mg a.s./L
Highest Test Concentration Without Toxic Effect (NOEC)	5.20 mg a.s./L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	7.20 mg a.s./L

Observations:
Statistically significant sublethal effects were noted at the 9.97 and 20.5 mg a.s./L test levels. The NOEC and LOEC listed above are based upon the statistically significant sublethal effects. These symptoms included abnormal position in the water column, erratic behavior, quiescence, loss of equilibrium, vertical orientation, and labored respiration. All fish at the control, solvent control and 2.48 mg a.s./L test level appeared normal during the exposure. No undissolved test substance was observed in the test chambers.

Conclusions:
Based on mortality and the mean measured concentrations, the Thiadone 96-hour LC_{50} was 15.3 mg a.s./L (95% confidence interval = 12.7 - 18.3 mg a.s./L) as determined by the Probit method. Thiadone is slightly toxic to sheepshead minnows.

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Report: **KCA 8.2.1/08; [redacted], A. T.; [redacted], C. V.; 1999**
Title: Acute toxicity of thiadone, a metabolite of FOE 5043, to the bluegill (*Lepomis macrochirus*)
Document No.: M-016583-01-1
Guidelines: FIFRA Guideline 72-1
GLP: yes (certified laboratory)

Objectives:
The purpose of this study was to determine the acute toxicity of thiadone, a metabolite of FOE 5043, to the bluegill sunfish (*Lepomis macrochirus*).

Materials and Methods:
Thiadone (a metabolite of FOE 5043), purity: 99.6%, Specification: (Batch No.: K778), tested young bluegill sunfish *(Lepomis macrochirus)*. 20 fish per test concentration (mean standard body length 30.55 mm, mean body weight 0.63 g) were control (<0.62), solvent control (<0.62), 7.5 (6.61), 15.0 (14.9), 30.0 (28.0), 60.0 (58.6) and 120 (115) mg a.s./L. The solvent used was dimethylformamide (DMF).
The test temperature during the 96-hour exposure ranged from 21.2 to 22.4°C (mean = 21.8°C) as measured hourly by the data logger. Dissolved oxygen (DO) concentrations ranged from 5.4 to 9.5 mg/L, representing 62 and 109 percent saturation at 22°C, respectively.
The primary measure for acute toxicity was mortality. Sublethal and behavioral effects were also assessed during the course of the study. Results of the test are expressed as a 96-hour median lethal concentration (LC_{50}) which is the concentration of thiadone estimated to be lethal to 50 percent of the test population of fish at the specified time.

Deviations: While the mean light intensity was 53 footcandles, the range of values recorded were 44 to 65 footcandles, which is below the 50 to 100 footcandle range designated in the protocol. While the mean water hardness was 60 mg $CaCO_3$/L, the range of hardness values were 58 to 66 mg $CaCO_3$/L, which is above the 40 to 60 hardness values designated in the protocol. There was no apparent relationship between water hardness and thiadone concentration. These two slight deviations had no impact on the validity of the test.

Findings:

Test substance	Thiadone (metabolite of FOE 5043)
Test object	Bluegill sunfish
Exposure	96 hour, Static
LC_{50} mg a.s./L	18.6 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	14.9 mg a.s./L
Highest Test Concentration Without Toxic Effect (NOEC)	6.61 mg a.s./L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	9.92 mg a.s./L

Analytical results:
The mean measured concentration of thiadone during the test period was 6.61, 14.9, 28.0, 58.6 and 115 mg a.s./L for the nominal concentrations of 7.5, 15.0, 30.0, 60.0, and 120 mg/L, respectively. The control solution consisted of dilution water only.

Method validation:
The method was validated by spiking process water with thiadone technical at concentrations of 0.62, 1.24, 6.18, 12.4, 61.8, and 124 mg/L. Fourteen spikes were prepared and analyzed during method validation: Three each at the 0.62, 1.24, 6.18, and 12.4 mg/L concentrations, one each at 61.8 and 124 mg/L concentrations. All spikes were analyzed using the above method. The average recovery from 14 spikes was 94 % with a relative standard deviation of 4 %.

Observations:
Thiadone was not detected in the control or solvent control at the limit of quantitation of 0.63 mg a.s./L. The compound was stable in the test system. No undissolved test substance was observed in the test chambers.

No behavioral or sublethal effects were observed in the control, solvent control or 6.61 mg a.s./L test level during the exposure period. All fish died within 24 hours of test initiation in the 28.0, 58.6 and 115 mg a.s./L test levels. One to two fish died within 24 hours of test initiation in the two replicates of the 14.9 mg a.s./L test level. The sublethal or behavioral observations of darkened coloration, loss of equilibrium, labored respiration and fish on the bottom of the aquaria were noted in the 14.9 mg a.s./L test level.

Conclusions:
Thiadone is slightly toxic to the bluegill sunfish. Based upon mortality and sublethal effects the lowest-observed effect-concentration (LOEC) was 14.9 mg a.s./L, and the no-observed effect-concentration (NOEC) was 6.61 mg a.s./L. The 96 hour LC_{50} was 18.6 mg a.s./L (95% confidence interval = 14.9 to 28.0 mg a.s./L).

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Report:	**KCA 8.2.1/10; [redacted], A.H.C., [redacted] de, H.A.M., [redacted], [redacted] G. (1992)**
Title:	The acute toxicity of Sodium Trifluoroacetate to the zebra fish *Brachydanio Rerio*
Document No:	M-247889-01-1
Guidelines:	OECD Guideline No. 203 (1984)
GLP:	Yes (certified laboratory)

Objective:

A limit test at 1200 mg test item / L was performed in order to demonstrate that the concentration which kills 50 percent of the fish (96h-LC_{50}) exceeds the limit test concentration. The limit test concentration was chosen based on a range-finder test with guppies.
The objective of the test was to determine the effects of **trifluoroacetic acid** on zebra fish. However, trifluoroacetic acid is strong acid (pKa=0.23), which means that the test solution must be neutralized before testing. Therefore it was decided to test the sodium salt of trifluoroacetic acid following OECD Guideline 203 (OECD 1984) according to OECD (1981) GLP-guidelines. Based on the molecular weights 1.0 g trifluoroacetic acid corresponds to 1.2 g of its sodium salt.

Materials and Methods:
Test material: Sodium trifluoroacetate analysed purity: 99 % was tested, specified by origin batch no.: ACA9135AB.

Test organism: Zebra fish (*Danio rerio*, formerly *Brachidanio Rerio*), body length 2.3 – 3.4 cm, mean body weight 0.23 g.
Fish were exposed in a limit test for 96 h under static test conditions to a nominal concentration of 1200 mg test item / L against a control 0 mg/L. Two test aquaria were used per concentration and to each aquarium 10 fishes were added.
The test aquaria were placed in a climate chamber where the temperature was maintained at 22 ± 1 °C. The fish were not fed during the test.
The test solutions were aerated during the test and the light regime was 16 h light and 8 h dark.
After 3,24,48,72 and 96 hours, mortality of the fish was recorded. Dead fish were removed each 24 hours. The fish were inspected for the following abnormalities: hyperactivity, hypoactivity, hyperventilation, uncontrolled movement, loss of equilibrium and discolouring.

Adequate sensitivity of the test-system was verified in the laboratory as follows: Once a year an acute toxiciry test with *Danio rerio* and the reference substance potassium bichromate was conducted. The most recent test was conducted in April 1992. The $EC_{50\ (96\ h)}$ found in this reference test was 142 mg/L (study number C.REF.51.006b).

During the test the pH, the dissolved oxygen concentration and the temperature were measured in all test solutions, at 0,24,48,72 and 96 hours.

Dates of experimental work: May 11 to May 15, 1992 (biological observations)
June 01 to June 03, 1992 (analytics)

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality in the control	≤ 10%	0%
Constant water quality and environmental conditions during the test	Yes	Yes
Concentration of dissolved oxygen	≥ 5.0 mg/L	8.3 – 8.7 mg/L
Concentration of test item	≥ 80%	Yes

All validity criteria for the study were met.

Analytical results:
The measured concentrations are well in agreement with the nominal ones, and the concentrations remained constant during the test. (Nominal concentration: 1200 mg/L, mean measured concentration during 96h period: 1210 mg/L). Therefore the conclusions are based on nominal values.
The pH of the test solutions ranged from 7.6 to 7.9 during the test.
The dissolved oxygen concentration was between 8.3 and 8.7 mg/L.
The temperature of the test solutions varied between 21.0 and 22.8°C

Biological results:
Mortalities: no mortalities in control or test groups

Conclusions:

The NOEC is 1200mg/L. Based on the molecular weights, a concentration of 1200 mg sodium trifluoroacetate/l corresponds to 1000 mg trifuoroacetate anion/L.

Comments by the Notifier:
The results of this study will be considered in the risk assessment. For details please refer to the respective section of the MCP document.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

Report: **KCA 8.2.2.1/02; [redacted], C. S.; [redacted], T. M.; [redacted], S.; 2013**
Title: Early life stage toxicity of flufenacet technical to the sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions
Document No.: M-464909-01-1
Guidelines: FIFRA Guideline 72-4 (1982)
OPPTS Guideline 850.1400 (1996 *draft*)
OECD Guideline 210 (1992)
GLP: Yes (certified laboratory)

Executive summary:

The objective of this study was to estimate the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) limits for Flufenacet technical.

Material and Methods:

Flufenacet Technical, purity: 98.83%, CAS No.: 142459-58-3, Batch No.: NK61CX0617.
The test temperature during the 35 days exposure ranged from 24.7 to 25.5°C. Dissolved oxygen concentrations ranged from 5.3 to 7.5 mg/L representing 70 to 99 percent saturation. The pH values ranged from 8.1 to 8.2, and the salinity was 18 to 22 ‰ (parts per thousand) throughout the test. Light intensity ranged from 736 to 805 lux (mean = 778 lux). The photoperiod was 16 hours light/ 8 hours dark (with 30 minute dawn/dusk transition period).

The Flufenacet technical exposures were conducted under flow-through conditions. Five concentrations of the test material (50, 100, 200, 400, and 800 µg a.s./L test solutions) were used for the test. 35 eggs per replicate were used at initiation, thinned to 20 alevin after hatching phase. On Day 0, impartially placed 5 eggs into each egg cup until 35 eggs were in each, egg cups were then placed in test chambers based on randomization sequence. When the hatch was completed, observations were made and the alevin were impartially thinned to 20 per replicate.
Fish were fed twice daily on weekends and two to three times daily on weekdays until approximately 24 hours prior to study termination with 24 to 48 hour old Brine shrimp nauplii (*Artemia salina*) starting on Day 5. Fish from the control and solvent control chambers were weighed and measured at test termination to determine the biomass loading factor.

Results:

Effects of Flufenacet Technical on the Sheepshead Minnow Early Life Stage

Test Substance	Flufenacet Technical			
Test Object	Sheepshead minnow (*Cyprinodon variegatus)*			
Exposure	35 Day, flow-through (ELS)			
Alevin Survival (Day 6):	NOEC	677 µg a.s./L	LOEC	> 677 µg a.s./L
Fry Survival (Day 35):	NOEC	677 µg a.s./L	LOEC	> 677 µg a.s./L
Percent Hatch:	NOEC	677 µg a.s./L	LOEC	> 677 µg a.s./L
Time to Hatch:	NOEC	677 µg a.s./L	LOEC	> 677 µg a.s./L
Growth (Length):	NOEC	49 µg a.s./L	LOEC	95 µg a.s./L
Growth (Dry Weight):	NOEC	49 µg a.s./L	LOEC	95 µg a.s./L
Morphological & Behavioral Effects:	Fish throughout all test levels, excluding the 677 µg a.s./L, appeared normal during the course of the study, with the exception of a few fish small in size being noted throughout various test levels. Fish in the 677 µg a.s./L test level were observed to be swimming at the bottom of the test vessel, except when being fed, beginning on study Day 32. All fish appeared normal in the 677 µg a.s./L test level on study Day 35.			

Observations:

Observations of fish were recorded daily throughout the study. Fish throughout all test levels, excluding the 677 µg a.s./L, appeared normal during the course of the study, with the exception of a few fish small in size being noted throughout various test levels. Fish in the 677 µg a.s./L test level were observed to be swimming at the bottom of the test vessel, except when being fed, beginning on study Day 32. All fish appeared normal in the 677 µg a.s./L test level on study Day 35. Additionally,

one fish in the 174 µg a.s./L test level was observed to have a blunt snout at termination, which appears to be incidental and not biologically significant.

Validity criteria:
Validity criteria for this study were met. The test is considered to be valid if the average hatchability in the controls is >75% and the average survival of controls is at least 80%.

Conclusions:
The 35-day exposure to Flufenacet technical resulted in a NOEC of 49 µg a.s./L and a LOEC of 95 µg a.s./L based on length and dry weight, which were the most sensitive endpoints analyzed.

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Report:	**KCA 8.2.2.1/01; [redacted], [redacted], [redacted], S., [redacted], 2013**
Title:	Comparison of developmental toxicity of seven perfluoroalkyl acids to zebrafish embryos
Source:	Environmental Toxicology And Pharmacology 36 (2013), 423-426
DOI No:	http://dx.doi.org/10.1016/j.etap.2013.05.004
Document No:	M-462660-01-1
Guidelines:	Not stated
GLP:	Not stated

EXECUTIVE SUMMARY
The toxicity of individual perfluoroalkyl acids (PFAAs) has been suggested to be determined by the carbon chain length as well as the functional group attached. In this study, seven different PFAAs including both sulfonic and carboxylic PFAAs were tested with different chain length to evaluate the developmental toxicity in zebrafish embryos. Generally, the acute toxicity of PFAAs including TFA is relatively low to zebrafish embryos. The LC_{50} values ranged from 1.5 to 2200 mg/L. A relationship between higher toxicity with longer carbon chain was observed. In addition, also a higher toxicity for sulfonic PFAAs than for carboxylic PFAAs was observed.

MATERIAL AND METHODS
Since the purpose of the literature review is to select literature relevant for the environmental risk assessment under Regulation (EC) No 1107/2009 for the metabolite trifluoroacetic acid (TFA), the study summary contains primarily the results for the compound of concern.

A. Material

1. Test material

Test item:	Perfluoroalkyl acids (PFAAs) including trifluoroacetic acid (TFA)
Active substance(s):	See above
Chemical state and description:	liquid
Source of test item:	[redacted], Germany
Batch number:	Not stated
Purity:	Not stated
Storage conditions:	Not stated
Water solubility:	Not stated

2. Test solutions

Vehicle/solvent:	Not stated
Source of vehicle/solvent:	Not stated
Concentration of vehicle/solvent:	Not stated
Method of preparation:	Not stated
Evidence of unsolved material:	Not stated

3. Test organism(s)

Species:	Zebrafish (*Danio rerio*)
Common name:	See above
Source of test species:	Not stated

4. Test conditions of test organism(s)

Culture medium:	Reconstituted water (ISO, 1996)
Temperature:	Not stated
Photoperiod:	Not stated
Light intensity:	Not stated
pH:	Not stated
Oxygen saturation:	Not stated
Food and feeding regime:	Not stated
Acclimatisation prior to testing:	Not stated
Observations during acclimatisation:	Not stated

B. Study design and methods

1. Test procedure

Test system:	Laboratory test, fish embryo acute toxicity
Test concentration(s):	TFAA: 10 - 3000 mg/L
Control(s):	Reconstituted water without test item
Number of replicates:	4 replicates with 6 embryos per replicate for each treatment group and control (= 168 embryos per PFAA)
Test conditions:	Zebrafish eggs within 15 min after collection were exposed to a series of concentrations of the test substance dissolved in reconstituted water. Fertilized eggs were randomly distributed individually into flat bottom, 48-well polystyrene plates along with 750 µL of the exposure medium. The PFAAs were tested at six consecutive concentrations differing by a factor of 3.3 based on logarithmic scale fitting. For each PFAA test four 48-well plates were used, with a total of 24 embryos per PFAA concentration as well as 24 in the water control group. The plates were covered with parafilm and the embryos were exposed to the chemical until 144 h post fertilization (hpf). Observations of mortality and sublethal endpoints (see below) were made after 24, 48, 120 and 144 hpf using a stereomicroscope according to endpoints presented in [redacted] et al. (2013). Test was done under the following environmental conditions: water

	temperature: 26± 1°C; pH: 7.2-7.6; 14 h light cycle.
Feeding:	Not stated
Medium renewal:	No renewal
Frequency of test item application:	One application
Test duration:	144 h
Endpoints:	Mortality and sublethal endpoints (presence of edema, malformations, not-hatched eggs, lack of circulation, reduced pigmentation)
Statistics:	The 50% effective concentration (EC_{50}) values with 95% confidence intervals were calculated for categorical data using probit analysis and defined as the concentration when 50% of the embryos displayed sublethal or lethal effects. The continuous data were analyzed using one-way ANOVA with two-sided Dunnett's post hoc test. LOEC and NOEC parameters were determined on the basis of Dunnett's test.

2. Measurements during the test

Water/medium parameters:	Not stated

3. Sampling

Sampling frequency:	No samples
Transport/storage of samples:	See above

4. Chemical analysis

Guideline/protocol:	No chemical analysis was done. Explanation given in the study: PFAA concentrations have been reported to be stable in similar exposure studies or considered so where actual concentrations were not measured.
Method:	See above
Pre-treatment of samples:	See above
Conduction:	See above
Reference item:	See above
Recovery:	See above
Limit of detection:	See above
Limit of quantification:	See above

RESULTS

1. Validity criteria:

An official OECD guideline for a fish embryo toxicity test (OECD 236) will be available soon. However, no information were given whether the study from [redacted] *et al.* (2013) meets the validity criteria set forth in the new guideline.

2. Analytical findings:
No chemical analysis was done. It was stated that PFAA concentrations have been reported to be stable in similar exposure studies or considered so where actual concentrations were not measured.

3. Other measurements:
Please refer to point 3 'Biological findings'. Measurement of other parameters was not reported.

4. Biological findings:
TFA and the other tested PFAAs are not highly toxic to early life stage zebrafish. Results are in agreement with those reported in the literature. Evaluation of the PFAAs in the present study followed established endpoints.

The statistical evaluations are based on the sum of total effects since the statistical power was too low for making correlations between individual endpoints and chemical concentrations. EC_{50} and NOEC/LOECS of TFAA and other PFAAs are presented in the table below.

Table 1 (taken from Uhlaq *et al.*, 2013): Chemical information and measurements of toxicity of PFAAs including TFA in zebrafish embryos

PFC	Chemical name	Formula	Cas#	Test range (mg/L)	LC_{50} 144 h (mg/L, 95% C.I.)	LC_{50} 144 h	NOEC/LOEC (mg/L)	
							Heart rate	Hatching time
TFAA	Trifluoroacetic acid	CF_3COOH	76 [illegible]	10–3000	700 (460–1000)	>3000	ne	300/1000
PFBA	Perfluorobutyric acid	C_3F_7COOH	3 [illegible] 4	10–3000	2200 (1300–2200)	>3000	ne	ne
PFOA	Perfluorooctanoic acid	$C_7F_{15}COOH$	335-67-1	3–300	430 (290–430)	430 (290–710)	ne	ne
PFNA	Perfluorononanoic acid	$C_8F_{17}COOH$	375-95-1	0.03–10	7.7 (7.7–450)	>10	ne	ne
PFDA	Perfluorodecanoic acid	$C_9F_{19}COOH$	335-76-2	0.03–10	3.0 (3.8–6[illegible])	8.4 (5.3–[illegible])	ne	ne
PFBS	Perfluorobutane sulfonic acid	$C_4F_9SO_3H$	375-73-5	10–3000	450 (350–600)	1500 (1100–1900)	300/1000	ne
PFOS	Perfluorooctane sulfonic acid	$C_8F_{17}SO_3H$	1763-23-1	0.03–10	1.5 (1.2–1.9)	>10	ne	ne

ne = no effect.

One commonly observed sublethal effect in the present study was pericardial edema, which was highly prevalent after exposure to TFAA and other PFAAs (PFBA, PFBS and PFOS). Also the heart rate was affected in case of TFAA. The order of toxicity for the PFAAs tested in the present study was calculated as: PFOS > PFDA > PFNA > PFOA > PFBS > TFAA > PFBA.

In addition, results of the study demonstrated that the length of the fluorinated carbon chain and the functional group seem to be related to the developmental toxicity of PFAAs in zebrafish embryos. Generally, PFAAs with longer carbon chain lengths had higher toxic potential than PFAAs with shorter chain length (e.g. TFAA). Further, PFAAs with a sulfonic group were more toxic than PFAAs with a carboxylic group of the same carbon chain length.

Comments by the Notifier:
The results of this study will be considered in the risk assessment. For details please refer to the respective section of the MCP document.

CA 8.2.2.2 Fish full life cycle test

Report:	**KCA 8.2.2.2/01; [redacted], A. T. & [redacted], C. V.; 2002**
Title:	Fathead minnow (*Pimephales promelas*) fish life cycle test with flufenacet (FOE 5043 technical)
Document No.:	M-082934-01-1
Guidelines:	FIFRA Guideline 72-4A
GLP:	Yes (certified laboratory)

Objective:
The purpose of this study was to conducted by Bayer Corporation's Research and Development Department to determine the toxicity of flufenacet (FOE 5043) technical to the early life stages and reproduction of the fathead minnow (*Pimephales promelas*).

Materials and Methods:
Flufenacet, Batch No. 803-1087, 95.6% a.s. purity. A fish life cycle test with fathead minnows exposed to nominal (mean measured) concentrations of control (<0.009), solvent control (<0.009), 0.087 (0.075), 0.175 (0.138), 0.33 (0.274), 0.70 (0.600) and 1.4 (1.211) mg a.s./L was conducted from June 16, 1998 to March 22, 1999.

Results:

Flufenacet Fish Life Cycle Test Endpoint	NOEC (mg a.s./L)	LOEC (mg a.s./L)	MATC (mg a.s./L)
FO Percent Hatch	1.211	>1.211	>1.211
FO Egg and Alevin Survivorship	1.211	>1.211	>1.211
FO Day 36 Survivorship	1.211	1.211	>1.211
FO Day 146 Survivorship	1.211	1.211	>1.211
FO Adult Survivorship on Day 237	1.211	1.211	>1.211
FO Adult Survivorship on Day 254	1.211	>1.211	>1.211
FO Day 36 Length	1.211	1.211	>1.211
FO Day 63 Length	0.600	1.211	0.852
FO Adult Female Length	1.211	>1.211	>1.211
FO Adult Female Weight	0.600	1.211	0.852
FO Adult Male Length	1.211	>1.211	1.211
FO Adult Male Weight	0.138	0.274	0.194
Egg Production between Days 148 and 237	1.211	1.211	>1.211
Egg Production between Days 148 and 254	0.600	1.211	0.852
Egg Viability between Days 148 and 237	0.600	1.211	0.852
Number of Eggs per Female	1.211	>1.211	>1.211
Number of Eggs per Spawn	1.211	>1.211	>1.211
Number of Spawns per Female	1.211	>1.211	>1.211
Fl Percent Hatch	0.600	1.211	0.852
Fl Egg and Alevin Survivorship	0.600	1.211	0.852
Fl Incubation Day 35 Survivorship	1.211	>1.211	>1.211
Fl Length	0.600	1.211	0.852
Fl Weight	0.600	1.211	0.852

Conclusions:

Flufenacet had minimal impact on the viability and hatching success of eggs, survivorship and reproduction (egg production, eggs per female, eggs per spawn, spawn per female) of the fathead minnow. The LOEC and NOEC for the endpoints related to these parameters equaled 1.211 and 0.600 mg a.s./L, respectively. The most sensitive endpoint in the test was growth. The lowest LOEC and NOEC obtained in the study were 0.274 and 0.138 mg a.s./L, respectively, which was for the FO adult male weight.

CA 8.2.2.3 Bioconcentration in fish

No new studies have been conducted with flufenacet since Annex I inclusion. For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda).

CA 8.2.3 Endocrine disrupting properties

Population relevant effects of Flufenacet on fish were studied in an early life-stage test (ELS) with rainbow trout and in a fish full life cycle test (FFLC) with fathead minnow (*P. promelas*). In the ELS the overall NOEC was 334 µg/L based on swim-up and dry weight. Transitional effects on length were observed at 334 µg/l (NOEC 179 µg/L), but not on other growth parameters and not later in the study.

In the FFLC after 279 days of flow-through exposure, a NOEC of 138 µg/L was obtained for effects on F0 adult male weight (but not on male length, nor on female weight or length). For all other endpoints, such as survival, reproduction and growth (other than male weight) higher NOECs of either 600 or 1211 µg/L were established. All chronic fish NOECs are more than an order of magnitude above regulatory acceptable concentrations of FFA, which are driven by algae and macrophytes.

Based on the absence of relevant effects it can be concluded that Flufenacet is not a (potential) endocrine disrupter.

No further testing is indicated to evaluate the endocrine disrupter potential of Flufenacet to fish.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Report: **KCA 8.2.4.1/03; [redacted], L. M.; [redacted], C. V.; 1998**
Title: Acute toxicity of thiadone (a metabolite of FOE 5043) to the waterflea *Daphnia magna* under static conditions
Document No.: M-093390-01-1
Guidelines: FIFRA Guideline 72-2
GLP: Yes (certified laboratory)

Objectives:
The objective of the study was to determine the acute toxicity of thiadone to the waterflea (*Daphnia magna*) during a 48-hour exposure period under static conditions.

Materials and Methods:
Thiadone (a metabolite of FOE 5043), purity: 99.4%, Reference No.: M-90-10-76. CAS number 84352-75-0.
The test temperature during the 48-hour exposure ranged from 19.9 to 20.3°C. The mean hardness and alkalinity of the test dilution water was 176 and 119 mg/L as CaCO3, respectively. The pH values ranged from 7.2 to 8.4. Dissolved oxygen concentrations ranged from 8.0 to 8.8 mg/L representing 88 to 97 % saturation respectively, at 20°C.

Less than 24-hour old *Daphnia magna* were exposed for 48 hours to concentrations (mean measured) from 8.7 to 119.7 mg a.s./L in a static test system. The nominal test concentrations were control, solvent control, 1.2, 12 and 120 mg/L. All organisms at the 120 mg/L level were dead after 24 hours. Based upon these results, the definitive test concentrations were set at 120, 60, 30, 15 and 7.5 mg/L and a solvent control and dilution water control.

Findings:
The measured thiadone concentrations were 119.7, 60.9, 30.1, 16.0 and 8.7 mg a.s./L.

Test substance	Thiadone (metabolite of FOE 5043)
Test object	*Daphnia magna*
Exposure	48 hour, Static
LC_{50} mg a.s./L	31.7 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	30.1 mg a.s./L
Highest Test Concentration Without Toxic Effect (NOEC)	16.0 mg a.s./L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	21.9 mg a.s./L

Observations:
No undissolved test substance was observed in any test chamber during the test period. The results are given on the basis of mean measured concentrations. The 48-hour EC_{50} value for *Daphnia magna* exposed to thiadone was 31.7 mg a.s./L. Sublethal effects included abnormal position at bottom of the water column and floaters.

Conclusions:
Based upon mortality and immobility during the 48-hour exposure of *Daphnia magna* to thiadone, a metabolite of FOE 5043, the EC_{50} was 31.7 mg a.s./L (95% confidence interval of 26.5 to 38.2 mg a.s./L). The no-observed-effect concentration (NOEC) was 16.0 mg a.s./L.

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Report:	**KCA 8.2.4.1/04; [redacted], A.H.C., [redacted], H.A.M., [redacted], [redacted] G. (1992)**
Title:	The Acute Toxicity of Sodium Trifluoroacetate to *Daphnia magna*
Document No:	M-247890-01-1
Guidelines:	OECD Guideline 202 (1984) EPA Guideline 72-2
GLP:	Yes (certified laboratory)

Objective:
The study was performed, to detect possible effects of TFA, trifluoroacetic acid. However, trifluoroacetic acid is strong acid (pKa=0.23), which means that the test solution must be neutralized before testing. Therefore it was decided to test the sodium salt of trifluoroacetic acid following OECD Guideline 202 (OECD 1984) according to OECD (1981) GLP-guidelines. Based on the molecular weights 1.0 g trifluoroacetic acid corresponds to 1.2 g of its sodium salt.
A limit test at 1200 mg test item / L was performed in order to demonstrate that the concentration which causes 50% immobilisation of *Daphnia magna* induced by 48 hours of exposure in a static laboratory test system (48h-EC_{50}) exceeds the limit test concentration.

The test concentration for the limit test was based on a range-finding test during which water fleas were exposed for 48 hours to various concentrations of sodium trifluoroacetate (0,10,30,100,300 and 1000 mg/L) without showing signs of immobilization at any test concentration.

Materials and methods:

Test material: TFA, Sodium trifluoroacetate analyzed purity: 99 % was tested, specified by origin batch no.: ACA9135AB.

Test organism: *Daphnia magna* (1st instars < 24 h old, 3 x 10 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0 and 1200 mg test item/L without feeding. The light regime during the study was 16h light and 8h dark. During the test the test solutions were neither aerated nor renewed. The test vessels (250 mL glasses with 200 mL test solution) were placed in a climate chamber where the temperature was maintained at 20 ± 1 °C.

The concentration of sodium trifluoroacetate remained constant during the test.

After 24 hours and 48 hours the water fleas were examined and immobility was recorded. The following abnormalities were recorded as well: slower movement, uncontrolled movement, floating on the surface, laying down on bottom of test vessel and abnormal shape. Water fleas were recorded as immobile if they did not move at all. Immobile daphnids were removed.

Adequate sensitivity of the test-system was verified in the laboratory as follows: Once a year an acute toxicity test with *Daphnia magna* and the reference substance potassium-bichromate was conducted. The most recent test was conducted in October 1991. The $EC_{50,48h}$ found in this reference test was 0.27 mg/L with a 95% confidence interval of 0.21-0.32 mg/L.

During the test the pH, the dissolved oxygen concentration and the temperature were measured at test initiation and termination in one test vessel per concentration.

Dates of experimental work: May 12 to May 14, 1992 (biological observations)
June 01 to June 03, 1992 (analytics)

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality in the control	≤ 10%	0%
Concentration of dissolved oxygen	≥ 5.0 mg/L	8.4 – 8.6 mg/L

All validity criteria for the study were met.

Analytical results:

The measured concentrations are well in agreement with the nominal ones, and the concentrations remained constant during the test. (Nominal concentration: 1200 mg/L, mean measured concentration during 48h period: 1215 mg/L). Therefore the conclusions are based on nominal values.

The pH of the test solutions ranged from 7.7 to 8.0 during the test.

The dissolved oxygen concentration was between 8.4 and 8.6 mg/L.

The temperature of the test solutions varied between 19.4 and 20.5°C

Biological results:
No immobilisation or other effects on behaviour occurred in nor the untreated control nor at the test concentration of 1200 mg test item/L within 48 hours of exposure.

Toxicity of sodium trifluoroacetate to *Daphnia magna*:

Nominal test concentration (mg/L)	No. of mobile Daphnids (0h)	No. of mobile Daphnids (24h)	No. of mobile Daphnids (48h)	Percentage (%) immobility after 48 hours
0	10	10	10	0
0	10	10	10	
0	10	10	10	
1200	10	10	10	0
1200	10	10	10	
1200	10	10	10	

Based on the results presented in the table above, it can be concluded that the EC_{50}(48h) is greater than 1200 mg/L. The NOEC is 1200 mg/L.
Based on the molecular weights, a concentration of 1200 mg sodium trifluoroacetate/L corresponds to 1000 mg trifluoroacetate anion/L.

Conclusions:
The NOEC for TFA, sodium trifluoroacetate is 1200 mg/L, the corresponding NOEC for trifluoroacetate is 1000 mg/L. The respective 48 h EC_{50} values are ≥ 1200 mg/L and ≥ 1000 mg/L respectively.

Comments by the Notifier:
The results of this study will be considered in the risk assessment. For details please refer to the respective section of the MCP document.

CA 8.2.4.2 Acute toxicity to Mysid species

Report:	**KCA 8.2.4.2/02; [redacted], S. J.; [redacted], H. O.; 1998**
Title:	Thiadone Metabolite of FOE 5043: A 96-Hour flow-through acute toxicity test with the saltwater mysid (*Mysidopsis bahia*)
Document No:	M-005110-01-1
Guidelines:	FIFRA Guideline 72-3
GLP:	Yes (certified laboratory)

Objectives:
The objective of the study was to evaluate the acute toxicity of thiadone to the saltwater mysid *(Mysidopsis bahia)* during a 96-hour exposure period under flow-through test conditions.

Materials and Methods:
Thiadone (a metabolite of FOE 5043), purity: 99.6%, Reference No.: M-90-10-76.
Adult mysids were held in water from the same source as used during the test. During the holding

period the adults showed no signs of disease or stress. During the 14-day holding period preceding the test, water temperatures ranged from 25.0 to 25.4°C. The pH of the water ranged from 7.9 to 8.0, salinity remained at 20 ‰ (parts per thousand) and dissolved oxygen ranged from 7.4 to 8.1 mg/L.
A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. Light intensity at test initiation was approximately 283 lux at the surface of the water. The target test temperature during the study was 25±1°C.

For the definitive study, saltwater mysids were exposed to a geometric series of five test concentrations, a negative (filtered saltwater) control and a solvent (0.10 mL dimethylformamide/L) control. Two replicate test chambers were maintained in each treatment and control group. One test compartment containing 10 mysids was suspended in each test chamber for a total of 20 mysids in each treatment and control group. Based upon the reported water solubility for thiadone of 56 ppm at 20°C and the maximum allowable solvent concentration of 0.1 mL/L, the highest achievable nominal test concentration was 15.0 milligrams of the active ingredient of thiadone per liter of test solution (mg a.s./L). Therefore, mysids were exposed to 0.38, 0.96, 2.40, 6.00 and 15.0 mg a.s./L in an exploratory thiadone range finding toxicity test.
Five mysids were placed in one test chamber at each test concentration. The nominal test concentrations selected for the definitive test were 1.94, 3.24, 5.40, 9.00 and 15.0 mg a.s./L. The mean measured test concentrations were determined from samples of test water collected from the treatment and control groups at the beginning and end of the test.

Findings:

Measurement of test concentrations:

The nominal concentrations selected for use in this study were 1.94, 3.24, 5.40, 9.00 and 15.0 mg a.s./L. Samples collected prior to test initiation had measured concentrations that ranged from 97 to 101% of nominal. Samples collected at 0 and 96 hours had measured concentrations that ranged from 99 to 104% of nominal. Measured concentrations of samples collected at 0 and 96 hours were averaged and the mean concentrations were 2.01, 3.36, 5.45, 9.09 and 15.1 mg a.s./L.
Observations of mortality and other clinical signs were made approximately 3.5, 24, 48, 72 and 96 hours after test initiation.
Cumulative percent mortality observed in the treatment groups was used to estimate LC_{50} values at 24, 48, 72 and 96 hours. The no-mortality concentration and no-observed-effect-concentration (NOEC) were determined by visual examination of the mortality and clinical observation data.

Observations:

Water temperatures were within the limits of the 25±1 °C range established for the test. Dissolved oxygen concentrations exceeded 80% of saturation throughout the test and pH ranged from 8.1 to 8.3. The salinity of the dilution water at test initiation and termination was 20‰.
Mysids in the negative control group and all treatment groups appeared normal and healthy throughout the test with no mortalities or clinical signs of toxicity evident. Except for one incidental mortality at 6.00 mg a.s./L at 96 hours of exposure, there were no mortalities in any of the treatment groups tested.

Conclusions:

The 96-hour LC_{50} value for saltwater mysids exposed to thiadone was greater than 15.1 mg a.s./L.
The no-mortality concentration and NOEC were 15.1 mg a.s./L.

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Report:	**KCA 8.2.4.2/03; [redacted] M. B., [redacted], K. H., [redacted], S. P., [redacted], H. O.; 2013**
Title:	Flufenacet: A 96-Hour static acute toxicity test with the saltwater mysid (*Americamysis bahia*)
Document No:	M-452205-01-1
Guidelines:	U.S. EPA OPPTS Number 850.1350
GLP:	Yes (certified laboratory)

Objective:

The objective of this study was to determine the acute effects of flufenacet on the saltwater mysid (*Americamysis bahia*) during a 96-hour exposure period under static test conditions.

Material and methods:

Test item: flufenacet technical; Batch No.: AE F133402-01-17; CAS number 142459-58-3; Purity: 97.49%.

Saltwater mysids were exposed to a geometric series of six test concentrations and a negative control (dilution water) for 96 hours under static conditions.

Nominal test concentrations selected were 0.31, 0.63, 1.3, 2.5, 5.0 and 10 mg active substance (a.s.)/L.

Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning, the approximate mid-point and the end of the test.

Observations of mortality and other signs of toxicity were made approximately 5, 24, 48, 72 and 96 hours after test initiation. Cumulative percent mortality observed in the treatment groups was used to determine LC_{50} values at 24, 48, 72 and 96 hours ± 1 hour. The no-mortality concentration and the no-observed-effect concentration (NOEC) were determined by visual interpretation of the mortality and biological observation data.

For analytical verification of the test item concentrations samples were taken at 48 and 96 hours from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The limit of quantification (LOQ) was 0.100 mg a.s./L.

Results:

<u>Analytical results</u>

Analytical verification of test solutions revealed measured concentrations of 0.29, 0.59, 1.2, 2.3, 4.7 and 9.5 mg a.s./L calculated as arithmetic mean.

<u>Validity criteria</u>

Test conditions met all validity criteria given by the guideline. There were no mortalities in the control group (≤ 10% required). The oxygen saturation in the test group was ≥ 73% at the end of the test (≥ 60% required)

Biological results:

Cumulative Mortality and Observations

Mean measured conc. (mg a.s./L)	Rep.	No. Exposed	Observation period					
			5 hours		24 hours		48 hours	
			No. Dead[1]	Obs.[2]	No. Dead[1]	Obs.[2]	No. Dead[1]	Obs.[2]
control	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
0.29	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
0.59	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
1.2	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
4.7	A	10	0	10 AN	0	10 AN	1	9 AN
	B	10	0	10 AN	0	10 AN	1	9 AN
9.5	A	10	0	10 AN	0	10 AN	3	7 AN
	B	10	0	10 AN	0	10 AN	7	2AN;1A

[1] Cumulative number of dead mysids.
[2] Observations: AN = appear normal; A = surfacing

Mean measured conc. (mg a.s./L)	Rep.	No. Exposed	Observation period				Cumulative Percent Mortality
			72 hours		96 hours		
			No. Dead[1]	Obs.[2]	No. Dead[1]	Obs.[2]	
control	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
0.29	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
0.59	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
1.2	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
4.7	A	10	1	10 AN	1	9 AN	30
	B	10	3	10 AN	1M + 4	2 AN;3 C	
9.5	A	10	6	10 AN	1M + 8	1 C	95
	B	10	8	10 AN	10	-	

1 Cumulative number of dead mysids; M = missing and assumed dead.
2 Observations: AN = appear normal; C = lethargy.

Conclusion:

Saltwater mysids (*Americamysis bahia*) were exposed for 96 hours under static conditions to six mean measured concentrations of Flufenacet ranging from 0.29 to 9.5 mg a.s./L.
The 96-hour LC_{50} value was 5.6 mg a.s./L, with a 95% confidence interval of 4.7 to 6.7 mg a.s./L. The slope of the concentration-response curve was 7.3.
The no-mortality concentration and the NOEC were both 2.3 mg a.s./L.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

No new studies have been concuted with flufenacet. For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda).

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

No new studies have been concuted with flufenacet. For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda).

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

Beside the studies provided in with the last Annex I inclusion studies on additional aquatic invertebrates were conducted. The summaries are presented below.

Report:	**KCA 8.2.5.2/01; [redacted] M. B., [redacted], K. H., [redacted], S. P., [redacted], H. O.; 2013**
Title:	Flufenacet: A flow-through life-cycle toxicity test with the saltwater mysid (*Americamysis bahia*)
Document No.:	M-452207-01-1
Guidelines:	U.S. EPA OPPTS Number 850.1350
GLP:	Yes (certified laboratory)

Objective:

The objective of this study was to evaluate the effects of flufenacet on the survival, reproduction and growth of the saltwater mysid (*Americamysis bahia*) during chronic exposure under flow-through test conditions.

Material and methods:

Test item: Flufenacet technical; Batch No.: AE F133402-01-17; CAS number 142459-58-3; Purity: 97.49%.

Saltwater mysids were exposed to a geometric series of five test concentrations, a negative control (dilution water) under flow-through conditions for 31 days.

Nominal test concentrations were 30, 60, 120, 240 and 480 µg active ingredient (a.s.)/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, approximately weekly during the test and at test termination.

Water temperatures were within the 25 ± 2°C range established for the test. Dissolved oxygen concentrations remained ≥99% of saturation (7.3 mg/L). Measurements of pH ranged from 7.9 to 7.9, and salinity ranged from 19 to 20‰ during the test. Light intensity at test initiation was 220 lux at the surface of the water of one representative test chamber.

Observations of mortality and signs of toxicity were conducted daily throughout the test. At test termination, the total body lengths and dry weights of all surviving first-generation mysids were measured.

Observations of the effects of flufenacet on survival, reproduction and growth were used to determine the no-observed-effect concentration (NOEC), the lowest-observed-effect concentration (LOEC), and the maximum acceptable toxicant concentration (MATC).

The analytical method consisted of diluting the samples in saltwater, as necessary, and analyzing by direct injection high performance liquid chromatography (HPLC) with UV detection at 220 nm. The limit of quantification (LOQ) was 10.0 mg a.s./L.

Results:

Analytical results

Analytical verification of test solutions revealed measured concentrations of 33, 68, 126, 221 and 469 µg a.s./L, representing 110, 113, 105, 92 and 98% of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.

Biological results:

Summary of survival of saltwater mysids exposed to flufenacet during a non-GLP pilot study

Nominal Concentration (µg a.s./L)	Survival of Saltwater Mysids					
	Juvenile Survival to Pairing on Day 14[1]			Adult Survival to Test Termination on Day 31[1]		
	Number Originally Exposed	Number Surviving	Percent Survival	Number Alive at Pairing[2]	Number Surviving	Percent Survival
Negative Control	30	26	86.7	17	13	76.5
20	30	29	96.7	16	15	93.8
50	30	28	93.3	25	24	96.0
500	30	28	93.3	25	23	92.0

[1]There were no statistically significant decreases in survival in comparison to the negative control using Fisher's Exact test ($p > 0.05$).

[2] The number alive at pairing may be less than the number surviving to Day 14 due to the fact that extra females that cannot be used to form pairs and any immature mysids are discarded at the time of pairing on Day 14.

Summary of reproduction of saltwater mysids exposed to flufenacet during a non-GLP pilot study

Mean Measured Concentration (µg a.i./L)	Mean Number of Young Produced Per Reproductive Day ± SD	Percent of Females Producing Young[1,2]	Average Number of Young Per Female[2]
Negative Control	0.433 ± 0.024	83.3	7.0 ± 0.354
20	0.777 ± 0.021	100	12.5 ± 0.354
50	0.478 ± 0.102	90.0	7.7 ± 1.63
500	0.234 ± 0.110*	77.5	3.8 ± 1.77*

* No statistical difference were noted between the control and treatment group, but there was an apparent decrease in reproduction and average number of young per female in comparison to the negative control.

[1] There were no statistically significant decreases in percent of females producing young in comparison to the negative control using Fisher's Exact test ($p > 0.05$).

[2] Calculated based on the total number of surviving females present at test termination. Females that died prior to test termination and the young that they produced were excluded from the calculation of the mean percent of females producing young and the mean number of young per female.

Summary of growth of saltwater mysids exposed to flufenacet during a non-GLP pilot study

Mean Measured Concentration (µg a.i./L)	Growth Parameters at Termination on Day 31[1]			
	Mean Total Length ± SD (mm)		Mean Dry Weight ± SD (mg)	
	Males	Females	Males	Females
Negative Control	8.41 ± 0.476	8.32 ± 0.278	1.18 ± 0.107	1.24 ± 0.268
20	8.15 ± 0.368	8.35 ± 0.103	0.91 ± 0.162	1.29 ± 0.093
50	8.14 ± 0.338	8.54 ± 0.024	1.07 ± 0.113	1.46 ± 0.042
500	8.38 ± 0.128	8.31 ± 0.177	1.08 ± 0.014	1.39 ± 0.127

[1] No statistically significant decreases in comparison to the negative control using Dunnett's test ($p > 0.05$).

Conclusion:

Saltwater mysids (*Americamysis bahia*) were exposed to flufenacet at mean measured concentrations of 33 to 469 µg a.i./L under flow-through conditions for 31 days, and were evaluated for survival, reproduction and growth. Reproduction, measured the mean number of young per surviving female and the mean number of young per reproductive day, was the most sensitive biological endpoint measured. While no statistical difference were noted in the reproduction endpoints, there was a decrease in reproduction in the 469 µg a.s./L treatment group, and hence a treatment-related effect could not be precluded for this level.

Consequently, the NOEC, based on reproduction, was 221 µg a.s./L.

The LOEC was 469 µg a.s./L and the MATC was 322 µg a.s./L.

CA 8.2.5.3 Development and emergence in Chironomus species

Report: **KCA 8.2.5.3/01; [redacted], E.; 2010**

Title: *Chironomus riparius* 28-day chronic toxicity test with flufenacet (tech.) in a water-sediment system using spiked water

Document No.: M-372857-01-1

Guidelines: OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (adopted 13 April 2004)

GLP: Yes (certified laboratory)

Objective: The aim of the study was to determine the influence of the test item on emergence and development of *Chironomus riparius* for 28-days in a static water-sediment-system (spiked water exposure).

Material and methods: Flufenacet (tech.), purity: 97.5 % w/w was tested, specified by batch-no.: K664078, TOX-No.: 07969-00, specification no.: 102000006978 and article-no.: 0157875.

First instar of *Chironomus riparius* larvae (4 beakers per test concentration and control with 20 animals each) were exposed for 28 days in a static test system to initial nominal concentrations in the overlying medium (spiked water application) of 1.25 – 2.50 – 5.00 – 10.0 and 20.0 mg a.s. /L of a water-sediment system.

The pH varied between 8.3 and 8.7. Dissolved oxygen concentration varied between 7.2 and 8.3 mg/L (7.2 mg O_2/L = 81% O_2-saturation) during the 28 days of the study. The water temperatures recorded were between 20.4 and 20.8°C. The mean light intensity was 826 Lux.

Findings:

Analytical findings: Chemical analysis of overlying water and pore water over time reflect expected aquatic fate data with high recoveries of 71 % to 87 % (mean 83 %) at the beginning of the exposure period in the overlying water.

Therefore, initial nominal concentrations were used for reporting and evaluation of the results. In the pore water of the sediment only low recoveries of 0.7 to 1.6 % (averages) of nominal initial test concentrations were detected.

Biological findings: Start of emergence was on day 14 for the control and test concentrations from 1.25 to 10.0 mg a.s./L. The start of emergence was reduced for four days at the highest test concentration of 20.0 mg a.s./L.
92.5 % of the inserted (n= 160) larvae maturated to adults in the controls after 28 days, fulfilling the guideline requirements.

Influence on the emergence and development after 28 days (based on nominal concentrations):

	NOEC (mg a.s./L)	LOEC (mg a.s./L)
Emergence ratio	5.0	10.0
Development rate	5.0	10.0

Conclusion: The NOEC for flufenacet in the 28 day study with *Chironomus riparius* was 5 mg/L. The LOEC was 10 mg a.s./L.

CA 8.2.5.4 Sediment dwelling organisms

No new studies have been concuted with flufenacet. For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda).

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Beside the studies provided in with the last Annex I inclusion additional studies on green algae were conducted. The summaries are presented below.

Report: **KCA 8.2.6.1/06; [redacted], A. T.; [redacted], C. V.; 1999**
Title: Toxicity of ^{14}C-Thiadone, a metabolite of FOE 5043, to the green alga *Selenastrum capricornutum*
Document No.: M-009214-01-1
Guidelines: FIFRA Guideline 123-2
GLP: Yes (certified laboratory)

Objectives:
The objective of the study was to determine the growth effects of 14C-Thiadone to the green alga *Selenastrum capricornutum* in a 96-hour exposure period under static conditions.

Materials and Methods:
^{14}C-Thiadone (a metabolite of FOE 5043), purity: 99.4%, Reference No.: M-90-10-76. CAS number 84352-75-0.
The test temperature during the 4-day exposure ranged from 23.1 to 23.8°C with a mean of 23.4°C as recorded hourly by the datalogger. The pH measurements ranged from 7.5 to 8.5 for all test levels

during the exposure period. Conductivity ranged from 75.2 to 77.6 µmhos/cm. The photoperiod was 24 hours light, and a light intensity of approximately 400 foot-candles (4.3 klux).

A preliminary test was performed at control, solvent control, 100, 10, 1.0, and 0.1 mg thiadone/L. The percent inhibition as compared to the solvent controls was 7.7% at 0.1 mg/L, 26.7% at 1.0 mg/L, and >99% at 10 and 100 mg/L.
In the definitive study each replicate was inoculated with *Selenastrum capricornutum* cells at a nominal density of 10,000 cells/ml using a standard glass pipe. Three replicate vessels were prepared for each concentration and used to determine daily cell density. All test solutions, including the controls, were prepared as uniform batches. All replicate test vessels were held under test conditions. The cell density, or standing crop, was determined daily by direct cell counts. The growth rate was analyzed by comparing the change in cell density from Day 0 to Day 4. The cumulative biomass, or area under the growth curve, was determined by plotting the daily cell density from Day 0 to Day 4. Day

Findings:
The mean measured concentrations of 14C-Thiadone were 0.06, 0.22, 0.66, 2.10 and 6.46 mg a.s./L which represents 100 to 110% of the nominal test concentrations.

Test substance	TG a.s.
Test object	*Selenastrum capricornutum*
Exposure	96 hour, Static
EC_{50} – cell desnity	6.5 mg a.s./L
EC_{50} – cumulative biomass	4.7 mg a.s./L
EC_{50} – growth rate	33.4 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	0.66 mg a.s./L
Highest Test Concentration Without Toxic Effect (NOEC)	0.22 mg a.s./L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	0.38 mg a.s./L

Statistical analysis of the 72- and 96-hour data showed that the cell density and growth rate data passed the criteria for normality and homogeneity of variance. No transformations were performed on the data prior to analysis. However, the cumulative biomass data did not pass the test for normality, and therefore nonparametric statistics were used to calculate the NOEC. No significant difference between the control and solvent control were detected at 72- or 96-hour for cell density, cumulative biomass or growth rate.

Observation:
The cells were observed each day during the cell counting procedure. No unusual observations were noted through 96 hours.

Conclusions:
Thiadone, a metabolite of FOE 5043, is moderately toxic to algae. Based on the mean measured concentration and cumulative biomass:
96-hour EC_{50} = 4.7 mg a.s./L (95% CI = 3.8 - 5.8 mg a.s./L)
96-hour NOEC = 0.22 mg a.s./L

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Report:	**KCA 8.2.6.1/07 [redacted] A.G., [redacted] J.A. 1993a**
Title:	The toxicity of sodium trifluoroacetate to the alga *Selenastrum capricornutum* at low concentrations
Document No.:	M-247818-02-1
Guidelines:	OECD Guideline 201 (1984)
GLP:	Yes (certified laboratory)

Material and methods:
Test substance: Sodium trifluoroacetate (NaTFA), purity >99%, batch number ACA9135AB.
*Pseudokirchneriella subcapitata (*formerly *Selenastrum capricornutum) were* exposed under static conditions for 72 hours to the following nominal concentrations: Control, 0.036, 0.12, 0.36 and 1.2 mg /L. Chemical analysis of the highest test concentration at day 0 and at day 3 and of the stock solution was conducted. The concentration of NaTFA remained constant during the test.
All reported toxicity values were calculated based on the nominal concentrations. Four replicates were prepared for each concentration. The pH values ranged from 7.2 (test initiation) to pH 7.2 (test termination). The mean measured air temperature was about 25°C. Initial cell density was 0.64 x 10^4 cells/mL. Each day, algal density was determined.

Findings:
The cell concentration of the control cultures increased by a factor of 200 during the test, clearly exceeding the validity criterion of the OECD guideline. For NaTFA no severe inhibition of the biomass integral or growth rate was found during the test.

Growth inhibition

Nominal concentration (mg /L)	Mean Cell density, day 0	Mean Cell density, day 3	Biomass integral, day 3	% biomass inhibition	% growth rate inhibition
Control	0.64 x 10^4	1.25 x 10^6	0.89 x 10^6	-	-
0.036	0.64 x 10^4	1.24 x 10^6	0.89 x 10^6	1	0.057
0.12	0.64 x 10^4	1.26 x 10^6	0.89 x 10^6	0.34	-0.28
0.36	0.64 x 10^4	1.11 x 10^6	0.78 x 10^6	12	2.3
1.20	0.64 x 10^4	0.901* x 10^6	0.64* x 10^6	29*	6.1*

* three replicates only

Conclusion:
The 72 hour growth rate EC_{50} value for NaTFA to *Pseudokirchneriella subcapitata* was estimated to be greater than 1.20 mg /L, the highest concentration tested. The determination of an ErC50 was not possible, because the choosen test concentrations were too low.

Comments by the Notifier:
The results of this study are in line with other results provided in this dossier and considered as supplemental information only.

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Report:	**KCA 8.2.6.1/08; [redacted], E., 2009**
Title:	*Pseudokirchneriella subcapitata* growth inhibition test with flufenacet-oxalate
Document No.:	M-358823-01-1
Guidelines:	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006)
GLP:	yes (certified laboratory)

Objective:
The aim of the study was to determine the influence of the test item on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume).

Material and methods:
Test item: Flufenacet-oxalate analysed purity: 95.3% was tested, specified by origin batch number: SES 10564-3-1, sample description: TOX08524-00 and LIMS number: 0910452
Test organism: *Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 6.25, 12.5, 25.0, 50.0 and 100 mg pure metabolite/L in comparison to the control.
The pH values ranged from 7.9 to 8.2 in the controls and the incubation temperature ranged from 21.6°C to 21.9°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7941 lux.
Quantitative amounts of flufenacet oxalate (calculated from flufenacet-oxalate hydrate) were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Findings:
Test conditions met all validity criteria, given by the mentioned guideline(s). Biomass increased in the control by more than 16-fold within the evaluation period, the mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35% and the mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35%.
The analytical findings of flufenacet-oxalate (calculated from flufenacet-oxalate hydrate) in the treatment levels found on day 0 were 104% to 107% of nominal (average 105%). On day 3 analytical findings of 102% to 117% of nominal (average 107%) were found. All results are based on nominal test concentrations of pure metabolite.

The static 72 hour algae growth inhibition test provided the following effects:

Nominal Concentration [mg p.m./L]	Cell Number after 72 h (means) per mL*	(0-72 h)-Average Specific Growth Rates [days^{-1}]	Inhibition of Average Specific Growth Rate [%]	Doubling time of algae cells [days]
Control	920000	1.507	-	0.460
6.25	994000	1.533	-1.7	0.452
12.5	962000	1.522	-1.0	0.455
25.0	983000	1.529	-1.5	0.453
50.0	1003000	1.536	-1.9	0.451
100	985000	1.530	-1.5	0.453

* test initiation with 10,000 cells/mL

-% inhibition: increase in growth relative to the control

Conclusions:

The (0-72 h)-E_rC_{50} for flufenacet-oxalate is > 100 mg p.m./L and the (0-72 h)-NOE_rC is ≥ 100 mg p.m./L.

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Report: **KCA 8.2.6.1/09; [redacted], E.; 2010**

Title: *Pseudokirchneriella subcapitata* growth inhibition test with flufenacet (tech.)

Document No.: M-363891-03-1

Guidelines: OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006)
JMAFF guideline (12 Nou(h)san No 8147 (2000))

GLP: yes (certified laboratory)

Objective:

The aim of the study was to determine the influence of the test item on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume).

Material and methods:

Flufenacet (tech.) analysed purity: 97.5% w/w was tested, specified by origin batch no.: K664078, customer order no.: TOX07969-01 and specification no.: 102000006978.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0.138, 0.416, 1.25, 3.71, 11.1, 34.4, 102, 322, 983, 3127 and 8605 µg active substance/L in comparison to controls. The pH values ranged from 7.8 to 8.5 in the controls and the incubation temperature ranged from 21.2°C to 22.5°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8313 lux.

Quantitative amounts of flufenacet were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Findings:

Test conditions met all validity criteria, given by the mentioned guideline(s).

The analytical findings of flufenacet in the treatment levels found on day 0 were 88% to 158% of nominal (average 110%). On day 3 analytical findings of 84% to 147% of nominal (average 113%) were found. Due to the analytical results, all results are based on geometric mean measured test concentrations.

The static 72 hour algae growth inhibition test provided the following effects:

Geometric mean measured concentration [µg a.s./L]	Cell Number after 72 h (means) per mL	(0-72 h)-Average Specific Growth Rates [days^{-1}]	Inhibition of Average Specific Growth Rate [%]	Doubling time of algae cells [days]
Control	801000	1.461	-	0.474
Solvent control	837000	1.475	-	0.470
Pooled controls	819000	1.468	-	0.472
0.138	791000	1.457	0.8	0.476
0.416	751000	1.440	1.9	0.481
1.25	712000	1.421	3.2	0.488
3.71	601000	1.364	7.1	0.508
11.1	117000	0.819	44.2	0.846
34.4	67000	0.632	57.0	1.100
102	65000	0.623	57.5	1.110
322	61000	0.602	59.0	1.150
983	56000	0.574	60.9	1.210
3127	41000	0.470	67.9	1.470
8605	37000	0.434	70.4	1.600

test initiation with 10,000 cells/mL

Observations:
The (0-72 h)-E_bC_{50} for flufenacet (techn.) is 6.694 µg a.s./L (95% CI: 3.342 – 13.499 µg a.s./L) and the (0-72 h)-NOE_bC is < 0.138 µg a.s./L.

Conclusions:
The (0-72 h)-E_rC_{50} for flufenacet (techn.) is 138 µg a.s./L (95% CI: 37.1 – 641 µg a.s./L) and the (0-72 h)-NOE_rC is 0.138 µg a.s./L.

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Report:	**KCA 8.2.6.1/10; [redacted], E.; 2010**
Title:	*Pseudokirchnerella subcapitata* growth inhibition test with flufenacet-methylsulfone
Document No:	M-364591-01-1
Guidelines:	OECD Guideline 201: “Freshwater Alga and Cyanobacteria, Growth Inhibition Test” (March 23, 2006)
GLP	Yes (certified laboratory)

Objective: The aim of the study was to determine the absence of influence of the test item on exponentially growing *Pseudokirchneriella subcapitata*.

Material and methods: Flufenacet-methylsulfone analysed purity: 97.6 % was tested, specified by batch number: SES 10623-5-1, TOX-no.: 08624-01 and LIMS no.: 0932397.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 10 mg pure metabolite (p.m.)/L in comparison to controls. The pH values ranged from 7.9 to 8.7 in the controls and the incubation temperature ranged from 22.0°C to 22.7°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8072 lux.
Quantitative amounts of flufenacet-methylsulfone were measured in the treatment group and in the controls on day 0 and day 3 of the exposure period.

Findings:

Effects on algal average growth rate

Test substance	Flufenacet-methylsulfone
Test object	*Pseudokirchneriella subcapitata*
Exposure	72h, static
E_rC_{50} [mg a.s./L]	> 10
LOE_rC [mg a.s./L] (Lowest tested concentration with effect)	> 10
NOE_rC [mg a.s./L] (Highest tested concentration without adverse effect)	10

Conclusions: The (0 - 72 h)-E_rC_{50} for flufenacet-methylsulfone is > 10 mg p.m./L based on nominal concentration.

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Report: **KCA 8.2.6.1/11; [redacted], E., 2013**
Title: *Pseudokirchneriella subcapitata* flow-through growth inhibition and recovery test with Flufenacet / AE F133402
Document No.: M-451057-01-1
Guidelines: None (no standardised test guideline available for this study)
GLP: Yes (certified laboratory)

Objective:

The purpose of the study was to determine the influence of variable test item concentrations on exponentially growing *Pseudokirchneriella subcapitata* under flow-through conditions.

Material and methods:

Flufenacet / AE F133402 analysed content: 97.5 % w/w was tested, specified by batch ID: NK61BX0367, sample description: TOX-09547-00 and specification no.: 102000006978.
Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed against 3 peaks of flufenacet using a flow through test system over a period of 35 days. The volume of the two chemostat reactors was 1.5 L each and the flow through was adjusted to 31 mL medium/h. The respective medium exchange corresponds to ca. 50% reactor volume per day.
The test started with a cell density of 40 000 cells/mL. After 5 days a steady state of about 400 000 cells/mL was reached. The reactors received nominal flufenacet peaks of 4.0, 23.0, and 12.0 µg/L test

item (measured 3.56; 21.8 and 6.40 µg/L) applied directly into the reactors using aqueous stock solution containing small amounts of DMF.
During the study period the cell number in the reactor outflows was determined daily. Orthophosphate und total phosphate were measured daily. The pH values measured in the sampled test medium at outflow ranged from 7.0 to 8.2 and the reactor temperature was 24°C during the entire test period.
To maintain the CO_2 level in the reactor sterile air (1 L min^{-1}) was added constantly. The reactors were illuminated with 13 LED panels placed directly at the reactor wall resulting in a light intensity of ca. 15500 lux (15.1-15.8 klux) in both reactors over the entire testing period.

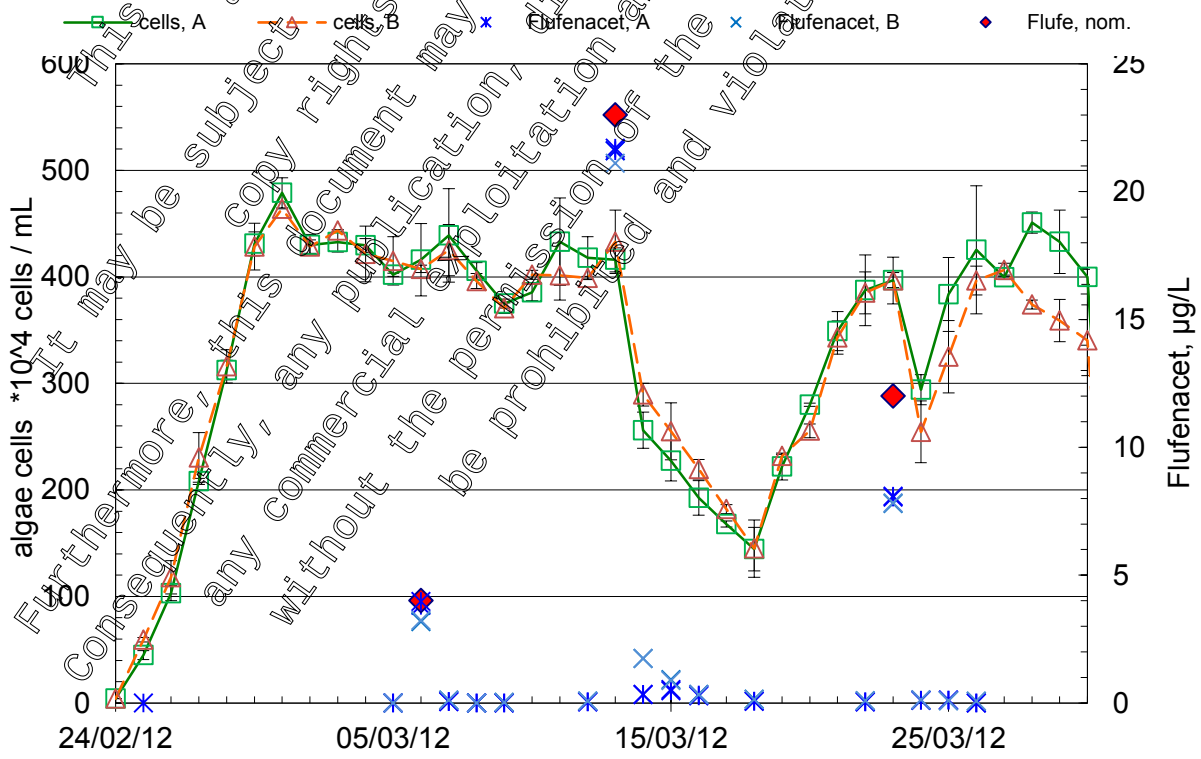
Results and discussion

The measured concentrations for the three peak exposure events ranged between 53.0 % and 94.0 % of nominal values of flufenacet.
The chemical analysis of the first peak (4.00 µg/L) resulted in a measured concentration of 3.56 µg/L for both reactors. The analysis of the second peak (nominal 23.0 µg/L) revealed a measured concentration of 21.6 µg/L on day one. On the following days decreasing concentrations of 1.03 µg/L, 0.701 µg/L and 0.0.311 µg/L were measured. The accompanying chemical analysis of the third peak (nominal 12.0 µg/L) resulted in 7.98 µg/L.
The first peak was applied after the cell density reached steady state on day 0. After exposure the cell density increased slightly on the next day, followed by a slight decline within the previously observed variability for two days (97.3 % on day 1 and 90.5 % on day two). On day 18 the second peak was applied, resulting in a decrease of cell density for five days (maximum growth inhibition 34.3 %). After five days the population recovered and reached the steady state cell density within five days. The last peak was applied on day 28, resulting in a cell density reduction of about 30 % (69.2 %) for one day, followed by a fast recovery of cell density one day later.
The observed results demonstrate the algistatic effect of flufenacet on the green algae *Pseudokirchneriella subcapitata*. A fast recovery of the algae was observed up to short term peak exposure concentrations of up to 21.6 µg/L. After three peaks of different heights recovery potential was still observed. The used peak exposure pattern was based on worst case assumption resulting from FOCUS exposure patterns.
After day 32 algal cell density in the two reactors started to differ slightly and also cell density declined therefore the experiment was ended on day 35.

Cell density

2012	Day	nominal concentration flufenacet [µg/L]	concentration flufenacet [µg/L]	mean cell density x 104 cells/ ml A + B	[%]*
06.03	0	4.00	3.56	412	100
07.03	1		0.111	432	105
08.03	2		<LoQ	401	92.8
09.03	3		<LoQ	373	86.3
10.03	4		-	394	91.2
11.03	5		-	418	101
13.03	7	23.0	21.6	425	100
14.03	8		1.83	273	64.2
15.03	9		0.701	242	56.9
16.03	10		0.311	207	48.7
17.03	11		-	175	41.2
18.03	12		<LoQ	145	34.1
19.03	13		-	227	53.4
20.03	14		-	268	63.1
21.03	15		-	346	81.4
22.03	16		-	387	93.4
23.03	17	12.0	7.98	397	100
24.03	18		0.107	275	69.2
25.03	19		0.106	355	84.2
13.03	20		<LoQ	412	104

* cell density in % compared to cell density of the related steady state



Test item analysis

Samples were analysed for the actual concentration of flufenacet present before the first peak and on the days of application as well as on the succeeding days following the three applications.

Conclusions: The results demonstrate the algistatic effect of flufenacet on the green algae *Pseudokirchneriella subcapitata*. Fast recovery of the algae was observed after short term peak exposure concentrations of up to 21.6 µg/L. After three peaks of different heights recovery potential was still observed.

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Report:	**KCA 8.2.6.1/12 [redacted], A.H.C., [redacted]de, H.A.M., [redacted], [redacted] G. (1992)**
Title:	The Toxicity of Trifluoroacetate to the Algae *Selenastrum capricornutum*
Document No.:	M-247820-01-1
Guidelines:	OECD Guideline 201 (1984)
GLP:	Yes (certified laboratory)

Objectives:

The aim of the study was to determine the influence of the test item trifluoroacetic acid on exponentially growing *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*). However, trifluoroacetic acid is strong acid (pKa=0.23), which means that the test solution must be neutralized before testing. Therefore it was decided to test the sodium salt of trifluoroacetic acid following OECD Guideline 201 (OECD 1984) according to OECD (1981) GLP-guidelines. Based on the molecular weights 1.0 g trifluoroacetic acid corresponds to 1.2 g of its sodium salt.

The results are expressed as EC_{50} values for growth rate and biomass.

Materials and Methods:

Test material: Sodium trifluoroacetate analysed purity: 99 % was tested, specified by origin batch no.: ACA9135AB.

Test organism: *Pseudokirchneriella subcapitata* were exposed for 3 days under static exposure conditions at nominal test concentrations of 1200, 360, 120, 36, 12, 3.6, 1.2, 0.36 and 0 mg per liter algal medium.

Samples of the test solutions were taken at day 0 and at day 3 and analysed by ion chromatography. The concentration of sodium trifluoroacetate remained constant during the test (97-111%). Therefore all results are based on nominal test concentrations.

Adequate sensitivity of the test-system was verified in the laboratory as follows: Once a year a toxicity test with *Pseudokirchneriella subcapitata* and the reference substance potassiumbichromate was conducted. The most recent test was conducted in January 1992. The EC_{50} (96h) based on biomass, found in this reference test was 1.0 mg/L. A ringtest between 10 laboratories revealed a mean EC_{50} of 1.1 m/L, which shows a good agreement between the results of our laboratory and the results of the ringtest.

The pH increased from 7.3 on day 0 to 9.4 on day 3 (in the control). Due to the increase in pH the experiment was not continued after 3 days. No test substance related effects on the pH were observed on day 0, but the increase in pH during the study is less at higher concentrations of sodium trifluoroacetate and is clearly related to algal growth.

The incubation temperature ranged from 24 ± 1°C to 23.6°C (calculated from temperature in shaking incubator). Over the whole period of testing at a continuous illumination of 7200 lux was maintained.

The test system consisted of four replicate vessels per test level and seven replicate vessels per control. The initial cell number was 10,000 cells/mL.

Dates of experimental work: August 10 1992 to August 19 1992

Results:
Validity of the study:

Validity Criteria:	**Obtained in this study:**
Increase of biomass:	Biomass increased in the control by more than 16-fold within the evaluation period.

In conclusion, it can be stated that the test conditions met all validity criteria given by the mentioned guideline (OECD 201, 1984).

Analytical results:
Samples of the test solutions were taken at day 0 and at day 3 and analysed by ion chromatography. The concentration of sodium trifluoroacetate remained constant during the test (97-111%). Therefore all results are based on nominal test concentrations.

Biological results:
Effects on biomass
At day 1 no inhibition above 50% is observed, even at 1200 mg/L, but the E_bC_{50} is 12 mg/L at day 2 and 4.8 mg/L at day 3. Based on the molecular weights, a concentration of 4.8 mg/L sodium trifluoroacetate corresponds to 3.6 mg/L trifluoroacetate anion (E_bC_{50} = 4.8 mg/L).
The results of the Williams test showed a statistically significant inhibition at all concentrations at day 3. Because the inhibition at 0.36 mg/L is only 11% this inhibition is considered to be of doubtful biological significance.

Effects on growth rate
The results show that the E_rC_{50} is 160 mg/L, which based on molecular weight corresponds to 130 mg/L trifluoroacetate anion.

In the following table effects on biomass and growth rate are summarized.

Effect of sodium trifluoroacetate on freshwater algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Nominal test concentration [mg p.m./L]	% biomass inhibition after a period of 72 h	% growth rate inhibition after a period of 72 h	µ growth rate
Control	0		1.72
0.36	11	2	1.69
1.2	36	9	1.57
3.6	47	13	1.50
12	59	19	1.39
36	75	30	1.20
120	87	46	0.93
360	92	59	0.71
1200	94	68	0.56

test initiation with 10,000 cells/mL

Morphology:

At day 3 a sample of the control and the highest concentration was examined by microscope. The control algae looked normal, while the algae exposed to the highest concentration (1200 mg/L) looked clearly affected.

Conclusions:

The (0 - 72h)-E_rC_{50} sodium trifluoroacetate is 160 mg p.m./L, corresponding to 130 mg/L for the trifluoroacetate anion.

Comment by the notifier:

As this is the only study with TFA resulting in a definitive EC50 (no > or < values) this endpoint will be used for risk assessment. All other endpoints will be used as supplemental information only.

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Report:	**KCA 8.2.6.1/13; [redacted] A.G., [redacted]-[redacted] W.A.J., [redacted] N.R.M. 1995a**
Title:	A comparison of the toxicity of sodium trifluoroacetate, sodium difluoroacetate, sodium monofluoroacetate and sodium fluoride to the alga *Scenedesmus supspicatus*
Document No.:	M-247825-01-1
Guidelines:	OECD Guideline 201 (1984)
GLP:	Yes (certified laboratory)

Material and methods:

Test substance: Sodium trifluoroacetate (NaTFA), purity 99%, batch number ACA9135AB.
Scenedesmus subspicatus were exposed under static conditions for 72 hours to the following nominal concentrations: Control, 0.12, 1.2, 12 and 120 mg /L. The following substances were tested in parallel: difluoroacetee acid, sodium monofluoroacetate, sodium fluoride and as reference potassium dichromate was used. No chemical analysis of the test solutions were conducted because previous algal studies with NaTFA showed a good agreement between nominal and measured concentrations.
All reported toxicity values were calculated based on the nominal concentrations. Two replicate vessels were prepared for each concentration. The pH values ranged from 7.8 (test initiation) to pH

7.2-7.4 (test termination). The incubation temperature varied between 22.5 and 24 °C over the whole period of testing. Initial cell density was 1.0 x 10^4 cells/mL. Each day, algal density was determined.

Findings:
The cell concentration of the control cultures increased by a factor of 55.6 during the test, which is in line with the OECD guideline. For NaTFA no severe inhibition of the biomass integral or growth rate was found during the test. The inhibition percentage was less than 35% at all concentrations.

Growth inhibition

Nominal concentration (mg /L)	Mean Cell density, day 0	Mean Cell density, day 3	Biomass integral, day 3
Control	1.00 x 10^4	60.3 x 10^4	51.96
0.12	1.00 x 10^4	63.0 x 10^4	46.5
1.2	1.00 x 10^4	58.3 x 10^4	42.3
12	1.00 x 10^4	53.8 x 10^4	39.3
120	1.00 x 10^4	52.9 x 10^4	38.6

Conclusion:
The 72 hour growth rate EC_{50} value for NaTFA to *Scenedesmus subspicatus* was estimated to be greater than 120 mg /L, the highest concentration tested.

Comment by the notifier:
The results of this study are in line with other data presented. Therefore the results are considered as of supplemental nature only and will hence not be considered in the risk assessment.

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Report: **KCA 8.2.6.1/14 [redacted] A.G. 1996a**
Title: The toxicity of sodium trifluoroacetate to algae Third Draft
Document No.: M-247822-01-1
Guidelines: n.a.
GLP: n.a.

This is a review of algal laboratory studies which were conducted with sodium trifluoroacetate (NaTFA), including M-247818-02-1 (C047121), M-247820-01-1 (C047124) and M-247825-01-1 (C047129). For 11 different algal species the available toxicity data are discussed.
Algal species: *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), *Chlorella vulgaris, Scenedesmus subspicatus, Chlamidomonas reinhardtii, Dunaliella tertiolecta, Euglena gracilis, Phaeodactylum tricornutum, Navicula pelliculosa, Skeletonema costatum, Anabaena flosaquae* and *Microcystis aeruginosa*.
The tests reported for *Pseudokirchneriella subcapitata,* in addition to M-247818-02-1 (C047121) and M-247820-01-1 (C047124) was only a preliminary test using 2 replicates per concentration. In another test the design was also limited to 2 replicates per concentration and in addition there was a large ratio (10) between the test concentrations. In a third test the growth rate of control algae decreased during the test due to a high initial cell density (4.9 x 10^4 cells/mL).
For the other algal species the ErC_{50} was reported to be between >112 to >2400 mg/L.

Anabaena		125h	E_rC_{50} >2400
Navicula		96h	E_rC_{50} >2400
Skeletonema costatum		96h	E_rC_{50} >2400
Chlorella vulgaris		72h	E_rC_{50} >1200
Chlamidomonas reinhardii	chronic, static	72 h	E_rC_{50} >120
Dunaliella tertiolecta		72 h	E_rC_{50} >124
Euglena gracilis		192 h	E_rC_{50} >112
Phaedactylum tricornutum		72 h	E_rC_{50} >117
Microcysstis aeruginosa		144 h	E_rC_{50} >117

Also included in this review is one semi-field study with mesocosm streams which had been conducted to study the potential effects of NaTFA on freshwater algal communities and primary productivity. Short term exposure to the highest concentration of 200 mg/L had no severe effect on the primary productivity. The long term exposure to a mean NaTFA concentration of 31-32 µg/L had no effect on the algal primary production in the mesocosm stream. Detrimental effects on the algal species composition of the stream mesocosm were not found.

<u>Comment by the notifier:</u>
As the resuts are all greater than (>) figures, these data will only be used as supplemental information on TFA.

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Report:	**KCA 8.2.6.1/16; [redacted], E.; 2011**
Title:	*Desmodesmus subspicatus* growth inhibition test with flufenacet (tech.)
Document No.:	M-415813-01-1
Guidelines:	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006)
GLP:	Yes (certified laboratory)

Objective: The aim of the study was to determine the effects of the test item on exponentially growing *Desmodesmus subspicatus*.

Material and methods: Flufenacet (tech.) analysed purity: 97.5 % was tested, specified by origin batch no.: K664078, customer order no.: TOX07969-01 and specification no.: 102000006978.
Desmodesmus subspicatus were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0.288, 0.921, 2.94, 9.40, 30.0, 96.0, 307, 980, 3130 and 10000 µg active substance (a.s.)/L in comparison to controls (3 replicates per treatment level, 6 replicates for dilution and solvent control). The pH values ranged from 7.8 to 7.9 in the controls and the incubation temperature ranged from 21.8°C to 21.9°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7258 Lux.
Quantitative amounts of flufenacet-methylsulfone were measured in the treatment group and in the controls on day 0 and day 3 of the exposure period.

Findings:

Effects on algal average growth rate

Test substance	Flufenacet tech.
Test object	*Desmodesmus subspicatus*
Exposure	72h, static
E_rC_{50} [µg a.s./L] (Confidence interval (95%))	675 (560 – 819)
LOE_rC [µg a.s./L] (Lowest tested concentration with effect)	18.7
NOE_rC [µg a.s./L] (Highest tested concentration without adverse effect)	8.4

Conclusions: The (0 - 72h)-E_rC_{50} for flufenacet (tech.) is 675 µg a.s./L.

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Report:	**KCA 8.2.6.1/15; [redacted], E.; 2012**
Title:	*Pseudokirchneriella subcapitata* growth inhibition test with BCS-CU62474 – limit test
Document No:	M-444217-01-1
Guidelines:	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006)
GLP	Yes (certified laboratory)

Objective: The objective of this 72 hour growth inhibition test was to verify the assumption that the test item will cause no adverse effects on the growth of the green algae *Pseudokirchneriella subcapitata*.

Materials and methods: BCS-CU62474 (analysed purity: 99.4 %) was tested, specified by origin batch no.: NLL 8865-4-1, customer order no.: TOX09477-00 and LIMS no.: 1140541.
Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentration of 100 mg pure metabolite (p.m.)/L in comparison to controls. The pH values ranged from 7.7 to 8.1 in the controls and the incubation temperature ranged from 21.4°C to 22.4°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7749 lux.
Quantitative amounts of BCS-CU62474 were measured in the treatment group and in the controls on day 0 and day 3 of the exposure period.

Results:
Test conditions met all validity criteria, given by the mentioned guideline(s).
The analytical finding of BCS-CU62474 in the treatment level found on day 0 was 103 % of nominal. On day 3 analytical finding of 99.4 % of nominal was found. All results are based on nominal test concentrations of the metabolite.

The static 72 hour algae growth inhibition test provided the following effects:

nominal concentration [mg p.m./L]	cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days-1]	inhibition of average specific growth rate [%]
control	807 000	1.463	--
100	913 000	1.504	-2.8

test initiation with 10,000 cells/mL
-% inhibition: increase in growth relative to the control

Conclusions: The (0 - 72h)-ErC$_{50}$ for BCS-CU62474 is > 100 mg p.m./L and the (0 - 72h) - NOErC is ≥100 mg p.m./L.

CA 8.2.6.2 Effects on growth of an additional algal species

Report:	**KCA 8.2.6.2/04; [redacted], L.; 2011**
Title:	*Synechococcus leopoliensis* growth inhibition test with flufenacet (tech.)
Document No.:	M-415814-01-1
Guidelines:	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006)
GLP:	Yes (certified laboratory)

Objective: The aim of the study was to determine the influence of the test item on exponentially growing *Synechococcus leopoliensis*.

Material and methods: Flufenacet (tech.) analysed purity: 97.5 % was tested, specified by origin batch no.: K664078, customer order no.: TOX07969-01 and specification no.: 102000006978.
Synechococcus leopoliensis were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0.0094, 0.03, 0.096, 0.307, 0.98, 3.13 and 10.0 mg active substance (a.s.)/L in comparison to controls (3 replicates per treatment group, 6 replicates per dilution and solvent control). The pH values ranged from 7.9 to 8.0 in the controls and the incubation temperature ranged from 21.4°C to 22.1°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8219 Lux.
Quantitative amounts of flufenacet were measured in the treatment group and in the controls on day 0 and day 3 of the exposure period.

Findings:

Effects on algal average growth rate

Test substance	Flufenacet tech.
Test object	*Synechococcus leopoliensis*
Exposure	72h, static
E_rC_{50} [mg a.s./L]	> 10
LOE$_r$C [mg a.s./L] (Lowest tested concentration with effect)	0.980
NOE$_r$C [mg a.s./L] (Highest tested concentration without adverse effect)	0.307

Conclusions: The (0 - 72h)-E_rC_{50} for flufenacet (tech.) is >10 mg a.s./L.

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Report:	**KCA 8.2.6.2/05; [redacted], E.; 2011**
Title:	*Chlorella vulgaris* growth inhibition test with flufenacet (tech.)
Document No.:	M-416169-01-1
Guidelines:	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006)
GLP:	Yes (certified laboratory)

Objective: The aim of the study was to determine the influence of the test item on exponentially growing *Chlorella vulgaris.*

Material and methods: Flufenacet (tech.) analysed purity: 97.5 % was tested, specified by origin batch no.: K664078, customer order no.: TOX07969-02 and specification no.: 102000006978.
Chlorella vulgaris were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0.00294, 0.0094, 0.03, 0.096, 0.307, 0.98, 3.13 and 10.0 mg active substance (a.s.)/L in comparison to controls (3 replicates per treatment and 6 replicates per dilution and solvent control). The pH values ranged from 7.8 to 7.9 in the controls and the incubation temperature ranged from 21.3°C to 22.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8141 Lux.
Quantitative amounts of flufenacet were measured in the treatment group and in the controls on day 0 and day 3 of the exposure period.

Findings:

Effects on algal average growth rate

Test substance	Flufenacet tech.
Test object	*Chlorella vulgaris*
Exposure	72h, static
E_rC_{50} [mg a.s./L] (Confidence interval (95%))	11.1 (10.3 – 12.0)
LOE_rC [mg a.s./L] (Lowest tested concentration with effect)	3.13
NOE_rC [mg a.s./L] (Highest tested concentration without adverse effect)	0.98

Conclusions: The (0 - 72h) E_rC_{50} for flufenacet (tech.) is 11.1 mg a.s./L.

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Report:	**KCA 8.2.6.2/06; [redacted], H.; 2011**
Title:	*Chlamydomonas terricola* growth inhibition test with flufenacet (tech.)
Document No.:	M-418627-01-1
Guidelines:	OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (March 23, 2006)
GLP:	Yes (certified laboratory)

Objective: The aim of the study was to determine the influence of the test item on exponentially growing *Chlamydomonas terricola.*

Material and methods: Flufenacet (tech.) analysed purity: 97.5 % was tested, specified by origin batch no.: K664078, customer order no.: TOX07969-01 and specification no.: 102000006978.
Chlamydomonas terricola were exposed in a chronic multigeneration test for 216 hours under static exposure conditions to nominal concentrations of 0.0094, 0.03, 0.096, 0.307, 0.98, 3.13 and 10.0 mg active substance (a.s.)/L in comparison to controls (3 replicates per treatment level and 6 replicates per dilution and solvent control). The pH values ranged from 5.9 to 6.6 in the controls and the incubation temperature ranged from 22.5°C to 22.8°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 5233 Lux.
Quantitative amounts of flufenacet were measured in the treatment group and in the controls on day 0 and day 4 and day 9 of the exposure period.

Findings:

Effects on algal average growth rate

Test substance	Flufenacet tech.
Test object	*Chlamydomonas terricola*
Exposure	216h, static
E_rC_{50} [mg a.s./L] (Confidence interval (95%))	0.657 (0.564 – 0.762)
LOE_rC [mg a.s./L] (Lowest tested concentration with effect)	0.307
NOE_rC [mg a.s./L] (Highest tested concentration without adverse effect)	0.096

Conclusions: The (0 - 216h)-E_rC_{50} for flufenacet (tech.) is 0.657 mg a.s./L.

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Report: **KCA 8.2.6.2/07; [redacted], M.; 1998**
Title: Toxicity of ^{14}C-FOE 5043 to the marine diatom *Skeletonema costatum*
Document No.: M-086470-01-1
Guidelines: U.S.-EPA-FIFRA § 123-2, Tier 2, Non-target Aquatic Plant Toxicity
OECD-Guideline No. 201: "Alga, Growth Inhibition Test" (June 7, 1984).
GLP: yes (certified laboratory)

Material and methods:
14C-FOE 5043; Vial C-583A; 99.4 % a.s.; Skeletonema costatum, strain SK30 was exposed under static conditions (shake cultures) for 120 h.

Findings and Observations:

Effects on algal average growth rate

Test substance	^{14}C-FOE 5043
Test object	*Skeletonema costatum*
Exposure	4 days, static
E_rC_{50} (0 - 96 h) in µg test substance/l	9.49
Lowest tested concentration with effect (LOErC, 0-96 h) in µg test substance/l	7.47
Highest tested concentration without adverse effect (NOErC, 0-96 h) in µg test substance/l	3.57
Threshold effect concentration, TEC (geometric mean LOE_rC - NOE_rC, 0-96 h) in µg test substance/l	5.16

Observations: Analytical determinations of ^{14}C-FOE 5043 revealed that all measured concentrations from day 0 and day 5 ranged 92 to 100 % of nominal. Calculations are based on mean measured values.

Comments: The cited study ran for a total exposure period of 5 days. Originally reported results (based on U.S.-specific data requirements) were: EC_{50} (5 days): 5.59 µg a.s./l and EC_{25} (5 days): 4.58µg a.s./l, NOEC (5 days): 3.57 µg a.s./l, based on cell density (standing crop). To fulfill the European reporting requirements an additional calculation of data was done based on raw data of this study.

New results are in accordance with the requirements of the Commision Directive 96/12/EC (March 8, 1996) and the OECD guideline No. 201. To fulfill reporting requirements of this OECD guideline, additional calculations for growth rate between 0 and 96 h were performed. An evaluation after 72 h is not possible, because the EC_{50}- values could not be calculated within the range of concentrations tested.

Conclusion:

The recalculated E_rC_{50} (0-96h) for flufenacet is 9.49 µg a.s./L.

CA 8.2.7 Effects on aquatic macrophytes

Report:	**KCA 8.2.7/03; [redacted], M.; 1998**
Title:	Acute toxicity of FOE 5043 (technical) to *Lemna gibba* G3
Document No.:	M-086479-01-1
Guidelines:	OECD Lemna Growth Inhibition Test (Draft of June 1998)
GLP:	Yes (certified laboratory)

Materials and methods:

FOE 5043, purity: 97.5 %, *Lemna gibba* G3, three plants, consisting of four fronds each (for a total of 12 frond) initially were counted after 7 days, to estimate the inhibition of growth rate, according to the OECD-Draft (1998). Plants were exposed for 7 days under static test conditions to nominal concentrations of 0.626, 1.25, 2.5, 5, 10 and 20 µg/L.

Findings and Observations:

Toxicity to *Lemna gibba* G3 (based on nominal concentrations)

Test substance	FOE 5043 (techn.)
Test object	*Lemna gibba* G3
Exposure	7 days, static
ErC_{50} in µg test substance/l (95 % C.I.)	31.8 (16.0-154)
ErC_{25} in µg test substance/l (95 % C.I.)	6.95 (4.22-13.0)
Lowest tested concentration with effect (LOEC) in µg test substance/l	1.25
Highest tested concentration without adverse effect (NOEC) in µg test substance/l	0.626
Threshold effect concentration, TEC (geometric mean LOEC - NOEC) in µg test substance/l	0.885

Observations: Analytical determinations of FOE 5043 revealed that all measured concentrations on day 0 ranged from 76 to 89 % of nominal (mean: 82.6 %); therefore all reported results are related to nominal concentrations.

Comments: The cited study ran for a total exposure period of 14 days from March 29, 1993 to April 12, 1993. Originally reported results were: EC_{50} (14 days): 2.43 µg a.s./l and EC_{25} (14 days): 1.01 µg a.s./l, NOEC (14 days): 0.44 µg a.s./l, based on biomass of fronds (mean measured). To fulfill the new European reporting requirements an additional calculation of data for growth rate inhibition in the first week was done based on raw data of this study. New results are in accordance with the requirements of the Commission Directive 96/12/EC (March 8, 1996) and the OECD-*Lemna*-Draft (June 1998).

Conclusion:
The recalculated E_rC_{50} for flufenacet is 31.8 µg a.s./L.

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Report:	**KCA 8.2.7/04; [redacted], D.V.; [redacted], R.S., [redacted], E. (1993)**
Title:	Sodium Trifluoroacetate: Toxicity to the duckweed (*Lemna gibba*)
Document No.:	M-247900-01-1
Guidelines:	ASTM (1991). E1415-91 Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3. American Society for Testing and Materials, Philadelphia, PA.
GLP:	Yes (certified laboratory)

Objectives:
The aim of the study was to determine the influence of the test item sodium trifluoroacetate on duck weed during a seven day exposure period. Effects on increase in frond number and increase in frond dry weight were determined. Effects on bioconcentration of the test substance in plant tissue were assessed.

Materials and Methods:
Test material: Sodium trifluoroacetate analyzed purity: 99 %. The sample of the test material was assigned the Brixham test substance number W907.
The test substance was mixed with radiolabelled trifluoro[2-^{14}C]acetic acid before use, to enable radiochemical analysis of the test solutions and Lemna tissues. The radiolabelled material was supplied by Amersham International pic, Amersham, Buckinghamshire with the reference CFQ7300

and was assigned the Brixham test substance number X188. The specific activity was 54 mCi/mmol (2.0 GBq/mmol) and the radiochemical purity was 99.6%.

Preparation of test solutions:
Stock solution: A primary stock solution was prepared containing 4.8 g of sodium trifluoroacetate and 0.0003 g of trifluoro[2-^{14}C]acetic acid in 25ml of deionised water (192,000 mg/L). The specific activity of this mixture was 1.0 Bq/µg.

Test solutions: A volume (20 mL) of the primary stock solution was sterilised and added to sterile culture medium to give a total volume of 1600 mL at a concentration of 2400 mg/L, which was the highest nominal concentration tested. The remaining test concentrations were prepared by the addition of aliquots of the nominal 2400 mg/L solution to sterile culture medium. The control consisted of culture medium only. 160 ml volumes of the appropriate test solution were dispensed to each of the triplicate test vessels and the remaining test solutions used for physical and chemical analysis.

Test organism: *Lemna gibba* (Strain G3) were grown in M-Hoagland Medium. Actively growing duck weed (3 plants with 4 fronds each per test vessel) was exposed for seven days to the following concentrations: control, 19, 38, 75, 150, 300, 600, 1200 and 2400 mg/L. The cultures (160 mL, 3 replicates per concentration) were incubated at 25 ± 1°C under continuous illumination using "warm-white" lights.
On days 2, 5 and 7 the number of plants and the number of fronds assessed for each test vessel. Any other symptoms of toxicity were recorded. At the end of the test the dry weight of the tissue was determined. The tissue was analysed for ^{14}C residues. The fresh weight/dry weight ratio of the tissue was determined.

The pH of each test solution was measured at the start of the test. The pH of 2 replicate test solutions was measured at the end of the test.
The temperature of the incubator was measured daily by thermometer and at hourly intervals using an automatic recording system. The light intensity was measured once during the study.
The concentration of the test solutions were analyzed at start and end of the test.

Dates of experimental work: May 05 1993 to May 12 1993

Results:
Validity of the study:

Validity Criteria:	Obtained in this study:
Increase in frond number in control:	Frond numbers increased in the control by more than 7-fold within the evaluation period of 7 days.

In conclusion, it can be stated that the test conditions met the validity criterion for Lemna.

Analytical results:
The measured concentrations were well in agreement with the nominal ones: The measured concentrations at the start of the test ranged from 102 to 113% of the nominal values, and the concentrations remained constant during the test. Therefore the conclusions are based on nominal values.

The pH of the test solutions ranged from 4.6 to 4.7 at the start, and from 5.0 to 5.6 at the end of the study.
The daily temperature measurements recorded, by thermometer, in the incubator ranged from 24.7 to 25.1°C. The hourly temperature measurements ranged from 25.0 to 25.8°C.
The light intensity was 9220 lux

Biological results:
Effects on frond growth
The increase in the number of fronds over the 7-day test period was calculated for each vessel. The mean is expressed as percentage inhibition, compared with the control.
The 7-day median effective concentration (EC_{50}) and its 95% confidence limits were calculated using the moving average angle method. The results, based on nominal concentrations, were as follows:

Frond increase, 7-day EC_{50} =1100 mg/L
95% confidence limits = 960 to 1200 mg/L

One-way analysis of variance, and Dunnett's procedure (P=0.05, one-sided revealed no significant decrease in frond growth compared to control at or below a nominal concentration of 300mg/L. Therefore:

Frond increase NOEC = 300 mg/L (nominal)

Although the purpose of the test was to detect inhibitory effects, the frond data were also examined using Dunnett's procedure (2-sided). At nominal concentrations of 75 and 150 mg/L, the increase in number of fronds was significantly greater (P=0.05) than in the control. This apparent stimulation should be interpreted with caution, since there was no evidence of stimulatory effects at 100 mg/L (nominal) in the preliminary range finding study.
No attempt was made to analyse the data for plant numbers, since frond number and weight increases were considered more reliable estimates of Lemna growth.

Effects on dry weight
The data for % inhibition of weight were analysed by the moving average angle method, to calculate the 7 day median effective concentration (EC_{50}) and its 95% confidence limits. The results, based on nominal concentrations, were:
Weight increase, 7-day EC_{50} = 1200 mg/L
95% confidence limits = 780 to 1900 mg/L

There was no significant decrease in dry weight at or below a nominal concentration of 300 mg/L. Therefore:
Weight increase NOEC = 300 mg/L (nominal concentration).

The weight data were also analyzed using Dunnett's procedure (2-sided). There were no significant increases (P=0.05) compared with the control.

Other symptoms of toxicity
From day 5 onwards, plants in the nominal 600, 1200 and 2400 mg/L exhibited pale, misshapen fronds with decreased root growth, compared with the control.

There were no observed symptoms at or below a nominal concentration of 300 mg/L compared with the control.

Tissue residues
Fresh/dry weight ratio: 19.0
The BCF values ranged from 1.0 to 1.6, indicating only slight bioconcentration above the ambient water concentration.

Conclusions:
The median effective concentrations (EC50s) for increase in frond number and increase in frond dry weight were as follows:

EC_{50} (frond increase) =	1100 mg/L
95% confidence limits =	960-1200 mg/L
EC_{50} (weight increase) =	1200 mg/L
95% confidence limits =	780-1900 mg/L

No significant inhibitory effects on frond or weight increase, at a nominal concentration of 300 mg/L (=NOEC).
Only slight bioconcentration of the test substance in tissues after 7 days, with bioconcentration factors ranging from 1.0 to 1.6, based on radiochemical analysis.

Comments by the Notifier:
This study does not contradict the results from existing study on effects of TFA on aquatic macrophytes. Thus, this study be used as supplemental information only and hence will not be further considered in the risk assessment.

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Report: **KCA 8.2.7/05: [redacted], E.; 2009**
Title: *Lemna gibba* G3 Growth inhibition test with flufenacet-oxalate under static conditions
Document No.: M-359515-02-1
Guidelines: OECD Guideline 221 (March 23, 2006)
GLP: Yes (certified laboratory)

Objective: The aim of the study was to determine the influence of the flufenacet-oxalate on exponentially growing *Lemna gibba* G3 expressed as NOEC, LOEC and EC_x for growth rate of the response variables, frond number and total frond area of plants.

Materials and methods:
Flufenacet-oxalate, analysed content of active substance: flufenacet-oxalate (BCS-AB16305): 95.3% w/w, specified by Batch code: BCS-AB16305-01-01, Tox No.: 08524-01.
3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for 7 days under static exposure conditions to the nominal concentrations of 1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg formulation/L in comparison to control. The pH values ranged from 7.5 to 8.7 and the incubation temperature ranged from 22.7 °C to 24.1 °C measured over the whole period of testing at a continuous illumination of 8090 lux (mean).
Quantitative amounts of flufenacet were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Results:
Test conditions met all validity criteria, given by the mentioned guideline.
The analytical findings of flufenacet-oxalate determined in all test levels on day 0 ranged between 100 and 106% (average 104%), on day 7 the analysed concentrations ranged between 103 and 132% (average 110%) of nominal concentrations.
As the toxicity has to be attributed to the tested formulation as a whole, all results submitted by this report are related to nominal test concentrations of the formulated product.

The static 7 day growth inhibition test provided the following tabulated effects:

Nominal test levels	Final frond number	Final total frond area of plants	% inhibition* of average growth rate of	
Flufenacet-oxalate [mg/L]	mean day 7	mean [mm2]	frond numbers	total frond area of plants
control	134	458	--	--
1.56	125	456	2.92	-4.94
3.13	130	462	1.15	-3.66
6.25	124	464	3.29	-3.35
12.5	131	407	0.88	-2.46
25.0	116	401	5.88	6.34
50.0	120	403	4.65	9.23
100	114	395	8.48	7.59

*negative values mean growth stimulation

Observed visual effects:

Test level (mg flufenacet-oxalate/L)	Observations
Control	no visual effects observed
0.156	no visual effects observed
3.13	no visual effects observed
6.25	no visual effects observed
12.5	no visual effects observed
25.0	no visual effects observed
50.0	some small fronds on day 7
100	some small fronds on day 7

Results are based on nominal concentrations of the flufenacet-oxalate:

End point (0-7 day)	Effect on frond no. [mg flufenacet-oxalate/L]	Effect on total frond area of plants [mg flufenacet-oxalate/L]
E_rC_{50} (CI 95%)	> 100 (n.d. – n.d.)	> 100 (n.d. – n.d.)
LOE_rC	100	> 100
NOE_rC	50.0	> 100

The LOE_rC and NOE_rC determinations are based on statistical data analysis, n.d. = not determined due to mathematical reasons

Conclusion: The most sensitive response variable was total frond number of plants resulting in (0-7-day)-E_rC_{50} of > 100 mg flufenacet-oxalate/L and a lowest (0-7-day)-NOE_rC of 50.0 mg flufenacet-oxalate/L.

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Report:	**KCA 8.2.7/06; [redacted], E.; 2010**
Title:	*Lemna gibba* G3 - Growth inhibition test with Flufenacet-methylsulfone (BCS-CO62475) under static conditions
Document No:	M-369703-01-1
Guidelines:	OECD Guideline 221 "*Lemna* sp. Growth Inhibition Test" (March 23, 2006)
GLP	Yes (certified laboratory)

Objective: The aim of this study was to demonstrate that concentrations which cause growth inhibition on exponentially growing *Lemna gibba* G3 are > 100 mg test item/L.

Materials and methods: Test item: Flufenacet-methylsulfone, analyzed content of active substance: Flufenacet-methylsulfone (BCS-CO62475): 97.67 % w/w, specified by origin batch no.: SES 10623-5-1, batch code: BCS-CO62475-01-01, Tox No.: 08624-01.
6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for 7 days under static exposure conditions to the nominal concentration of 100 mg pure metabolite in comparison to control. The pH values ranged from 7.5 to 8.9 and the incubation temperature ranged from 23°C to 26°C measured over the whole period of testing at a continuous illumination of 8260 Lux (mean).
Quantitative amounts of Flufenacet-methylsulfone were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Findings: The chemical analysis of Flufenacet-methylsulfone revealed recoveries of 102% of the nominal concentration on day 0 and 99% of the nominal concentration on day 7. As the toxicity has to be attributed to the tested pure metabolite as a whole, all results submitted by this report are related to nominal test concentrations of the formulated product.

Results are based on nominal concentrations of the test item:

Test item	Flufenacet-methylsulfone	
Test system	*Lemna gibba*	
Exposure	7 d, static	
	Effect on frond number	Effect on total frond area of plants
E_rC_{50} (dry weight, day 0-7) [mg/L]	100	> 100
(95% confidence limits)	n. d.	n.d.

n.d.: could not be determined

Observations: No visual effects were observed in control or treatment group.

Conclusion: Since the highest tested concentration of 100 mg a.s./L caused no growth inhibition effects, statistical evaluations were not applicable.
The E_rC_{50} was determined to be > 100 mg metabolite/L.

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Report:	**KCA 8.2.7/07; [redacted], E.; 2010**
Title:	*Lemna gibba* G3 - Growth inhibition test with flufenacet-methylsulfide under static conditions
Document No:	M-393709-01-1
Guidelines:	OECD Guideline 221 "*Lemna* sp. Growth Inhibition Test" (March 23, 2006)
GLP	Yes (certified laboratory)

Objective: The aim of this study was to determine the influence of the test item on exponentially growing *Lemna gibba* G3.

Materials and methods: Test item: Flufenacet-methylsulfide, analyzed content of active substance: Flufenacet-methylsulfide (BCS-CP38571): 98.0 % w/w, specified by origin batch no.: SES 11158-2-4, Tox No.: 09042-00.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for 7 days under static exposure conditions to the nominal concentrations of 8.78, 13.2, 19.8, 29.6, 44.4, 66.7 and 100 mg pure metabolite in comparison to control. The pH values ranged from 7.5 to 8.9 and the incubation temperature ranged from 23.9°C to 24.4°C measured over the whole period of testing at a continuous illumination of 8010 Lux (mean).

Quantitative amounts of Flufenacet-methylsulfide were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Findings: The analytical findings of flufenacet determined in all test levels on day 0 ranged between 101 and 103 % (average 102 %), on day 7 the analysed concentrations ranged between 102 and 109 % (average 105 %) of nominal concentrations.

As the toxicity has to be attributed to the test item as a whole and due to the high observed recoveries, all results submitted by this report are related to nominal test concentrations of the formulated product.

Results are based on nominal concentrations of the test item:

Test item	Flufenacet-methylsulfide	
Test system	*Lemna gibba*	
Exposure	7 d, static	
	Effect on frond number	Effect on total frond area of plants
E_rC_{50} (dry weight, day 0-7) [mg/L] (95% confidence limits)	125 (104 – 171)	106 (95.1 – 122)
LOE_rC [mg/L]	44.4	19.8
NOE_rC [mg/L]	29.6	13.2

Observations: No visual effects were observed in control or any treatment group.

Conclusion: The most sensitive response variable was total frond area of plants resulting in (0-7-day)-E_rC_{50} of 106 mg a.s./L and a lowest (0-7-day)-NOE_rC of 13.2 mg a.s./L.

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Report:	**KCA 8.2.7/08; [redacted], E.; 2010**
Title:	*Lemna gibba* G3 - Growth inhibition test with Flufenacet-thiadone under static conditions
Document No:	M-393718-01-3
Guidelines:	OECD Guideline 221 "*Lemna* sp. Growth Inhibition Test" (March 23, 2006)
GLP	Yes (certified laboratory)

Objective: The aim of this study was to determine the influence of the test item on exponentially growing *Lemna gibba* G3.

Materials and methods: Test item: Flufenacet-thiadone, analyzed content of active substance: Flufenacet-thiadone (AE 1258593): 98.6 % w/w specified by origin batch no.: SES 10558-3-5, Tox No.: 09021-00.
3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for 7 days under static exposure conditions to the nominal concentrations of 1.25, 2.50, 5.0, 9.6, 10.0, 20.0, 40.0 and 80 mg test item in comparison to control. The pH values ranged from 7.4 to 8.8 and the incubation temperature ranged from 23.7°C to 26.0°C measured over the whole period of testing at a continuous illumination of 8390 Lux (mean).
Quantitative amounts of Flufenacet-thiadone were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Findings: The analytical findings of flufenacet-thiadone determined in all test levels on day 0 ranged between 100 and 104 % (average 102 %), on day 7 the analysed concentrations ranged between 104 and 107 % (average 105 %) of nominal concentrations.
As the toxicity has to be attributed to the test item as a whole and due to the high observed recoveries, all results submitted by this report are related to nominal test concentrations of the formulated product.

Results are based on nominal concentrations of the test item:

Test item	Flufenacet-thiadone	
Test system	*Lemna gibba*	
Exposure	7 d, static	
	Effect on frond number	Effect on total frond area of plants
E_rC_{50} (dry weight, day 0-7) [mg/L] (95% confidence limits)	20.8 (15.9 – 27.3)	18.3 (14.9 – 22.7)
LOE_rC	< 1.25	2.50
NOE_rC	1.25	1.25

Observations: No visual effects were observed in control or any treatment group.

Conclusion: The most sensitive response variable was total frond area of plants resulting in (0-7-day)-E_rC_{50} of 18.3 mg a.s./L and a lowest (0-7-day)-NOE_rC of 1.25 mg a.s./L.

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Report:	**KCA 8.2.7/09; [redacted], C.S., [redacted], T.M., [redacted], C.V.; 2011**
Title:	Toxicity of Flufenacet (FOE 5043) to the aquatic macrophyte, *Myriophyllum spicatum*
Document No.:	M-408819-01-1
Guidelines:	OECD Guideline 221
GLP:	Yes (certified laboratory)

Material and methods: Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed for 14 days under static conditions. Nominal (mean measured) concentrations were Control (<LOQ), Solvent Control (<LOQ), 2.4 (1.8), 7.8 (5.7), 25 (18.8) and 80 (59.6) µg a.s./L. Mean measured recoveries are based on day 0, day 7 and day 14 sampling events and were within the range of 73 to 76% of the nominal concentrations. The toxicity values were calculated based on the mean measured concentrations.

Findings:

Toxicity to *Myriophyllum spicatum*

Test Substance	Flufenacet technical		
Test Object	*Myriophyllum spicatum*		
Exposure	14 Day - Static Exposure		
Endpoint Unit	(µg a.s./L)		
Endpoint results	Day 14 Shoot Length Yield	Day 14 Wet Weight Yield	Day 14 Dry Weight Yield
Highest Concentration Without an Effect (NOEC)	18.8	18.8	18.8
Lowest Concentration With an Effect (LOEC)	59.6	59.6	59.6
E_yC_{50} (95% C.I.)	26.2 (15.7 to 43.7)	18.8 to 59.6 (not applicable)	18.8 to 59.6 (not applicable)

Observations: Plants in the control vessels and all treatment groups appeared normal throughout the study. At study termination roots and shoots appeared normal in controls and all treatment groups with one exception. In the control group, five plants were observed to have underdeveloped roots and eight plants were observed to have shoots with light red colored tips. However, growth data for all plants was included in the data analysis.
Due to the nature of the wet weight and dry weight yield data, the EC_{50}, NOEC and LOEC could not be calculated for these endpoints. However, it was empirically determined that an adverse effect on plant growth occurred at the highest test concentration. Thus the EC_{50}, NOEC and LOEC estimates in the table above where empirically determined based on this observation.

Conclusion: The most sensitive endpoint in the 14 day exposure of Flufenacet technical to the rooted aquatic macrophyte *Myriophyllum spicatum* was Shoot Length Yield. The statistical NOEC, LOEC and E_yC_{50} for this endpoint was 18.8, 59.6 and 26.2 µg a.s./L, respectively.

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Report:	**KCA 8.2.7/10; [redacted] A.; 2013**
Title:	*Lemna gibba* G3 – growth inhibition test with BCS-CU62474 (potassium salt of trifluoroethanesulfonic acid, metabolite of flufenacet) under static conditions
Document No.:	M-445884-01-1
Guidelines:	OECD 221 (2006)
GLP:	Yes (certified laboratory)

Objective:

The objective of this growth inhibition test was, to verify the assumption that the test item will cause no adverse effects on the growth of *Lemna gibba G3* up to a test item concentration of 10 mg pure metabolite / L.

Materials and Methods:

Test item: BCS-CU62474 (metabolite of flufenacet); Batch No.: BCS-CU62474-01-01; Origin Batch No.: NLL 8865-4-1; Customer Order No.: TOX 09477-02; Analyzed purity: 94.7 % a.i.

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a water control. The pH values ranged from 8.0 to 9.0 in the control and the incubation temperature ranged from 24.6°C to 24.8°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7948 lux. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. Quantitative amounts of BCS-CU62474 were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Factor of increase of the frond number of the control	≥ 7	19.2
Doubling time in days	≤ 2.5	1.6

All validity criteria for the study were met.

Analytical results:

Analytical findings on Day 0 and Day 7

Nominal concentration in mg p.m./L	actual concentration (mg BCS-CU62474/L)			
Day 0				
	1. determination	2. determination	average	%
control	<0.101	<0.101	<0.101	--
10.0	8.78	8.85	8.81	88.1
Day 7				
	1. determination	2. determination	average	%
control	<0.101	<0.101	<0.101	--
10.0	10.3	10.3	10.4	104

Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

Biological results:

Neither frond numbers nor frond area were significantly affected of the exposure to BCS-CU62474. Furthermore, there were no visual signs of toxicity caused by the test item.

Frond counts, doubling time, percent inhibition of average growth rate, and visual effects during the exposure of *Lemna gibba* G3 to BCS-CU62474

Nominal concentration [mg p.m./L]	Replicate	Frond counts and visual effects				Growth rate µ [1/d] (0→7 d)	Doubling time [d]	Inhibition of µ [%]
		Day 0	Day 2	Day 5	Day 7			
Control	A	12	25	93	214	0.412	1.7	
	B	12	31	99	255	0.437	1.6	
	C	12	28	92	217	0.414	1.7	
	D	12	30	92	232	0.423	1.6	
	E	12	28	101	231	0.423	1.6	
	F	12	29	96	230	0.422	1.6	
	Mean	12	28.5	95.5	229.8	0.422	1.6	--
	%CV		7.3	4.0	6.3	2.1	2.1	
10.0	A	12	34	109	271	0.445	1.6	
	B	12	25	109	247	0.432	1.6	
	C	12	31	108	210	0.409	1.7	
	D	12	32	124	311	0.465	1.5	
	E	12	31	109	262	0.440	1.6	
	F	12	32	113	290	0.455	1.5	
	Mean	12	30.8	112	265.2	0.441	1.6	- 4.6
	%CV		9.9	5.5	13.2	4.4	4.5	

Total frond area and percent inhibition of their average growth rate effects during the exposure of *Lemna gibba* G3 to BCS-CU62474

Nominal concentration [mg p.m/L]	Replicate	Total frond area [mm²]				Growth rate μ (0→7) [1/d]	Inhibition of μ [%]
		Day 0	Day 2	Day 5	Day 7		
Control	A	81	163	625	1447	0.412	
	B	93	193	762	1807	0.424	
	C	81	172	620	1455	0.413	
	D	86	178	665	1520	0.410	
	E	87	181	661	1586	0.415	
	F	93	189	675	1613	0.408	
	Mean	86.8	179.3	668.0	1571.3	0.413	---
	%CV	6.2	6.1	7.7	8.5	1.4	
10.0	A	106	243	794	1784	0.405	
	B	89	205	723	1663	0.418	
	C	97	209	661	1589	0.380	
	D	118	257	915	2259	0.422	
	E	109	242	826	1780	0.399	
	F	109	240	840	2157	0.426	
	Mean	105	232	793	1838	0.408	1.3
	%CV	9.8	9.2	11.3	17.5	4.2	

Conclusions:
BCS-CU62474 caused no adverse effects on the growth of *Lemna gibba G3* up to a test item concentration of 10 mg pure metabolite/L. The EC_{50} was determined to be >10.0 mg p.m./L, the LOEC > 10.0 mg p.m./L and the NOEC ≥ 10.0 mg p.m./L (based on mean growth rates as well was yield).

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Report: **KCA 8.2.7/11, [redacted], E., 2013**
Title: *Lemna gibba* G3 - Growth inhibition test with flufenacet (technical substance) under static conditions
Document No.: M-451198-01-1
Guidelines: OECD Guideline 221 "Lemna sp. growth inhibition test" (2006)
GLP: yes (certified laboratory)

Objective:
The aim of the study was to determine the influence of the test item on exponentially growing *Lemna gibba G3* expressed as NOEC, LOEC and ECx for growth rate of both response variables, frond number and total frond area of plants.

Material and Methods:
Flufenacet (tech.) analysed purity: 97.49 % w/w was tested, specified by origin batch no: NK61BX0367, certificate no.: MZ 00466, customer order no.: TOX 09547-00 and specification no.: 102000006978.
6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to nominal concentrations of 0.658, 1.50, 3.40, 7.73, 17.6 and 39.9 µg a.s./L in comparison to a water control. The pH values ranged from 7.5 to 8.0 in the

control and the incubation temperature ranged from 24.6°C to 25.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 9031 lux.
Quantitative amounts of flufenacet were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Findings and observations:
The study met all validity criteria, requested by the mentioned guideline. The analytical determination of flufenacet revealed mean recoveries of 99% of nominal on day 0 and 94 % of nominal on day 7. The analytical findings confirm the nominal concentrations. Therefore the results of this study are given based on nominal concentrations of the test substance.

The static 7 day growth inhibition test provided the following tabulated effects:

Nominal test levels	Final frond number	Final total frond area of plants	% inhibition* of average growth rate of	
formulation [µg/L]	mean day 7	mean [mm^2]	frond numbers	total frond area of plants
control	212.3	1726.0	--	--
0.658	220.7	1711.3	-1.4	-1.9
1.50	161.0	1284.7	9.8	6.6
3.40	172.7	1376.0	7.2	9.8
7.73	135.7	1037.0	15.7	20.7
17.6	36.0	280.0	62.3	67.7
39.9	23.7	198.0	76.3	80.2

Observed visual effect:
No morphological change in *Lemna gibba* was observed at any test concentration.

Results are based on nominal concentrations of the test item:

Endpoint (0-7 day)	Effect on frond no. [µg formulation/L]	Effect on total frond area of plant [µg formulation/L]
E_rC_{50} (Cl 95%)	16.1 (10.4 – 25.8)	13.9 (9.71 – 20.0)
LOE_rC	1.50	1.50
NOE_rC	0.658	0.658

The LOE_rC determination is based on statistical data analysis

Conclusion:
The most sensitive response variable in this study was total frond area of plants resulting in a (0-7 day) E_rC_{50} of 13.9 µg a.s./L.
The NOE_rC was 0.658 µg a.s./L and was based on statistical data analysis of the total frond area of plants and frond numbers.

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Report:	**KCA 8.2.7/13; [redacted] .P, 2014**
Title:	Flufenacet: rationale for the replacement of the old 14-day Lemna growth inhibition study ([redacted] & [redacted] 1993; M-002418-02-1) with the new 7-day Lemna study ([redacted] 2013; M-451198-01-1)
Document No.:	M-478762-01-1
Guidelines:	-
GLP:	no

Introduction

Two static *Lemna*-studies have been conducted with flufenacet a.s (see table 1). The first one is a 14-day study conducted in 1993 by [redacted] & [redacted] according to FIFRA Guideline 123-2 (tier 2 non-target aquatic plant toxicity). In this study only frond number was determined on days 2, 4, 7, 9, 11 and 14. A second endpoint like frond dry weight or frond area, which is mandatory according to OECD 221 (2006), has not been determined. Moreover, inhibition percentages were calculated by using the absolute frond counts in the treatments compared to the control, while nowadays a 7-day ErC50 based on growth rate inhibition is used for risk assessments.

For this study the 7-day growth rates were calculated from the respective frond counts by [redacted] (1998; M-086479-01-1). Nevertheless, the 14-day EC_{50} of 2.43µg/L based on frond counts was still used as an EU-agreed endpoint.

The second study ([redacted] 2013) was performed according to the currently valid guideline OECD 221 (2006) measuring two endpoints, frond number and frond area. This study can be considered as fully valid study without restrictions. The determined NOEC was in the same range as was observed in the old study performed by [redacted] & [redacted] (1993). However the endpoint relevant for risk assessment – the 7-day E_rC_{50} – was by more than a factor of 2 lower in the new study than the one recalculated by [redacted] (1998) out of the 14-day study.

The endpoints from both studies are listed in the table below.

Table 1: survey of results obtained from static *Lemna*-growth inhibition tests conducted with flufenacet a.s.

Test species	Test system	Duration of exposure	Results (µg a.s./L)	Reference
Lemna gibba	chronic, static	14 d	14d-NOEC: 0.44 µg/L (frond counts) 14d-EC_{50}: **2.43 µg/L (frond counts) [EU agreed endpoint]** recalculated as 7d-E_rC_{50}: 31.8 µg/L	[redacted] & [redacted], 1993; M-002418-02-1 recalculated: [redacted], 1998; M-086479-01-1
Lemna gibba	chronic, static	7 d	NOEC: 0.658 µg/L E_rC_{50} (frond number): 16.1 µg/L **E_rC_{50} (frond area): 13.9 µg/L**	[redacted] (2013); M-451198-01-1

Conclusion

The new *Lemna* study ([redacted] 2013; M-451198-01-1) shall replace the old study mentioned above for the following reasons:

1. In the new study two endpoints, frond number and frond area, where measured.
2. The new study has been conducted on the currently valid guideline OECD 221 (2006).

3. The growth rate related endpoints have been used already in the past but a lot of regulators were using the biomass related values because they are lower. Nevertheless the scientific community in Europe was already convinced since a long time that the focus should be on the growth rate related endpoints. This is as well reflected in the current versions of the OECD guidelines for algae and *Lemna*. In these guidelines it is stated that the growth rate related endpoints are preferred. Within a risk assessment sensitivities of different plant species are compared. As their growth, the test durations and the test designs are different a comparison of sensitivities only makes sense when growth rate related endpoints are used.
4. The no observed effect concentrations (NOECs) from both studies reveal that the test organisms were of equal sensitivity (0.44 and 0.658 µg/L from the old and new study, respectively). The comparison of NOECs from different endpoints (frond counts vs. growth rate) is justified, because a NOEC is based on the comparison of variations and overlap of individual figures between the control and treatment levels.

In addition, it should be emphasized that the E_rC_{50} of 13.9 µg/L is lower than the re-calculated E_rC_{50} (=31.8 µg/L) from the old study (re-calculation by [redacted] 1998).

Overall, it can be concluded that the new fully valid and according to current state of the science performed 7-day *Lemna*-study supersedes the old 14-day Lemna study, based on biomass solely. Consequently the EU-agreed endpoint of 2.43 µg/L, based on frond counts shall be replaced by the new 7-day E_rC_{50} of 13.9 µg a.s./L based on growth rate. This new E_rC_{50} is even by a factor of 2 lower than the E_rC_{50} re-calculated by [redacted] (1993) based on the old 14-day study.
The OECD 221 test guideline states that even though the results based on yield or frond counts are often lower than the endpoints based on the average specific growth rate "this should not be interpreted as a difference in sensitivity between the two response variables" but is "due to the mathematical basis of the respective approaches".

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Report:	**KCA 8.2.7/12: [redacted], E., 2013**
Title:	*Lemna gibba* G3 - Growth inhibition test with flufenacet (technical substance) under static conditions
Document No.:	M-452567-01-1
Guidelines:	OECD Guideline 221 "Lemna sp. growth inhibition test" (2006)
GLP:	yes (certified laboratory)

Objective

The aim of the study was to determine the influence of the test item after short term exposure (peak exposure) on exponentially growing *Lemna gibba G3* expressed as NOEC, LOEC and ECx for growth rate of the response variables, frond number and total frond area of plants.

Material and Methods

Test item: flufenacet tech. (AE F133402), analyzed content of active substance: Flufenacet tech. (AE F133402): 97.5 % w/w, specified by origin batch no: NK61BX0367, specification number 102000006978, Tox No.: 09547-00.
Exponentially growing cultures of *Lemna gibba* were investigated under defined conditions for 2 x 7 days. The plants were exposed in week one for 1 day (approx. 24 h) followed by a 6 day period

without exposure in which the plants were growing in untreated growth media. The second week started again with 1 day of exposure (approx. 24 h) after which the plants were transferred again into untreated media in which they were lasting for another 6 day period. Following peak concentrations were tested 12, 21.6, 39.0, 70.0 and 126 µg/L. The controls were treated in the same way as the test concentrations.
In addition to the 2 peak design the same concentrations were used for a single peak design. In this case the plants were exposed for the initial 24 hours followed by a 13 day growth period in untreated growth medium

Findings

Test conditions met all validity criteria, given by the mentioned guideline.
The analytical findings of flufenacet detected in all freshly prepared test levels on day 0 ranged between 102 and 105 % of nominal. In aged test solutions on day one analytical results ranged between 100 and 105 % of nominal. For the second peak on day seven the analytical findings ranged between 99.0 and 105 % of nominal peak concentrations. In the aged media on day 8 the chemical analysis revealed recoveries between 96.0 and 108 %. Therefore the study results are presented based on nominal peak concentrations. As the initial measurements demonstrated the correct dosing of the test item all reported results are based on nominal peak concentrations.
No effects on the growth form of *Lemna gibba* were observed.

The evaluation of the observed growth data for *Lemna gibba* resulted in the following values:

nominal test levels flufenacet tech. [µg /L]	week 1 one peak	week 2 two peaks rep A-C	week 2 one peak rep D-F
control	--	--	--
Solvent control	--	--	--
12.0	--	--	--
21.6	--	--	--
39.0	--	--	--
70.0	--	--	--
126	--	--	--

- - No effects

Results are based on nominal concentrations of the test item.

Single peak [24 h] (0-7 day)	effect on mean growth rate of frond no. [µg a.s./L]	effect on mean growth rate of total frond area of plants [µg a.s./L]
E_rC_{10} (CI 95%)	> 126 (87.5 - 397239.6)	100.9 (64.6-278.7)
LOE_rC	39.0	21.6
NOE_rC	21.6	12.0

two peaks [each 24 h] (7-14 day)	effect on mean growth rate of frond no. [µg a.s./L]	effect on mean growth rate of total frond area of plants [µg a.s./L]
E_rC_{10} (CI 95%)	106.1 (8.21 - 155.7)	70.3 (12.2 – 170518.5)
LOE_rC	70.0	21.6
NOE_rC	39.0	12.0

Single peak [24 h] (7-14 day)	effect on mean growth rate of frond no. [µg a.s./L]	effect on mean growth rate of total frond area of plants [µg a.s./L]
E_rC_{10} (CI 95%)	n.d.	n.d.
LOE_rC	> 70.0	> 70.0
NOE_rC	> 70.0	> 70.0

n.d. = not determined due to mathematical reasons or inappropriate data
The LOE_rC determination is based on statistical data analysis.

Conclusion

Two short term peaks of up to 12 µg flufenacet a.s./L, each lasting 24 h, with a seven-day interval did not result in significant effects on the growth of *Lemna gibba*. A single one-day peak of up to 70 µg flufenacet a.s./L did not result in adverse effects on the growth of *Lemna gibba* within the 13-day period following the peak exposure.

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Report: **KCA 8.2.7/14; [redacted], [redacted] L.; [redacted], [redacted] R., 2004**
Title: Haloacetic acids in the aquatic environment - Part I: macrophyte toxicity
Source: Environmental Pollution 130(3), 371-383
Document No.: M-455787-01-1 (doi:10.1016/j.envpol.2003.12.016)
Guidelines: *Lemna*: Greenberg et al. (1992), ASTM (2000); *Myriophyllum* spp.: ASTM (1999)
GLP: No (not stated)

EXECUTIVE SUMMARY

Laboratory tests were conducted with 3 macrophytes (*Lemna gibba*, *Myriophyllum sibiricum*, and *Myriophyllum spicatum*) to assess the toxicity of 5 HAAs. The HAAs in the present experiments were monochloroacetic acid (MCA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), trifluoroacetic acid (TFA), and chlorodifluoroacetic acid (CDFA). MCA was the most toxic to *Myriophyllum* spp. with EC_{50} values ranging from 8 to 12.4 mg/L depending on the endpoint, followed by DCA (EC_{50} range 62-722.5 mg/L), TCA (EC_{50} range 49.5-1702.6 mg/L), CDFA (EC_{50} range 105.3 to greater than 10,000 mg/L), and with TFA (EC_{50} range 222.1 to 10,000 mg/L) the least toxic. Generally, *L. gibba* was less sensitive to HAA toxicity than *Myriophyllum* spp., with the difference in toxicity between them approximately 3-fold. The range of toxicity within *Myriophyllum* spp. was normally less than 2-

fold. Statistically, plant length and node no. were the most sensitive endpoints as they had the lowest observed coefficients of variation, but they were not the most sensitive to HAA toxicity. Toxicological sensitivity of endpoints varied depending on the measure of effect chosen and the HAA, with morphological endpoints usually an order of magnitude more sensitive than pigments for all plant species. Overall, mass and root measures tended to be the most sensitive indicators of HAA toxicity.

MATERIAL AND METHODS

Since the purpose of the literature review is to select literature relevant for the environmental risk assessment under Regulation (EC) No 1107/2009 for the metabolite trifluoroacetic acid (TFA), the study summary contains only the results for the compound of concern.

A. Material

1. Test material

Test item:	Haloacetic acids (HAAs) including TFA, tested as neutralized sodium salts
Active substance(s):	See above
Chemical state and description:	Not stated
Source of test item:	[redacted], USA)
Batch number:	Not stated
Purity:	99 + % (spectrophotometric grade)
Storage conditions:	Not stated
Water solubility:	Not stated

2. Test solutions

Vehicle/solvent:	Not stated
Source of vehicle/solvent:	Not stated
Concentration of vehicle/solvent:	Not stated
Method of preparation:	Not stated
Evidence of undissolved material:	Not stated

3. Test organism(s)

Species:	*Myriophyllum spicatum* L., *M. sibiricum*, *Lemna gibba*
Common name:	Not stated
Source of test species:	Not stated

4. Culture conditions of test organism(s)

Culture medium:	*Myriophyllum* spp. cultured according to standard methods (ASTM, 1999); *L. gibba* cultured axenically according to Greenberg et al. (1992) with Hunter's media containing 10 g/l sucrose.
Temperature:	25:20°C during light and dark phases
Photoperiod:	16 h light:8 h

Light intensity:	Not stated
pH:	pH 5.8
Oxygen saturation:	Not stated
Acclimatisation prior to testing:	The test conditions appear to be similar to the culture conditions, thus acclimatization was not necessary. However, approximately 10 days prior to a *L. gibba* toxicity test, plants were transferred from growth media containing sucrose to media without sucrose. This was done so that the plants would switch from heterotrophic to autotrophic energy production.
Observations during acclimatisation:	Not stated

B. Study design and methods

1. Test procedure

Test system:	
Test concentration(s):	*Myriophyllum* spp.: 10, 30, 100, 300, 1000, 3000, 10,000 mg/L. *Lemna gibba*: 10, 30, 100, 300, 1000, 3000 mg/L
Control(s):	Yes: Test media without test item
Number of replicates:	*Myriphyllum* spp.: Controls: n = 10; exposed plants: n = 5 per treatment. L*emna gibba*: Controls: n = 5; treated plants: n = 3
Test conditions:	*Myriophyllum* spp.: Conducted axenically in the environmental growth chamber for 14 days and under the environmental conditions described above. All plants were trimmed to a 3 cm apical length so that all plants would have the same initial status, with no roots or side shoots evident. Range-finding studies were conducted and used to determine the final range of concentrations chosen for the definitive tests (see above). At the end of the 14-day test period, plants were evaluated for several parameters (see below). *Lemna*: Each test solution (see above) was transferred to a 10-ml plastic Petri dish and two plants, each with four fronds, for a total of eight fronds, were introduced and monitored. Tests were conducted in the growth chamber for 7 days and under environmental conditions described above.
Medium renewal:	*Myriophyllum* spp: No renewal reported *Lemna gibba*: Test solutions were changed on day 3 and 5 to maintain consistent levels of the compound under study.
Frequency of test item application:	See above
Test duration:	*Myriophyllum* spp.: 14 days *Lemna gibba*: 7 days
Endpoints:	*Myriophyllum* spp.: Plant length, node number, root number, total root length, longest root length, wet mass, drymass, and chlorophyll a, chlorophyll b, and carotenoid concentrations *Lemna gibba*: frond number, colony number, wet mass, frond mass, frond growth rate and chlorophyll a, chlorophyll

b, and total chlorophyll concentrations.

Statistics: Regression analysis: Data evaluated from toxicity testing with all three plant species were evaluated using non-linear regression techniques described in Stephenson et al. (2000). Only new growth (e.g., shoot length, wet/dry mass, nodes) was used in the models so that a more sensitive and conservative estimate of toxicity was obtained.

NOEC / LOEC calculations: NOEC and LOEC were calculated with a one-way ANOVA in a completely randomized design in SAS Version 8.2 (SAS Institute, Cary NC, USA) using General Linear Models with no adjustments for new growth as was done for the nonlinear regression analysis.

2. Measurements during the test

Water/medium parameters: Not stated

3. Sampling

Sampling frequency: *Myriophyllum* spp.: Endpoints were evaluated at the end of the test (after 14 days).

Lemna gibba: Not stated / most probably endpoints were only evaluated at the end of the test (after 7 days)

Transport/storage of samples: Not stated

4. Chemical analysis

Guideline/protocol: Concentrations of HAAs could not be verified analytically due to interference by the growth media with the ion chromatographic methods used to quantify the HAAs in other studies

Method: See above

Pre-treatment of samples: See above

Conduction: See above

Reference item: See above

Recovery: See above

Limit of detection: See above

Limit of quantification: See above

RESULTS

1. Validity criteria:

Not stated

2. Analytical findings:

Concentrations of HAAs could not be verified analytically due to interference by the growth media with the ion chromatographic methods used to quantify the HAAs in other studies.

3. Other measurements:

Please refer to point 3 ‘Biological findings’. Measurement of other parameters was not reported.

4. Biological findings:

TFA was the least toxic compound to *Myriophyllum* spp. with EC_{50} values ranging from 222.1 to > 10000 mg/L depending on the endpoint. *L. gibba* was less sensitive to TFA toxicity than *Myriophyllum* spp., with EC_{50} values ranging from 618.3 to > 3000 mg/L. Overall, mass and root measures tended to be the most sensitive indicators of HAA toxicity.

Table 1 (taken from [redacted] & [redacted], 2004): Laboratory-derived EC_x values with 95 % confidence intervals for 14 day *Myriophyllum sibiricum* toxicity tests with TFA

Endpoint	EC_{10}	EC_{25}	EC_{50}	Model	Variables	r^2
Plant length	31.8 (0, 64.1)	155.9 (53.0, 258.7)	765.0 (444.7, 1085.3)	Logistic	$t=4.943$ $x=765.001$ $b=0.691$	0.88
Node number	97.1 (0, 203.2)	392.2 (121.1, 633.3)	1583.6 (897.5, 2269.7)	Logistic	$t=17.876$ $x=1583.253$ $b=0.787$	0.83
Root number	90.5 (24.0, 157.0)	251.7 (130.5, 372.9)	700.0 (477.9, 922.1)	Logistic	$t=8.446$ $x=700.020$ $b=1.074$	0.91
Root length	81.7 (18.7, 144.7)	166.9 (83.6, 250.1)	340.7 (224.4, 456.9)	Logistic	$t=34.163$ $x=340.657$ $b=1.539$	0.88
Longest root length	91.0 (26.2, 155.9)	237.2 (126.1, 348.3)	618.1 (425.6, 810.7)	Logistic	$t=6.806$ $x=618.135$ $b=1.147$	0.91
Wet mass	36.3 (3.5, 69.1)	113.8 (45.8, 181.8)	357.0 (216.3, 497.6)	Logistic	$t=436.080$ $x=356.991$ $b=0.961$	0.88
Dry mass	21.9 (0, 52.7)	134.1 (12.5, 255.6)	822.6 (354.0, 1291.2)	Logistic	$t=73.885$ $x=822.621$ $b=0.606$	0.80
Chlorophyll *a*	4460.3 (1849.8, 7070.7)	7890.4 (6082.0, 9698.8)	13,958.4 (9702.7, 18214.2)	Logistic	$t=0.749$ $x=13958.416$ $b=1.926$	0.66
Chlorophyll *b*	> 10,000	> 10,000	> 10,000	nc[a]	nc	nc
Carotenoids	> 10,000	> 10,000	> 10,000	nc	nc	nc

[a] The effect measure could not be calculated for these endpoints.

Table 2 (taken from [redacted] & [redacted], 2004): Laboratory-derived EC_x values with 95 % confidence intervals for 14 day *Myriophyllum spicatum* toxicity tests with TFA

Endpoint	EC_{10}	EC_{25}	EC_{50}	Model	Variables	r^2
Plant length	43.4 (15.7, 71.1)	196.2 (115.3, 227.1)	886.6 (654.9, 1118.3)	Logistic	$t=6.698$ $x=886.599$ $b=0.728$	0.95
Node number	53.8 (1.6, 106.0)	225.8 (84.1, 367.6)	947.9 (570.5, 1325.3)	Logistic	$t=18.201$ $x=947.871$ $b=0.766$	0.87
Root number	88.5 (7.9, 169.1)	243.2 (97.9, 388.4)	668.0 (404.5, 931.6)	Logistic	$t=7.142$ $x=668.032$ $b=1.087$	0.87
Root length	37.9 (15.8, 59.9)	91.7 (56.8, 126.7)	222.1 (166.1, 278.1)	Logistic	$t=31.467$ $x=222.137$ $b=1.242$	0.95
Longest root length	52.4 (23.8, 81.0)	129.3 (83.0, 175.5)	318.8 (242.4, 395.1)	Logistic	$t=7.731$ $x=318.790$ $b=1.217$	0.95
Wet mass	41.8 (8.8, 74.8)	114.4 (55.0, 173.8)	312.9 (205.0, 420.8)	Logistic	$t=377.373$ $x=312.908$ $b=1.092$	0.90
Dry mass	46.3 (0, 95.4)	144.5 (51.6, 237.3)	450.3 (265.1, 635.5)	Logistic	$t=72.078$ $x=450.311$ $b=0.966$	0.77
Chlorophyll *a*	672.4 (0, 1478.7)	5052.5 (2343.9, 7761.2)	37965.4 (2877.0, 73053.7)	Logistic	$t=0.0963$ $x=37965.380$ $b=0.545$	0.68
Chlorophyll *b*	>10,000	>10,000	>10,000	nc[a]	nc	nc
Carotenoids	>10,000	>10,000	>10,000	nc	nc	nc

[a] The effect measure could not be calculated for these endpoints.

Table 3 (taken from [redacted] & [redacted], 2004): Laboratory-derived EC_x values with 95 % confidence intervals for 7 day *Lemna gibba* toxicity tests with TFA

Endpoint	EC_{10}	EC_{25}	EC_{50}	Model	Variables	r^2
Frond number	388.8 (306.9, 470.8)	512.3 (407.9, 616.8)	884.0 (654.3, 1113.6)	Hormetic	$t=59.415$ $h=0.011$ $x=883.961$ $b=0.829$	0.94
Colony number	541.1 (407.2, 675.0)	693.2 (516.3, 870.1)	1140.4 (757.5, 1524.3)	Hormetic	$t=17,876$ $h=0.009$ $x=1140.410$ $b=0.897$	0.87
Wet mass	192.8 (104.1, 281.5)	298.5 (191.0, 406.0)	618.3 (421.1, 815.5)	Hormetic	$t=265.412$ $h=0.009$ $x=618.269$ $b=0.662$	0.91
Frond mass	11.2 (0, 44.2)	596.6 (0, 1189.8)	22965.3 (0, 70230.3)	Logistic	$t=3.940$ $x=22965.257$ $b=0.288$	0.71
Growth rate	445.2 (342.8, 547.6)	790.4 (638.5, 942.3)	2505.2 (1761.1, 3249.3)	Hormetic	$t=0.445$ $h=0.017$ $x=2505.208$ $b=0.361$	0.95
Chlorophyll *a*	>3000	>3000	>3000	nc [a]	nc	
Chlorophyll *b*	>3000	>3000	>3000	nc	nc	
Total chlorophyll	>3000	>3000	>3000	nc	nc	

[a] The effect measure could not be calculated for these endpoints.

Table 4 (taken from [redacted] & [redacted], 2004): NOEC for *Myriophyllum sibiricum* exposed to HAAs including TFA. Values in brackets are the percent change from control as either stimulation (+) or inhibition (-) for untransformed data.

Endpoint	MCA	DCA	TCA	TFA	CDFA
Plant length	10 (−44)[a]	10 (−7)[b]	10 (+1)	100 (−6)	30 (−7)
Node number	10 (−38)[a]	10 (−2)	10 (−4)	100 (+1)	30 (−5)
Root mumber	5 (−22)[a]	100 (−41)[a]	100 (−51)[a]	100 (−7)	300 (−58)[a]
Root length	5 (−32)[a]	100 (−51)[a]	100 (−57)[a]	100 (−12)[a]	300 (−76)[a]
Longest root length	5 (−14)[a]	100 (−34)[c]	30 (−19)	100 (−3)	300 (−45)[a]
Wet mass	2.5 (−4)	10 (−9)	3 (+7)	100 (−10)[b]	10 (+4)
Dry mass	5 (−17)	10 (−11)	10 (−9)	100 (−7)	10 (+2)
Chlorophyll *a*	10 (−54)[a]	100 (+4)	1000 (−49)[a]	3000 (−5)	1000 (0)
Chlorophyll *b*	10 (−58)[a]	100 (+6)	1000 (−34)[a]	3000 (+113)	3000 (−5)
Carotenoids	10 (−53)[a]	100 (+4)	1000 (−31)[a]	3000 (0)	3000 (−1)

Values in parentheses are the percentage change from control as either stimulation (+) or inhibition (−) for untransformed data.
[a] This analysis was run as a non-parametric Kruskal–Wallis on Ranks.
[b] The data were ln transformed.
[c] The data were square transformed.

Table 5 (taken from [redacted] & [redacted], 2004): NOEC for *Myriophyllum spicatum* exposed to HAAs including TFA. Values in brackets are the percent change from control as either stimulation (+) or inhibition (-) for untransformed data.

Endpoint	MCA	DCA	TCA	TFA	CDFA
Plant length	5 (−6)	10 (−4)	30 (−20)[a]	30 (−5)	10 (+1)
Node number	5 (−6)	10 (−7)	3 (0)	100 (−2)	30 (−5)
Root number	2.5 (−12)	10 (−23)[a]	10 (−4)	100 (−18)	300 (−63)[a]
Root length	5 (−33)[a]	3 (−3)	10 (−17)[a]	30 (−7)[a]	30 (−24)[b]
Longest root length	10 (−49)[a]	10 (−13)	300 (−43)	30 (−1)	300 (−49)[a]
Wet mass	5 (−17)[b]	3 (−1)	10 (−12)	30 (−3)	10 (0)
Dry mass	10 (−45)[a]	3 (+4)	10 (−4)	100 (−16)	30 (−5)
Chlorophyll *a*	10 (−31)[a]	300 (−18)	30 (−15)	300 (−56)	1000 (−13)
Chlorophyll *b*	10 (−30)[a]	300 (−4)	300 (−20)	>10000 (0)	3000 (−10)
Carotenoids	10 (−32)[a]	300 (−8)	300 (−20)	>10000 (+2)	3000 (−5)

[a] This analysis was run as a non-parametric Kruskal–Wallis on Ranks.
[b] The data were square root transformed.

Table 6 (taken from [redacted] & [redacted], 2004): NOEC for *Lemna gibba* exposed to HAAs including TFA. Values in brackets are the percent change from control as either stimulation (+) or inhibition (-) for untransformed data.

Endpoint	MCA	DCA	TCA	TFA	CDFA
Frond number	10 (−6)	50 (+9)	30 (+8)	300 (+5)	30 (+1)
Colony number	10 (−3)	100 (−21)	100 (−19)	<1000	100 (+2)[e]
Wet mass	3 (−9)	50 (−16)	100 (−17)	100 (+6)	30 (0)[c]
Frond mass	3 (−12)	25 (0)	>800 (+19)[b]	30 (−11)	100 (−11)
Growth rate	10 (−3)	50 (+5)	30 (+9)[c]	300 (+3)	30 (0)
Chlorophyll *a*	20 (−16)[a]	400 (0)[a]	nc[d]	3000 (+9)[b]	1000 (+5)
Chlorophyll *b*	20 (−7)[a]	400 (0)[a]	nc[d]	3000 (+7)	1000 (+4)
Total chlorophyll	20 (−14)[a]	400 (0)[a]	nc[d]	3000 (+9)[b]	1000 (+5)

[a] This analysis was run as a non-parametric Kruskal–Wallis on Ranks.
[b] The data were reciprocal transformed.
[c] The data were square transformed.
[d] Only the 100 mg/l TCA showed a significant difference from control, with concentrations on both sides not being significantly different from controls.
[e] The data were ln transformed.

RESULTS SUMMARY

Under the conditions of this study, the overall lowest 14 day EC_{50} of *Myriophyylum* spp. was 222.1 mg TFA/L (based on root length) and the NOEC was established at 30 mg TFA/L. For *Lemna gibba*, the overall lowest 7 day EC_{50} was 618.3 mg TFA/L (based on wet mass) and the NOEC was established at 30 mg TFA/L (based on front mass). In conclusion, tested HAAs including TFA do not exhibit a high

degree of toxicity to *Myriophyllum* spp. or *L. gibba* under laboratory conditions. In general, *L. gibba* was less sensitive to TFA toxicity than *Myriophyllum* species.

Comments by the Notifier:
The results of this study will be considered in the risk assessment. For details please refer to the respective section of the MCP document.

CA 8.2.8 Further testing on aquatic organisms

Report:	**KCA 8.2.8/01; [redacted], J., [redacted], J., 1993**
Title:	Acute effects of FOE 5043 (technical) on new shell growth of the eastern oyster (*Crassostrea virginica*)
Document No.:	M-002427-01-1
Guidelines:	FIFRA Guideline 72-3 Oyster Shell Growth Toxicity Test
GLP:	Yes (certified laboratory)

Objectives:
The objective of this study was to evaluate the acute toxicity of FOE 5043 on shell deposition of the eastern oyster (*Crassostrea virginica*) during a 96-hour exposure period under flow-through test conditions.

Materials and Methods:
BAY FOE 5043, purity: 98.2%, batch #: 2030019, sec.Reference No.: 89811/3006.
Water temperatures were within the limits of the 20.3±0.4 °C range established for the test. Dissolved oxygen concentrations exceeded 26% of saturation throughout the test, and pH ranged from 7.5 to 8.0. The salinity of the dilution water ranged from 29 to 320‰.

Eastern oysters were exposed to a series of six test concentrations, a negative (unfiltered saltwater) control and a solvent (0.50 mL dimethylformamide/L) control. A 96-hour flow-through screening test was conducted at a nominal BAY FOE 5043 concentration of 20 mg/L. Eighty-eight percent reduction in new shell growth occurred at this concentration. Based upon results of this screening test, nominal BAY FOE 5043 Technical concentrations selected for definitive testing were 1.2, 1.9, 3.2, 5.4, 9.0 and 15 mg a.s./L.

Findings:
Measurement of Test Concentrations:
The nominal concentrations selected for use in this study were 1.2, 1.9, 3.2, 5.4, 9.0 and 15 mg a.s./L. The samples collected prior to test initiation had measured concentrations that ranged from 86 to 103% of nominal. The measured concentrations of the samples collected at 0 and 96 hours were averaged and the mean concentrations were 1.2, 1.7, 3.0, 4.9, 8.4 and 13.9 mg a.s./L. The mean measured concentrations were used in the determination of EC_{50} values.

Observations:
After 96 hours mean new shell growth was 2.15 mm and 1.93 mm for the control and solvent control, respectively. These two means were not significantly different therefore they were pooled. The mean new shell growth for the pooled controls was 2.04 mm. Mean new shell growth of oysters exposed to BAY FOE 5043 ranged from 0.76 mm at 13.9 mg/L to 2.17 mm at 1.2 and 3.0 mg a.s./L.

Survival of oysters was 100 percent in the control and all test concentrations except 13.9 mg/L which had a survival of 95 percent. Mean new shell growth was statistically reduced from that measured for the pooled control oysters at a concentration of 13.9 mg a.s./L. The 96-hour EC50 was calculated by binomial probability based on mean measured BAY FOE 5043 concentrations.
The EC_{50} was 12.6 mg/L with 95 percent confidence limits of 8.37 and 13.9 mg/L. The no-observed-effect concentration was 8.4 mg a.s./L based upon the lack of statistically significant reduction in new shell growth at this concentration.

Conclusions:
Shell growth was statistically reduced from that of the pooled controls at a concentration of 13.9 mg/L. The EC_{50} was 12.6 mg/L. The NOEC was 8.4 mg/L.

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Report:	**KCA 8.2.8/02; [redacted], S. J.; [redacted], H. O.; 1998**
Title:	Thiadone Metabolite of FOE 5043: A 96-Hour shell deposition test with the Eastern Oyster (*Crassostrea virginica*)
Document No.:	M-005108-01-1
Guidelines:	FIFRA Guideline 72-3
GLP:	Yes (certified laboratory)

Objectives:
The objective of this study was to evaluate the acute toxicity of thiadone on shell deposition of the eastern oyster (*Crassostrea virginica*) during a 96-hour exposure period under flow-through test conditions.

Materials and Methods:
Thiadone (a metabolite of FOE 5043), purity: 99.6%, Reference No.: M-90-10-76.
Water temperatures were within the limits of the 22±1 °C range established for the test. Dissolved oxygen concentrations exceeded 83% of saturation throughout the test, and pH ranged from 7.7 to 8.1. The salinity of the dilution water measured at test initiation and termination was 20‰.

Eastern oysters were exposed to a geometric series of five test concentrations, a negative (unfiltered saltwater) control and a solvent (0.50 mL dimethylformamide/L) control. One test chamber was maintained for each treatment and control group, with 20 oysters in each test chamber. Based upon the reported water solubility for thiadone of 56 ppm at 20 °C and the maximum allowable solvent concentration of 0.5 mL/L, the highest achievable nominal test concentration was 55.0 milligrams of the active ingredient of thiadone per liter of test solution (mg a.s./L). Therefore, oysters were exposed to 0.45, 1.49, 5.00, 16.5 and 55.0 mg a.s./L in an exploratory thiadone range finding toxicity test. After 96 hours of exposure, shell growth inhibition in comparison to the solvent control group was 3.2, 24.7, 25.0, 43.6 and 95.0% for the 0.45, 1.49, 5.00, 16.5 and 55.0 mg a.s./L treatment groups. The nominal concentrations selected for the definitive test were 2.50, 5.00, 10.0, 20.0 and 40.0 mg a.s./L. The mean measured test concentrations were determined from samples of test water collected from the treatment and control groups at the beginning and end of the test.

Findings:

Measurement of Test Concentrations:

The nominal concentrations selected for use in this study were 2.50, 5.00, 10.0, 20.0 and 40.0 mg a.s./L. The samples collected prior to test initiation had measured concentrations that ranged from 104 to 112% of nominal. Samples collected at 0 and 96 hours had measured concentrations that ranged from 106 to 122% of nominal. The measured concentrations of the samples collected at 0 and 96 hours were averaged and the mean concentrations were 2.71, 5.51, 10.7, 22.1 and 47.0 mg a.s./L. The mean measured concentrations were used in the determination of EC_{50} values.

Observations:

Oysters in both control groups and all of the treatment groups appeared normal and healthy throughout the test. There were no mortalities or sublethal effects observed at any concentration tested. When the shell deposition data for the negative (dilution water) control was compared with that in the solvent control, no statistically significant differences were found at the 95% level of confidence.

Therefore, the control groups were pooled and percent inhibition was calculated relative to the pooled control data. Inhibition for the 2.71, 5.51, 10.7, 22.1 and 47.0 mg a.s./L treatment groups was 11.7, 15.8, 23.4, 50.2 and 77.4%, respectively. When compared to the pooled control group, the inhibition of shell growth in the 5.51, 10.7, 22.1 and 47.0 mg a.s./L treatment groups were statistically significant ($p < 0.05$).

Oysters were visually observed at approximately 1.75, 24, 48, 72 and 96 hours after test initiation for mortality and clinical signs of toxicity. At the end of the test, the longest finger of new shell growth was measured to the nearest 0.05 mm using calipers.

Conclusions:

The 96-hour EC_{50} value for eastern oysters exposed to thiadone was 22.0 mg a.s./L with 95% confidence limits of 17.8 and 29.9 mg a.s./L. Based upon statistical analysis of the dose response data, and an evaluation of the dose-response pattern, the 96-hour no-observed-effect-concentration was 2.71 mg a.s./L.

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Report:	**KCA 8.2.8/04; [redacted], C. S.; [redacted], T. M.; [redacted], S., 2013**
Title:	Acute toxicity of flufenacet technical to the African clawed frog (*Xenopus laevis*) under static conditions
Document No:	M-471899-01-1
Guidelines:	No formal English guideline exists for this test protocol. Methodologies from USEPA, OPPTS Guideline 850.1075, USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1, and OECD Guideline 203 were considered in the development of this protocol. Scientific discretion was implemented where guideline parameters do not fully converge.
GLP:	Yes (certified laboratory)

Objective:

The aim of the study was to determine the acute toxicity of the test item to African clawed frog (*Xenopus laevis*), expressed as 48 h-LC_{50} for mortality.

Material and methods:

Test item: flufenacet (tech.), analyzed content of active substance: 97.49% w/w, specified by Batch code: AE F133402-01-17, Origin batch number: NK61BX0367, tox no.: 09547-00.

Xenopus laevis tadpoles were exposed under static conditions to determine the 48-hour LC_{50}, up to the functional limit of solubility. The following nominal (mean measured) concentrations were included in the study: Control (>0.05), Solvent Control (>0.05), 0.63 (0.68), 1.25 (1.19), 2.5 (2.4), 5.0 (4.5), and 10 (8.7) mg a.s./L. There were three replicates of 10 tadpoles each in the controls and toxicant levels. The mean measured recovery of solutions analysed on day 0 and day 2 ranged from 87 to 107% of the nominal concentrations. Since the concentration of the test solution was stable and within 20% of the nominal concentrations, the results of the study are based on the nominal test concentration.

Findings:

Nominal Concentration (mg a.s./L)	Hour 6		24 Hour		48 Hour	
	Dead	Obs	Dead	Obs	Dead	Obs
Control	0	30 N	1	29 N	1	29 N
Solvent Control	0	30 N	1	29 N	1	29 N
0.63	0	30 N	1	29 N	1	29 N
1.25	0	30 N	0	30 N	1	29 N
2.5	0	30 N	0	30 N	0	30 N
5.0	0	30 N	0	30 N	1	29 N
10	1	29 N	1	29 N	1	29 N

Obs = Observations (number of individuals observed plus observation)
Dead = Cumulative number of dead
N = Normal

Note: There were 30 organisms present in each test concentration at the start of the test. Mortality remained below 4% for any given test concentration and does not follow a dose response trend. There were no sublethal effects noted during the test, therefore, these deaths are considered incidental and not indicative of a toxic response.

Test Substance	flufenacet technical
Test Object	*Xenopus laevis*
Exposure	48-Hour, Static
LC_{50} 48 hours	> 10 mg a.s./L
Lowest Concentration With an Effect (LOEC)	> 10 mg a.s./L
Highest Concentration Without Toxic Effect (NOEC)	10 mg a.s./L
Highest Concentration Causing No Mortality (NOLEC)	10 mg a.s./L
LC_{50} = concentration estimated to be lethal to 50 percent of the test population; NOEC = No Observed Effect Concentration; NOLEC = No Observed Lethal Effect Concentration; LOEC = Lowest Observed Effect Concentration	

Validity criteria for this study were met: mortality rate during domestication period did not exceed 5%; mortality rate of the blank reference group did not exceed 10%; dissolved oxygen content in the test solution was not less than 5.8 mg/L during the test; the test solution maintained a constant pH value during the test.

The analytical determination of flufenacet revealed mean recoveries of 87 to 107%. The analytical measurements revealed maximum concentrations of flufenacet in the test media of 9.4 mg/L. Prior to the definitive study; multiple trials were performed to determine the solubility of flufenacet technical

in dilution water. An initial trial was conducted at concentrations of 25 and 50 mg/L using overnight mixing with an electric mixer. Analytical samples were collected and the results showed little to no recoveries, indicating that the test material was not properly mixing into solution at these concentrations. A second trial was performed at four concentrations: 1.0, 2.5, 5.0, and 10 mg/L. These solutions were mixed for approximately 18 hours, and no precipitates were observed; with the exception of white foamy bubbles at the surface of the 10 mg/L solution. Analytical verification of the samples was performed to determine if the samples were above the limit of solubility, and it was found that the recoveries were 90%, 87%, 79% and 75% of nominal, respectively. Since the percent recoveries were increasing at the lower concentrations, and only 75% at the 10 mg/L concentration, it was determined that this was a good approximation of the functional limit of solubility in dilution water, and was used as the high test concentration for the study.

Conclusions:

The final results for the test are based on the nominal concentrations of flufenacet technical in the test system.

Based on mortalities and sublethal effects:

48 Hour NOEC	10 mg a.s./L
48 Hour NOLEC	10 mg a.s./L
48 Hour LOEC	> 10 mg a.s./L
48 Hour LC_{50}	> 10 mg a.s./L (functional limit of solubility)

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Report: **KCA 8.2.8/03, [redacted], E., 2009**

Title: Statement on the suitability of the microcosm study "The fate and biological effects of Flufenacet WG 60 in aquatic indoor microcosms" for the use in higher tier risk assessments with special focus on algal species and aquatic macrophytes

Document No: M-329959-01-1

Guidelines: OECD Guidance Document "Freshwater Lentic Field Tests", July 1996 (Draft) Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991);none

GLP: no

The relevance of the results of the microcosm study, [redacted] & [redacted] (1999, M-023412-01) is supported by an expert statement.

A NOEAEC was not reported, as up to the highest concentration tested no significant effects but some trends only have been observed. If the study results are translated into the actually used effect class system by Theo Brock et al., than all observed parameters would be described by the effect classes 1 and 2. No adverse long term effect on the investigated biocoenosis was observed and could be expected in the environment based on the outcome of this microcosm study. Due to the fact that several phytoplanktonic algae species, periphyton and three aquatic macrophytes have been investigated, the study was suitable to investigate potential direct adverse effects on aquatic plants. The testing of a biocoenosis enables the use of this study as well for the determination of indirect effects on zooplankton and/or the macrofauna.

The highest test concentration of 24 µg/L showed only minor, non significant, differences compared to the control and can be seen as EAC.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda). These studies are listed in grey in the table below.

In addition to these already available acute laboratory studies with technical flufenacet, a further laboratory study on acute oral and contact toxicity to honey bees has been performed with technical flufenacet according to current guidelines and requirements (KCA 8.3.1.1.1/03). Moreover, an acute contact toxicity study in bumble bees has been conducted (KCA 8.3.1.1.2/01) in order to benchmark potential sensitivity differences to honey bees.

In addition, a chronic 10 day adult feeding limit test was conducted with technical flufenacet (KCA 8.3.1.2/01) as well as bee brood feeding test in order to investigate potential side effects of flufenacet on immature honey bee life stages (KCA 8.3.1.3/01). The respective study summaries are presented below

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity (laboratory) in honey bees			
Flufenacet, tech.	LD_{50}-contact 24 h	> 25 µg a.s./bee	[redacted] (1994) M-004922-01-1
Flufenacet, tech.	LD_{50}-oral 48 h LD_{50}-contact 48 h	> 140.4 µg a.s./bee > 400 µg a.s./bee	[redacted] (1995) M-004920-01-1
Flufenacet, tech.	LD_{50}-oral 48 h LD_{50}-contact 48 h	> 170.36 µg a.s./bee > 200 µg a.s./bee	[redacted] (1995) M-004919-01-1
Flufenacet, tech.	LD_{50}-contact 48 h	> 25 µg a.s./bee	[redacted] (1996) M- 004918-01-1
Flufenacet, tech.	LD_{50}-oral, 48 h LD_{50}-contact, 48 h	> 109.2 µg a.s./bee > 100 µg a.s./bee	[redacted] (2011) M-421687-01-1 KCA 8.3.1.1.1/03 KCA 8.3.1.1.2/03
Acute contact toxicity (laboratory) in bumble bees			
Flufenacet, tech.	LD_{50}-contact, 48 h	LD_{50} > 100 µg a.s./bee	[redacted] (2014) M-478564-01-1 KCA 8.3.1.1.2/04
Chronic toxicity in adult honey bees (laboratory)			
Flufenacet, tech.	10 d chronic adult feeding study	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted] (2014) M-477339-01-1 KCA 8.3.1.2/01
Bee brood feeding test			
Flufenacet SC 508.8	Honey bee brood feeding ([redacted] *et al.*, 1992)	No adverse effects on mortality, bee brood development (eggs, young larvae, old larvae, pupae) and colony development by feeding honey bee colonies sugar syrup with a flufenacet - concentration typical for/exceeding the concentration of flufenacet in the spray tank (1500 ppm)	[redacted]-[redacted] (2012) M-456504-01-1 KCA 8.3.1.3/01

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	**KCA 8.3.1.1.1/03; [redacted]; 2011**
Title:	Effects of flufenacet tech. (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Document No:	M-421687-01-1
Guidelines:	OECD Guideline 213 and 214 (1998)
GLP	Yes (certified laboratory)

Objective: Honey bees (*A. mellifera*) can be affected by pesticide residues as a result of indirect contact on plant surfaces, via oral intake of contaminated food or water, via inhalation of vapour or by direct overspray in the course of an application in the field according to normal agricultural practice. If the proposed use pattern of flufenacet tech. indicates such a possible exposure of honey bees, acute contact and oral toxicity data is necessary for the registration of the pesticide use in question. This study provides:
• the acute toxicity levels of the test item to honey bees;
• toxicity information comparable to expected residues from standard rates, for assessment of the potential hazard to honey bees;
• information to support precautionary label statements;
• information to indicate the need for further testing e.g. semi-field or field studies.

Material and methods: Test item: Flufenacet tech. (Specification; Batch Code.: AE F133402-01-02, Origin Batch No.: K664078, Customer Order No.: TOX 07969-02, Specification No.: 102000006978); content: 97.5% w/w analytical.
Test organism: Honey bee (*Apis mellifera* L.), female worker bees, obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.
Under laboratory conditions *Apis mellifera* (30 worker bees per dose; 10 individuals in 3 replicates per test item dose level, controls and reference item doses) were exposed for 48 hours to a single dose of 100.0, 50.0, 25.0, 12.5 and 6.3 µg a.s. per bee for topical application (contact) and feeding (oral value based on the actual intake of the test item) with a single dose of 109.2, 54.3, 26.9, 13.8 and 6.8 µg a.s. per bee.

<u>Oral toxicity study</u>
Appropriate amounts of the test item and reference item were prepared in such a way that they had the respective target concentration of the test item once they were subsequently mixed with sugar syrup at a ratio of 1 + 1. After mixing of these test solutions with ready-to-use sugar syrup (composition of the sugar component: 30 % saccharose, 31 % glucose, 39 % fructose) the final concentration of sugar syrup in the test item solutions offered to the bees was 50 % (45 % water, 50 % syrup and 5 % acetone (w/w)).
For the solvent control, the same proportion of syrup, water and acetone was used; in the water control water and sugar syrup was used at the ratio 1 + 1.
The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake ranged from 1 hour 25 minutes to 2 hours + 5 minutes for the test item treatments). After a maximum of 2 hours + 5 minutes, the food uptake was complete, weighed and replaced by ones containing fresh, untreated food.

The target dose levels (e.g. 100.0 µg a.s./bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested, in practice, higher (or lower) dose levels were obtained as the bees had a higher or lower uptake of the test solutions than the nominal 20 mg/bee.
The measured dose level was 109.2 µg a.s./bee.
The test was conducted in darkness, temperature was 25°C and humidity between 48 and 83%.
Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing. Results are based on measured concentrations of the a.s. per bee.

Contact toxicity study
A single 5 µL droplet of flufenacet tech. in an appropriate carrier (Acetone) was placed on the dorsal bee thorax.
For the control one 5 µL droplet a) of tap water containing 0.5 % Adhäsit and b) pure acetone was used. The reference item was also applied in 5 µL tap water (dimethoate made up in acetone).
A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.
The test was conducted in darkness, temperature was 25°C and humidity between 48 and 83%. Biological observations, including mortality and behavioural changes were recorded at 4, 24 and 48 hours after application. Results are based on nominal concentrations of the a.s. per bee.

Findings: The results can be considered as valid, as all validity criteria of the test were met: water and solvent control mortality is 0% with one exception for water control mortality of 3.3% in the contact test, LD_{50} (24 h) of the toxic standard in the oral test equals 0.11 µg a.s./bee, the LD_{50} (24 h) of the toxic standard in the contact test equals 0.21 µg/bee.
A summary of effects of the test item on mortality and behavioural abnormalities of the bees is given below for both tests:

Mortality and behavioural abnormalities of the bees in the oral toxicity test

ingested dosage [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %
test item						
109.2	0.0	0.0	0.0	0.0	0.0	0.0
54.3	0.0	0.0	0.0	0.0	0.0	0.0
26.9	0.0	0.0	0.0	0.0	0.0	0.0
13.8	0.0	0.0	0.0	0.0	0.0	0.0
6.8	0.0	0.0	0.0	0.0	0.0	0.0
water control	0.0	0.0	0.0	0.0	0.0	0.0
solvent control	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.27	0.0	100.0	100.0	0.0	100.0	0.0
0.16	0.0	23.3	100.0	0.0	100.0	0.0
0.08	0.0	0.0	0.0	0.0	6.0	30.0
0.06	0.0	0.0	0.0	0.0	0.0	0.0

results are averages from three replicates (ten bees each) per dosage / control

Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dose [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %
test item 100.0	0.0	0.0	0.0	0.0	0.0	0.0
50.0	0.0	3.3	3.3	0.0	3.3	0.0
25.0	0.0	0.0	3.3	0.0	3.3	0.0
12.5	0.0	0.0	0.0	0.0	0.0	0.0
6.3	0.0	0.0	0.0	0.0	0.0	0.0
water control	0.0	0.0	3.3	0.0	3.3	0.0
solvent control	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.30	3.3	53.3	100.0	0.0	100.0	0.0
0.20	6.7	20.0	43.3	13.3	73.3	6.7
0.15	3.3	13.3	26.7	0.0	40.0	6.7
0.10	0.0	3.3	3.3	0.0	3.3	0.0

results are averages from three replicates (ten bees each) per dosage / control

Observations: Actual oral doses of 109.2, 54.3, 26.9, 13.8 and 6.8 µg a.s./bee resulted in no mortality in any of the dose levels until the end of the test (48 hours after application). Also no mortality occurred in the solvent control group and in the water control group, respectively.
In the contact toxicity test, mortality occurred in the 50.0 and 25.0 µg a.s./bee dose levels, when one out of the 30 treated bees were found dead, respectively. In the other dose levels (100.0, 12.5 and 6.3 µg a.s./bee) no mortality occurred. 3.3 % mortality occurred in the water control group (water + 0.5 % Adhäsit) and there was no mortality in the solvent control group (acetone).

Conclusion:

Toxicity to Honey Bees; laboratory tests

Test Item	Flufenacet tech.	
Test object	*Apis mellifera*	
Application rate (µg a.s./bee)	109.2, 54.3, 26.9, 13.8 and 6.8	100.0, 50.0, 25.0, 12.5 and 6.3
Exposure	oral (sugar/acetone solution)	contact (solution in acetone)
LD_{50} µg a.s./bee	> 109.2	> 100.0

The toxicity of flufenacet tech. was tested in both an acute contact and an oral toxicity test on honey bees.

The LD_{50} (48 h) value was > 100.0 µg a.s./bee in the contact toxicity test.
The LD_{50} (48 h) value was > 109.2 µg a.s./bee in the oral toxicity test.

CA 8.3.1.1.2 Acute contact toxicity

For data on honey bees please refer to the MCA section CA 8.3.1.1.1.
In addition a study on the acute contacti toxicity to bumble bees was performe. The summary is presented below.

Report:	**KCA 8.3.1.1.2/04; [redacted], E.; 2014**
Title:	Flufenacet (tech.): Acute contact toxicity to the bumble bee, *Bombus terrestris* L. under laboratory conditions
Document No:	M-478564-01-1
Guidelines:	No specific guidelines available, based on OEPP/EPPO 170 (4) (2010), OECD Guideline No. 214 (1998) and on the review article of VAN DER STEEN (2001).
GLP	Yes (certified laboratory)

Material and methods:

Test item:	Name:	Flufenacet (tech.)
	TOX-No:	10011-00
	Origin Batch No.:	NK61CX0650
	Purity:	98.18 % w/w (analysed)

The contact toxicity of flufenacet (tech.) to the bumble bee (*Bombus terrestris* L.) was determined in a limit test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).

In the laboratory, bumble bees were exposed to 100 µg flufenacet a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after application. The control groups were exposed for the same period of time under identical conditions to tap water and acetone, respectively.

Dates of work: 09 October 2013 – 11 October 2013

Findings:

In both control groups, treated either with tap water or acetone, no mortality was observed during the 48 h test period. In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

Flufenacet (tech.)	Contact toxicity test [µg a.s./bumble bee]
LD_{50} (24 h)	> 100
LD_{50} (48 h)	> 100

In the test item treatment group, no mortality and no sub-lethal effects were observed until the final assessment 48 hours after start of the experimental phase. Thus, it can be concluded that the topical application of flufenacet (tech.) on bumble bees at the treatment level of 100 µg flufenacet a.s./bumble bee, caused no adverse effects regarding mortality, sub-lethal effects and behaviour.

Conclusion:

The 48 hour contact LD_{50} value for flufenacet (tech.) was determined to be > 100 µg flufenacet a.s./bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

Report:	**KCA 8.3.1.2 /01; [redacted], A., 2014**
Title:	Flufenacet (tech.) - Assessment of chronic effects to the honeybee, *Apis mellifera* L., in a 10 days continuous laboratory feeding limit test
Document No:	M-477339-01-1
Guidelines:	No agreed and ring tested guideline available
GLP:	yes

Material and methods:

Test item:	Name:	Flufenacet (tech.)
	TOX-No:	10011-00
	Origin Batch No.:	NK61CK0650
	Purity:	98.18 % w/w (analysed)

The chronic effects of the test item flufenacet (tech.) on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding in the laboratory.

Over a period of 10 days, honey bees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing nominally 120 mg a.s./kg of the test item flufenacet (tech.) by continuous and ad libitum feeding. Because the test item was first dissolved in acetone and then diluted with aqueous sucrose solution, the final test item application (feeding) solution contained 3 % acetone. The control group was exposed for the same period of time under identical exposure conditions to untreated 50 % (w/v) aqueous sucrose application (feeding) solution, also containing 3 % acetone. Mortality, sub-lethal effects and behavioural observations were assessed every day throughout the 10 days exposure period. Furthermore, the daily food uptake was determined.

Dates of work (biology): 14 May 2013 – 09 July 2013

Findings:

After 10 days of continuous exposure, mortality at the test item treatment level of 120 mg a.s./kg of flufenacet (tech.) was not statistically significantly different when compared to the control group.

The cumulative control mortality was 0.0 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg flufenacet (tech.) was 3.0 % at the final assessement.

At 120 mg a.s./kg flufenacet (tech.), no remarkable sub-lethal effects or behavioural abnormalities were observed throughout the entire observation period of 10 days.

After 10 days of continuous exposure, by considering the actual food consumption of the honey bees, the accumulated nominal intake of the test item flufenacet (tech.) at the treatment level of 120 mg a.s./kg was 44.2 µg a.s./bee, the corresponding average daily dose was therefore 4.4 µg a.s./bee.

The overall mean daily consumption of the application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different (lower) when compared to the untreated control group (36.8 mg/bee at 120 mg a.s./kg, compared to 38.4 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose application (feeding) solution was not statistically significantly different (lower) between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison), except for the first day and the 8th day of exposure.

Mean consumption of application solution, mean nominal intake of test item accumulated over all test days, average daily dose, cumulative mortality after ten days of continuous exposure (test end) as well as the LC_{50} and NOEC

Treatment Level	Control [1]	Flufenacet (tech.) at 120 mg a.s./kg (nominal) [2]
Cumulative mortality after ten days of continuous exposure [%]	0.0	3.0
Overall mean daily consumption of application (feeding) solution [mg/bee] [3]	38.4	36.8
Mean nominal intake accumulated over ten test days [µg a.s./bee/10d]	-	44.2
Average daily dose (nominal) throughout ten days of continuous exposure [µg a.s./bee/d]	-	4.4
LC_{50}	> 120 mg a.s./kg (nominal)	
NOEC [4]	120 mg a.s./kg (nominal)	

[1] Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing 3 % acetone
[2] Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing 3 % acetone and flufenacet (tech.)
[3] The mean values per replicate over the test period (non-rounded values) were used for the calculation of the overall mean daily consumption of application (feeding) solution per treatment
[4] Determined to be the NOEC based on mortality (not statistically significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided, $p \leq 0.05$)
a.s. = active substance

Conclusions:

It can be concluded that the continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item flufenacet (tech.) at the treatment level of 120 mg a.s./kg caused no adverse effect regarding mortality, sub-lethal effects and behaviour.

The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly lower compared to the untreated control group. Further on every single day during the 10 day continuous exposure period the mean food consumption per bee was not statistically significantly different (lower) in the test item treatment group compared to the control group, except for the first day and the 8th day of exposure.

As the overall mean daily food uptake in the test item treatment group was not statistically significantly lower compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 120 mg a.s./kg.

The NOEC for mortality was determined at the end of the test period to be 120 mg a.s./kg (nominal).

The LC_{50} after 10 days of continuous exposure was determined to be > 120 mg a.s./kg (nominal).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	**KCA 8.3.1.3/01; [redacted], S.; 2012**
Title:	Flufenacet SC 508.8: A honeybee brood feeding study to evaluate the effects on brood development of the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae)
Document No.:	M-456504-01-1
Guidelines:	EPPO Bulletin 22 ([redacted] *et al*., 1992)
GLP:	Yes (certified laboratory)

Objective

The purpose of the honeybee brood feeding study was to evaluate the effect of Flufenacet SC 508.8 on brood development and mortality of adult worker honeybees, *Apis mellifera* L. (Hymenoptera Apida). The colonies were freely flying with access to natural nectar and pollen sources, however, the study was conducted at a time without mass flowering plants/agricultural crops in the study region, so that the nectar flow of natural sources was low at the time of treatment administration.

Material and methods

Test item:

Flufenacet SC 508.8 (active ingredient: flufenacet (BAY005NOR); Batch ID.: EFKF001049, Sample Description: TOX09446-00, Specification No.: 102000007779-02; Analytical content: 42.8% w/w; 519.2 g flufenacet/L; Density: 1.213 g/mL at 20 °C).

Test species:

Honey bees (*Apis mellifera* L.); honey bee colonies were maintained according to normal beekeeping practice, containing two magazines with 12 combs, each. The colonies were freely flying with access to natural nectar and pollen sources, however, the study was conducted at a time without mass flowering plants/agricultural crops in the study region, so that the nectar flow of natural sources was low at the time of treatment administration.

Endpoints:

- Bee mortality of adult worker bees, pupae and larvae before (DAT[2] -3 to 0) and after treatment/feeding (DAT 1 to 21), in dead-bee traps
- Flight activity shortly before (DAT 0) and on the day after treatment/feeding (DAT 1)
- Condition of the colonies at study initiation (DAT -2/0[3]) and at study termination (DAT 21)

Test concentrations:

Control: 1 L untreated commercial ready-to-use sugar syrup (Apiinvert; 30% sucrose, 31% glucose, 39% fructose) per colony.

[2] DAT = days after treatment

[3] On DAT 0 the intended colony 1C was replaced by one of the back-up colonies (old larvae stage was missing). Since the colony was assessed and replaced before treatment/feeding (also the mortality was assessed during the pre- treatment/feeding period), this operation had no impact on the study result.

Test Item: Colonies were fed with 1.5 g flufenacet a.i./L, corresponding to 2.89 mL Flufenacet SC 508.8 in 1 L 50% (w/v) aqueous sucrose solution. Each colony in the test item group was fed with 1 L test item fortified 50% (w/v) aqueous sucrose solution.

Reference Item: 1.6 g reference item (Insegar; 25% fenoxycarb) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to a nominal active substance concentration of 0.4 g fenoxycarb a.s./L.

Results

Honeybee mortality

Date	Mortality [mean daily number of dead bees[1)] per replicate ± SD]		
	Control	Treatment◦	Reference item
Ø DAT –2 to 0	30.2	24.9	30.8
Ø DAT 1	92.3	84.7	126.3*
Ø DAT 1 to 21	49.5	53.2	104.4**
$Q_{M(0(at))}$	3.1	3.4	4.1
$Q_{M(mean)}$	1.6	2.1	3.4

DAT = days after treatment
SD = standard deviation
QM(0(at)) = Ø mortality on the day after treatment/feeding 1 ÷ Ø pre-application mortality (per treatment group)
QM(mean) = Ø post-treatment mortality ÷ Ø pre-treatment mortality (per treatment group)
1) including adult worker bees, freshly emerged bees, pupae and larvae
* statistically significantly different when compared to the control
** statistically significantly different when compared to the pre-phase (DAT -2 to 0)

Colony conditions

Date	Mean percentage [%] of comb covered by brood stages (egg, larvae, pupae)		
	Control	Treatment◦	Reference item
Ø DAT –2 to 0	22.2	16.7[n.s.]	22.7[n.s]
Ø DAT 1	23.3	26.7[n.s]	23.3[n.s]

DAT = days after treatment/feeding
n.s. not statistically significantly different when compared to the control

Detailed brood development of observed eggs

Date	Brood termination rate [%] n.s.		
	Control	Treatment	Reference item
BFD0/DAT0	0.0	0.0	0.0
BFD6/DAT6	25.1	9.1	63.3
BFD10/DAT10	27.8	9.3	64.9
BFD16/DAT17	32.0	10.7	67.6
BFD21/DAT21	32.0	34.2	67.6
Brood Index n.s.			
BFD0/DAT0	1.0	1.0	1.0
BFD6/DAT6	2.5	2.9	1.0
BFD10/DAT10	2.9	3.6	1.4
BFD16/DAT17	2.7	3.6	1.3
BFD21/DAT21	3.4	4.5	1.6
Compensation Index n.s.			
BFD0/DAT0	1.0	1.0	1.0
BFD6/DAT6	2.5	2.9	1.1
BFD10/DAT10	2.9	3.6	1.8
BFD16/DAT17	3.0	3.6	2.1
BFD21/DAT21	4.0	4.5	3.5

BFD = brood fixing day

DAT = days after treatment

ns not statistically significantly different when compared to the control

Detailed brood development of observed young larvae

Date	Brood termination rate [%] n.s.		
	Control	Treatment	Reference item
BFD0/DAT0	0.0	0.0	0.0
BFD6/DAT6	35.6	14.9	70.2
BFD10/DAT10	38.0	17.6	72.2
BFD16/DAT17	38.0	17.6	72.2
BFD21/DAT21	38.0	50.0	72.2
Brood Index n.s.			
BFD0/DAT0	2.0	2.0	2.0
BFD6/DAT6	2.6	3.4	1.2
BFD10/DAT10	2.5	3.3	1.1
BFD16/DAT17	3.1	4.1	1.4
BFD21/DAT21	3.1	4.1	1.3
Compensation Index n.s.			
BFD0/DAT0	2.0	2.0	2.0
BFD6/DAT6	2.6	3.4	1.3
BFD10/DAT10	2.6	3.3	1.6
BFD16/DAT17	3.8	4.2	2.6
BFD21/DAT21	4.1	4.3	3.0

BFD = brood fixing day

DAT = days after treatment

ns not statistically significantly different when compared to the control

Detailed brood development of old larvae

Date	Brood termination rate [%] n.s.		
	Control	Treatment	Reference item
BFD0/DAT0	0.0	0.0	0.0
BFD6/DAT6	10.2	3.8	8.3
BFD10/DAT10	10.4	5.2	61.9
BFD16/DAT17	10.4	5.2	61.9
BFD21/DAT21	10.4	5.2	61.9
Brood Index n.s.			
BFD0/DAT0	3.0	3.0	3.0
BFD6/DAT6	3.6	3.5	3.7
BFD10/DAT10	3.6	3.8	1.5*
BFD16/DAT17	4.5	4.7	1.9*
BFD21/DAT21	4.4	4.7	1.8*
Compensation Index n.s.			
BFD0/DAT0	3.0	3.0	3.0
BFD6/DAT6	3.6	3.8	3.7
BFD10/DAT10	3.6	3.8	1.5*
BFD16/DAT17	4.7	4.8	3.1*
BFD21/DAT21	4.8	4.9	3.8*

BFD = brood fixing day
DAT = days after treatment
*
not statistically significantly different when compared to the control

Conclusion

The consumption of the test item by honey bee colonies at a concentration of 1.5 g flufenacet a.s./L, corresponding to 2.89 mL Flufenacet SC 508.8 in 1 L 50% (w/v) aqueous sucrose solution, had no adverse effects on the colony conditions and survival of honeybee life stages (eggs, young larvae and old larvae), developing in brood cells within the hives. Also, the test item had no adverse effects on the survival of the exposed adult worker bees. Overall, it can be concluded according to the results of this study that Flufenacet SC 508.8 does neither adversely affect honey bee colonies nor bee brood development.

CA 8.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess “sub-lethal effects” in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

CA 8.3.2 Effects on non-target arthropods other than bees

In the first Annex I listing process non-target arthropod data for a different formulation of flufenacet were submitted and evaluated. The formulation FFA WG60 is no longer considered to be the representative formulation, therefore only data on the new representative formulation Flufenacet + Diflufenican SC 600 (Herold SC 600) for the Annex I renewal process will be presented with this dossier. For details on the extended laboratory and aged residue study summaries please refer to the respective sections in the MCP “Section 10 Ecotoxicological Studies”.

Table 1: Flufenacet + Diflufenican SC 600: Ecotoxicological endpoints for arthropods other than bees

Test species, references	Tested Formulation, study type, exposure	Ecotoxicological endpoint		
Typhlodromus pyri M-058604-01-1 Rep.No.: 9352063 [redacted], A.; 2001 KCP 10.3.2.1/01	FFA+DFF SC 600 Laboratory, glass plates 22.5 mL prod./ha 45 mL prod./ha 90 mL prod./ha 180 mL prod./ha 360 mL prod./ha	LR_{50} 81.8 mL prod./ha Corr. Mortality [%] 1.9 9.2 61.1 92.6 100	 Effect on Reproduction [%] 1.3 -12.5[A] n.a. n.a. n.a.	
Typhlodromus pyri M-034242-01-1 Rep.No.: 01TYBYL12 [redacted], M.P.; 2002 KCP 10.3.2.2/01	FFA+DFF SC 600 Extended lab., exposure on detached bean leaves 9.9 mL prod./ha 28.7 mL prod./ha 83.2 mL prod./ha 241.4 mL prod./ha 700 mL prod./ha	LR_{50} 110.2 mL prod./ha Corr. Mortality [%] 0 0 17.1 94.3 100	 Effect on Reproduction [%] 4.4 13.3 -17.8[A] n.a. n.a.	
Typhlodromus pyri M-355238-01-1 Rep.Nr.: CW09/026 [redacted], D.; 2009 KCP 10.3.2.2/04	FFA+DFF SC 600 Aged residues, spray deposits on maize plants, 1 appl. of 0.7 L prod./ha Residues aged for 0 days: Residues aged for 14 days: Residues aged for 28 days:	Corr. Mortality [%] 98.9 87.1 9.5	Effect on Reproduction [%] n.a. n.a. 8.4	
Aphidius rhopalosiphi M-058618-01-1 Rep.No.: 9351001 [redacted], M.; [redacted], R.; 2001 KCP 10.3.2.1/02	FFA+DFF SC 600 Laboratory, glass plates 500 mL prod./ha 600 mL prod./ha 700 mL prod./ha	LR_{50} > 700 mL prod./ha Corr. Mortality [%] 0 2.0 2.0	 Effect on Reproduction [%] 9.0 14.0 3.5	
Chrysoperla carnea M-352372-01-1 Rep.No.: CW09/010 [redacted], J.; 2009 KCP 10.3.2.2/02	FFA+DFF SC 600 Extended lab., exposure on detached maize leaves Control 30 mL prod./ha 63 mL prod./ha 134 mL prod./ha 284 mL prod./ha 600 mL prod./ha	LR_{50} > 600 mL prod./ha Corr. Mortality - 0.0 7.7 2.6 7.7 20.5	 Eggs/Female/Day 26.4 24.1 23.9 27.5 28.4 27.6	 Hatching [%] 79.9 81.4 80.7 83.4 82.5 82.7
Aleochara bilineata M-353760-01-1 Rep.No.: 09 10 48 027 A [redacted], U.; 2009 KCP 10.3.2.2/03	FFA+DFF SC 600 Extended lab., spray deposits on soil (LUFA 2.1) 60 mL prod./ha 107 mL prod./ha 190 mL prod./ha 337 mL prod./ha 600 mL prod./ha	ER_{50} > 600 mL prod./ha Effect on Reproduction [%] 4.3 -2.3[A] 1.7 5.8 7.9		

A: A negative value indicates a higher reproduction rate in the treatment than in the control.
n.a.: not assessed

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Please refer to the respective section in the MCP "Section 10 Ecotoxicological Studies".

CA 8.3.2.2 Effects on Typhlodromus pyri

Please refer to the respective section in the MCP "Section 10 Ecotoxicological Studies".

CA 8.4 Effects on non-target soil meso and macrofauna

For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. its Addenda). These studies are listed in grey in the tables below.

Effects on earthworms

Test species	Test design	Ecotoxicological endpoint			Reference
Flufenacet					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50} **LC_{50}**	219 **109.5***	mg a.s./kg dws **mg a.s./kg dws**	[redacted] (1995) M-004876-01-2
Flufenacet WG 60					
Eisenia fetida	chronic, 56 d (10% peat in test soil)	NOEC amended **$NOEC_{refined}$**	3.0 3.0 0.605 **1.2[1]***	kg a.s./ha mg a.s./kg dws kg a.s./ha mg a.s./kg dws	[redacted] (2011) M-004878-02-1 KCA 8.4.1/04
FFA SC 500					
Natural earthworm fauna	field study 1 year, spray	**NOEAER**	1.2 **0.6**	l prod./ha kg a.s./ha	[redacted] (2008) M-307211-01-1 KCA 8.4.1/11
DFF+FFA SC 600					
Natural earthworm fauna	field study 1 year, spray	**NOEAER**	**1.8**	l prod./ha	[redacted] (2014) M-478092-01-1 KCP 10.4.1.2/01
FOE oxalate					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 1000	mg p.m./kg dws	[redacted] (1999) M-008793-01-1
Eisenia fetida	chronic, 56 d (10% peat in test soil)	NOEC	**≥100**	mg p.m./kg dws	[redacted] (2010) M-398163-01-1 KCA 8.4.1/07
FOE sulfonic acid-Na-salt					
Eisenia fetida	acute, 14 d (10% peat in test soil)	LC_{50}	> 1000	mg p.m./kg dws	[redacted] (1999) M-008794-01-1
Eisenia fetida	chronic, 56 d (5% peat in test soil)	NOEC	**500**	mg p.m./kg dws	[redacted] (2009) M-358264-01-1 KCA 8.4.1/05
FOE methylsulfone					
Eisenia fetida	chronic, 56 d (5% peat in test soil)	NOEC	**62.5***	mg p.m./kg dws	[redacted] (2010) M-362081-01-1 KCA 8.4.1/06
TFA					
Eisenia fetida	chronic, 56 d (10% peat in test soil)	NOEC	**320[2)]**	mg p.m./kg dws	[redacted] (2005) M-251328-01-1 KCA 8.4.1/09
FOE 5043-trifluoroethane sulfonic acid					
Eisenia fetida	chronic, 56 d (5% peat in test soil)	NOEC	**≥100**	mg p.m./kg dws	[redacted] (2012) M-436340-01-1 KCA 8.4.1/10
FOE-Thiadone					
Eisenia fetida	chronic, 56 d (5% peat in test soil)	NOEC	**3.2**	mg p.m./kg dws	[redacted] (2012) M-442579-01-1 KCA 8.4.1/08

* endpoints corrected to allow for log P_{ow} > 2
dws = dry weight soil, pm = pure metabolite
1) based on 605 g flufenacet/10000 m², size of test boxes = 198 cm² and 500 g dry weight substrate per test box
2) NOEC reduced to 320 mg/kg based on effects on the body weight in the concentration 1000 mg/kg.
Bold values: Endpoints considered relevant for risk assessment

Effects on non-target soil meso and macrofauna (other than earthworms)

Test species	Test design	Ecotoxicological endpoint	Reference
Flufenacet			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **31.5*** mg a.s./kg dws	[redacted] (2010) M-363896-01-1 KCA 8.4.2.1/02
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **281*** mg a.s./kg dws	[redacted] (2013) M-455214-01-1 KCA 8.4.2.1/12
FOE oxalate			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 100** mg p.m./kg dws	[redacted] (2010) M-394712-01-1 KCA 8.4.2.1/04
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 100** mg p.m./kg dws	[redacted] (2010) M-393634-01-1 KCA 8.4.2.1/03
FOE sulfonic acid-Na-salt			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{repro}}$ **≥ 100** mg p.m./kg dws	[redacted] (2010) M-396039-01-1 KCA 8.4.2.1/05
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 100** mg p.m./kg dws	[redacted] (2013) M-455654-01-1 KCA 8.4.2.1/13
FOE methylsulfone			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 50*** mg p.m./kg dws	[redacted] (2010) M-392345-01-1 KCA 8.4.2.1/14
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **250*** mg p.m./kg dws	[redacted] (2009) M-357707-01-1 KCA 8.4.2.1/01
TFA			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥100** mg pm/kg dws	[redacted] (2012) M-436127-01-1 KCA 8.4.2.1/06
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 100** mg p.m./kg dws	[redacted] (2012) M-436326-01-1 KCA 8.4.2.1/09
FOE 5043-trifluoroethane sulfonic acid			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 100** mg p.m./kg dws	[redacted] (2012) M-436128-01-1 KCA 8.4.2.1/07
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **≥ 100** mg p.m./kg dws	[redacted] (2012) M-436315-01-1 KCA 8.4.2.1/08
FOE-Thiadone			
Folsomia candida	chronic, 28 d (5% peat in test soil)	**NOEC**$_{\mathbf{reproduction}}$ **1.8** **mg p.m./kg dws**	[redacted] (2012) M-440372-01-1 KCA 8.4.2.1/10

Test species	Test design	Ecotoxicological endpoint	Reference
Hypoaspis aculeifer	chronic, 14 d (5% peat in test soil)	**$NOEC_{reproduction}$ 32 mg p.m./kg dws**	[redacted] (2012) M-442897-01-1 KCA 8.4.2.1/11

* endpoints corrected to allow for log P_{ow} > 2
Bold values: Endpoints considered relevant for risk assessment

CA 8.4.1 Earthworm, sub-lethal effects

Report: **KCA 8.4.1/04; [redacted], M.-A., 2011**
Title: Influence of FOE 5043 WG 60 on the reproduction of earthworms (*Eisenia fetida*)
Document No: M-004878-02-1
Guidelines: ISO/DIS 11268-2 (1995): Part 2 ; ISO/DIS 11268-2 (1995)
GLP yes

Objective: New statistical calculation with the data obtained in [redacted] (1997, M-004878-01-1).

Results

	Number of adult worms		Mean weight of worms [g]		weight change
	Day 0	Day 28	Day 0	Day 28	[%]
Control	10	10	0.36	0.55	52.78
	10	10	0.40	0.62	55.00
	10	10	0.36	0.55	52.78
	10	10	0.38	0.51	34.21
mean	10	10	0.375	0.558	48.692
stabw	0	0	0.019	0.046	9.711
1 x 1	10	10	0.39	0.58	48.72
	10	10	0.40	0.59	47.50
	10	10	0.36	0.54	50.00
	10	10	0.36	0.54	50.00
mean	10	10	0.378	0.563	49.054
stabw	0	0	0.021	0.026	1.200
1 x 2	10	10	0.37	0.50	35.14
	10	10	0.39	0.52	33.33
	10	10	0.36	0.49	36.11
	10	10	0.35	0.48	37.14
mean	10	10	0.368	0.498	35.431 *
stabw	0	0	0.017	0.017	1.621
1 x 5	10	10	0.40	0.50	25.00
	10	10	0.35	0.48	37.14
	10	10	0.37	0.47	27.03
	10	10	0.37	0.49	32.43
mean	10	10	0.373	0.485	30.401 *
stabw	0	0	0.021	0.013	5.481

	Box Number	Number of juvenile worms
Control	1	54
	2	49
	3	35
	4	59
mean		49.3
stabw		10.3
1 x 1	1	56
	2	57
	3	47
	4	36
mean		49.0
stabw		9.8
1 x 2	1	48
	2	51
	3	49
	4	47
mean		48.8
stabw		1.7
1 x 5	1	50
	2	49
	3	47
	4	48
mean		48.5
stabw		1.3

Mortality

No mortality of adult earthworms was observed after 28 days of exposure at any test concentration of the test item in this study.

Effects on growth

Changes in body weight values of the surviving test organisms of the treatment groups during the test period were compared to the values of the control group. The normal distribution of the data was tested by Kolmogorov-Smirnov test. The normality hypothesis was accepted. The homogeneity of variances of the data was checked by Cochran's test. The homogeneity hypothesis was rejected. Therefore the data were transformed ($y' = \ln(y)$). The homogeneity of variances of these transformed data was given. The data were statistically evaluated by means of a Williams multiple sequential t-test, two-sided, $\alpha = 0.05$. The data for 2 and 5 kg test item/ha was statistically significant different to the control. The statistical software package ToxRatPro Version 2.09 @ was used for the calculation.

Therefore:

NOEC related to growth: 1 kg test item/ha

LOEC related to growth: 2 kg test item/ha

Effects on reproduction

The reproduction of the surviving test organisms per test vessel at the end of the study was compared to the control values. The normal distribution of the data was tested by Kolmogorov-Smirnov test. The

normality hypothesis was accepted. The homogeneity of variances of the data was checked by Cochran's test. The homogeneity hypothesis was accepted. The homogeneity hypothesis was accepted. The data were statistically evaluated by means of a Williams multiple sequential t-test, one – sided smaller, α = 0.05. The statistical software package ToxRatPro Version 2.09 @ was used for the calculation. No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at all test concentrations.
Therefore, based on statistical significance:
NOEC related to reproduction: ≥ 5 kg test item/ha
LOEC related to reproduction: > 5 kg test item/ha

Conclusion
Overall, based on the biological and statistical significance of the effects observed on growth or reproduction, it is concluded, that the NOEC for this study is 1 kg test item/ha and the overall LOEC is determined to be 2 kg test item/ha.

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Report:	**KCA 8.4.1/05; [redacted], T., 2009**
Title:	Flufenacet (FOE 5043) – Sulfonic acid Na-salt: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5% peat.
Document N°:	M-358264-01-1
Guidelines:	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004
GLP	yes (certified laboratory)

Objective:
The purpose of this study was to assess the effect of Flufenacet-Sulfonic acid Na-salt, on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil at 5 different test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2; 1998 (E) and OECD 222: April 13, 2004).

Materials and Methods:
Test item: Flufenacet-Sulfonic acid Na-salt, Batch code: AE 0841914-01-03, Origin Batch No.; SES 10294-6-2. TOX No. 08523-00, content of as (analysed): 92.4%.
Reference Item: Carbendazim
Control: same application as test item but with deionised water.
Test organism: Adult earthworms (*Eisenia fetida*). The mean body wet weight of the test organisms at the start of the test ranged from 0.3 to 0.5 g per worm. The worms were adult with a well developed clitellum and approximately 8 months old.
Adult *Eisenia fetida* (approx. 8 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 62.5 - 125 - 250 - 500 and 1000 mg test item/kg dry weight artificial soil. The test item was mixed into the soil.
The test vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 - 800 Lux.
During the test period, the temperature was in the range of 18 to 22°C. The measured mean light intensity was 552 Lux at day 0, 560 Lux at day 28 and 646 Lux at day 56 of the study.

After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:

The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 10% (0% in this study), reproduction of the control was ≥ 30 worms per container (161.8 worms in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (10.4% in this study).

Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the table below.

Test object	***Eisenia fetida***					
Test item	**Control**	**Flufenacet-Sulfonic acid Na-salt**				
Test concentration (mg test item/kg dws*)	---	62.5	125	250	500	1000
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	5
Mean change of body weight of the adults from day 0 to day 28 [%]	+ 20.0	+ 40.9	+ 44.3	+ 44.4	+ 34.2	+ 36.7
Standard Deviation	± 2.1	± 9.6	± 1.8	± 9.0	± 5.9	± 3.2
Statistical comparison to the control **	—	s.	s.	s.	s.	s.
Mean number of offspring per test vessel after 56 days	161.8	153.5	163.8	155.0	167.5	120.8
Standard Deviation	± 16.8	± 25.0	± 15.5	± 10.7	± 24.3	± 5.1
Statistical comparison to the control ***	—	n.s.	n.s.	n.s.	n.s.	s.

Values in table are rounded
* dws = Dry weight artificial soil
** Result of a Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$
*** Result of a Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$
n.s.: mean value not statistically significant different compared to the control ($p \geq 0.05$)
s.: mean value statistically significant different compared to the control ($p < 0.05$)

Observations:

Mortality

Mortality of adult earthworms was observed after 28 days of exposure only at the highest test concentration of 1000 mg test item/kg dry weight artificial soil. 5% mortality is below the allowed maximum mortality for the control and is therefore not considered as an adverse effect.

Effects on growth

Statistically significant different values for the growth relative to the control were observed at all tested concentrations. Since the growth in all tested concentrations of the test item were higher than in the control this was not considered as an adverse effect.

NOEC related to growth: ≥ 1000 mg test item/kg dry weight artificial soil
LOEC related to growth: > 1000 mg test item/kg dry weight artificial soil

Effects on reproduction

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations up to and including 500 mg test item/kg dry weight artificial soil.

A statistically significant different value for the number of juveniles per test vessel relative to the control was observed at the test concentrations of 1000 mg test item/kg dry weight artificial soil.

NOEC related to reproduction: 500 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 1000 mg test item/kg dry weight artificial soil

Conclusions:

Overall, based on the biological and statistical significance of the effects, it is concluded that the NOEC for this study is 500 mg test item/kg dry weight artificial soil. The overall LOEC is determined to be 1000 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.1/06; [redacted], T.; 2010**
Title:	Flufenacet (FOE 5043) – Methylsulfone: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5% peat.
Document No:	M-362081-01-1
Guidelines:	ISO 11268-2 (1998) OECD Guideline 222 (2004)
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet-methylsulfone, on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil at 5 different test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004).

Materials and Methods:

Test item: Flufenacet-methylsulfone, Origin Batch No.: SES 10623-5-1; Material No.: BCS-CO62475; Batch code: BCSCO62475-01-01; customer order No.: TOX 08624-00; content of a.s. (analysed): 97.6 % w/w.

Reference Item: Carbendazim

Control: same application as test item but with untreated quartz sand only.

Test organism: Adult earthworms (*Eisenia fetida*). The mean body wet weight of the test organisms at the start of the test ranged from 0.25 to 0.45g per worm. The worms were adult with a well developed clitellum and approximately 8 months old.

Adult *Eisenia fetida* (8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 62.5 - 125 - 250 - 500 and 1000 mg test item/kg dry weight artificial soil. The test item was mixed into the soil.

The test vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 - 800 Lux.

During the test period, the temperature was in the range of 18 to 22°C. The measured mean light intensity was 538 Lux at day 0, 58 Lux at day 28 and 556 Lux at day 56 of the study.
After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 10% (0% in this study), reproduction of the control was ≥ 30 worms per container (103 worms in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (27.4% in this study).
Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the table below.

Test object	***Eisenia fetida***					
Test item	**Control**	**Flufenacet-Methylsulfone**				
Test concentration (mg test item/kg dws*)	--	62.5	125	250	500	1000
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	75
Mean change of body weight of the adults from day 0 to day 28 [%]	+ 62.7	+ 61.2	+ 63.0	+ 45.6	+ 10.1	-64.6
Standard Deviation	± 5.2	± 15.3	± 9.7	± 5.4	± 5.7	± 9.6
Statistical comparison to the control **	—	n.s.	n.s.	s.	s.	s.
Mean number of offspring per test vessel after 56 days	103.3	115.5	101.5	51.0	3.5	0.0
Standard Deviation	± 28.3	± 12.0	± 23.3	± 25.1	± 5.1	± 0.0
Statistical comparison to the control ***	—	n.s.	n.s.	s.	s.	s.

* dws = Dry weight artificial soil
** Result of a Bonferoni-Holm Multiple Sequential u-test
*** Result of a Williams Multiple Sequential t-test, one-sided smaller, α = 0.05
n.s.: mean value not statistically significant different compared to the control (p ≥ 0.05)
s.: mean value statistically significant different compared to the control (p < 0.05)

Observations:
Mortality
No mortality of adult earthworms was observed after 28 days of exposure at the control group and at the test concentrations 62.5, 125, 250 and 500 mg test item/kg dry weight artificial soil. In the highest test concentration 1000 mg test item/kg dry weight artificial soil 75% (30 worms) died.

Effects on growth
No statistically significant different value for the growth relative to the control were observed at the tested concentrations 62.5 and 125 mg test item/kg dry weight artificial soil.
A statistically significant different value for the growth relative to the control were observed at the tested concentrations 250, 500 and 1000 mg test item/kg dry weight artificial soil.

NOEC related to growth: 125 mg test item/kg dry weight artificial soil
LOEC related to growth: 250 mg test item/kg dry weight artificial soil

Effects on reproduction

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations up to and including 62.5 and 125 mg test item/kg dry weight artificial soil.

A statistically significant different value for the number of juveniles per test vessel relative to the control was observed at the test concentrations of 250, 500 and 1000 mg test item/kg dry weight artificial soil.

NOEC related to reproduction: 125 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 250 mg test item/kg dry weight artificial soil

Conclusions:

Overall, based on the biological and statistical significance of the effects, it is concluded that the NOEC for this study is 125 mg test item/kg dry weight artificial soil. The overall LOEC is determined to be 250 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.1/07; [redacted], T.; 2010**
Title:	FOE 5043 – oxalate: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 10% peat.
Document No:	M-398163-01-1
Guidelines:	ISO 11268-2 (1998) OECD Guideline 222 (2004)
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of FOE 5043-oxalate, on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure into an artificial soil with one test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004).

Materials and Methods:

Test item: FOE 5043-oxalate, Batch code: BCS-AB16305-01-01; Origin Batch No.: SES 10564-3-1; LIMS No.: 1027926; Sample Ident.: TOX 08524-03 content of p.m. (analyzed): 92.2 % (w/w).
Reference Item: Carbendazim
Control: same application as test item but with untreated quartz sand only.
Test organism: Adult earthworms (*Eisenia fetida)*. The mean body wet weight of the test organisms at the start of the test ranged from 0.25 to 0.44 g per worm. The worms were adult with a well developed clitellum and approximately 6 months old.
Adult *Eisenia fetida* (8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentration of 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil.
The test vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 - 800 Lux.

During the test period, the temperature was in the range of 18 to 22°C. The measured mean light intensity was 539 Lux at day 0, 472 Lux at day 28 and 479 Lux at day 56 of the study.
After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:

The results can be considered as valid, as all validity criteria of the test were met: Mortality in the control was ≤ 10% (0% in this study), reproduction of the control was ≥ 30 worms per container (76.4 worms in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (15.6% in this study).
Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the table below.

Test object	***Eisenia fetida***	
Test item	Control	FOE 5043-oxalate
Test concentration (mg p.m.*/kg dry weight soil)	---	100
Mortality of adult earthworms [%] after 28 days	0	0
Mean change of body weight of the adults from day 0 to day 28 [%]	21.2	24.5
Standard Deviation	4.8	4.8
Statistical comparison to the control **	—	n.s.
Mean number of offspring per test vessel after 56 days	76.4	68.1
Standard Deviation	11.9	18.3
Statistical comparison to the control ***	—	n.s.

* p.m. = pure metabolite
** Result of a Student-t-test for Homogeneous Variances, two-sided, α = 0.05
*** Result of a Student-t-test for Homogeneous Variances, one-sided smaller, α = 0.05
n.s.: mean value not statistically significant different compared to the control ($p > 0.05$)

Observations:

Mortality

No mortality was observed after 28 days of exposure at the control group and at the tested concentration of 100 mg test item/kg dry weight artificial soil.

Effects on growth

No statistically significant different values for the growth relative to the control were observed at the tested concentration of 100 mg test item / kg dws.
Therefore:
NOEC related to growth: ≥ 100 mg FOE 5043-oxalate/kg dry weight artificial soil
LOEC related to growth: > 100 mg FOE 5043-oxalate/kg dry weight artificial soil.

Effects on reproduction

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the tested concentration of 100 mg FOE 5043-oxalate/kg dry weight artificial soil.

NOEC related to reproduction: ≥ 100 mg FOE 5043-oxalate/kg dry weight artificial soil
LOEC related to reproduction: > 100 mg FOE 5043-oxalate/kg dry weight artificial soil.

Conclusions:

Overall, based on the biological and statistical significance of the effects, it is concluded that the NOEC for this study is ≥ 100 mg FOE 5043-oxalate/kg dry weight artificial soil. The overall LOEC is determined to be >100 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.1/08; [redacted], M.-A..; 2012**
Title:	Flufenacet-thiadone (AE 1258593, BCS-AA 41715): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Document No:	M-442579-01-1
Guidelines:	ISO 11268-2 (1998) OECD 222: April 13, 2004
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet-thiadone (AE 1258593, BCS-AA 41715) on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil. In a 1st test run this was started with a control and one test concentration (limit test with 100 mg test item/kg dry weight soil). Since all adult worms were dead at day 28 this 1st run was terminated and will not be reported. All raw data of the 1st test run will be archived with the study. A 2nd test run was conducted with 5 different test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004).

Materials and Methods:

Test item: Flufenacet-thiadone (AE 1258593, BCS-AA 41715); (Sample description: TOX09021-03; LIMS No.: 1219379; Batch Code: AE 1258593-01-01; Origin Batch No.: SES 10558-3-5; content: 98.6 % w/w).

Test organism: Adult earthworms (*Eisenia fetida*). The mean body wet weight of the test organisms at the start of the test ranged from 310 to 510 mg per worm. The worms were adult with a well-developed clitellum and not older than 1 year.

Adult *Eisenia fetida* were exposed in an artificial soil (5 % peat content) to the nominal test concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg test item/kg dry weight artificial soil. In this test 8 x 10 animals, approximately five months old, for the control group and 4 x 10 animals per test concentration of the treatment groups were used. The test item was mixed into the soil.

The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 - 800 Lux.

After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:

Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table (values in this table are rounded values).

Test object	*Eisenia fetida*					
Test item	**Control**	**Flufenacet-thiadone (AE 1258593, BCS-AA 41715)**				
mg test item/kg dry weight artificial soil	---	1.0	1.8	3.2	5.6	10.0
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	29.55	29.66	37.90	40.34	36.40	36.80
Standard Deviation	4.05	5.80	8.51	5.97	11.77	3.32
Mean number of offspring per test vessel after 56 days	340.1	324.5	338.3	303.0	274.8 **	271.0 **
Standard Deviation	33.8	43.1	72.4	30.6	16.5	20.1
Coefficient of variance (%)	9.9	13.3	21.4	10.1	6.0	7.4
% of control	---	95.4	99.4	89.1	80.8	79.7

* no statistical significance compared to the control (Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$)

** statistical significance compared to the control (Welch-t-test for inhomogeneous variance with Bonferroni-Holm adjustment, one-sided smaller, $\alpha = 0.05$)

The validity criteria of the test according to the guideline were fulfilled.

Validity criteria	**Recommended**	**Obtained**
Mortality of the adults in the control	≤ 10 %	0
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	340.1
Coefficient of variance of reproduction in the control	≤ 30 %	9.9 %

The results of the reference test item indicated that the test system was sensitive to the reference test item.

Observations:

Mortality

After 28 days of exposure no worms died in the control group and no mortality was observed at all test item concentrations.

Effects on growth

In all tested concentrations, no statistically significant different values for the growth relative to the control were observed (Williams multiple sequential t-test, two-sided, $\alpha = 0.05$.).

Therefore, based on biological and statistical significance:

NOEC related to growth: ≥ 10.0 mg test item/kg dry weight artificial soil

LOEC related to growth: > 10.0 mg test item/kg dry weight artificial soil

<u>Effects on reproduction</u>

Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the two highest test concentrations 5.6 and 10.0 mg test item/kg dry weight artificial soil (Welch-T test for inhomogeneous variances with Bonferroni-Holm adjustment, one-sided smaller, $\alpha = 0.05$).

Therefore, based on biological and statistical significance:
NOEC related to reproduction: 3.2 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 5.6 mg test item/kg dry weight artificial soil

Conclusions:

Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 3.2 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 5.6 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.1/09; [redacted]; 2005**
Title:	Effects of AE C502988 00 1B99 0001 on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Document No:	M-251328-01-1
Guidelines:	ISO 11268-2 (1998) BBA 1994: "Effects of pesticides on the reproduction and growth of *Eisenia fetida /Eisenia andrei*".
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to investigate the effects of AE C502988 00 1B99 0001 (trifluoroacetic acid, TFA) on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida*. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and BBA 1994).

Materials and Methods:

Test item: trifluoroacetic acid, Batch code: AE C502988 00 1B99 0001, Origin Batch No.; 18921, TOX No. 08523-00, content of a.s. (analysed): 98.8%.
Reference Item: Carbendazim
Control: untreated (and moistened with deionised water).
Test organism: Adult earthworms (*Eisenia fetida*). The mean body wet weight of the test organisms at the start of the test ranged from 321 to 521 mg per worm. The worms were adult with a well developed clitellum and approximately 11 months old.
Adult *Eisenia fetida* (4 x 10 animals per test concentration of the treatment group and 4 x 10 for the control) were exposed in an artificial soil to the nominal test concentrations of 10 - 32 - 100 - 320 and 1000 mg test item/kg dry weight artificial soil. The test item was mixed into the soil.
The test vessels were kept in a temperature-controlled room at 19-21°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 480 - 790 Lux.
During the test period, the temperature was in the range of 18 to 22°C. The measured mean light intensity was 552 Lux at day 0, 560 Lux at day 28 and 646 Lux at day 56 of the study.

After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 10% (5% in this study), reproduction of the control was ≥ 30 worms per container (246 - 375 worms in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (19.8% in this study).
Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the table below.

Test object	*Eisenia fetida*					
Test item	**Control**	**trifluoroacetic acid**				
Test concentration (mg test item/kg dws*)	---	10	32	100	320	1000
Mortality of adult earthworms [%] after 28 days	5	0	2.5	5	0	0
Standard Deviation	± 5.8	± 0	± 5.0	± 5.8	± 0	± 0
Statistical comparison to the control **	—	n.s.	n.s.	n.s.	n.s.	n.s.
Mean change of body weight of the adults from day 0 to day 28 [%]	+ 42.7	+ 36.3	+ 39.0	+ 34.2	+ 35.9	+ 28.4
Standard Deviation	± 4.5	± 6.0	± 3.3	± 8.1	± 8.5	± 5.7
Statistical comparison to the control **	—	n.s.	n.s.	n.s.	n.s.	s.
Mean number of offspring per test vessel after 56 days	291	307	377	304	322	309
Standard Deviation	± 58	± 89	± 31	± 97	± 28	± 20
Statistical comparison to the control ****	—	n.s.	n.s.	n.s.	n.s.	n.s.

Values in table are rounded
* dws = Dry weight artificial soil
** Result of a Fisher exact test, two-sided, α = 0.05
*** Result of a Dunnett test, two-sided, α = 0.05
**** Result of a Dunnett test, one sided smaller, α = 0.05
n.s.: mean value not statistically significant different compared to the control (p ≥ 0.05)
s.: mean value statistically significant different compared to the control (p < 0.05)

Observations:
Mortality
A mortality of 5% was observed in the control and at the concentration of 100 mg test item/kg soil and 2.5% of mortality were observed at 32 mg test item/kg soil. The mortality in the test item treated group was not significantly different compared to the control (Fisher exact test, α = 0.05) and is not considered to be treatment related since at the two highest concentrations no mortality was observed.

Effects on growth
The body weight changes of the test item treated groups were not significantly different compared to the control up to and including the concentration of 320 mg test item/kg soil (Dunnett test, α = 0.05, two sided). At 1000 mg test item/kg soil the body weights showed a weight increase of 28.4% which,

however, was statistically significantly lower compared to the control (Dunnett test, a = 0.05, two sided)

Effects on reproduction

The reproduction rates were not significantly different compared to the control in any test item treated groups (Dunnett test, α = 0.05, one sided smaller).

Conclusions:

Overall, based on the biological and statistical significance of the effects, it is concluded that the NOEC for this study is 1000 mg test item/kg dry weight artificial soil. Due to effects on body weighth changes, the NOEC for effects on growth is 320 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.1/10; [redacted], M. A.; 2012**
Title:	Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial
Document No:	M-436340-01-1
Guidelines:	ISO 11268-2 (1998) OECD Guideline 222 (2004)
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474) on survival, growth, and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with 2 different test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004).

Materials and Methods:

Test item: Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474); (Customer Order No. TOX 09477-00; Batch code: BCS-CU62474-01-01; Material BCS-CU62474; Origin Batch No.: NLL 8865-4-1; purity: 99.4 % w/w). Due to its pka-value < 2 FOE 5043-trifluroethanesulfonic acid is deprotonated under environmental conditions and hence the deprotonated form, FOE 5043-trifluoroethanesulfonate ($CF_3CH_2SO_3^-$) is used to test the toxicological properties of this metabolite.

Principles of the testing procedure: Adult *Eisenia fetida* (approx. 5 months old, 8 x 10 animals for the control group and treatment group) were exposed in an artificial soil (with 5 % peat content) to the nominal test concentration of 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Results:

Validity of the study:

Validity criteria	Recommended by the guideline	Obtained in this study
Mortality of the adults in the control	≤ 10 %	0
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	322.5 (294 – 365)
Coefficient of variance of reproduction in the control	≤ 30 %	6.3 %

All validity criteria were met. Therefore this study is valid.

The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table (values in this table are rounded values).

Effect of flufenacet-trifluoroethanesulfonic acid Na-salt on *Eisenia fetida* in a 56-day reproduction study

Test object	*Eisenia fetida*	
Test item	Control	Flufenacettrifluoroethanesulfonic acid Na-salt (BCS-CU62474)
mg test item/kg dry weight artificial soil	--	100
Mortality of adult earthworms [%] after 28 days	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	21.68	24.24
Standard Deviation	4.34	4.12
Mean number of offspring per test vessel after 56 days **	322.5	312.9
Standard Deviation	20.2	58.9
Coefficient of variance (%)	6.3	18.8
% of control	--	97.0

* statistical significance compared to the control (Student-t test for homogeneous variances, two-sided, α = 0.05)
** statistical significance compared to the control (Welch-t test for inhomogeneous variances, one-sided smaller, α = 0.05)

<u>Mortality</u>
After 28 days of exposure no worms died in the control group and no mortality was observed at any test item concentration.

<u>Effects on growth</u>
Statistically significant different values for the growth relative to the control were not observed.
Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 100 mg test item/kg dry weight artificial soil
LOEC related to growth: > 100 mg test item/kg dry weight artificial soil

<u>Effects on reproduction</u>
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed.
Therefore, based on biological and statistical significance:

NOEC related to reproduction: ≥ 100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: > 100 mg test item/kg dry weight artificial soil

Conclusions:
Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is ≥ 100 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be > 100 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.1/11; [redacted], T., 2008**
Title:	Flufenacet SC 500: effect on the earthworm fauna of a grassland area within one year
Document No:	M-307211-01-1
Guidelines:	BBA (Federal Biological Research Centre for Agriculture and Forestry, Germany): Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2 - 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field ISO (International Standard Organisation): Guideline CD 11268-3 (E), Soil Quality - Effects of pollutants on Earthworms, Part 3: Guidance on the determination of effects in field situations (1999);
GLP	Yes (certified laboratory)

Material and methods:
The effects of Flufenacet SC 500 (content of Flufenacet. (analysed): 499.9 g/L, Batch-No.: EFKF000175, TOX-No.: 07958-00) on earthworm populations under field conditions were studied. To ensure an abundant earthworm population, an area was selected which was used as grassland for several years, located in [redacted] (Germany). The soil was characterized as loamy sand. On April 19, 2007 a presampling of earthworms was conducted to ensure a sufficient number of earthworms being present at the test plot. Four selected plots within this area were treated with 1.2 l Flufenacet SC 500/ha on May 22, 2007. Four untreated plots served as negative controls, as positive control 4 plots were treated with Carbendazim (8 kg/ha). Within three days after application 14.5 mm of precipitation was measured. All plots were screened for alive and dead earthworms on the soil surface within three days after the applications. For chemical verification of the exposure soil samples from the control and from the treated plots were taken on May 22, 2007 after the applications and analysed for the presence of Flufenacet. On treated plots Flufenacet was detected on average in a concentration of 0.438 mg/kg dry weight soil, assuming a soil depth of 10 cm and a soil density of 1.5 g/cm³. This is equivalent to 110% of the nominal application rate of 1.2 l Flufenacet SC500/ha resulting in a nominal concentration of 0.399 Flufenacet mg/kg dry weight soil.

The earthworm numbers and biomass were determined nine weeks (July 25, 2007), five months (October 30, 2007) and eleven months (April 22, 2008) after application by sampling earthworms using formalin method. At each sampling time 16 samples per treatment (4 plots, 4 samples per plot) were collected.

Findings and observations:

Earthworm number and diversity in pre-sampling and in the control plots:

The abundance of earthworms at the study site was determined 5 weeks before the application of the test substance (April 19, 2007) by pre-sampling using the formalin method. The mean total abundance of earthworms determined was 196 worms/m^2. The five species *Lumbricus terrestris, Lumbricus rubellus, Lumbricus castaneus, Aporrectodea caliginosa,* were found. Nine weeks after the application the mean number of earthworms in the control plots, sampled with the formalin method, was determined to be 113 earthworms/m^2, five months after the application 164 earthworms/m^2 and eleven months after the application 306 earthworms/m^2., respectively

Six different earthworm species were identified in the test area at different abundances: *Lumbricus terrestris, Lumbricus rubellus, Lumbricus castaneus, Aporrectodea caliginosa, Allolobophora chlorotica* and *Aporrectodea terrestris longa*.

These data indicate, that the earthworm population of the selected test area can be assumed to be quite high (BAUCHHENSS 1982, EDWARDS & LOFTY 1977, KENNEL & NIKLAS 1980).

Adult and juvenile earthworms, changes in numbers and biomass:

Data for category "**adult and juvenile**" and for the species classes "total", "total anecic", "total endogeic" and "total epigeic" earthworms. The values are replicate means (n = 4) and standard deviations per 0.25 m². Values between parentheses are relative differences to the control in %:

Treatment group	9 weeks after the application		5 months after the application		11 months after the application	
	Numbers (n) / replicate					
	Total earthworms					
Control	28.31 ± 3.46		40.88 ± 2.99		76.50 ± 14.86	
Flufenacet	20.75 ± 3.69	(-27%) *	39.81 ± 8.61	(-3%)	76.19 ± 5.54	(0%)
Carbendazim	13.88 ± 2.92	(-51%) *	40.88 ± 8.61	(0%)	54.00 ± 6.07	(-29%)*
	Total of anecic earthworms					
Control	10.06 ± 1.55		25.25 ± 2.35		17.06 ± 3.13	
Flufenacet	9.63 ± 1.16	(-4%)	24.56 ± 3.64	(-3%)	19.75 ± 1.34	(+16%)
Carbendazim	3.38 ± 1.05	(-66%) *	20.06 ± 2.49	(+21%) *	15.63 ± 4.09	(-8%)
	Total of endogeic earthworms					
Control	8.81 ± 3.99		5.44 ± 2.81		53.63 ± 13.68	
Flufenacet	5.13 ± 2.72	(-42%)	6.81 ± 4.52	(+25%)	48.88 ± 5.11	(-8%)
Carbendazim	2.81 ± 1.71	(-68%) *	9.69 ± 2.38	(+78%)	26.69 ± 4.93	(-50%)*
	Total of epigeic earthworms					
Control	9.44 ± 1.48		10.19 ± 3.45		6.31 ± 2.15	
Flufenacet	6.00 ± 1.15	(-36%) *	8.44 ± 1.13	(-17%)	7.56 ± 2.68	(+20%)
Carbendazim	7.69 ± 3.15	(-19%)	11.43 ± 7.74	(+9%)	11.69 ± 3.78	(+85%)
	Biomass (g) / replicate					
	Total earthworms					
Control	18.21 ± 3.31		36.46 ± 9.78		44.79 ± 5.64	
Flufenacet	15.83 ± 4.86	(-13%)	36.45 ± 4.32	(0%)	47.64 ± 2.47	(+6%)
Carbendazim	5.74 ± 1.25	(-68%) *	28.34 ± 6.23	(-22%)	32.84 ± 2.67	(-27%)*
	Total of anecic earthworms					
Control	16.42 ± 3.08		33.34 ± 9.81		24.01 ± 4.22	
Flufenacet	15.13 ± 4.75	(-8%)	32.54 ± 2.54	(-2%)	28.00 ± 3.47	(+17%)
Carbendazim	4.39 ± 1.34	(-73%) *	18.96 ± 4.74	(-43%)	16.11 ± 4.44	(-33%)
	Total of endogeic earthworms					
Control	1.12 ± 0.54		2.09 ± 0.98		20.23 ± 4.36	
Flufenacet	0.40 ± 0.23	(-64%) *	2.86 ± 1.48	(+37%)	18.86 ± 2.96	(-7%)
Carbendazim	0.70 ± 0.53	(-38%)	7.11 ± 2.03	(+241%) *	15.19 ± 1.63	(-25%)
	Total of epigeic earthworms					
Control	0.67 ± 0.26		1.04 ± 0.31		0.55 ± 0.24	
Flufenacet	0.29 ± 0.26	(-56%) *	1.06 ± 0.50	(+2%)	0.78 ± 0.12	(+41%)
Carbendazim	0.66 ± 0.28	(-2%)	2.27 ± 1.54	(+119%) *	1.54 ± 0.27	(+180%)*

* indicates a statistically significant difference between treatment and control (Wilcoxon, Mann and Withney U-Test, p = 0.05)

An application of 1.2 l product/ha Flufenacet SC 500 has no statistically significant effect on the parameters "numbers" and "biomass" of all tested categories earthworms five and 11 months after the application, indicating no effect of Flufenacet on the earthworm community. However nine weeks after application for the category "total earthworms" a statistically significant reduction in number of -27% and a statistically insignificant reduction of the biomass of -13 % were observed. The group of anecic earthworms was not affected on Flufenacet treated plots nine weeks after application (Numbers -4 %; biomass -8%). The ecological groups of endogeic (Number -42%; biomass -64 %) and epigeic (Number -36 %; biomass -56%) earthworms were reduced on Flufenacet treated plots nine weeks after application. A possible explanation for this observation is the influence of Flufenacet acting as herbicide on the vegetation of the treated plots. Although all plots were treated with Glyphos before start of the test, untreated plots showed a regrowing of weeds. Especially in the dry summer period this has a strong influence on the water regime of the soil thereby affecting the habitat of the endo- and epigeic earthworms. Therefore this variation is not considered to be a compound related effect but rather a secondary effect of the herbicide Flufenacet on the earthworm community.

Adult earthworms; changes in numbers and biomass:
Data for category "**adult**" and for the species classes "total", "total anecic", "total endogeic" and "total epigeic" earthworms. The values are replicate means (n = 4) and standard deviations per 0.25 m². Values between parentheses are relative differences to the control in %:

Treatment group	9 weeks after the application		5 months after the application		11 months after the application	
	Numbers (n) / replicate					
	Total earthworms					
Control	5.25 ± 1.46		14.88 ± 3.11		20.94 ± 6.62	
Flufenacet	4.56 ± 1.61	(-13%)	14.81 ± 1.36	(0%)	21.63 ± 2.90	(+3%)
Carbendazim	2.00 ± 0.61	(-62%) *	19.25 ± 6.00	(+29%)	20.13 ± 2.22	(-4%)
	Total of anecic earthworms					
Control	4.94 ± 1.03		10.44 ± 3.07		7.38 ± 2.39	
Flufenacet	4.56 ± 1.61	(-8%)	10.31 ± 0.90	(-1%)	8.63 ± 1.11	(+17%)
Carbendazim	1.44 ± 0.69	(-71%) *	5.00 ± 1.06	(-52%) *	3.63 ± 1.74	(-51%) *
	Total of endogeic earthworms					
Control	0.13 ± 0.16		1.88 ± 1.70		12.38 ± 5.04	
Flufenacet	0 ± 0	(-100%)	2.44 ± 1.18	(+30%)	10.56 ± 2.13	(-15%)
Carbendazim	0.31 ± 0.38	(+150%)	7.38 ± 2.66	(+293%)*	11.94 ± 1.82	(-4%)
	Total of epigeic earthworms					
Control	0.19 ± 0.24		2.56 ± 1.74		1.19 ± 0.69	
Flufenacet	0 ± 0	(-100%)	2.06 ± 0.97	(-20%)	2.44 ± 0.92	(+105%)
Carbendazim	0.25 ± 0.20	(+33%)	6.88 ± 6.47	(+168%)	4.56 ± 1.60	(+284%) *
	Biomass (g) / replicate					
	Total earthworms					
Control	11.20 ± 2.28		25.63 ± 9.26		24.50 ± 4.72	
Flufenacet	10.10 ± 3.96	(-10%)	25.40 ± 3.63	(-1%)	26.19 ± 3.71	(+7%)
Carbendazim	3.11 ± 1.08	(-72%) *	18.48 ± 3.99	(-28%)	18.02 ± 2.86	(-26%)
	Total of anecic earthworms					
Control	11.05 ± 2.06		24.29 ± 9.07		16.51 ± 4.05	
Flufenacet	10.10 ± 3.96	(-9%)	23.44 ± 3.28	(-3%)	19.42 ± 2.94	(+18%)
Carbendazim	2.83 ± 1.20	(-74%) *	10.77 ± 3.30	(-56%) *	7.93 ± 4.17	(-52%)*
	Total of endogeic earthworms					
Control	0.08 ± 0.16		0.82 ± 0.62		7.78 ± 3.38	
Flufenacet	0 ± 0	(-100%)	1.43 ± 0.61	(+74%)	6.35 ± 1.33	(-18%)
Carbendazim	0.19 ± 0.24	(+136%)	5.83 ± 2.07	(+610%) *	9.05 ± 1.68	(+16%)
	Total of epigeic earthworms					
Control	0.07 ± 0.12		0.53 ± 0.32		0.21 ± 0.18	
Flufenacet	0 ± 0	(-100%)	0.54 ± 0.37	(+2%)	0.42 ± 0.04	(+103%)
Carbendazim	0.09 ± 0.11	(+19%)	1.89 ± 1.49	(+259%) *	1.04 ± 0.16	(+401%) *

*) indicates a statistically significant difference between treatment and control (Wilcoxon, Mann and Withney U-Test, p= 0.05)

An application of 1.2 L product/ha Flufenacet SC 500 has no statistically significant effect on the parameters "numbers" and "biomass" of the categories "total", "total anecic", "total endogeic" and "total epigeic" adult earthworms compared to control plots five and 11 months after the application.
Nine weeks after application also no statistically significant differences between Flufenacet and control plots were found. However the number of earthworms identified in the categories epigeic and endogeic were less than 0.31 earthworm/m². This abundance is too low to perform an appropriate statistical analysis of the data. In addition this data also indicates that the analysis for the 9 week sampling should not be overestimated.

Juvenile worms; changes in numbers and biomass:
Data for category "**juvenile**" and for the species classes "total", "total anecic", "total endogeic" and "total epigeic" earthworms. The values are replicate means (n = 4) and standard deviations per 0.25 m². Values between parentheses are relative differences to the control in %.

Treatment group	9 weeks after the application		5 months after the application		11 months after the application	
	Numbers (n) / replicate					
	Total earthworms					
Control	23.06 ± 2.92		26.00 ± 2.39		55.56 ± 9.64	
Flufenacet	16.19 ± 3.36	(-30%) *	25.00 ± 7.43	(-4%)	54.56 ± 7.63	(-2%)
Carbendazim	11.88 ± 2.66	(-49%) *	21.63 ± 3.00	(-17%)	33.88 ± 4.09	(-39%)*
	Total of anecic earthworms					
Control	5.13 ± 0.60		14.81 ± 1.31		9.69 ± 1.14	
Flufenacet	5.06 ± 0.63	(-1%)	14.25 ± 3.52	(-4%)	11.15 ± 1.05	(+15%)
Carbendazim	1.94 ± 0.47	(-62%) *	15.06 ± 1.66	(+2%)	12.00 ± 3.33	(+24%)
	Total of endogeic earthworms					
Control	8.69 ± 4.05		3.56 ± 1.39		40.75 ± 9.43	
Flufenacet	5.13 ± 2.72	(-41%)	4.38 ± 3.62	(+23%)	38.31 ± 7.80	(-6%)
Carbendazim	2.50 ± 1.34	(-71%) *	2.31 ± 1.71	(-35%)	14.75 ± 3.38	(-64%)*
	Total of epigeic earthworms					
Control	9.25 ±1.34		7.63 ± 1.76		5.13 ± 2.05	
Flufenacet	6.00 ±1.15	(-35%) *	6.38 ± 0.72	(-16%)	5.13 ± 2.11	(0%)
Carbendazim	7.44 ± 3.11	(-20%)	4.25 ± 1.66	(-44%)*	7.13 ± 2.24	(+39%)
	Biomass (g) / replicate					
	Total earthworms					
Control	7.01 ±1.07		10.83 ± 1.15		20.30 ± 2.16	
Flufenacet	5.73 ±1.40	(-18%)	11.05 ± 1.99	(+2%)	21.44 ± 2.07	(+6%)
Carbendazim	2.65 ±0.31	(-62%) *	9.86 ± 2.41	(-9%)	14.82 ± 1.54	(-27%)*
	Total of anecic earthworms					
Control	5.38 ±1.20		9.05 ± 1.31		7.50 ± 1.16	
Flufenacet	5.03 ±1.20	(-6%)	9.10 ± 1.69	(+1%)	8.58 ± 1.40	(+14%)
Carbendazim	1.56 ±0.18	(-71%) *	8.19 ± 1.68	(-9%)	8.18 ± 1.16	(+9%)
	Total of endogeic earthworms					
Control	1.04 ±0.61		1.27 ± 0.56		12.45 ± 1.29	
Flufenacet	0.40 ±0.23	(-61%)	1.43 ± 1.00	(+13%)	12.51 ± 3.24	(0%)
Carbendazim	0.50 ±0.30	(-55%)	1.35 ± 0.97	(+6%)	6.24 ± 1.05	(-50%)*
	Total of epigeic earthworms					
Control	0.60 ±0.16		0.51 ± 0.09		0.34 ± 0.18	
Flufenacet	0.29 ±0.07	(-51%) *	0.52 ± 0.20	(+1%)	0.36 ± 0.09	(+4%)
Carbendazim	0.57 ±0.23	(-5%)	0.38 ± 0.13	(-26%)	0.50 ± 0.15	(+46%)

*) indicates a statistically significant difference between treatment and control (Wilcoxon, Mann and Withney U-Test, p= 0.05)

An application of 1.2 L product/ha Flufenacet SC 500 has no statistically significant effect on the parameters “numbers” and “biomass” of the categories “total”, “total anecic”, “total endogeic” and “total epigeic” juvenile earthworms five and 11 months after the application. Nine weeks after application also no statistically significant differences in number and biomass between Flufenacet and control plots for the categories “total anecic” and “total endogeic” were found. For the category “total” the number of earthworm was reduced by 30 % and for the group of “total epigeic” earthworms the biomass was reduced by 51 %. A possible explanation for this observation is the influence of Flufenacet acting as herbicide on the vegetation of the treated plots. Although all plots were treated with Glyphos before start of the test, untreated plots showed a regrowing of weeds. Especially in the dry summer period this has a strong influence on the water regime of the soil thereby affecting the habitat of the endo- and epigeic earthworms. Therefore this variation is not considered to be a compound related effect but rather a secondary effect of the herbicide Flufenacet on the earthworm community.

Conclusions:
The present earthworm field study shows, that Flufenacet SC 500 applied at a rate of 1,2 l product/ha on grassland has no adverse effect on the population of earthworms 11 months after the application date (Table 1). Compared to the control plots, plots treated with Flufenacet SC 500 showed changes of the relative abundance of adult & juvenile earthworms relative to control of 0% (number) and +6% (biomass) 11 months after application. 5 months after application plots treated with Flufenacet SC500 showed a reduction in the total number of juvenile and adult earthworms by -3 % and no change in the biomass compared to control plots. Nine weeks after application of Flufenacet SC500 a relative reduction of adult & juvenile earthworms of -27 % (number) and -13 % (biomass) was observed.

Changes in numbers and biomass for juvenile & adult earthworms, summary
The values are replicate means (n = 4) and standard deviations per 0.25 m². Values between parentheses are relative differences to the control in %;

Treatment group	9 weeks after the application		5 months after the application		11 months after the application	
	Relative **number** of juvenile & adult earthworms in the study plots (from replicate means)					
	Total earthworms					
Control	28.31 ± 3.46		40.88 ± 2.99		76.50 ± 14.86	
Flufenacet	20.75 ± 3.69	(-27%) *	39.81 ± 8.61	(-3%)	76.19 ± 5.54	(0%)
Carbendazim	13.88 ± 2.92	(-51%) *	40.88 ± 8.61	(0%)	54.00 ± 5.07	(-29%) *
	Relative changes of **biomass** of juvenile & adult earthworms in the study plots (from replicate means)					
Control	18.21 ± 3.31		36.46 ± 9.78		44.79 ± 5.64	
Flufenacet	15.83 ± 4.86	(-13%)	36.45 ± 4.32	(0%)	47.64 ± 2.47	(+6%)
Carbendazim	5.74 ± 1.25	(-68%) *	28.34 ± 6.23	(-22%)	32.84 ± 2.67	(-27%) *

*) Significant difference from control according to the U-test, two sided at the significance level alpha = 0.05 (U-test from Wilcoxon, Mann and Whitney after SACHS 1978).

Overall no effect according to the criteria defined by the EPPO standards (2003) of more than 30 % difference between control and Flufenacet SC 500 treated plots was observed at nine weeks, 5 months or 11 months after application of Flufenacet SC500.
In addition, there were no negative findings within three days directly after the application.
Considering the variability of earthworm abundances in natural soils, this study indicates that earthworm populations were not adversely affected by the application of Flufenacet SC 500 of 1.2 L product/ha.

CA 8.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

Report:	**KCA 8.4.2.1/01, [redacted], M.-A., 2009**
Title:	Flufenacet-methylsulfone: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil with 5 % peat
Document No.:	M-357707-01-1
Guidelines:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP	yes (certified laboratory)

Objectives:

The purpose of the study was to assess the effects of Flufenacet-methylsulfone on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil with 5% peat comparing control and treatment.

Material and Methods:

Test item: Flufenacet-methylsulfone, Batch Code BCS-CO62475-01-01, Origin Batch No. SES 10623-5-1, TOX 08624-00, analysed content of 97.6% Flufenacet-methylsulfone.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each application rate) were exposed to control (water treated), 63, 125, 250, 500 and 1000 mg test item/kg dry weight artificial soil. The test item was applied by mixing into the artificial soil. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (35 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2°C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2 % Calcium carbonate ($CaCO_3$).

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Test item Test object Exposure	Flufenacet-methylsulfone *Hypoaspis aculeifer* Artificial Soil		
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**
Control	3.8	355.5 ± 31.1	-
63	5.0	370.8 ± 22.8	104.3
125	0.0	387.0 ± 28.2	108.9
250	5.0	390.5 ± 16.8	109.8
500	0.0	374.5 ± 24.6	105.3
1000	5.0	304.3 ± 10.9	85.6*
			Reproduction
NOEC (mg test item/kg dry weight artificial soil)			500 mg test item /kg
LOEC (mg test item/kg dry weight artificial soil)			1000 mg test item/kg

* statistical significance (Williams Test one-sided smaller, $\alpha = 0.05$)

Observations:

In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is within the recommended range of ≤ 20 % mortality. An LC_{50} cannot be calculated and is considered to be >1000 mg test item/kg dry artificial soil.

Concerning the number of juveniles statistical analysis (Williams Test, one-sided smaller, $\alpha = 0.05$) revealed significant differences between the control and 1000 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 500 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect- Concentration (LOEC) for reproduction is 1000 mg test item/kg dry weight artificial soil. An EC_{50} could not be calculated and is considered to be >1000 mg test item/kg dry artificial soil.

Conclusions:

NOEC: 500 mg test item/kg dry weight artificial soil.
LOEC: 1000 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.2.1/02, [redacted] U., 2010**
Title:	Flufenacet a.s.: Influence on the reproduction of the collembola species *Folsomia candida* tested in artificial soil with 5% peat
Document No.:	M-363896-01-1
Guidelines:	ISO 11267 (1999)
GLP	yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet a.s. on survival and reproduction of the collembola species *Folsomia candida* during an exposure of 28 days in an artificial soil at 5 different test concentrations.

Materials and Methods:

Flufenacet a.s., 97.5 % w/w analysed content, origin batch no.: K664078, customer order no: TOX 07969-01, specification no.: 102000006978, LIMS no.: 0906063.

Toxic standard: Betosip, active ingredient: Phenmedipham (153 g/L).
Control: same application as test item but with deionised water and quartz sand only.
Ten Collembola (10-12 days old) per replicate (5 replicates per treatment group) were exposed to control (water treated), 32, 63, 125, 250 and 500 mg test item/kg artificial soil dry weight at 18–22°C, 400 – 800 Lux, 16h light : 8h dark, 5 % peat in the artificial soil. During the test they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 10% (8% in this study), reproduction of the control was ≥ 100 juveniles per control vessel (1050 juveniles in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (6.7% in this study).

Test item **Test object** **Exposure**	**Flufenacet a.s.** ***Folsomia candida*** **Artificial Soil**		
mg test item/kg soil (dw)[1)] nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	8	1050 ± 71	-
32	12	973 ± 156	93 n.s.
63	2	1181 ± 153	113 n.s.
125	8	665 ± 262	63 *
250	8	301 ± 57	29 *
500	12	156 ± 17	15 *
NOEC (mg test item/kg soil (dw)) LOEC (mg test item/kg soil (dw))			63 125

1) Dry weight
* Statistically significant (Dunnett's Test one-sided-smaller, α = 0.05)
n.s. = statistically not significant (Holms Bonferroni U-test one-sided-smaller, α = 0.05)

Observations:
The highest mortality rate of 12 % was found in the test with 32 and 500 mg test item/kg artificial soil dry weight. Concerning the number of juveniles statistical analysis revealed significant differences between the control and the treatment groups with 125, 250 and 500 mg test item/kg artificial soil dry weight.

Conclusions:
$NOEC_{reproduction}$: 63 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: 125 mg test item/kg artificial soil dry weight.

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Report:	**KCA 8.4.2.1/03, [redacted], M.-A., 2010**
Title:	Flufenacet-oxalate: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil with 5 % peat
Document No.:	M-393634-01-1
Guidelines:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP	yes (certified laboratory)

Objectives:

The purpose of the study was to assess the effects of Flufenacet-oxalate on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil with 5% peat at 100 mg test item/kg dry weight artificial soil and control.

Material and Methods:

Test item: Flufenacet-oxalate, Batch Code BCS-AB16305-01-01, Origin Batch No. SES 10564-3-1, Material BCS AB16305, technical substance; Customer Order No. Tox 08524-02; Purity 95.3 % w/w.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 8 replicates treatment replicates) were exposed to control (water treated) and 100 mg test item/kg dry weight artificial soil. The test item was applied by mixing a test item-quartz sand-mixture into the artificial soil. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (29 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2°C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2 % Calcium carbonate ($CaCO_3$).

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Test item **Test object** **Exposure**	**Flufenacet-oxalate** ***Hypoaspis aculeifer*** **Artificial Soil**				
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**			**Reproduction (% of control)**
Control	7.5	288.1	±	55.3	-
100	15.0	248.1	±	53.5	86.1
					Reproduction
NOEC (mg test item/kg dry weight artificial soil)					≥100
LOEC (mg test item/kg dry weight artificial soil)					>100

No statistical significance (Student-t test one sided smaller, α = 0.05)

Observations:

In the control group 7.5 % of the adult *Hypoaspis aculeifer* died which is within the allowed range of ≤ 20 % mortality. An LC_{50} cannot be calculated and is considered to be >100 mg test item/kg dry artificial soil.

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant differences between the control and treatment. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥100 mg test item/ kg dry weight artificial soil. The Lowest-Observed-Effect- Concentration (LOEC) for reproduction is >100 mg test item/ kg dry weight artificial soil. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg dry artificial soil.

Conclusions:
NOEC: ≥100 mg test item/kg dry weight artificial soil.
LOEC: >100 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.2.1/04; [redacted], U.; 2010**
Title:	Flufenacet-oxalate: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Document No:	M-394712-01-1
Guidelines:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
GLP	Yes (certified laboratory)

Objective:
The purpose of this study was to assess the effect of Flufenacet-oxalate on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test item: Flufenacet-oxalate analysed content 95.3 % w/w, batch code: BCS-AB 16305-01-01, origin batch no.: SES 10564-3-1, LIMS No.: 1012990, Customer order no.: TOX 08524-02, certificate no.: MZ 00288.
10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 Lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 20% (3.8% in this study), reproduction of the control was ≥ 100 juveniles per control vessel (1450 juveniles in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (5.2% in this study).

Test item		Flufenacet-oxalate	
Test object		*Folsomia candida*	
Exposure		Artificial Soil	
Mg test item/kg soil dry weight (nominal concentration)	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	3.8	1450 ± 76	-
100	12.5	1487 ± 116	103 [n.s.]
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			> 100

The calculations were performed with unrounded values
n.s. = statistically not significant (Student-t-test, one-sided smaller, $\alpha = 0.05$)

Observations:

Mortality:

In the control group 3.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Reproduction:

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between the control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Conclusions:

$NOEC_{reproduction}$: ≥ 100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: > 100 mg test item/kg artificial soil dry weight.

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Report:	**KCA 8.4.2.1/05; [redacted], U.; 2010**
Title:	Flufenacet-sulfonic acid Na-salt: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Document No:	M-396039-01-1
Guidelines:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet-sulfonic acid Na-salt on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Flufenacet-sulfonic acid Na-salt (analytical findings: 92.4 % w/w Flufenacet-sulfonic acid Na-salt (AE 0841914), origin batch no.: SES 10294-6-2, customer order no.: TOX 08523-03, batch code: AE F 0841914-01-03, LIMS no.: 1017204.

Toxic standard: Boric acid.
Control: same application as test item but with quartz sand only.
Ten Collembola (10-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 Lux, 16h light : 8h dark, 5 % peat in the artificial soil. During the test they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 20% (12.5% in this study), reproduction of the control was ≥ 100 juveniles per control vessel (1283 juveniles in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (8.3% in this study).

Test item **Test object** **Exposure**	**Flufenacet-sulfonic acid Na-salt** ***Folsomia candida*** **Artificial Soil**		
mg test item/kg soil (dw)[1)] nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	12.5	1283 ± 107	-
100	8.8	1382 ± 63	108 n.s.
NOEC (mg test item/kg soil (dw))			≥100
LOEC (mg test item/kg soil (dw))			>100

1) Dry weight
n.s. = statistically not significant (Student-t-test, one-sided-smaller, $\alpha = 0.05$)

Observations:
Concerning the number of juveniles statistical analysis revealed no significant difference between control and treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is considered to be ≥100 mg test item/kg artificial soil dry weight and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >100 mg test item/kg artificial soil dry weight.

Conclusions:
$NOEC_{reproduction}$: ≥100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: >100 mg test item/kg artificial soil dry weight.

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Report:	**KCA 8.4.2.1/06, [redacted],U.; 2012**
Title:	Trifluoroacetic acid Na-salt (BCS-AZ56567): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Document No.:	M-436127-01-1
Guidelines:	OECD 232 (2009)
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of trifluoroacetic acid Na-salt (BCS-AZ56567) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Test item: Trifluoroacetic acid Na-salt (BCS-AZ56567); Report name: Natrium-trifluoroacetat; Material: AE 1046319; Batch code: AE 1046319-01-01; Origin batch No.: SES 11755-1-1; Customer order no.: TOX 09476-01; Analyzed content: 95.1 % w/w. Due to its pk_a-value < 2 trifluoroacetic acid is deprotonated under environmental conditions and hence the deprotonated form, trifluoroacetate (CF_3COO^-) is used to test the toxicological properties of this metabolite.

10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2 °C, 400 – 800 lux and 16h light to 8h dark. During the study, collembolans were fed with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	16.3%
Average reproduction rate in the control	≥ 100	1132.6
Coefficient of variation of reproduction	< 30%	9.7%

All validity criteria for the study were met.

Reference test:

The most recent non-GLP-test (Bayer Report No.: FRM-Coll-Ref-19/12, May 25, 2012) with the reference item boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95 % confidence limits from 98 mg to 137 mg boric acid/kg artificial soil dry weight), which is in the recommended range of the guideline (OECD 232, 2009) of about 100 mg boric acid/kg artificial soil dry weight showing that the test organisms were sufficiently sensitive.

Mortality:

In the control group 16.3% of the adult *Folsomia candida* died, while the mortality rate in the test group was 10%.

Reproduction:

The mean number of juveniles in the control was 1132.6 ± 110.4 and 1051.9 ± 133.4 in the test group. Statistical analysis (Student's t-test, one-sided smaller, α = 0.05) of the number of juveniles revealed no significant difference between control and the treatment group.

Survival and reproduction of collembolans after 4 weeks of treatment with natrium-trifluoroacetat

Test item	**Trifluoroacetic acid Na-salt (BCS-AZ56567)**		
Test object	***Folsomia candida***		
Exposure	**Artificial soil**		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	16.3	1132.6 ± 110.4	-
100	10.0	1051.9 ± 133.4	92.9 $^{n.s.}$
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			> 100

The calculations were performed with un-rounded values
SD = standard deviation
n.s. = statistically not significant (Student's t-test one-sided-smaller, α = 0.05)

Conclusions:

$NOEC_{reproduction}$: ≥ 100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: > 100 mg test item/kg artificial soil dry weight.

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Report:	**KCA 8.4.2.1/07; [redacted], U.; 2012**
Title:	Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Document No:	M-436128-01-1
Guidelines:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474,) analytical findings: 99.4 % w/w, origin batch no.: NLL 8865-4-1, customer order no.: TOX 09477-00, batch code: BCS-CU62474-01-01, material: BCS-CU62474. Due to its pk_a-value < 2 FOE 5043-trifluroethanesulfonic acid is deprotonated under environmental conditions and hence the deprotonated form, FOE 5043-trifluoroethanesulfonate ($CF_3CH_2SO_3^-$) is used to test the toxicological properties of this metabolite.
The most recent non-GLP-test (FRM-Coll-Ref-19/12, U. [redacted], May 25, 2012) with the reference item Boric acid showed that that the test organisms were sufficiently sensitive.
10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 lux, 16h light: 8h dark. During the study, they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Findings:

Validity criteria for the untreated control of the study according OECD 232 from September 07, 2009

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20 %	16.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1132.6
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	9.7 %

The results can be considered as valid, as all validity criteria of the test were met.

Survival and reproduction of collembolans after 4 weeks of treatment

Test item	**Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474)**		
Test object	***Folsomia candida***		
Exposure	**Artificial soil**		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	16.3	1132.6 ± 110.4	-
100	12.5	1066.0 ± 64.9	94.1 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			> 100

The calculations were performed with un-rounded values
n.s. = statistically not significant (Student-t test one-sided-smaller, α = 0.05)

Observations:

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight.

Conclusions:

$NOEC_{reproduction}$: ≥ 100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: > 100 mg test item/kg artificial soil dry weight

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Report: **KCA 8.4.2.1/08, [redacted], M. A., 2012**
Title: Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Document No.: M-436315-01-1
Guidelines: OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals – Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP: yes (certified laboratory)

Objectives:

The purpose of the study was to assess the effects of Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and methods:

Test item: Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474); (Batch code: BCS-CU62474-01-01; Origin Batch No.: NLL 8865-4-1; Material: BCS-CU62474; Certificate No.: MZ 00482; Customer order No.: TOX 09477-00; purity: 99.4 %w/w)

Due to its pk_a-value < 2 FOE 5043-trifluroethanesulfonic acid is deprotonated under environmental conditions and hence the deprotonated form, FOE 5043-trifluoroethanesulfonate ($CF3CH2SO3^-$) is used to test the toxicological properties of this metabolite.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 100 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$).

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water, 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Results:

Validity of the study:

Validity criteria (control values)	Recommended by the guideline	Obtained in this study
Mean adult female mortality	≤ 20 %	2.5 %
mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	346.5
coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	6.8 %

All validity criteria were met. Therefore this study is valid.

The most recent non-GLP-test ([redacted]-[redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate showed that the test organisms are sufficiently sensitive according to the guideline.

Effect of flufenacet-trifluoroethanesulfonic acid Na-salt on soil mite species *Hypoaspis aculeifer* in a 14-day reproduction study

Test item	**Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474)**		
Test object	***Hypoaspis aculeifer***		
Exposure	**Artificial Soil**		
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	2.5	346.5 ± 23.5	---
100	5	387.9 ± 36.8	111.9
NOEC (mg test item/kg dry weight artificial soil)			≥ 100
LOEC (mg test item/kg dry weight artificial soil)			> 100

No statistical significance (Student t-test for homogeneous variances, one-sided smaller, $\alpha = 0.05$) was found.

Mortality

In the control group 2.5 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction

Concerning the number of juveniles statistical analysis (Student t-test for homogeneous variances, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil.

Conclusions:

NOEC: ≥ 100 mg test item/kg dry weight artificial soil.
LOEC: > 100 mg test item/kg dry weight artificial soil.

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Report: **KCA 8.4.2.1/09, [redacted], M. A., 2012**
Title: Trifluoroacetic acid Na-salt (BCS-AZ56567): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Document No: M-436326-01-1
Guidelines: OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP yes (certified laboratory)

Objectives:

The purpose of the study was to assess the effects of trifluoroacetic acid Na-salt on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil with 5% peat comparing control and treatment.

Materials and methods:

Test item: Trifluoroacetic acid Na-salt (BCS-AZ56567); (Batch code: AE 1046319-01-01; Origin Batch No. SES 11755-1-1; Material: AE 1046319; Certificate No.: MZ 00513; Customer order No.: TOX 09476-01; purity: 95.1 %w/w). Due to its pka-value < 2 trifluoroacetic acid is deprotonated under environmental conditions and hence the deprotonated form, trifluoroacetate (CF3COO-) is used to test the toxicological properties of this metabolite.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 100 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and approximately 0.2 % Calcium carbonate (CaCO3).

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Results:

Validity of the study:

Validity criteria (control values)	Recommended by the guideline	Obtained in this study
Mean adult female mortality	≤ 20 %	2.5 %
mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	346.5
coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	6.8 %

All validity criteria were met. Therefore this study is valid.

The most recent non-GLP-test ([redacted]-[redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate showed that the test organisms are sufficiently sensitive according to the guideline.

Effect of trifluoroacetic acid Na-salt on soil mite species *Hypoaspis aculeifer* in a 14-day reproduction study

Test item **Test object** **Exposure**	**Trifluoroacetic acid Na-salt (BCS-AZ56567)** ***Hypoaspis aculeifer*** **Artificial Soil**		
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)
Control	2.5	346.5 ± 23.5	---
100	0.0	372.1 ± 19.1	107.4
NOEC (mg test item/kg dry weight artificial soil) LOEC (mg test item/kg dry weight artificial soil)			≥ 100 > 100

No statistical significance (Student *t*-test for homogeneous variances, one-sided smaller, α = 0.05) was found.

Mortality

In the control group 0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction

Concerning the number of juveniles statistical analysis (Student t-test for homogeneous variances, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >100 mg test item/kg dry weight artificial soil.

Conclusions:

NOEC: ≥ 100 mg test item/kg dry weight artificial soil.
LOEC: > 100 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.2.1/10, [redacted], U., 2012**
Title:	Flufenacet-thiadone (BCS-AA41715): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Document No.:	M-440372-01-1
Guidelines:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of flufenacet-thiadone (BCS-AA41715) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Test item: Flufenacet-thiadone, Synonyms: AE 1258593, BCS-AA41715; Batch code: AE 1258593-01-01; Origin batch No.: SES 10588-3-5; Customer order No.: TOX 09021-02 (first run), TOX 09021-03 (second run); Analyzed content: 98.6 % w/w; LIMS No.: 1119471 (first run), 1219379 (second run).

Since in the first test run the NOEC for reproduction could not be determined, a second test run was started testing lower concentrations. In the first test run 10 collembolans (11-12 days old) per replicate (8 replicates for the control group and the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight. In the second test run 10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 1.0, 1.8, 3.2, 5.6 and 10 mg test item/kg artificial soil dry weight. Both test runs at 20 ± 2°C, 400 – 800 lux, 16h light to 8h dark. During the study, collembolans were fed with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained	
		1st run	2nd run
Mean adult mortality	< 20%	16.3	6.3
Average reproduction rate in the control	≥ 100	1132.6	1196.1
Coefficient of variation of reproduction	< 30%	9.7	10.6

All validity criteria for the study were met.

Reference test:
The most recent non-GLP-test (Bayer Report No.: FRM-Coll-Ref-19/12, May 25, 2012) with the reference item boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95 % confidence limits from 98 mg to 137 mg boric acid/kg artificial soil dry weight), which is in the recommended range of the guideline (OECD 232, 2009) of about 100 mg boric acid/kg artificial soil dry weight showing that the test organisms were sufficiently sensitive.

Biological results:
Mortality:
In the control group 16.3% (first run) and 6.3% (second run) of the adult *Folsomia candida* died. In the first run all adult collembolans died in the treatment group with 100 mg test item/kg artificial soil dry weight. In the second run the highest mortality rate of 25% was observed in the treatment group with 5.6 test item/kg artificial soil dry weight.

Reproduction
In the first test run no juveniles were found in the treatment group with 100 mg test item/kg artificial soil dry weight. Concerning the number of juveniles statistical analysis (Welch's t test, one-sided, smaller, $\alpha = 0.05$) revealed statistically significant difference between control and the treatment groups with 10, 5.6 and 3.2 mg test item/kg artificial soil dry weight in the second test run.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 1.8 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 3.2 mg test item/kg artificial soil dry weight.

Survival and reproduction of collembolans after 4 weeks of treatment with flufenacet-thiadone

Test item Test object Exposure	Flufenacet-thiadone (BCS-AA41715) *Folsomia candida* Artificial soil		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
1st run			
control	16.3	1132.6 ± 110.4	-
100	100	0 ± 0	0
2nd run			
control	6.3	1196.1 ± 126.7	-
10	20.0	690.8 ± 497.1	57.7*
5.6	25.0	954.5 ± 126.6	79.8*
3.2	20.0	881.3 ± 95.0	73.7*
1.8	10.0	1156.3 ± 77.4	96.7 n.s.
1.0	2.5	1125.0 ± 93.6	94.1 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			1.8
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			3.2

The calculations were performed with un-rounded values
SD = Standard deviation
* = statistically significant (Welch's t-test one-sided-smaller, $\alpha = 0.05$)
n.s. = statistically not significant (Welch's t-test one-sided-smaller, $\alpha = 0.05$)
n.s.* = statistically not significant (Welch's t-test one-sided-smaller, $\alpha = 0.05$) due to high SD in this treatment group 42.3% difference to control confirms the effect on the reproduction of juveniles.

Conclusions:
$NOEC_{reproduction}$: 1.8 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: 3.2 mg test item/kg artificial soil dry weight.

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Report: **KCA 8.4.2.1/11, [redacted] M.-A., 2012**

Title: Flufenacet-thiadone (BCS-AA41715): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Document No.: M-442897-01-1
Guidelines: OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP yes (certified laboratory)

Objectives:
The purpose of the study was to assess the effects of Flufenacet-thiadone (BCS-AA41715) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Material and Methods:
Test item: Flufenacet-thiadone (BCS-AA41715)
1st Test run (AE 1258593; Batch code: AE 1258593-01-01; Customer Order No.: TOX 09021-02; Origin Batch Code SES 10558-3-5; Certificate No.: MZ 00417; LIMS No. 1119471; Purity: 98.6 %w/w)

2nd and 3rd Test run (AE 1258593; Batch code: AE 1258593-01-01; Customer Order No.: TOX 09021-03; Origin Batch Code SES 10558-3-5; Certificate No.: MZ 00532; LIMS No.1219379; Purity: 98.6 %w/w)

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate were exposed to control and treatments. In the 1st test run 8 control replicates and 8 treatment replicates were tested and in the 2nd and 3rd test run 8 control replicates and 4 replicates for each test item concentration were tested.
In the first test run a concentration of 100 mg test item/kg dry weight artificial soil was tested. Since the first run did not provide a final result, a second test run was performed studying lower test concentrations. In the 2nd test run concentrations of 1.0, 1.8, 3.2, 5.6 and 10 mg test item/kg dry weight artificial soil were tested. Since the 2nd test run did not provide a final result a 3rd test run was performed studying higher concentrations. In the 3rd test run concentrations of 18, 32 and 56 mg test item/kg dry weight artificial soil were tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (29 days in the 1st and 2nd test run, and 35 days in the 3rd test run after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine Quartzsand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

1st Test run :

Test item Test object Exposure	Flufenacet-thiadone (BCS-AA41715) *Hypoaspis aculeifer* Artificial Soil			
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	2.5	346.5 ± 23.5		
100	100	0 ± 0.0	0.0	

(*)= no statistical calculations were performed

2[nd] Test run:

Test item Test object Exposure	Flufenacet-thiadone (BCS-AA41715) *Hypoaspis aculeifer* Artificial Soil			
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	3.8	367.8 ± 44.6		
1.0	7.5	402.5 ± 22.6	109.4	-
1.8	7.5	385.0 ± 30.7	104.7	-
3.2	7.5	360.5 ± 59.1	98.0	-
5.6	0.0	382.0 ± 45.7	103.9	-
10.0	2.5	418.5 ± 12.0	113.8	-
			Reproduction	
NOEC (mg test item/kg dry weight artificial soil) LOEC (mg test item/kg dry weight artificial soil)			**≥ 10** **> 10**	

(*)= Williams-t.-test one sided smaller; α=0.05

3[rd] Test run:

Test item Test object Exposure	Flufenacet-thiadone (BCS-AA41715) *Hypoaspis aculeifer* Artificial Soil			
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	1.3	346.8 ± 24.2		
18	5.0	339.8 ± 17.3	98.0	-
32	22.5	245.3 ± 95.4	70.7	-
56	100.0	4.5 ± 4.2	1.3	+
			Reproduction	
NOEC (mg test item/kg dry weight artificial soil) **LOEC (mg test item/kg dry weight artificial soil)**			**32** **56**	
		Adult mortality	Reproduction	
LC_{10} / EC_{10} (mg test item/kg dry weight artificial soil) LC_{20} / EC_{20} (mg test item/kg dry weight artificial soil) LC_{50} / EC_{50} (mg test item/kg dry weight artificial soil)		**30** **32** **35**	**28** **30** **36**	

(*)= Welch-t.-test one sided smaller; α=0.05

The most recent non-GLP-test ([redacted]-[redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate showed that the test organisms are sufficiently sensitive according to the guideline.

Validity of the study:

Validity criteria	**Recommended by the guideline**	**Obtained in this study**		
		1[st] run	2[nd] run	3[rd] run
Mean adult mortality	≤ 20 %	2.5 %	3.8 %	1.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	346.5	367.8	346.8
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	6.8 %	12.1%	7.0 %

All validity criteria were met. Therefore these test runs are valid.

Observations:

Mortality

In the control group 2.5 % (1st run), 3.8 % (2nd run) and 1.3 % (3rd run) of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The highest mortality rate of 100 % was observed in the treatment groups with 56 and 100 mg test item/kg dry weight artificial soil. The LC_{50} for adult mortality is 35 mg test item/kg dry weight artificial soil. The confidence limits could not be determined due to mathematical reasons or inappropriate data.

Reproduction

In the 1st test run no NOEC could be determined and a 2nd test run with lower concentrations was performed. In this 2nd test run no LOEC could be determined and a 3rd test run with concentrations lower than the 1st test run and higher than the 2nd test run was performed. In this 3rd test run the highest test concentration of 56 mg/kg test item dry weight artificial soil was statistically significant concerning the number of juveniles whereas the test concentration of 32 mg/kg test item dry weight artificial soil revealed no statistically significant difference compared to the control (Welch t-test for inhomogeneous variances with Bonferroni-Holm adjustement, one-sided smaller, $\alpha = 0.05$). Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 32 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 56 mg test item/kg dry weight artificial soil.

Conclusions:

$NOEC_{reproduction}$: 32 mg test item/kg dry weight artificial soil.
$LOEC_{reproduction}$: 56 mg test item/kg dry weight artificial soil.

$LC_{10\ (adult\ mortality)}$: 30 mg test item/kg dry weight artificial soil
$LC_{20\ (adult\ mortality)}$: 32 mg test item/kg dry weight artificial soil
$LC_{50\ (adult\ mortality)}$: 35 mg test item/kg dry weight artificial soil
The confidence limits could not be determined due to mathematical reasons or inappropriate data.

$EC_{10\ (reproduction)}$: 28 mg test item/kg dry weight artificial soil
$EC_{20\ (reproduction)}$: 30 mg test item/kg dry weight artificial soil
$EC_{50\ (reproduction)}$: 36 mg test item/kg dry weight artificial soil
The confidence limits could not be determined due to mathematical reasons or inappropriate data.

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Report: **KCA 8.4.2.1/12, [redacted], M-A.; 2013**

Title: Flufenacet a.s.: Influence on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested in artificial soil

Document No.: M-455214-01-1

Guidelines: OECD 226 (2008) Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil

GLP: Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Flufenacet a.s. on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:

Flufenacet a.s.: analytical findings: 98.18 % w/w AE F133402, batch ID: AE F133402- 01-18, customer order no.: TOX 10011-00, specification no.: 102000006978, LIMS no.: 1301045.

Ten adult, fertilized, female *Hypoaspis aculeife*r per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight were tested. During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 20% (3.8 % in this study), Mean number of juveniles per replicate was ≥ 50 (272) and the coefficient of variation ≤ 30% (20.6% in this study).

Test item	Flufenacet a.s.			
Test organism	*Hypoapsis aculeifer*			
Exposure	Artificial soil			
mg test item/kg dry weight artificial soil	% Mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	3.8	272.3 ± 56.1	-	
100	15.0	311.5 ± 65.4	114.4	-
178	0.0	322.8 ± 5.7	118.5	-
316	10.0	294.5 ± 52.0	108.2	-
562	12.5	265.5 ± 30.6	97.5	-
1000	20.0	198.3 ± 31.5	72.8	+
NOECreproduction (mg test item/kg dry weight artificial soil) LOECreproduction (mg test item/kg dry weight artificial soil)			562 1000	
			Adult mortality	Reproduction
LC_{10}/EC_{10}(mg test item/kg dry weight artificial soil) [1)] LC_{20}/EC_{20}(mg test item/kg dry weight artificial soil) [1)] LC_{50}/EC_{50}(mg test item/kg dry weight artificial soil) [1)]			- - -	751.21 905.60 1294.90

(*)=William's-t.-test one sided smaller; α=0.05: - : non-significant; + : significant

1) Probit analysis (95 % confidence limits could not be determined due to mathematical reasons).

Observations:

Mortality:

In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg artificial soil dry weight.

Reproduction:

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed a significant difference between control and the highest treatment group of 1000 mg test item/kg artificial soil dry weight.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg test item/kg artificial soil dry weight. The EC_{10}, EC_{20} and EC_{50} values determined by Probit analysis are 751.21, 905.60 and 1294.90 mg test item/kg artificial soil dry weight, respectively. The 95% confidence limits could not be determined due to mathematical reasons

Conclusions

$NOEC_{reproduction}$: 562 mg test item/kg artificial soil dry weight

$LOEC_{reproduction}$: 1000 mg test item/kg artificial soil dry weight

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Report:	**KCA 8.4.2.1/13, [redacted], M-A.; 2013**
Title:	Flufenacet-sulfonic acid Na-salt (BCS-AZ23374): Influence on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Document No.:	M-455654-01-1
Guidelines:	OECD 226 (2008) Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the effect of flufenacet-sulfonic acid Na-salt (BCS-AZ23374) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Flufenacet-sulfonic acid Na-salt (BCS-AZ23374): analytical findings: 93.4 % w/w AE 0841914; origin batch no.: NLL8839-6-7, customer order no.: TOX 09486-01, batch code: AE 0841914-01-05, LIMS no.: 1304576.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatment. One single concentration of 100 mg pure metabolite (107 mg test item)/kg artificial soil dry weight was tested. During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution

(20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 20% (3.8 % in this study), Mean number of juveniles per replicate was ≥ 50 (272) and the coefficient of variation ≤ 30% (20.6% in this study).

Test item	Flufenacet-sulfonic acid Na-salt (BCS-AZ23374)			
Test object	*Hypoaspis aculeifer*			
Exposure	Artificial Soil			
mg pure metabolite/kg d.w. artificial soil	% motrality (adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	3.8	272.3 ± 20.6	-	
100	6.3	264.9 ± 19.1	97.3	-
NOECreproduction (mg pure metabolite/kg dry weight artificial soil)			100	
LOECreproduction (mg pure metabolite/kg dry weight artificial soil)			100	

Observations:
Mortality:
In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect- Concentration (LOEC) for reproduction is > 100 mg pure metabolite/kg artificial soil dry weight.

Conclusions:
$NOEC_{reproduction}$: ≥ 100 mg pure metabolite/kg artificial soil dry weight
$LOEC_{reproduction}$: > 100 mg pure metabolite/kg artificial soil dry weight

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Report: **KCA 8.4.2.1/14; [redacted] T.; 2010**
Title: Flufenacet-methylsulfone (BCS-CO62475): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Document No: M-392345-01-1
Guidelines: OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
GLP Yes (certified laboratory)

Objective:
The purpose of this study was to assess the effect of Flufenacet-methylsulfone (BCS-CO62475) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Flufenacet-methylsulfone, 97.6 % w/w analysed content, origin batch no.: SES 10623-5-1, customer order no: TOX 08624-02, LIMS no.: 1013480, batch code: BCS-CO62475-01-01.
Toxic standard: Boric acid.
Control: same application as test item but with deionised water and quartz sand only.
Ten Collembola (10-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 Lux, 16h light : 8h dark, 5 % peat in the artificial soil. During the test they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Findings:

The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 10% (3.8% in this study), reproduction of the control was ≥ 100 juveniles per control vessel (1470 juveniles in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (10.2% in this study).

Test item **Test object** **Exposure**	**Flufenacet-methylsulfone** ***Folsomia candida*** **Artificial Soil**		
mg test item/kg soil (dw)[1] nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	3.8	1470 ± 150	-
100	1.3	1341 ± 106	91.2 *
NOEC (mg test item/kg soil (dw))			≥100
LOEC (mg test item/kg soil (dw))			>100

1) Dry weight
* Statistically significant (Dunnett's Test one-sided smaller, α = 0.05)

Observations:

Mortality:
In the control group 3.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. In the treatment group a very low mortality rate of 1.3 % was observed.

Reproduction:
In the treatment group Student-t test, one-sided smaller, α = 0.05 revealed a significant difference to the control. Because of the low difference of the treatment group to the control group (8.8 %) in relation to a coefficient of variation of 10.2 % concerning the number of juveniles the effect is not considered to be test item related, but is in the range of the biological variability of the test system.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is considered to be ≥100 mg test item/kg artificial soil dry weight and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >100 mg test item/kg artificial soil dry weight.

Conclusions:

$NOEC_{reproduction}$: ≥100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: >100 mg test item/kg artificial soil dry weight.

CA 8.5 Effects on soil nitrogen transformation

For information on studies already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. it's Addenda). These studies are listed in grey in the table below.

Test species	Test item	Test design	Ecotoxicological endpoint	Reference
Flufenacet				
C-cycle	a.s.	2 soils, 28 d	no sign. influence at 0.62 and 3.1 kg a.s./ha	[redacted] (1994) M-003872-01-2
N-cycle	a.s.	2 soils, 28 d	no sign. influence at 0.62 and 3.1 kg a.s./ha	[redacted] (1994) M-003871-01-2
C-cycle	WG 60	2 soils, 28 d	no sign. influence at 0.6 and 3.0 kg a.s./ha	[redacted] (1995) M- 003888-01-1
N-cycle	WG 60	2 soils, 56 d	no sign. influence at 0.6 and 3.0 kg a.s./ha	[redacted] (1995) M- 003891-01-1
FOE oxalate				
N-cycle	p.m.	1 soil, 28 d	no sign. influence at 1.86 kg/ha (equiv. to 2.48 mg p.m./kg dws)	[redacted]-[redacted] (2005) M-250511-01-1 KCA 8.5/04
FOE sulfonic acid-Na-salt				
N-cycle	p.m.	1 soil, 28 d	no sign. influence at 2.455 kg a.s./ha (equiv. to 3.27 mg p.m./kg dws)	[redacted]-[redacted] (2005) M-250265-01-1 KCA 8.5/03
FOE methylsulfone				
N-cycle	p.m.	1 soil, 28 d	0.451 and 4.51 kg/ha (equiv. to 0.6 and 6.0 kg FFA/ha) - no relevant influence	[redacted] (2010) M-398568-01-1 KCA 8.5/05
TFA				
N-cycle	p.m.	1 soil, 28 d	no sign. influence at 0.24 and 1.2 kg p.m./ha	[redacted] (2013) M-444423-01-1 KCA 8.5/06
FOE 5043-trifluoroethane sulfonic acid				
N-cycle	p.m.	1 soil, 28 d	0.123 and 0.615 kg p.m./ha no relevant influence	[redacted] (2013) M-457331-01-1 KCA 8.5/08
FOE-Thiadone				
N-cycle	p.m.	1 soil, 28 d	0.112 and 0.562 kg p.m./ha no relevant influence	[redacted] (2013) M-457326-01-1 KCA 8.5/07

Report: **KCA 8.5/03, [redacted]-[redacted], C., 2005**
Title: Metabolite Flufenacet-Sulfonic acid Na-salt: Determination of effects on nitrogen transformation in soil
Document No: M-250265-01-1
Guidelines: OECD No. 216, Adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test
GLP yes (certified laboratory)

Material and Methods:

A high dosage of Flufenacet-Sulfonic acid Na-salt, a metabolite of Flufenacet, was used in the tests. The purity of the metabolite was 99.5% (development No.: 3000289445, batch No.: KTS9465-3-3). A loamy sand soil was exposed for 28 d to 3.27 mg Metabolite Flufenacet-Sulfonic acid Na-salt/kg d.wt. soil, which is equivalent to 2.455 kg Metabolite Flufenacet-Sulfonic acid Na-salt/ha. This quantity was determined by taking the recommended field rate of the parent compound (0.6 kg a.s./ha), multiplying this by 5 (3 kg a.s./ha), and converting the resulting quantity into the molecular weight equivalent of metabolite. The molecular weight of Flufenacet is 363.36 g/mole and the meolecular weight of the Metabolite Flufenacet-Sulfonic acid Na-salt is 297.3 g/mole. Lucerne-grass-green meal was added to soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

Results:

During the 28-day tests, the metabolite Flufenacet-Sulfonic acid Na-salt (2.455 kg metabolite Flufenacet-Sulfonic acid Na-salt/ha, based on the 5-fold overdose of the field rate of the parent compound) had no influence on the nitrogen transformation in a loamy sand soil amended with luzerne grass-green-meal (5g/kg dry weight soil). Under field conditions this metabolite should not have an impact on nitrogen transformation in soils.

Effects on non-target soil micro-organisms

Test item	Metabolite Flufenacet-Sulfonic acid Na-salt (Molecular Weight = 297.3 g/mole)
Test object	Soil Micro-organisms Nitrogen-Transformation (loamy sand soil)
Exposure	28days
mg metabolite/kg dry weight soil	3.27
kg metabolite/ha (molecular equivalent)	2.455 (corresponding to 3 kg a.s./ha of the parent compound)
Final Result after 28 days	Difference to Control < 25 %

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Report: **KCA 8.5/04, [redacted], 2005**

Title: Metabolite flufenacet-oxalate hydrate: determination of effects on nitrogen transformation in soil

Document No.: M-250511-01-1

Guidelines: OECD No. 216, Adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test

GLP: yes (certified laboratory)

Material and Methods:

A high dosage of Metabolite Flufenacet-oxalate hydrate, a metabolite of Flufenacet, was used in the test. The purity of the metabolite was 99 % (batch No.: 921103ELB01). A loamy sand soil was exposed for 28 d to 2.48 mg Metabolite Flufenacet-oxalate hydrate/kg d.wt. soil, which is equivalent to 1.86 kg Metabolite Flufenacet-oxalate hydrate/ha. This quantity was determined by taking the recommended field rate of the parent compound (0.6 kg a.s./ha), multiplying this by 5 (3 kg a.s./ha), and converting the resulting quantity into the molecular weight equivalent of metabolite. The molecular weight of Flufenacet is 363.34 g/mole and the molecular weight of the metabolite Flufenacet-oxalate hydrate is

225.2 g/mole. Lucerne-grass-green meal was added to soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

Results:
During the 28-day tests, Metabolite Flufenacet-oxalate hydrate had no influence on the turnover of nitrogen to a Metabolite Flufenacet-oxalate hydrate soil amended with lucerne-grass-green meal. Under field conditions, this metabolite should not have an impact on nitrogen transformation in soils.

Effects on non-target soil micro-organisms

Test item	Metabolite Flufenacet-oxalate hydrate (Molecular Weight = 225.2 g/mole g/mole)
Test object	Soil Micro-organisms Nitrogen-Transformation (loamy sand soil)
Exposure	28days
mg metabolite/kg dry weight soil	2.48
kg metabolite/ha (molecular equivalent)	1.86 (corresponding to 3 kg a.s./ha of the parent compound)
Final Result after 28 days	Difference to Control < 25 %

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Report: **KCA 8.5/05; [redacted], U.; 2010**
Title: Metabolite flufenacet-methylsulfone (BCS-CO62475): Determination of effects on nitrogen transformation in soil
Document No: M-398568-01-1
Guidelines: OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test.
GLP Yes (certified laboratory)

Objectives: The objective of the test was to determine the influence of 0.60 mg and 6.01 mg of Metabolite flufenacet-methylsulfone (BCS-CO62475)/kg dry weight soil on nitrogen transformation in an agricultural soil

Material and Methods: Metabolite flufenacet-methylsulfone (BCS-CO62475), analytical findings: 97.6 % w/w, batch code: BCS-CO62475-01-01, origin batch no.: SES 10623-5-1, LIMS no.: 1027925, customer order no.: TOX 08624-03, was used in the test. A loamy sand soil (according to DIN 'mittel lehmiger Sand') was exposed for 28 d to 0.60 and 6.01 mg test item/kg dry weight soil, which is equivalent to 0.451 and 4.51 kg/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation
The coefficients of variation in the control at the end of the study were between 3% and 11%. Therefore the validity criteria for the study, which requires a coefficient of variation ≤ 15% in the control, was fulfilled.

Findings: Effects on non-target soil microorganisms

Time Interval (days)	Application rates										
	Metabolite flufenacet-methylsulfone (BCS-CO62475)										
	Control			0.60 mg/kg dry weight soil				6.01 mg/kg dry weight soil			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	-0.81	±	0.05	-0.77	±	0.05	5 n.s.	-0.72	±	0.12	11 n.s.
7-14	1.79	±	0.03	1.71	±	0.13	5 n.s.	1.82	±	0.14	2 n.s.
14-28	1.22	±	0.08	1.27	±	0.11	4 n.s.	1.16	±	0.08	5 n.s.

[1] Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation.
n.s. = No statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$).

Observations: During the 28-day test, 0.60 mg Metabolite flufenacet-methylsulfone (BCS-CO62475)/kg dry weight soil and the 10-fold dose of the test item had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeds the trigger value of 25 %.

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Report: **KCA 8.5/06: [redacted], 2013**
Title: Trifluoroacetic acid Na-salt (BCS-AZ56567): Effects on the activity of soil microflora (Nitrogen transformation test)
Document No: M-444423-01-1
Guidelines: OECD 216 (2000)
GLP Yes (certified laboratory)

Objective:

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and Methods:

Test item: Trifluoroacetic acid Na-salt, Substance code: AE 1046319, BCS-code: BCS-AZ56567, Batch code: AE 1046319-01-01, Origin Batch No.: SES 11755-1-1, CAS. No.: 2923-18-4, LIMS No.: 1226556, Customer order No.: TOX 09476-02, analysed purity: 95.1 % w/w sodium trifluoroacetate.
A loamy sand soil (DIN 4220) was exposed for 28 days to 0.32 and 1.60 mg test item/kg soil dry weight. Application rates were equivalent to 0.24 and 1.20 kg test item/ha. Determination of the nitrogen transformation (NO_3-N production) in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-N, NO_3- and NO_2-N were determined using the Autoanalyzer (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment). The soil of each treatment was incubated as a series of 3 replicates.

The control was prepared with quartz meal only (3 replicates). As toxic reference was used dinoterb in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)).

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Variation between replicate control samples	< 15%	2.4%

All validity criteria were met.

Reference test:
In the most recent test, dated 13.01. - 10.02.2012, the toxic standard dinoterb caused an effect of +40.4%, +68.1% and +83.5% (required ≥ 25%) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Biological results:
No adverse effects of trifluoroacetic acid Na-salt on nitrogen transformation in soil could be observed in both test concentrations (0.32 mg/kg dry soil and 1.60 mg/kg dry soil) after 28 days. Differences from the control of +3.1% (test concentration 0.32 mg/kg dry soil) and +24.2% (test concentration 1.60 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Effects of trifluoroacetic acid Na-salt on nitrogen transformation in soil (based concentrations of the test item [mg test item/kg soil dry weight])

Time Interval (days)	Control			0.32 mg test item/kg soil dry weight equivalent to 0.24 kg test item/ha				1.60 mg test item/kg soil dry weight equivalent to 1.20 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	1.79	±	0.10	1.62	±	0.06	**-9.1** n.s.	1.76	±	0.48	**-1.6** n.s.
7-14	0.80	±	0.11	0.85	±	0.02	**+5.3** n.s.	0.70	±	0.35	**-13.0** n.s.
14-28	0.61	±	0.08	0.63	±	0.15	**+3.1** n.s.	0.76	±	0.04	**+24.2** *s

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
*s = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusion:
Trifluoroacetic acid Na-salt caused no adverse effects on the soil nitrogen transformation at the end of the 28-day incubation period.

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Report:	**KCA 8.5/07; Schulz, N.; 2013**
Title:	Flufenacet-thiadone (BCS-AA41715): Effects on the activity of soil microflora (Nitrogen transformation test)
Document No.:	M-457326-01-1
Guidelines:	OECD 216 (2000)
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and Methods:

Flufenacet-thiadone, BCS-code: BCS-AA41715, Batch code: AE 1258593-01-01, Origin Batch No.: SES 10558-3-5, LIMS No.: 1311098, Customer order No.: TOX 09021-04, analysed purity: 98.6 % w/w 5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-one.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.149 and 0.749 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH4-nitrogen, NO3- and NO2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

The coefficients of variation in the control (NO3-N) were maximum 3.0 % and thus fulfilled the demanded range (≤15 %).

Findings:

The coefficients of variation in the control for NO_3-N were maximum 3.0 % and thus fulfilled the demanded range (≤15 %).
In the most recent test with the toxic standard Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Effects on nitrogen transformation in soil after treatment with Flufenacet-thiadone (BCS-AA41715)

Time Interval (days)	Control			0.149 mg test item/kg soil dry weight equivalent to 0.112 kg test item/ha				0.749 mg test item/kg soil dry weight equivalent to 0.562 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.84	±	0.05	3.86	±	0.24	+0.4 [n.s.]	4.19	±	0.15	+9.0 [*s.]
7-14	1.40	±	0.20	1.03	±	0.08	-26.8 [*s.]	1.20	±	0.09	-14.6 [n.s.]
14-28	1.21	±	0.11	1.45	±	0.22	+19.7 [n.s.]	1.17	±	0.15	-3.2 [n.s.]

The calculations were performed with unrounded values
[1)]Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
[n.s.]= No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)
[*s.] = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

Observations:
The test item Flufenacet-thiadone (BCS-AA41715) caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.149 mg/kg at time interval 7-14 days after application.
However, no adverse effects of Flufenacet-thiadone (BCS-AA41715) on nitrogen transformation in soil could be observed at both test concentrations (0.149 mg/kg dry soil and 0.749 mg/kg dry soil) at the end of the 28- day experiment. Differences from the control of +19.7 % (test concentration 0.149 mg/kg dry soil) and -3.2 % (test concentration 0.749 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Conclusions:
Flufenacet-thiadone (BCS-AA41715) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.749 mg test item/kg soil dry weight.

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Report:	**KCA 8.5/08; [redacted]; 2013**
Title:	Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474): Effects on the activity of soil microflora (Nitrogen transformation test)
Document No.:	M-457331-01-1
Guidelines:	OECD 216 (2000)
GLP:	Yes (certified laboratory)

Objectives:
The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and methods:
Flufenacet-trifluoroethanesulfonic acid Na-salt, BCS-code: BCS-CU62474, Batch code: BCS-CU62474-01-02, Origin Batch No.: NLL 8865-7-1, LIMS No.: 1311096, Customer order No.: TOX 09484-01, analysed purity: 98.4 % w/w sodium 2,2,2-trifluoroethanesulfonate.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.164 and 0.820 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH4-nitrogen, NO3- and NO2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

The coefficients of variation in the control (NO_3-N) were maximum 2.1 % and thus fulfilled the demanded range (≤15 %).

Findings:
The coefficients of variation in the control for NO_3-N were maximum 2.1 % and thus fulfilled the demanded range (≤15 %).

In the most recent test with the toxic standard, Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Effects on nitrogen transformation in soil after treatment with trifluoroethanesulfonic acid Na-salt (BCS-CU62474)

Time Interval (days)	Control			0.164 mg test item/kg soil dry weight equivalent to 0.123 kg test item/ha				0.820 mg test item/kg soil dry weight equivalent to 0.615 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	4.06	±	0.12	3.72	±	0.14	-8.2 [*s.]	3.82	±	0.26	-5.8 [n.s.]
7-14	1.35	±	0.16	1.40	±	0.10	+4.2 [n.s.]	0.96	±	0.11	-29.0 [*s.]
14-28	1.22	±	0.09	1.19	±	0.13	-2.3 [n.s.]	1.41	±	0.08	+15.4 [n.s.]

The calculations were performed with unrounded values

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

Observations:

The test item flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474) caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.820 mg/kg dry soil at time interval 7-14 days after application.

However, no adverse effects of flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474) on nitrogen transformation in soil could be observed at both tested concentrations (0.164 mg and 0.820 mg test item/kg dry soil) at the end of the 28-day experiment. Differences from the control of -2.3 % (test concentration 0.164 mg/kg dry soil) and +15.4 % (test concentration 0.820 mg/kg dry soil) were measured at the end of the 28- day incubation period (time interval 14-28).

Conclusions:

Flufenacet-trifluoroethanesulfonic acid Na-salt (BCS-CU62474) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.820 mg test item/kg soil dry weight.

CA 8.6 Effects on terrestrial non-target higher plants

In the first Annex I listing process non-target plant data for a different formulation of flufenacet were submitted and evaluated. The formulation FFA WG60 is no longer supported, therefore only data on the new representative formulation Flufenacet + Diflufenican SC 600 (Herold SC 600) for the Annex I renewal process will be presented with this dossier. For details on the study summaries please refer to the respective sections in the MCP "Section 10 Ecotoxicological Studies".

Flufenacet & Diflufenican SC 600 (Herold SC 600)

Test organism	Study type	Test duration	Lowest ER_{50}	Most sensitive species	References
Terrestrial non-target plants; 6 species	vegetative vigour; Tier 2 dose response	21 days	23.82 g a.i./ha = 0.039 L/ha	Allium cepa	[redacted], 2002; M-071692-01-1 KCP 10.6.2/01
Terrestrial non-target plants; 6 species	seedling emergence; Tier 2 dose response	21 days	190.43 g a.i./ha = 0.311 L/ha	Lycopersion esculentum	[redacted], 2002; M-072308-01-1 KCP 10.6.2/02

CA 8.6.1 Summary of screening data

For herbicides and plant growth regulators, it is considered unprofitable to conduct tier 1 screening studies as it is inevitable that these will lead to tier 2 or dose response studies in order to generate data suitable for deterministic or probabilistic risk assessments, i.e. ER_{50} values for 6-10 species, representing a broad range of plant species. Therefore, no screening studies were conducted for flufenacet or its representative formulation.

CA 8.6.2 Testing on non-target plants

Please refer to the comment under CA 8.6.
For details on studies performed please refer to the respective section in the MCP "Section 10 Ecotoxicological Studies".

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

No studies on other terrestrial organisms were necessary. However three articles on the metabolite TFA were found in the open literature, which are considered reliable with some restrictions. Summaries are presented below.

Report:	**KCA 8.7/03; [redacted], J.A., [redacted], M.S., [redacted], G.R., [redacted], T.M. (2002)**
Title:	Investigation of effects of trifluoroacetate on vernal pool ecosystems
Source	Environmental Toxicology and Chemistry, Vol. 21, No. 3, pp. 640 - 647, 2002
DOI No:	Not stated
Document No:	M-455780-01-1
Guidelines:	Not stated
GLP:	Not stated

EXECUTIVE SUMMARY

This study focused on assessing the impact of TFA on vernal pool soil microbial communities as well as vernal pool and wetland plant species. Microbial respiration for three vernal pool soils and an agricultural soil was not affected by TFA exposures (0, 10, 100, 1000, and 10000 µg/L), and degradation of TFA by microbial communities was not observed in soils incubated for three months. TFA accumulated in foliar tissue of wetland plant species as a function of root exposure concentration (100 and 1000 µg/L TFA), and accumulation was found to stabilize or decrease after the second or third month of exposure. Seeds accumulated TFA as a function of root exposure concentration;

however, germination success was not affected. No adverse physiological responses, including general plant health and photosynthetic and conductance rates, were observed for root exposures at the TFA concentrations used in this study.

Based on the soils and plant species used in this study, predicted TFA concentrations will not adversely affect the development of soil microbial communities and vernal pool plant species.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	NaTFA (CF_3COO-Na)
Active substance(s):	See above
Adjuvant / Surfactant:	Not stated
Source of test item:	[redacted], USA (chemicals supplier)
Lot/Batch number:	Lot 106H3462
Purity:	Not stated
Storage conditions:	

2. Test solutions

Vehicle/solvent:	Not stated
Source of vehicle/solvent:	Not stated
Concentration of vehicle/solvent:	Not stated

3. Test organism(s)

Species:	Microbial soil communities: MOs from three natural vernal pool soil and one agricultural soil; Plants: (1) TFA uptake via roots: *Polypogon monspeliensis* (annual beardgrass), *Deschampsia elongata* (vernal pool hairgrass), *Lasthenia californica* (small sunflower), *Oryza sativa* (rice, M-201); (2) Biomass experiment: *D. elongate*, *O. Sativa and P. monspeliensis;* (3) Germination experiments: *Eryngium vaseyi* (Coyote thistle), *Epilobium densiflora* (Fleshy owl's clover), *L. californica* and *D. elongata*.
Cultival:	Not stated except for rice (M-201)
Source of test species:	Microbial soil communities: natural soil collected from vernal pools on the properties of the Rancho Seco Power Plant and Beale Air Force Base near Sacramento (CA, USA); Red Rock Playa, Stead (NV, USA; and agricultural soil from the University of Nevada Agriculture Experiment Station, Reno (NV, USA) Plants: S&S Seed, Capenteria, CA, USA; Pacific Coast Seed, Livermore, CA, USA; University of California, Davis, CA, USA)

Age of test organisms at study initiation / Crop growth stage at treatment:	Microbial soil communities: (1) Exposure experiments: TFA was added to MOs at the beginning of the experiment rather than after microbial respiration had established. Plants: (1) TFA uptake via roots: plants were 1.25 ± 0.25 cm in height; (2) Biomass experiment: plants were 1.5 ± 0.5 cm in height; (3) Germination experiments: Seeds of several wetland plant species
Holding conditions prior to test / Preparation before experiments:	Microbial soil communities: (1) Exposure experiments: Soils air-dried, homogenized, and sieved to 0.2 mm before test start; (2) Microbial degradation of TFA: no further preparation. Plants: (1) TFA uptake via roots: 4 species germinated and grown in 0.25 Hoagland's solution (pH 6.0 ± 0.5). Silicon was added (10 µmol/L Na_2SiO_3) to the solution; (2) Biomass experiment: (a) *Deschampsia* seeds germinated in rock wool immersed in aerated hydrophonic solutions until plants were 1.5 ± 0.5 cm in height; (b) *Oryza* and *Polypogon* seeds germinated in vermiculite until plants were 1.5 ± 0.5 cm in height; (3) Germination experiments: (a) first-generation seeds (seeds obtained from 4 plant species that had not been grown in TFA-containing solution) no preparation needed before test start, (b) second-generation seeds of had developed from *Lasthenia* and *Oryza* plants growing in solutions of 0, 100 and 1000 µg/L TFA and accumulated TFA.
Acclimatisation:	Not stated

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory studies assessing effects of TFA on vernal pool soils microbial communities and vernal pool and wetland plant species
Guideline deviation:	Not stated
Duration of study:	See below (treatment)

Treatments: Microbial soil communities:

(1) Exposure experiments: Glass microcosms (250-ml volume) fitted with gastight lids containing a septa port for sampling headspace gas using a gastight syringe, were filled with 50 g of dry soil. Solutions with different TFA concentrations (see below) were added to achieve an 80% saturation level (by weight). Microcosm headspace analysis of carbon dioxide was conducted by collection of triplicate samples of 500 ml of microcosm air that was immediately injected into a CO2 analyzer. Methods and procedures for this study are similar to those described by Walton et al. (1989) and Taylor et al. (1996). After headspace sampling, the microcosms were opened for 30 min and allowed to degas. This procedure was maintained for 25 d for the first experiment that utilized all soils. The procedure was repeated for the second experiment for 14 d but utilized only the agricultural, Beale, and Red Rock soils.

(2) Microbial degradation of TFA: One-gram samples of each soil type were placed into glass vials (n = 72 per soil type) and spiked in the same manner as the two studies described above with solutions containing different TFA concentrations (see below). Twenty-four vials were used for each exposure concentration for each soil type. Test conditions are described below. Vials were incubated in 10-gal aquaria with 3 L of distilled water to maintain a relative humidity of 85 ± 5% and temperatures of 23.5°C for the first month and 20 ± 2.58C for the remaining two months. Six vials from each concentration and soil type were collected at zero, one, two, and three months and placed into a -20°C freezer. Three vials containing MilliQ-filtered ultra-high-purity water, which were incubated in the aquaria, were collected at each sample time from each aquaria to verify that TFA contamination had not occurred. Ten vials of each soil exposure were frozen at the initiation of the experiment, and 10 vials containing MilliQ ultra-high-purity water were also collected at the beginning of the experiment.

Plants:

(1) TFA uptake via roots: Two hundred plants of *Deschampsia*, *Lasthenia*, and *Oryza* were germinated under 60 µmol/m²/s fluorescent lighting rockwool immersed in aerated hydroponic solutions of different TFA concentrations (see below). After seedlings were 1.25 ± 0.25 cm tall (14 d), they were randomly placed into triplicate Rubber-maidy plastic tubs (23 L) containing the same respective concentration of TFA so that each tub contained 25 plants of the three species. Hydroponic solutions were replaced weekly. Plants were then grown in a greenhouse (25 ± 15°C) under 175 µmol/m²/s cool-white florescent lighting supplementing natural greenhouse lighting for a 14-h/d light cycle. Individual plants of *Oryza* and *Deschampsia* were sampled from each tub 21, 42, and 84 d after germination. After 150 d, dry seeds from *Oryza* were collected. *Lasthenia* plants and flowers were collected at 21 and 42 d after germination, and at 72 d seeds were collected. After 84 d, the photosynthetic and conductance rates for six plants of *Oryza* and *Deschampsia* in each tub were measured using a LI-COR 6400 Photosynthetic System.

(2) Biomass experiment: Plants with 1.5 6 0.5 cm height were randomly placed in tubs with aerated hydroponic solutions containing different TFA concentrations. After 57 d, height and total foliar biomass was determined for each plant. Biomass and leaf length were also monitored for *Oryza* and *Polypogon* exposed to different TFA concentrations (see below). Plants were germinated in vermiculite, and after 7 d, five 1.5 6 0.5-cm seedlings of each species were transferred into hydroponic systems containing Hoagland's solution amended with TFA. After two months, leaf length wasmeasured, as was root and leaf biomass. For both experiments, solutions were replaced weekly, solution pH was maintained at 5.55 ± 0.20, and plants were grown under a 14-h/d light cycle (70 µmole/m²/s) as described previously.

(3) Germination experiments: One first-generation germination experiment. Fifty seeds of each species were placed atop pieces of rockwool in tubs (3.5 L) containing Hoagland's solution spiked with different TFA concentrations (see below). The number of germinated seeds was counted daily until .50% had germinated. Seeds were germinated under the same lighting conditions as the biomass experiments. These germination experiments were performed twice. The temperatures for the ®rst and second germination experiments were 25 ± 4°C and 24.5 ± 2.5°C, respectively. An additional first-generation germination experiment was conducted using *Oryza*, *Lasthenia*, and *Deschampsia* seeds. This germination experiment followed the same protocol as the one described previously, except 200 seeds of

	each species were used. Second-generation germination experiments utilized *Lasthenia* and *Oryza* seeds that had developed from plants growing in solutions of different TFA contrations (see below). Seeds were collected after they reached full development and foliar tissue had dried. Two hundred *Lasthenia* seeds from each exposure concentration were germinated in triplicate in solutions at the same concentration as the parent plants had been grown. Fifty *Oryza* seeds were germinated similarly. In addition, 50 *Oryza* and 200 *Lasthenia* second-generation seeds were germinated in Hoagland's solution containing no TFA. These experiments were replicated twice.
Test concentrations	Microbial soil communities: (1) Exposure experiments: 0, 10, 100, 1000 and 10000 µg/L TFA; (2) Microbial degradation of TFA: 0, 0.3 and 1.5 µg/L TFA. Plants: (1) TFA uptake via roots: 0, 100 and 1000 µg/L TFA; (2) Biomass experiment: (a) *Deschampsia* seedlings: 0 and 100 µg/L TFA; (b) *Oryza* and *Polypogon* seedlings: 0, 10, 100 and 1000 µg/L TFA; (3) Germination experiments: (a) first generation experiment: 0, 10, 100, 1000 and 10000 µg/L TFA; (b) second generation experiment: 0, 100 and 1000 µg/L TFA
Number of replicates:	See above (treatments)
Individuals per replicate:	See above (treatments)
Test conditions:	See above (treatments)
Test units (type and size):	See above (treatments)
Application / device / nozzles:	See above (treatments)
Water volume:	See above (treatments)
Calibration of sprayer:	Not stated

2. Environmental conditions

Test medium:	See above (treatments)
Temperature / relative humidity:	See above (treatments)
Photoperiod:	See above (treatments)
Lighting	See above (treatments)
pH:	See above (treatments)
Organic matter (C_{org}):	Not stated
$CaCO_3$	Not stated
Cation exchange capacity:	Not stated
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	Not stated
Fertilization:	Not stated

3. Observations and measurements:

Analytical parameters measured:	Analysis of TFA in solutions, soil and plant tissues was done using the method by [redacted] et al. (1999)
Biological parameters measured:	Microbial soil communities: Soil respiration; microbial degradation of TFA.

	Plants: Uptake of TFA via root; morphology and biomass development; photosynthetic and conductance rates; germination success.
Measurement frequency:	See above (treatments)
Statistical analyses:	Data were evaluated using analysis of variance techniques (one-way, two-way). For biomass experiments, one-way ANOVA and two-tailed *t* tests, assuming equal variance, were used to compare leaf length, leaf weight, and root weight of exposed plants in comparison to control plants. A one-way ANOVA was used to compare soil TFA concentrations as a function of time. Germination and microbial results were compared using two-way ANOVA.

RESULTS

1. Validity criteria:
No test guideline and no validity criteria were stated in this study.

2. Other measurements:
Please refer to point 3 'Biological findings'. Measurement of other parameters was not reported.

3. Biological findings:

Microbial experiments:

Respiration in microcosms containing vernal pool soils treated with TFA was not affected over time. Microbial respiration stabilized on approximately day 8, and respiration ranged between 75 and 300 µmole CO_2/mol air/g soil/d. No significant difference was observed in the decline in respiration rates to day 8 as a function of TFA exposure concentration with time.

Vernal pool soils exhibited higher respiration rates (> 100 µmole CO_2/mol air/g soil/d) than the agricultural soils (< 100 µmole CO_2/mol air/g soil/d). No significant difference was observed in measured respiration as a function of TFA concentration for any of the soils exposed, except for the control agricultural soils that exhibited lower respiration than agricultural soil exposed to TFA. This experiment was replicated using agricultural, Beale, and Rancho Seco soils, and again no significant trends in respiration were observed as a function of exposure concentration.

In a further experiment, microbial degradation of TFA over a three-month time period was investigated. As a result, no significant difference was observed in the soil TFA concentrations at 0, 1, 2 and 3 months.

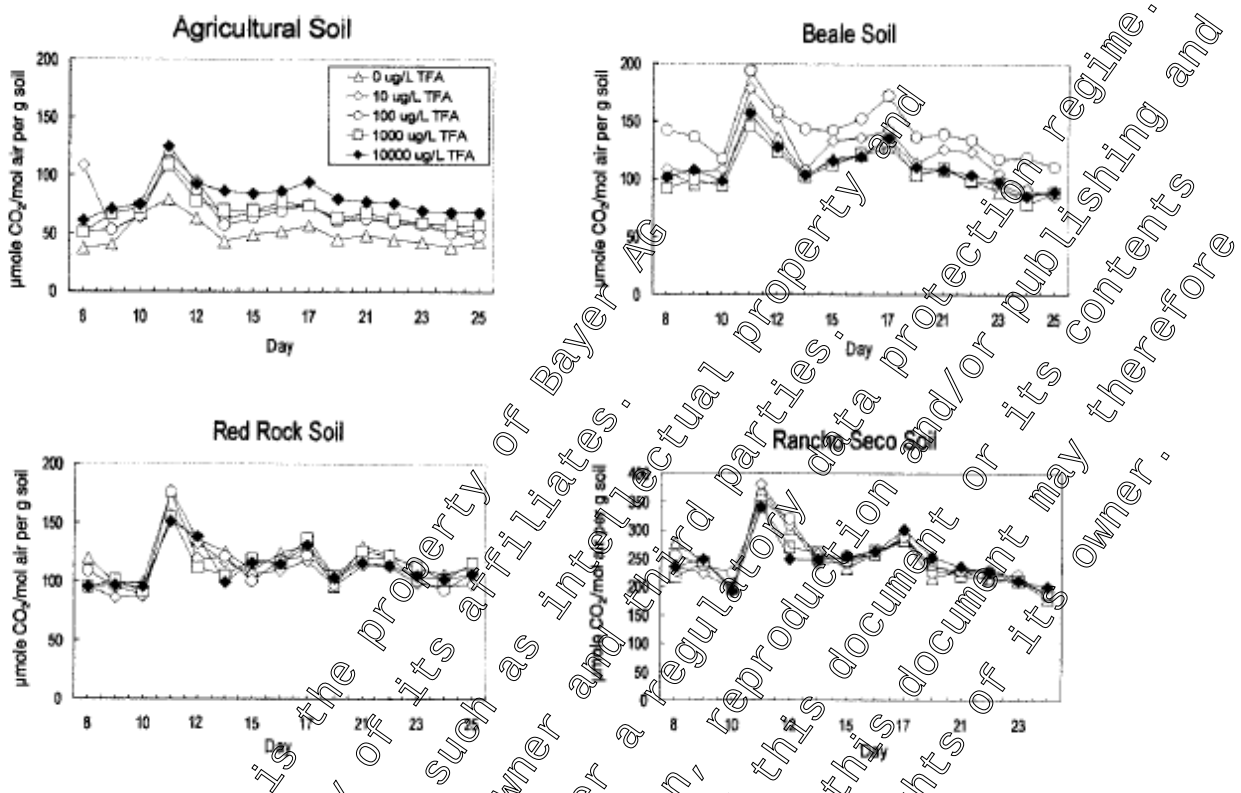

Figure 1 (taken from [redacted] et al., 2002): Microbial respiration (µmol CO2/mol air/g soil) in microcosms amended with TFA as a function of time, starting with day 8, when respiration had stabilized. Each point represents a mean of three CO_2 measurements for three replicate microcosms.

TFA uptake via roots

At TFA concentrations of 100 and 1,000 µg/L, TFA taken up by plant roots was found to accumulate in foliar tissue as a function of concentration and time in the leaves of plants grown in aqueous medium. However, TFA concentrations in foliar tissue leveled off and/or declined with time.

	100-µg/L TFA root exposure				1,000-µg/L TFA root exposure				
Species	42 d	63 d	72 d	105 d	150 d	42 d	63 d	72 d	105 d	150 d
Oryza leaves	26 ± 5 *n* = 9	33 ± 10 *n* = 9*		56 ± 6 *n* = 9*		118 ± 27 *n* = 9	289 ± 92 *n* = 9*		234 ± 75 *n* = 9	
Oryza seeds						18 ± 5 *n* = 9				17 ± 3 *n* = 9
Lasthenia leaves	35 ± 14 *n* = 18	76 ± 19 *n* = 17*				159 ± 33 *n* = 9	295 ± 50 *n* = 9*			
Lasthenia flowers	12 ± 2 *n* = 3	30 ± 7 *n* = 3				81 ± 27 *n* = 3	108 ± 32 *n* = 3			
Lasthenia seeds				22 ± 3 9				17 ± 2 *n* = 9		
Deschanmpsia leaves	27 ± 7 *n* = 9	38 ± 5 *n* = 9*		30 ± 7 *n* = 18*		210 ± 80 *n* = 9	171 ± 52 *n* = 9		248 ± 50 *n* = 9*	

Table 1 (taken from [redacted] et al., 2002): Mean bioaccumulation factor ([BCF] = µg trifluoroacetate [TFA]/g dry plant weight divided by µg TFA/g solution) values of *Oryza* leaves and seeds; *Lasthenia* leaves, flowers, and seeds; and *Deschanmpsia* leaves for the 100- and 1000-µg/L exposures as a function of time. Data presented are mean 6 standard deviation of BCF value calculated for *n* plants. To convert BCF values to µg TFA/g dry weight for the 100-µg/L exposure concentration, divide by a factor of 10. The BCF values listed for the 1000-µg/L exposure are equivalent to µg TFA/g dry-weight concentrations. Asterisks indicate that data are statistically different ($p < 0.05$) than prior sampling.

After 105 d, *Oryza* grown in 100 µg/L TFA had accumulated 5.6 ± 0.9 µg/g TFA (n = 9) in leaf tissue, whereas controls had < 0.05 µg/g TFA (n = 9). After 63 d, leaf tissue of *Oryza* grown in 1000 µg/L TFA exposure had accumulated 289 ± 92 µg/g TFA (n = 9), and at 105 d concentration had declined by 19 % ($p < 0.05$; 234 ± 75 µg/g, n = 9). *Deschampsia* also accumulated TFA as a function of exposure concentration; however, at 42, 63 and 105 d, foliar concentrations were roughly the same as reflected in the bioconcentration factors. The mean foliar concentration was 3.0 ± 0.7 µg/g (n = 18) for the 100-µg/L exposure and 248 ± 50 µg/g (n = 9) for the 1000-µg/L exposure at 105 d (controls contained < 0.02 µg/g). *Lasthenia* plants did not live as long *Oryza* and *Deschampsia* and by day 63 had developed seeds and were beginning to die. After 42 d, their mean foliar concentration was 7.5 ± 1.9 µg/g (n = 17) for the 100-µg/L exposure and 295 ± 50 µg/g (n = 9) for the 1000-µg/L exposure (controls contained < 0.04 µg/g). *Lasthenia* flowers also bioaccumulated TFA but to a lesser amount than the foliar tissue.

Oryza seeds accumulated 1.8 ± 0.5 µg/g for the 100-µg/L exposure and 17 6 3 µg/g for the 1000-µg/L (controls contained < 0.07 µg/g). *Lasthenia* seeds had TFA concentra-tions of 2.2 6 0.3 µg/g for the 100-µg/L exposure and 17 ± 2 µg/g for the 1000-µg/L exposure (controls contained < 0.01 µg/g). It is noteworthy that *Oryza* and *Lasthenia* seeds had similar TFA concentrations and bioconcentration factors de-spite the fact that they required different amounts of time to fully develop. No adverse physiological effects were observed for plants exposed to TFA concentrations as high as 1000 µg/L. Photosynthetic and conductance rates for exposed plants did not differ significantly ($p < 0.05$) from the controls. Mean photosynthetic rates were 19.0 ± 3.6 and 11.1 ± 4.2 mmol CO_2/ mol air for *Oryza* and *Deschampsia*, respectively.

Mean conductance rates were 0.4 ± 0.2 and 0.20 ± 0.09 mol H_2O/mol air for *Oryza* and *Deschampsia*, respectively. Photosynthetic rates reflect the plant's ability to fix CO_2, and conductance rates reflect the plant's ability to transpire water.

Biomass

After 57 d, *Deschampsia* exhibited no significant ($p < 0.05$) difference in the plant height and biomass for the control versus the treatment plants (100 µg/L TFA). Leaf and root biomass and leaf length of *Polypogon* and *Oryza* harvested after two months of growth in 10-, 100-, and 1000-µg/L exposure concentrations were not significantly different from those plants grown in solutions containing no TFA with one exception. *Polypogon* exhibited a slight decline in leaf length with long-term exposure of 1000 µg/L TFA; however, no significant reduction was observed in development of biomass.

Germination experiments

The first-generation germination experiments showed no significant effect at any TFA exposure concentration (100, 1000, and 10000 µg/L TFA) for Eryngium and Epilobium. In fact, Eryngium and Epilobium seeds exposed to solutions without TFA exhibited less germination success than those seeds exposed to TFA. In replication of this experiment, Eryngium and Epilobium seeds in control solutions exhibited better germination success for the first 9 d than seeds germinating in the 10000-µg/L TFA solution. Lasthenia seeds in control solutions exhibited significantly better germination success (~5-10 %) than seeds exposed to TFA in both replicate experiments. Deschampsia germination success was significantly better (~10-30 %) for the first 9 d for seeds in the 0- and 100-µg/L exposures than higher exposures in the first experiment.

However, in the duplicate experiment, *Deschampsia* seeds germinated in the 1000- and 10000-µg/L solutions exhibited greater success than the 0- and 100-µg/L exposures.

In the third first-generation germination experiment, which utilized 200 seeds of *Oryza sativa*, *Lasthenia californica* and *Deschampsia elongata*, both *Lasthenia* and *Deschampsia* seeds in 0-µg/L TFA solutions exhibited significantly higher germination success than respective seeds in solutions containing TFA. For *Oryza*, no statistically significant difference was observed between success of seeds grown in the presence or absence of TFA.

Experiments in which *second-generation seeds* were germinated in solutions of the same concentration as the parent plants exhibited inconsistent results. In the first experiment, control seeds of *Lasthenia* exhibited significantly better germination success than seeds in the TFA-containing solutions. These results were not observed in the duplicate experiment, where the mean success for the three control exposures was not significantly different from germination success of seeds in the 1000-µg/L exposures.
The germination success of second-generation *Oryza* seeds in solutions of 0 µg/L TFA was less than for seeds exposed to TFA. In addition, *Lasthenia* and *Oryza* second-generation seeds germinated in solutions without TFA showed no significant difference in germination as a function of seed TFA concentration.

RESULTS SUMMARY

Based on the results of this study investigating (a) vernal pool soil microbial communities with respect to soil respiration and (b) vernal pool and wetland plant species with respect to morphology and biomass development, photosynthetic and conductance rates, and germination success, no adverse effects as a consequence of environmentally relevant TFA exposures or even concentrations one order of magnitude higher need to be expected. In conclusion, it is unlikely that vernal pool microbial community and plant growth, development, and health will be impacted by the predicted TFA concentrations.

Comments by the Notifier:
This study confirms the results from an existing study on effects of TFA on microbial nitrogen transformation. The microbial degradation is not affected due to the presence of TFA in soil. Thus, this study will not be further considered in the risk assessment.

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Report:	**KCA 8.7/02; [redacted], M.F., van [redacted], P.D.R., [redacted], J.J., [redacted], L., [redacted], R.J., [redacted], G.H.J. (2009)**
Title:	Effect of trifluoroacetate, a persistent degradation product of fluorinated hydrocarbons, on *Phaseolus vulgaris* and *Zea mays*
Source	Plant Physiology and Biochemistry 47 (2009) 623–634
DOI No:	doi:10.1016/j.plaphy.2009.02.003
Document No:	M-455801-01-1
Guidelines:	Not stated
GLP:	Not stated

EXECUTIVE SUMMARY

The aim of this study was to quantify the effect of the pollutant, trifluoroacetate (TFA), on growth and photosynthesis of *Phaseolus vulgaris* (C3) and *Zea mays* (C4) in order to elucidate the physiological and biochemical basis of its inhibitory action. In whole plant studies, photosynthetic gas exchange, fast phase fluorescence kinetics and Rubisco activity were measured in parallel over a 14-day period in plants cultivated in a water culture system with NaTFA added at concentrations ranging from 0.625 to 160 mg L^{-1}. Although initial stimulation of some photosynthetic parameters was observed at low TFA concentrations early on in the experiment, marked inhibition occurred at higher concentrations. In general *Z. mays* was affected more severely than *P. vulgaris* showing a large TFA-induced decrease in both apparent carboxylation efficiency and in vitro Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity. Analysis of photosynthetic gas exchange revealed that besides constraints on mesophyll processes such as Rubisco activity, stomatal limitation also increased with increasing TFA concentration, especially in *P. vulgaris*. In depth analysis of the fast phase fluorescence transients pointed at TFA-induced uncoupling of the oxygen evolving complex and inhibition of electron transport beyond Q_α including possible constraints on the reduction of end electron acceptors of photosystem I.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Trifluoroacetate
Active substance(s):	See above
Chemical state and description:	Liquid
Source of test item:	Not stated
Batch number:	Not stated
Purity:	Not stated
Storage conditions:	Not stated
Water solubility:	Not stated

2. Test solutions

Vehicle/solvent:	Not stated / not used
Source of vehicle/solvent:	See above
Concentration of vehicle/solvent:	See above

Method of preparation:	See above
Evidence of unsolved material:	See above

3. Test organism(s)

Species:	*P. vulgaris* (genotype: Panthera); *Z. mays* (genotype: Jenny)
Common name:	Not stated
Source of test species:	Not stated

4. Culture conditions of test organism(s)

Culture medium:	Hoagland's nutrient solution (pH 6.8)
Temperature:	Unclear if culture conditions differ from test conditions (see below). Plants were cultured according to the method described in Hoagland & Arnon (1950)
Photoperiod:	See above
Light intensity:	See above
pH:	See above
Oxygen saturation:	See above
Food and feeding regime:	Hoagland's nutrient solution was changed every 3 days
Acclimatisation prior to testing:	A few days after germination in vermiculite plants were transferred to the water culture system, consisting of aerated opaque glass bottles filled with nutrient solution also used in the test
Observations during acclimatisation:	Not stated

B. Study design and methods

1. Test procedure

Test system:	Laboratory test, water culture system
Test concentration(s):	0.625, 2.5, 10, 40 and 160 mg TFA L-1
Control(s):	Water culture solution without test item
Number of replicates:	4 replicates per treatment group and control
Treatments / Test conditions:	Experiments were carried out over a 14-day treatment period on plants grown in growth chambers under vigorously controlled conditions, i.e.: 15-h photoperiod and 26°C /20°C day/night temperatures. The irradiance intensity of 1000 µmol m^{-2} s^{-1} at the level of the plant canopy in the chambers was provided by a combination of fluorescent (Sylvania Cool White VHO, 215 W) and incandescent (Sylvania, 100 W) lamps. The CO_2 concentration inside the chambers was controlled at 350 µmol mol^{-1} by a built-in infrared gas analyser connected to CO_2 gas cylinders. When the third leaves of *P. vulgaris* and *Z. mays* reached maturity chlorophyll a fluorescence, photosynthetic gas

	exchange and the chlorophyll content index were measured in these leaves. In addition, the plastochron index (in the case of *P. vulgaris*) of each plant was determined. Thereafter, NaTFA was applied to the water culture solution at different concentrations (see above). Measurements were taken 4, 8 and 12 days after application. Measurements throughout the experiment were done on the same mature leaves.
Feeding:	Fresh nutrient solutions were applied on days 5 and 9
Medium renewal:	See above
Frequency of test item application:	NaTFA was applied at test start and on days 5 and 9 (together with the exchange of the nutrient solution)
Test duration:	14 day treatment period
Endpoints:	Measurement of plant development (plastochron index), biomass; CO_2 assimilation; determination of chlorophyll content index; measurements of oxygen evolution/consumption on isolated thylakoids, chlorophyll a fluorescence and rubisco activity [for details on methods, please refer to the study]
Statistics:	In data sets with parametric distribution, significant differences between treatment means were determined using Student's t-test.

2. Measurements during the test

Water/medium parameters:	Not stated. However, nutrient solution was exchanged on days 5 and 9.

3. Sampling

Sampling frequency:	Measurements were taken 4, 8 and 12 days after application.
Transport/storage of samples:	Not stated

4. Chemical analysis

Guideline/protocol:	Concentrations of the test item were not confirmed by appropriate analytical verification.
Method:	See above
Pre-treatment of samples:	See above
Conduction:	See above
Reference item:	See above
Recovery:	See above
Limit of detection:	See above
Limit of quantification:	See above

RESULTS

1. Validity criteria:

Study was not conducted according to an official test guideline, e.g. OECD or EU guideline. No validity criteria were determined.

2. Analytical findings:

Concentrations of the test item were not confirmed by appropriate analytical verification. Nutrient solutions together with the test item were exchanged on days 5 and 9.

3. Other measurements:

Please refer to point 3 'Biological findings'. Measurement of other parameters was not reported.

4. Biological findings:

Effects on plant growth: From day 7 to 14 growth rates of *P. vulgaris* (measured by the plastochron index, in ΔPI units per day) declined with increasing concentration of TFA ranging from 0.625 to 160 mg NaTFA L^{-1}. The respective growth rate reductions were 13 %, 12 %, 48 %, 48 % and 76 %. The reductions in growth at the 0.625 and the 2.5 mg L^{-1} concentrations were not statistically significant. At the end of the treatment period significant differences occurred in the final PI values corresponding to decreases of 11 %, 30 %, 27 % and 38 % for the NaTFA concentration of 2.5, 10, 40 and 160 mg L^{1}, respectively. *Z. mays* plants also displayed a reduction in plant height and growth rate with increasing TFA concentration. Due to their monocotyledonous growth form, however, no PI values could be measured.

TFA treated *Z. mays* plants displayed signs of increasing chlorosis and reduction in plant height with increasing TFA concentration. The visible chlorotic symptoms in *Z. mays* corresponded to the actual decreases in measured chlorophyll content index values ranging from 17 % to 70 % for the 0.625 – 160 mg L^{-1} treatments, respectively. In contrast to *Z. mays*, in *P. vulgaris* no significant chlorosis occurred at any TFA level applied. Severe epinasty, wrinkling and necrosis of young *Z. mays* and *P. vulgaris* leaves were observed in the 40 and 160 mg L^{-1} treatments. No visual symptoms were, however, observed on the mature leaves which were used for physiological measurements.

Observations of reduction in plant growth and development also correlated with the shoot and root biomass data: Shoot growth was stimulated (although not statistically significantly; $p > 0.05$) at 0.625 and 2.5 mg L^{-1} in *P. vulgaris*, but was significantly inhibited at all higher concentrations in both species. Since root growth was inhibited much more than shoot growth in both species, increased shoot:root ratios occurred. *Z. mays* however displayed a larger inhibition of root growth than *P. vulgaris*.

Inhibition of photosynthetic CO_2 assimilation by TFA

Inhibition of photosynthetic CO_2 assimilation: The constraints imposed by TFA on photosynthetic gas exchange of the test plants were evaluated by analysis of CO_2 response curves, i.e. CO_2 assimilation rate plotted vs. intercellular CO_2 concentration response curves. The data of the study revealed that *P. vulgaris* and *Z. mays* responded differently to TFA treatment. The initial slope of the demand function, which is a measure of the apparent carboxylation efficiency, was much more effected in *Z. mays* (69 % decrease at the 160 mg L^{-1} concentration) than in *P. vulgaris*. On the other hand the supply function, which is related to the stomatal conductance, was inhibited more in *P. vulgaris* (58 % decrease) at the

160 mg L^{-1} concentration than in *Z. mays* (43 % decrease). Early on, after 4 days of treatment at 0.625 mg L^{-1}, an increase of 55 % in stomatal conductance was apparent in *P. vulgaris*.

This initial increase in stomatal conductance however soon gave way to a decrease in stomatal conductance at all TFA concentrations. In *Z. mays*, a C_4 plant, J_{max} which is determined by either Rubisco activity, PEP regeneration capacity or photosynthetic electron transport rate, was already reached at a Ci value of below 500 mmol mol^{-1}, a phenomenon typical of C_4 plants. A very pronounced decrease in J_{max} of up to 33 % at the highest TFA concentration occurred. In *P. vulgaris*, the corresponding TFA induced changes in J_{max}, which is an indicator of RuBP regeneration capacity, were much less pronounced, showing only a 19 % decrease at the highest concentration. From the calculated intercellular CO_2 concentration (C_i) values, corresponding to the respective actual CO_2 assimilation rate, it was evident that in the case of *Z. mays*, C_i almost remained constant, while in the case of *P. vulgaris*, C_i decreased with increasing TFA concentration.

Inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity. In *P. vulgaris* statistically non-significant decreases in total Rubisco activity, calculated on a leaf area basis, namely 8 %, 14 %, 29 %, 27 % and 15 % occurred at the 0.625, 2.5, 10, 40 and 160 mg L^{-1} NaTFA treatments respectively. In *Z. mays* on the other hand decreases of 20 %, 8 %, 32 %, 52 % and 46 % were observed at the corresponding concentrations. Since the initial Rubisco activity changed in parallel with total Rubisco activity in both *P. vulgaris* and *Z. mays*, no significant change in Rubisco activation state occurred.

Inhibition of photosynthetic electron transport in thylakoids of *P. vulgaris*

TFA had marked concentration dependent effects on the electron transport of isolated thylakoid membranes in the system, H_2O – PSII - FeCy. In this case, the oxygen evolution rate was used as measure of electron transport rate. At the lowest TFA treatment of 0.00005 mmol L^{-1}, a significant stimulation of 9 % occurred in the oxygen evolution rate, while a significant decrease ranging from 10 % to 52 % occurred at increasing concentrations ranging from 0.005 to 100 mmol L^{-1} respectively. TFA also had marked concentration dependent effects on electron transport of isolated thylakoid membranes in the system, DCPIP/Asc – PSI - MV/NaN_3. In this case the oxygen consumption rate was used as measure of electron transport rate. At the lowest TFA treatment of 0.0001 mmol L^{-1} no significant inhibition occurred in oxygen consumption rate, while a significant decrease ranging from 1 % to 33 % occurred at concentrations ranging from 0.001 to 100 mmol L^{-1} respectively.

Inhibition of PSII function and photosynthetic electron transport in vivo

Analysis of the recorded chlorophyll a fluorescence transients showed that TFA-concentration dependant changes occurred in both the specific (per reaction centre) and the phenomenological (per cross-section) energy fluxes through PSII in both *P. vulgaris* and *Z. mays*. In *P. vulgaris* significant decreases occurred in the electron transport per cross-section of 2 %, 3 % and 12 % as well as concurrent decreases in density of reaction centres of 3 %, 6 % and 7 % at the 10, 40 and 160 mg L^{-1} concentrations, respectively. Concomitantly significant increases occurred in ‘‘antenna size’’ of 5 %, 9 %, 16 % and 13 % as well as decreases of 4 %, 3 %, 14 % and 10 % in the specific trapping flux from the 2.5 to the 160 mg L^{-1} concentration, respectively.

Also *Z. mays* displayed significant decreases in the electron transport per cross-section namely 8 %, 11 % and 15 % with concomitant decreases of 11 %, 12 % and 8 % in density of reaction centres at the 10, 40 and 160 mg L^{-1} concentrations, respectively. Concurrently a significant increase in ''antenna size'' of 10 %, 15 % and 17 % and an increase of 8 %, 12 % and 14 % in the specific trapping flux occurred for the 10, 40 and 160 mg L^{-1} concentrations.

The performance index calculated on an absorption basis (PI_{total}) was found to be a very sensitive parameter for quantification of TFA-effects in both *P. vulgaris* and *Z. mays*. For *P. vulgaris*, changes in PI_{total} after 12 days of treatment corresponded well to the corresponding gas exchange data. The PI_{total} of treated *P. vulgaris* plants decreased significantly between 10 % and 35 % for concentrations ranging from 0.625 to 160 mg L^{-1} respectively.

The individual effect on the component parameters of PI_{total} was as follows: the efficiency of absorption of light decreased significantly by 7 %, 15 % and 11 % in the range 10–160 mg L^{-1} respectively; the performance due to the quantum efficiency of primary photochemistry decreased significantly by 2 %, 7 %, 8 % and 13 % from the 2.5 to the 160 mg L^{-1} concentrations; the performance due to the quantum efficiency of the conversion of excitation energy to electron transport decrease by 7 %, 6 %, 13 %, 22 % and 30 % from the 0.625 to the 160 mg L^{-1} treatment; the performance due to the quantum efficiency of the reduction of end acceptors decreased by 11 % at the 10 mg L^{-1} concentration. At the 40 – 160 mg L^{-1} concentrations, it showed a significant increase of 10 % and 19 % respectively.

In *Z. mays* the PI_{total} decreased significantly by between 6% and 48 % from the 0.625 to the 160 mg L^{-1} concentrations respectively. The effect on the component parameters of PI_{total} was as follows: the efficiency of light absorption decreased significantly by 9 %, 13 % and 14 % from the 10 to the 160 mg L^{-1} concentrations respectively; the performance due the quantum efficiency of primary photochemistry displayed a significant decrease of 9 %, 13 % and 10 % from the 10 to the 160 mg L^{-1} concentrations; the performance due to the quantum efficiency of the conversion of excitation energy to electron transport displayed a decrease of 10 %, 20 % and 30 % from the 10 to the 160 mg L^{-1} concentrations; the performance due to the quantum efficiency of the reduction of end acceptors decreased by 8 % at the 0.625 mg L^{-1} concentration and showed a maximum decrease of 18 % at the 40 mg L^{-1} concentration.

RESULTS SUMMARY

This study reported on adverse effects on growth as well as the physiological and biochemical basis of the inhibition of photosynthesis in *P. vulgaris* and *Z. mays* plants which were induced by NaTFA applied to growth medium (water culture system instead of soil culture system). However, TFA levels tested in this study are much greater (by orders of magnitude) than the levels currently found in the environment.

Comments by the Notifier:

This study reports physiological effects of TFA in two plant species. These endpoints are not comparable to endpoints obtained from tests with non-target plants (i.e. emergence, survival, biomass). Thus, this study will not be further considered in the risk assessment.

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Report:	**KCA 8.7/01; [redacted], N.[redacted], L.S., [redacted], D.B., [redacted], D.W. (2004)**
Title:	The HFC/HCFC breakdown product trifluoroacetic acid (TFA) and its effects on the symbiosis between *Bradyrhizobium japonicum* and soybean (*Glycine max*)
Source:	Soil Biology & Biochemistry 36 (2004) 333–342
DOI No:	doi:10.1016/j.soilbio.2003.10.00
Document No:	M-455785-01-1
Guidelines:	Not stated
GLP:	Not stated

EXECUTIVE SUMMARY

The study was performed in accordance with the Alternative Fluorocarbon Environmental Acceptability Study (AFEAS). Those results are presented in addition to the findings of further experimentation on the initial interaction of *B. japonicum* with soybean. Three levels of TFA (0.67, 6.74 and 67.40 µL TFA kg^{-1} soil; 0.003, 0.031 and 0.314 µL TFA L^{-1}) were used for soil and hydroponics conditions and three levels (10, 100 µM and 1 mM) in bacterial culture. The results demonstrate that TFA affects growth of *B. japonicum* significantly, but does not affect PHB accumulation. Also no F⁻ was found in cultures grown on TFA. Attachment of *B. japonicum* to soybean roots was enhanced with the lowest level of acetate or TFA and was significantly reduced with 1 mM acetate or TFA. Cultures grown on acetate or acetate with TFA do not attach well, with those grown with 1 mM TFA the least. Both effects may be attributed to pH. Soybean seedlings had significantly retarded development with levels of TFA at or above 6.74 µL TFA kg^{-1} soil and 0.031 µL TFA L^{-1} nutrient solution. No nodules formed on those plants treated with these levels of TFA except in the hydroponics trials. Nodule location was not affected regardless of the TFA level. At the lowest level used we found no effects on soybean or symbiotic nitrogen fixation. In some cases, nodulation was enhanced, but nodule weight reduced. Anaerobically isolated bacteroids had normal levels of acetylene reduction activity regardless of the level of TFA used.

In summary, soybean is much more sensitive to low levels of TFA than its symbiotic counterpart *B. japonicum*. No detrimental effects on symbiotic nitrogen fixation in soybean should be expected unless large bioaccumulation of TFA occurs in agricultural areas.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Trifluoroacetic acid (TFA)
Active substance(s):	See above (MW 114.03)
Adjuvant / Surfactant:	Gluconate or acetate as carbon source
Source of test item:	[redacted]

	[redacted]
Lot/Batch number:	Not stated
Purity:	Not stated
Storage conditions:	Not stated

2. Test solutions

Vehicle/solvent:	Not stated
Source of vehicle/solvent:	Not stated
Concentration of vehicle/solvent:	Not stated

3. Test organism(s)

Species:	*Bradyrhizobium japonicum* strains USDA 110, 2143 and 184; *G. max* seedlings (cv Williams 82)
st species:	Not stated
Age of test organisms at study initiation / Crop growth stage at treatment:	Not relevant / not stated
Holding conditions prior to test:	See below (treatments)
Acclimatisation:	See below (treatments)

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory study investigating effects of TFA on symbiotic nitrogen fixation in soybeans
Guideline deviation:	No official test guideline available
Duration of study:	Unclear. Approximately 40 days
Treatments:	TFA in culture (free living state): Strains grown in Tully's (T.) defined liquid media without vitamins (exact composition is given in the study) with acetate, pH 6.8 (T. acetate). Liquid cultures were grown at 28 8C, monitored over time and sampled for optical density (O.D.) readings at A_{630} using a Cary 1Bio UV–Visible. Growth curves (three trials) were performed on *B. japonicum* 2143 using three different starting O.D. (5× 10, 1×10^7 and 5×10^7 cells mL^{-1}) and were monitored periodically at A_{630} until stationary growth phase. To test the effects of TFA on growth of this strain, three different concentrations (10, 100 µM and 1 mM) of TFA were added to T. acetate. Inorganic acids, organic acids and free fluoride content of B. japonicum 2143 grown on gluconate, acetate and acetate þ TFA, were analyzed in duplicate cultures which had reached late-log phase (method is described in the study). The extent of PHB accumulation within *B. japonicum* strain 2143 grown on gluconate, acetate and acetate + TFA, was performed with UV detection of crotonic acid (from PHB acid-catalyzed chemical depolymerization) at 210 nm (method is described in the study).

Attachment of B. japonicum to soybean roots: *B. japonicum* strain 2143, either grown on T. gluconate, T. acetate or T. acetate plus TFA were diluted to a standard 1× 10^7 cells ml^{-1} with a buffered solution, then incubated with the roots of whole soybean seedlings (cv Williams 82) and the cells were allowed to attach to the roots for 3 or 6 min. Cells were removed with low intensity sonication, aliquots plated in replicate and colonies counted to quantify the number of cells attached to the roots. In a separate experiment, strain 2143 grown in T. gluconate was compared for attachment in the presence of three levels of TFA in the attachment buffer with the controls having equal amounts of acetate. For this experiment the attachment buffer (which has a low buffering capacity) was allowed to undergo a pH change from the addition of the three levels of acetate or TFA. The pH of each solution of acetate or TFA dissolved in the attachment media was 10 mM acetate (pH 6.8), 100 mM acetate (pH 6.7), 1 mM acetate (pH 4.6), 10 µM TFA (pH 6.8), 100 µM TFA (pH 6.5) and 1mM TFA (pH 3.4). Results are presented as the number of cells attached per root from three separately inoculated seedlings, done in replicate.

Symbiosis: The effect of TFA on symbiosis was tested in two different growth regimes. First regime utilized a sterile Missouri silt loam soil (< 10 % organic matter) with TFA incorporated at levels of 0.674, 6.74 and 67.4 µL TFA kg^{-1} of dry soil. Second regime utilized a hydroponics system where the root system was bathed in a nitrogen free plant nutrient solution contained within sterile clear plastic growth pouches with TFA incorporated at levels of 0.003, 0.031 and 0.314 µL TFA L^{-1}. Both experiments done with soybean cv Williams 82. Environmental conditions: experiments utilized in a growth chamber with 50 % relative humidity and a 16 h light/8 h dark cycle. Plants were inoculated with *B. japonicum* strain 110, 2143 or 184 depending on the experimental parameter.

Test concentrations: See above (Treatments)
Number of replicates: See above (Treatments)
Individuals per replicate: See above (Treatments)
Test conditions: See above (Treatments)
Test units (type and size): See above (Treatments)
Application / device / nozzles: See above
Water volume: See above
Calibration of sprayer: Not relevant / not stated

2. Environmental conditions: See above (Treatments)
Test medium: See above
Temperature / relative humidity: See above
Photoperiod: See above
Lighting: See above
pH: See above

Organic matter (C_{org}):	See above
$CaCO_3$	See above
Cation exchange capacity:	See above
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	See above
Fertilization:	See above

3. Observations and measurements:

Analytical parameters measured:	Concentrations of the test item were not confirmed by appropriate analytical verification.
Biological parameters measured:	See above (Treatments)
Measurement frequency:	See above (Treatments)
Statistical analyses:	Statistical significance for the majority of experiments was determined using the t-test for significance as control and experimental standard deviation values were re-evaluated for each experimental trial. Chi^2 analysis was performed on those experiments where the control was replicated enough to serve as an expected value for each data point.

RESULTS

1. Validity criteria:
No official test guideline available and thus no validity criteria.

2. Other measurements:
Please refer to point 3 'Biological findings'. Measurement of other parameters was not reported.
3. Biological findings:

TFA in culture (free living state): *B. japonicum* 2143 grown in liquid culture with 10 mM gluconate as the carbon source had a doubling time of 10 h. The same strain grown both on 10 mM acetate or 10 mM acetate with TFA added revealed that growth was slower with increasing amounts of TFA. The doubling times were acetate (14 h), acetate with 10 µM TFA (15 h), acetate with 100 µM TFA (20 h) and acetate with 1 mM TFA (28 h). Analysis of PHB content of *B. japonicum* 2143 grown in the presence of TFA revealed that accumulation of PHB is three times higher in those cells grown on acetate compared to those grown on gluconate. In addition, the presence of TFA had no affect on PHB accumulation in those cells grown only on acetate, regardless of the level of TFA in addition to the acetate. Fluoride was not detected in any cultures grown in the presence of TFA, however, small levels of fluoride were detected in those grown on acetate and gluconate.

Attachment of *B. japonicum* to soybean roots: The effect of TFA on attachment of *B. japonicum* to soybean roots was determined under two conditions. The first condition was the attachment of *B. japonicum* grown on gluconate and then assayed for attachment in the presence of acetate or TFA (see Fig. 1). When cells were grown on gluconate and then exposed to low levels of TFA or acetate during the attachment assay, the number of cells adhering to the root increased. The number of cfu significantly increased after a 6 min incubation; the trend was obvious at 3 min but not significant. *B. japonicum* incubated with the highest level of acetate or TFA demonstrated reduced attachment. This reduction could have been the result of a drop of pH in the attachment medium, since authors of

this study have found correlation between low rhizosphere pH and reduced attachment (unpublished results).

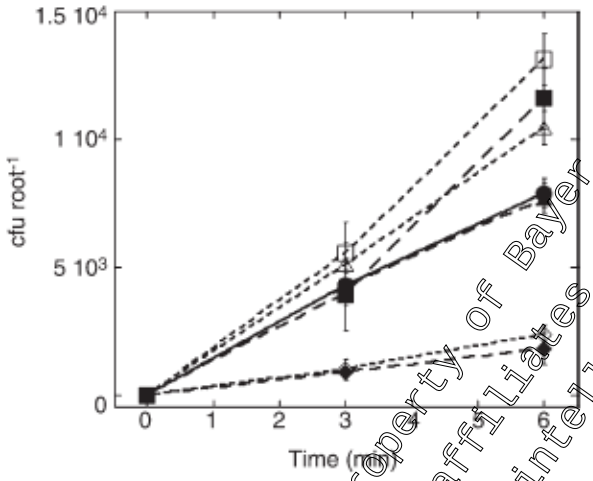

Fig. 1. Attachment (in cfu root^{-1}) of *B. japonicum* strain 2143 grown on T. gluconate to soybean roots with three levels of acetate or TFA. Control (●), 10 µM acetate (■), 100 µM acetate (▲), 1 mM acetate (◆), 10 µM TFA (□), 100 µM TFA (○) and 1 mM TFA (◇). Data point error is reported as SEM.

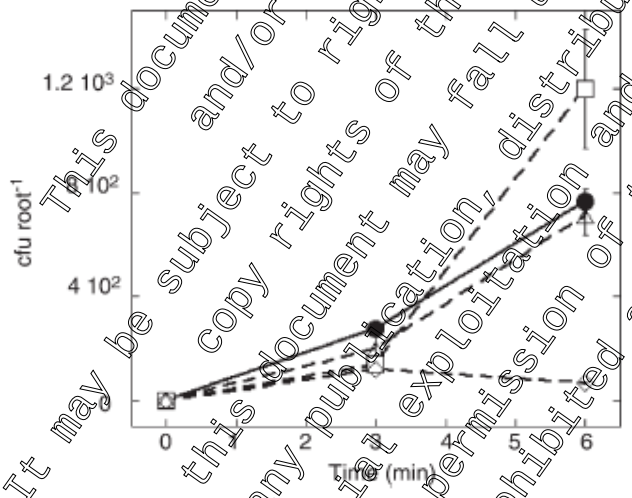

Fig. 2. Attachment (in cfu root^{-1}) of *B. japonicum* strain 2143 grown on T. acetate (●), and T. acetate plus 100 µM TFA (□), 1 mM TFA (△) or 1 mM TFA (◇). Data point error is reported as SEM.

The second condition was attachment of *B. japonicum* grown on acetate in the absence or presence of TFA (see Fig. 2). TFA was removed immediately prior to the assay. Growth on acetate markedly reduced the number of cells capable of attachment. This result is in contrast to the effect of acetate in the attachment medium of cells grown on gluconate. This demonstrates that acetate affects the attachment process differentially depending on whether acetate is the primary carbon source for growth or is an exogenous effector. Growth on acetate in the presence of low levels of TFA enhanced

attachment at 6 min. In the first attachment experiment, acetate and TFA yielded similar effects, but in this experiment different results were obtained suggesting that acetate and TFA may have different mechanisms of action. The highest level of TFA reduced attachment significantly. The effects of TFA observed here were not due to pH as the growth medium was highly buffered, but TFA did significantly increase culture doubling time.

Symbiosis: Effects of TFA on early plant growth (post-germination) were examined when both the soybean seedling and *B. japonicum* were pre-incubated for 1 h with the three levels of TFA. There was no statistically significant difference in the fresh weights of plants between controls not treated with TFA and those treated with 0.674 µl TFA kg^{-1} soil (see Figure 3A). However, those plants treated with the two highest levels of TFA were developmentally stunted and had shoot weights that were significantly reduced. Those plants treated with 0.674 µl TFA kg^{-1} soil developed root systems similar to those of the control plants and they developed very normal nodules capable of nitrogen fixation. The nodule weight (see Fig. 3B) of these plants was not significantly different from the control plants nor was the acetylene reduction activity (see Fig. 3C) of these plants different from control plants: However, higher levels of TFA significantly affected plant development and shoot fresh weight when pre-incubated with TFA. Those plants treated with 6.74 µL kg^{-1} soil developed secondary shoots with small leaf-like structures at the internodes, but these structures remained small and never developed into mature leaves. Internodal expansion was much less than those of the untreated control, resulting in stunted growth. The growth of most of these plants halted between eight and ten days. The root systems of these plants were considerably shorter and less developed compared to untreated plants. These plants occasionally developed root nodules, but they were small and ineffective. Those plants treated with 67.4 µL TFA kg^{-1} soil never progressed beyond the cotyledon stage of plant development. That is, these plants germinated and the seeds opened to expose the cotyledons as they normally do, but the secondary shoot never emerged. All growth ceased at three days but the cotyledons remained green and succulent throughout the experiment. Root development was also severely reduced. None of these plants developed root nodules.

Additional experiments were performed in which *B. japonicum* and soybean seedlings were allowed to begin the infection process before being planted into soil containing TFA. The plants grown in soil with the two highest levels of TFA (6.74 and 67.4 µL TFA kg^{-1} soil) showed very similar developmental effects to those plants described in the previous experiment, so little or no nodule data could be collected. The 0.674 µL TFA kg^{-1} treatment was reduced in terms of acetylene reduction activity relative to the control at 32 dpi. This difference was not significantly less in conjunction with all other time points, which were very similar. The decrease in acetylene reduction activity at the peak time was unexpected since the number of nodules formed per plant was significantly higher. Consequently, the average nodule weight of those grown with TFA was much less of those harvested at 32 and 35 dpi, respectively.

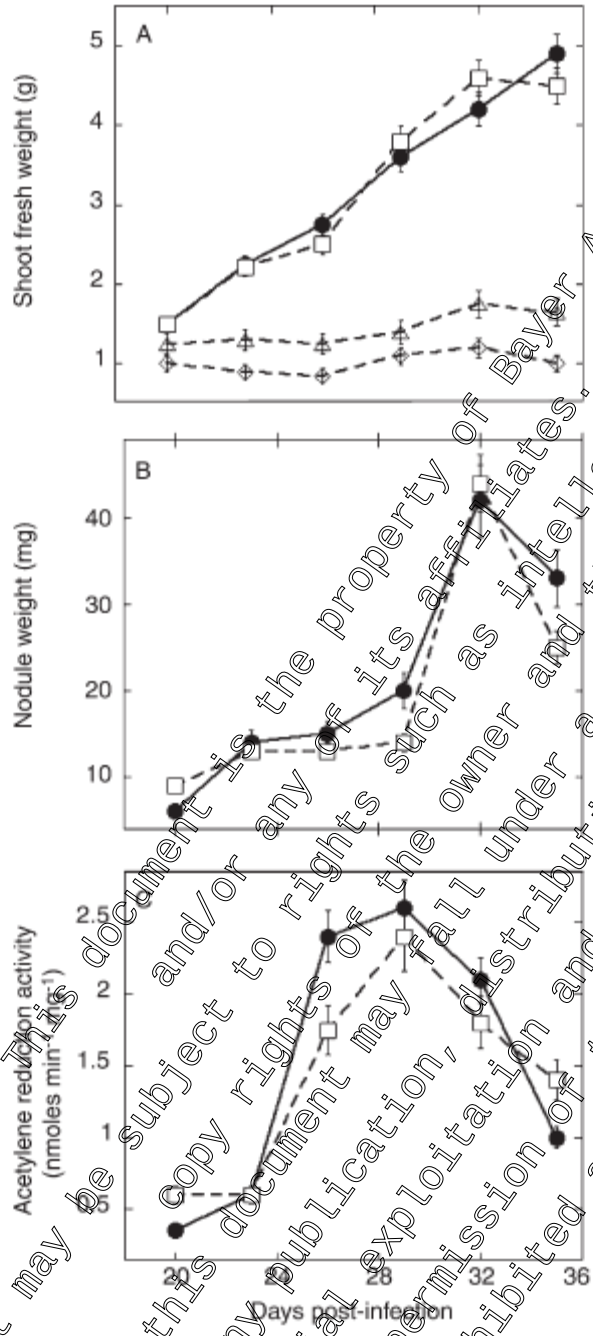

Fig. 3. (A) Shoot weights of soybean when both plant and bacteria were pre-incubated with TFA for 1 h before inoculating and planting in soil containing either no TFA (●), 0.674 µl TFA kg^{-1} soil (□), 6.74 µl TFA kg^{-1} (▲) and 67.4 µl TFA kg^{-1} (◇). (B) The nodule weight of those plants in (A) not treated (●) or treated with the lowest level of TFA (□). (C) Acetylene reduction activity of those nodules collected in (B) not treated with TFA (●) or treated with the lowest level of TFA (□). Data point errors are reported as SEM.

To determine whether greater concentrations of TFA could affect the reduction of atmospheric dinitrogen, bacteroids, the symbiotic form of *B. japonicum*, were isolated from 4-week old nodules on plants grown in soil without TFA. The bacteroids were measured ex planta in the presence of TFA by the acetylene reduction technique, which serves as an index of nitrogen fixation activity. The bacteroid acetylene reduction activity for each level of TFA tested were statistically no different from control plants suggesting that TFA has no direct effect on the nitrogenase enzyme complex.

The results from using TFA with strain 110 and soybean under hydroponics conditions were different from those obtained from both soil experiments. As with the soil experiments, 0.003 µL TFA L^{-1} of solution had no measurable effect on plant growth whereas 0.031 and 0.314 µl TFA L^{-1} of solution retarded plant development. However, these conditions were sufficiently different from the soil trials in that nodules were able to form on the roots of all plants regardless of treatment. The pronounced effects of the two highest TFA levels on plant development in soil conditions precluded obtaining nodule number, mass or development. With these plants however, nodulation onset was normal regardless of treatment. These plants were not pre-incubated with TFA and were only subjected to it under growth conditions. The average nodule weight from these same plants (see Fig. 4) indicates that TFA at all three levels had some effect on nodule mass, especially in the latter portion of the nitrogen fixation time course. At 24 and 27 dpi there was a statistically significant reduction in nodule mass with the lowest level of TFA. At the two higher levels of TFA, even though the plants had the same number of nodules, they were significantly reduced in mass.

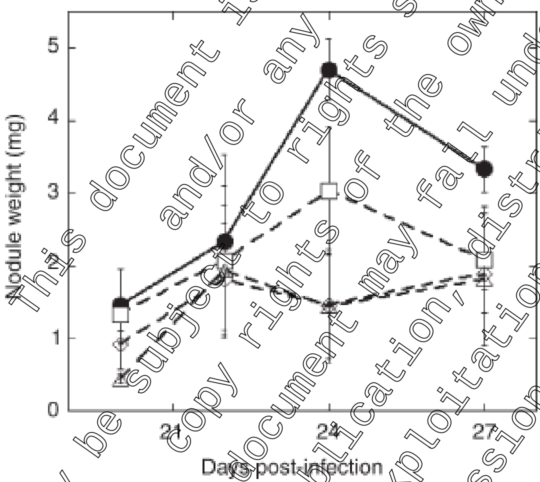

Fig. 4. Nodule weight of soybean seedlings grown hydroponically after inoculation with *B.japonicum* strain 110. Seedling were grown with no TFA (●), 0.003 µl TFA l^{-1} nutrient solution (□), 0.031 µl TFA l^{-1} (△) and 0.314 µl TFA l^{-1} (◇). Data point errors are reported as SEM.

By measuring nodule appearance, a judgment can be made as to the whether TFA affects infection. These studies in conjunction with analysis of attachment provide information as to when an exogenous substance has an effect on symbiosis. In a separate experiment, strains 110, 2143 and 184 were each used as inoculum and the plants monitored within clear plastic growth pouches. The presence of 0.003 µL TFA L^{-1} did not affect the rate of appearance of nodules by inoculation with strain 110, but did cause a slight delay in the appearance of nodules with strain 2143.

The delay observed with strain 2143 in the presence of TFA was similar to that with strain 184, a mutant that consistently demonstrates a delay in nodulation. Though nodule appearance was slightly delayed with strain 2143, the presence of TFA did increased the number of nodules per plant to a significant degree. This was also true with strain 110.

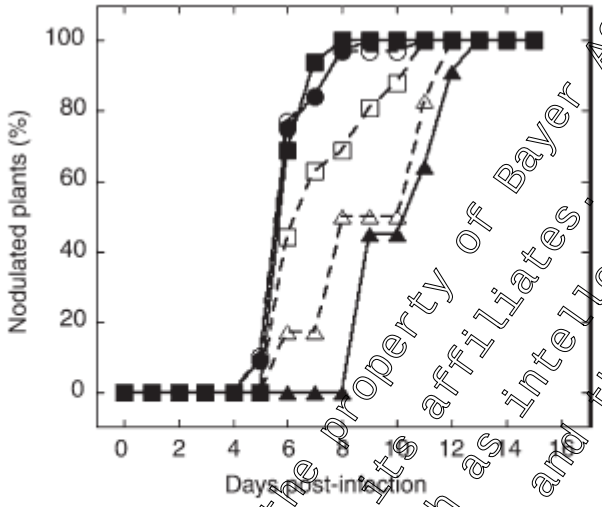

Fig. 5. Nodulation kinetics of *B. japonicum* strain 110 (●), 2143 (■) and 184 (▲) in the absence of TFA in hydroponically grown soybean seedlings. In the presence of 0.003 μl TFA l^{-1} nutrient solution, strain 110 (○) was unaffected, strain 2143 (□) had reduced nodulation and mutant strain 184 (△) nodulated earlier.

In the presence of the lowest level of TFA, the mutant that normally displays a delay in nodule appearance had a slightly earlier appearance. The location of the first nodule relative to the root tip at the time of inoculation (nodule geometry) was also examined during all the hydroponics experiments. The results demonstrated a slight downward trend in nodule location in the presence of all three levels of TFA and with all strains (data not shown), however none of these differences were significant.

RESULTS SUMMARY

In summary, at very low levels TFA has little or no effect on either symbiosis or the two partners involved. As the level of TFA increase, the effects become detrimental, with the plant being more affected at lower levels. However, the lowest concentration of TFA used in the study (0.674 μL kg^{-1}) is at least 25 times greater than the levels currently found in some contaminated surface waters and is 1000 to 10000 times greater than the projected for the near future. Thus, TFA at the levels currently found in the environment will not have an adverse effect on symbiotic nitrogen fixation in soybeans.

Comment of the notifier:

The treatment level in the study mentioned above are by far higher than the maximum PECsoil-figures for TFA, which occur after the applicaton of flufenacet. Thus, the study is not relevant for the risk assessment.

CA 8.8 Effects on biological methods for sewage treatment

For information on the study already evaluated during the first EU review of flufenacet, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph (incl. its Addenda). The study is listed in grey in the table below.
One additional study was performed. The respective summary is listed below.

Study type	EC_{50}	References
Activated sludge	>10000 mg/L	[redacted], R., 1989; M-004740-02-1
Activated sludge	>10000 mg/L	[redacted] A., 2007 M-283846-01-1 KCA 8.8/02

Report: **KCA 8.8/02; [redacted], A (2007)**
Title: Flufenacet TC - Toxicity to bacteria
Source: [redacted] Germany
Document No: M-283846-01-1
Guidelines: Not stated
GLP: yes

A study was performed to assess the toxicity of flufenacet techn. to bacteria.

Material and methods
The activated sludge was exposed to flufenacet (Batch-ID: EDHB001715, purity 97.0%) at 3 different concentrations, 100, 1000 and 10000 mg/L. As a toxic reference 3.5-Dichlorophenol was tested at concentrations of 5, 10 and 20 mg/L. The respiration rate of each mixture was determined after aeration periods of 3 hours.

Results

	Test concentration [mg/L]	O_2 start [mg O_2/L]	O_2 end [mg O_2/L]	Time (start-end) [min.]	Temp. [°C]	pH
Test item	100	4.4	2.7	3	19.2	7.9
	1000	4.1	2.9	2	19.2	7.9
	10000	4.8	3.3	3	19.3	7.9
Control 1	-	4.8	3.3	3	18.8	7.9
Control 2	-	5.5	3.8	3	19.5	7.9
Physico-chemincal oxygen consumption control	10000	7.6	7.6	9.	19.2	7.2
Reference substance	5	5.3	4.1	3	18.8	7.9
	10	5.8	4.8	3	18.9	7.9
	20	7.1	6.5	3	19.0	7.9

Test concentration [mg/L]	Respiratory rate [mg/L x h]	Phys.-chem. O_2-consumption [mg/L x h]	Respiratory rate - phys.-chem. O_2-consumption [mg/L x h]	Inhibition [%]
Test item				
100	34.0	*0.0	34.0	0.0
1000	36.0	*0.0	36.0	0.0
10000	30.0	0.0	30.0	6.3
Reference substance				
5	24.0			25.0
10	20.0			37.5
20	7.2			77.5
Control				
Control mean	32.0			
Control 1	30.0			
Control 2	34.0			

* The physico-chemical oxygen consumption has been determined at 10000 mg/L test item concentration. As no physico-chemical oxygen consumption was observed at that test item concentration this observation also holds true for the lower test item concentrations.

Flufenacet showed 6.3% respiration inhibition of activated sludge at a test item concentration of 10000 mg/L. The e ffect value relates to nominal concentration (no analytical monitoring).

Conclusion

The EC_{50} is higher than 10000 mg/L.

CA 8.9 Monitoring data

Monitoring data are considered not necessary.