Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Flurtamone

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013

Document KCA

Section 8: Ecotoxicological studies

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

Date

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Version history

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

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

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Section 8 - ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Introduction

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

This Supplemental Dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of flurtamone and were, therefore, not evaluated during the first EU review of this compound. 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.

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

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

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 8-2). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartments.

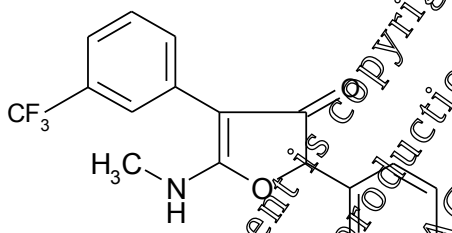
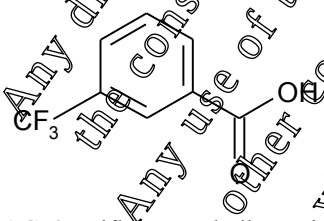
List of synonyms and codes

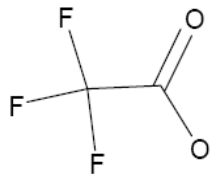
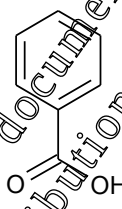
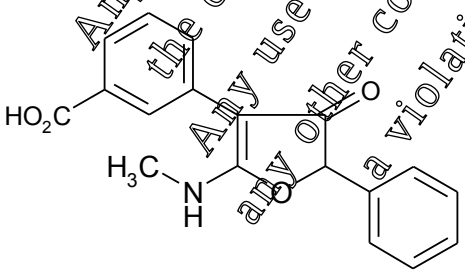
Flurtamone is a compound that was originally developed by the Chevron Chemical Company. It was purchased by Rhône-Poulenc Agriculture, which later merged with a division of Schering Agrochemicals to form Aventis CropScience. Aventis CropScience was purchased by Bayer to form part of Bayer CropScience,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

The original code number for flurtamone was RE 40885 and for its metabolites the codes were also RE followed by a five-digit number. In some reports there is a hyphen between the RE and the digits (eg RE-40885). When the molecule was owned by Rhône-Poulenc the compounds were given new (RPA) codes. In addition, metabolites not previously identified were detected and these were given

RPA codes. When Aventis CropScience was formed the compounds were given another set (AE) of codes. The AE codes continued to be used by Bayer CropScience except where studies conducted since the molecules' acquisition have resulted in additional metabolites (for which there was no AE code existing). More recently Bayer CropScience codes (BCS) have also been used. The key codes and standardized names for flurtamone and its major environmental degradates/metabolites relevant for ecotoxicology are summarized in the table below. A full list containing structural formula, various names, short forms, codes and occurrences of degradation products is provided in Document N3.

Table 8-1 : Flurtamone and its metabolites (including Aventis and/or BCS [a], Chevron [b] and Rhone-Poulenc [c] codes)

No.	Name, Structure IUPAC name CAS name, CAS number (if known)	Molecular formula molar mass Other names / codes	Occurrence Major/Minor Compartment(s)
AS	FLURTAMONE  Name IUPAC: 5-Methylamino-2-phenyl-4-(3-trifluoromethylphenyl)-3(2H)-furanone Name CAS: 3(2H)-Furanone, 5-(methylamino)-2-phenyl-4-[3-(trifluoromethyl)phenyl]-, (±)- CAS No.: 96525-23-4	$C_{18}H_{14}F_3NO_2$ 333.3 g mol^{-1} [a] AE B107587 [a] BCS-AD26195 [b] RE 40886 [c] RPA 590515 (also 201918 and 304563) Report name: flurtamone	Active substance
M04	SM4/PM11/AM30  Name IUPAC: 3-Trifluoromethylbenzoic acid Name CAS: Benzoic acid, 3-(trifluoromethyl)- CAS No.: 454-92-2 Sodium salt: Name IUPAC: sodium 3-(trifluoromethyl)benzoate CAS No.: 69226-41-1	$C_8H_5F_3O_2$ 190.1 g mol^{-1} [a] AE C518919 [a] BCS-AA52670 [a] BCS-CX97256 (sodium salt) [b] RE 54488 [c] RPA 025905 Common abbreviation: TFMBA Report name: TFMBA	**Major in soil** **Aerobic soil – max. 24.7%** Soil photolysis – max. 3.8% Water/sediment total – max. 4.1% Cereals, Sunflower Rat, Hen, Goat

No.	Name, Structure IUPAC name CAS name, CAS number (if known)	Molecular formula molar mass Other names / codes	Occurrence Major/Minor Compartment(s)
M05	SM5/PM12/  Name IUPAC: Trifluoroacetic acid Sodium trifluoroacetate Name CAS: Trifluoroacetic acid Sodium trifluoroacetate CAS No.: 76-05-1 (acid) 2923-18-4 (sodium salt)	$C_2HF_3O_2$ 114.0 g mol^{-1} [a] AE C502988 (acid) [a] BCS-AL85845 (acid) [b] none given [c] RPA 017505 (acid) [a] AE1046319 (sodium salt) [a] BCS-AZ56567 (sodium salt) Common abbreviation: TFA (or TFAA) Report name: Trifluoroacetic acid or trifluoroacetate	**Major in soil** **Aerobic soil – max. 9.8%** **Confined rotational crops**
M06	SM6  Name IUPAC: Benzoic acid Name CAS: Benzoic acid CAS No.: 65-85-0	$C_7H_6O_2$ 122.1 g mol^{-1} [a] BCS-AG74706 [b] none given [c] RPA 73543 Report name: Benzoic acid	**Major in soil:** **Soil photolysis – max 7.2%**
M07	AQM1  Name IUPAC: 3-(2-Methylamino-4-oxo-5-phenyl-4,5-dihydrofuran-3-yl)benzoic acid Name CAS: Benzoic acid, 3-[4,5-dihydro-2-(methylamino)-4-oxo-5-phenyl-3-furanyl]- CAS No.: 148681-60-1	$C_{18}H_{15}NO_4$ 309.3 g mol^{-1} [a] AE 1083976 [a] BCS-BA29451 [b] none given [c] RPA 203597 Report name: flurtamone-carboxylic acid	**Major in Aqueous photolysis – max. 33.5%**

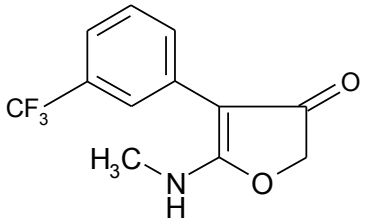
No.	Name, Structure IUPAC name CAS name, CAS number (if known)	Molecular formula molar mass Other names / codes	Occurrence Major/Minor Compartment(s)
M08	AQM2  Name IUPAC: 5-methylamino-4-(3-trifluoromethylphenyl)-3(2H)-furanone Name CAS: 3(2H)-Furanone, 5-(methylamino)-4-[3-(trifluoromethyl)phenyl]- CAS No.: 96525-53-0	$C_{12}H_{10}F_3NO_2$ 257.2 g mol^{-1} [a] AE 2093305 [a] BCS-BT61400 [b] none given [c] RPA 59-1720 Report name: flurtamone-desphenyl	**Major in Aquatic** Water – max. 7.8% Sediment – max.3.6% **Total max. 10.7%**

Table 8- 2: Definition of the residue for risk assessment*

Compartment	Compound /Code
Soil	Flurtamone, M04 TFMBA and M05 TFA
Groundwater	Flurtamone and M05 TFA
Surface water	Flurtamone, M07 flurtamone-carboxylic acid and M08 flurtamone-desphenyl.
Plant material	Flurtamone and M05 TFA

*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. The soil photolysis metabolite M06 benzoic acid has been considered as non-relevant for risk assessment as outlined in the position paper under KCP-9.1 /01; Lowden P. 2013.

CA 8.1 - Effects on birds and other terrestrial vertebrates

In addition to the parent compound flurtamone, studies performed for one metabolite, namely trifluoroacetic acid (TFA) are presented. TFA has been identified as an environmental metabolite of different chemicals, including pesticide active substances as e.g. flurtamone. 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 studies 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 screening assessment of omnivorous and herbivorous birds in the MCP documents. Nevertheless, the endpoints will only be listed in endpoint lists under 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 flurtamone, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph. The studies are listed in the table below. No new studies have been conducted.

Table 8.1.1- 1: Avian toxicity data of flurtamone

Test species	Study	Ecotoxicological endpoint		Reference
Bobwhite quail	acute oral	LD_{50} > 2530 [1)] LD_{50} = 4777 [2)]	mg/kg bw	[redacted] 1988, M-160680-01-1
Bobwhite quail	5-day dietary	LC_{50} > 6000[1)] ≙ LDD_{50} > 1535[3)]	ppm mg/kg bw/day	[redacted] 1989, M-160689-01-1
Mallard duck		LC_{50} = 2000 ≙ LDD_{50} = 545	ppm mg/kg bw/day	[redacted] 1989, M-160687-01-1 [redacted] 2005, M-247726-01-1 [4)]
Bobwhite quail	1-generation reproduction (21-weeks feeding)	NOAEL = 80 ≙ NOAEL = 7.3	ppm mg/kg bw/day	[redacted] *et al.* 1990, M-203211-01-1
Mallard duck	1-generation reproduction (22-weeks feeding)	NOAEL = 200 ≙ NOAEL = 28	ppm mg/kg bw/day	[redacted] *et al.* 1990, M-203217-01-1

[1)] 10 birds per group; no mortality occurred during study
[2)] LD_{50} extrapolated according EFSA, JD birds & mammals (2009)
[3)] Parameters over 5-day exposure period (6000 ppm group): mean feed consumption: 8.7 g/bird /day; mean bodyweight: 34g
[4)] Calculation of daily dietary dose in amendment

CA 8.1.1.1 - Acute oral toxicity to birds

No new studies have been conducted.

CA 8.1.1.2 - Short-term dietary toxicity to birds

No new studies have been conducted.

CA 8.1.1.3 - Sub-chronic and reproductive toxicity to birds

No new studies have been conducted.

CA 8.1.2 - Effects on terrestrial vertebrates other than birds

For information on studies already evaluated during the first EU review of flurtamone, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and to the Monograph. For details on the studies please refer to the respective section in the MCA Section 5 “Summary of the toxicological and metabolism studies for Flurtamone”.

Table 8.1.2- 1: Summary of effects of flurtamone to mammals

Test species	Study	Ecotoxicological endpoint	Reference
Rat	acute oral	LD_{50} > **5000**[1)] mg/kg bw	[redacted] 1989, M-160698-01-1 See KCA 5.2.1.
Rabbit	developmental toxicity	NOAEL = **20** mg/kg bw/day	[redacted] 1989, M-160636-01-1 See KCA 5.6.2
Rat	2-generation reproduction	NOAEL = 500 ppm NOAEL = 25 mg/kg bw/day	[redacted] 1992, M-203254-01-1 See KCA 5.6.1

[1)] 10 rats per group; no mortality occurred during study

Table 8.1.2- 2: Summary of effects of metabolite M05 TFA to mammals

Test species	Study	Ecotoxicological endpoint	Reference [1)]
Rat	acute, oral	LD_{50} > **2000** mg p.m./kg bw	[redacted] (2013) M-444479-01-1 See KCA 5.8.1
Rat	28 days dietary	NOEL ♂ 1315 / ♀ 1344 — 1329 [2)] mg p.m./kg bw/d	[redacted] (2005) M-259106-01-1 See KCA 5.8.1
Rat	90 days dietary	NOEL ♂ 98 / ♀ 123 — **110** [2)] mg p.m./kg bw/d	[redacted] (2007) M-283994-01-1 See KCA 5.8.1

[1)] re-evaluation of endpoints by [redacted] 2014, M-477154-01-1, **KCA 8.1.2.2/01**)
[2)] Geometric mean of male and female

CA 8.1.2.1 - Acute oral toxicity to mammals

No new studies have been conducted with flurtamone.

CA 8.1.2.2 - Long-term and reproduction toxicity to mammals

No new studies have been conducted with flurtamone. New studies with the metabolite TFA are summarised in Section 5. The ecotoxicological relevance of endpoints for risk assessments derived from these studies is summarised in the following position paper.

Report:	**KCA 8.1.2.2/01; [redacted], L.; 2014**
Title:	Trifluoroacetate (TFA) Toxicity Endpoint for Terrestrial Vertebrate Risk Assessment
Document No:	M-477154-01-1
Guidelines:	Not applicable
GLP:	no

Objective

The present paper reviews the ecotoxicologically relevant studies available for TFA and proposes suitable endpoints for the acute and long-term/reproductive risk assessment.

Material and methods
Toxicological endpoints for TFA derived in the following studies were evaluated.

- acute oral toxicity on rats ([redacted], 2013; 42461)
- 28-day toxicity in the rat by dietary administration ([redacted], 2005; M-259106-01-1)
- 90-day toxicity in the rat by dietary administration ([redacted], 2007; M-283994-01-1)
- Embryo-fetal oral gavage toxicity in rats ([redacted], 2010; M-411209-01-1)

Findings

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: **$NOAEL_{ecotox}$: 1600 ppm, equivalent to 98 mg/kg bw/day.**

With TFA no toxicity studies 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

No new studies have been conducted with flurtamone.

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

No new studies have been conducted.

CA 8.1.5 - Endocrine disrupting properties

WHO/IPCS (2002)[1] provided the currently widely accepted definition "An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations." An adverse effect has been defined also by WHO/IPCS (2009)[2]: "Change in the morphology, physiology, growth,

[1] WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2002. Global Assessment of the State-of-the-science of Endocrine Disruptors. WHO/PCS/EDC/02.2, 180 pp.

[2] WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. 689 pp.

development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences."
Both definitions were used as the basis for evaluating the potential impact of flurtamone to wildlife.

Birds

The population relevant effects of flurtamone on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. For both species there were no statistically significant effects on adult birds, offspring or reproductive parameters up to and including the highest test level of 800 ppm a.s.. However, there seemed to be a treatment level dependent trend in the number of eggs laid in bobwhite quail which is why the NOEL for this species was set to 50 ppm. At the highest test level there was also a slight but not statistically significant decrease in egg shell thickness. In the absence of any indication of endocrine activity of flurtamone in mammalian studies the effect on egg shell thickness - if it were a true effect - is unlikely endocrine mediated.

For mallard duck the NOEL was set to 200 ppm a.s. based on (not statistically significant) lower terminal body weight gain in females and suspected increased food consumption at the highest test level of 800 ppm a.s..

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 flurtamone. No further testing for endocrine disrupting properties is warranted.

Wild Mammals

A detailed analysis of all the apical toxicological studies (subchronic, chronic / oncogenicity, reproduction and developmental toxicity) on flurtamone revealed no evidence of any reproducible endocrine effect. Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of flurtamone in mammals.

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test exists, this test was developed to evaluate potential effects on the thyroid system and not to measure population relevant effects. Therefore no further studies can be suggested at this time for these groups of organisms.

CA 8.2 - Effects on aquatic organisms

Table 8.2- 1: Toxicity of flurtamone to aquatic organisms

Test species	Test system	Duration of exposure	Toxicity [mg/L]	Reference
Pimephales promelas (Fathead minnow)	acute, semi-static	96 h	LC_{50} > 6.64 * NOEC 6.64 *	[redacted], 2012a; M-424825-01-1 KCA 8.2.1/01
	ELS, flow-through	35 d	NOEC 0.188	[redacted], 2012b; M-443591-01-1 KCA 8.2.2.1/01
Oncorhynchus mykiss (Rainbow trout)	acute, static	96 h	LC_{50} 7.0	[redacted], 1989; M-160659-01-1
	chronic, juvenile	28 d	NOEC 0.63	[redacted] et al., 1994;

Test species	Test system	Duration of exposure	Toxicity [mg/L]	Reference
	growth, flow through			M-162489-01-1
Lepomis macrochirus (Bluegill sunfish)	acute, static	96 h	LC_{50} 11	[redacted], 1989; M-160664-01-1
	bioaccumulation	28 d	BCF = 27-28	[redacted], 1994 M-162223-01-1
Xenopus laevis (amphibian)	acute, static	48 h	LC_{50} > 20	[redacted], 2013 M-475146-01-1 KCA 8.2.3/02
Daphnia magna (Waterflea)	acute, static	48 h	EC_{50} 13.0	[redacted], 1989; M-160662-01-1
	acute, static	48 h	EC_{50} 25.1	[redacted], 2011 M-420504-01-1 KCA 8.2.4.1/01
	chronic, flow through	21 d	NOEC 0.071	[redacted], 1992; M-203224-01-1
Chironomus riparius (Chironomid)	chronic, static, spiked water	22 d	NOEC 0.1	[redacted], 1997; M-247873-01-1
Pseudokirchneriella subcapitata (Green algae)	chronic (growth inhibition test), static	96 h	E_bC_{50} 0.020	[redacted] et al, 1992; M-203220-01-1
		72 h	recalculation based on new OECD 201: E_rC_{50} 0.038	[redacted], 2005; M-247782-01-1 KCA 8.2.6.1/01
		72 h	E_rC_{50} 0.053 NOE_rC 0.010	[redacted], 2013 M-473178-01-1 KCA 8.2.6.1/05
	chronic, flow-through, variable exposure	one pulse at 0.04 mg/L day 7: one pulse at 0.02 mg/L day 14: one pulse at 0.035 mg/L	$EC_{50(population)}$ >0.04	[redacted], 2014 M-474520-01-1 KCA 8.2.6.1/04
Navicula pelliculosa (Diatom)	chronic (growth inhibition test), static	72 h	E_bC_{50} 0.011 E_rC_{50} 0.024	[redacted], 1997; M-242493-01-1
Lemna gibba (Duck weed)	chronic (growth inhibition test), static renewal	14 d	E_rC_{50} 0.0140 (frond density) E_bC_{50} 0.0099	[redacted], 1997; M-244591-01-1
			recalculation based on new OECD 221: E_rC_{50} 0.0445 (frond no.) E_rC_{50} 0.0429 (dry weight)	[redacted], 2005; M-258189-01-1 KCA 8.2.7/01
	chronic, static	7 d	E_rC_{50} 0.014 NOE_rC 0.000916	[redacted], 2013 M-470528-01-1 KCA 8.2.7/09
	peak exposure	One 48h peak and two 48h peaks;	Day 0-7 **after single peak**: E_rC_{50} 0.124 (frond	[redacted], 2014 M-475376-01-1 KCA 8.2.7/10

Test species	Test system	Duration of exposure	Toxicity [mg/L]	Reference
		total test duration 14 d	number) E_rC_{50} 0.0618 (frond area) NOE_rC < 0.01 day 7-14 **after two peaks at 7 d-interval**: E_rC_{50} 0.0719 (frond number) E_rC_{50} 0.0608 (frond area) NOE_rC < 0.01	
Myriophyllum spicatum (higher aquatic plant)	acute,static	14 d	E_yC_{50} > 0.123 mm NOEC 0.0071 mm LOEC 0.015 mm	[redacted], 2012 M-431579-01-1 KCA 8.2.7/04
Mesocosm Lentic freshwater community	chronic, static	68 d	No Observed Ecologically Adverse Effect Concentration NOEAEC 0.003	[redacted], 2010 M-389526-01-1 KCA 8.2.7/05
Outdoor potted plant *Potamogeton crisprus* *Elodea canadensis*	chronic, static	42 d	*Potamogeton*: NOEC 0.003 *Elodea*: NOEC 0.001	[redacted] & [redacted] 2013 M-469643-01-1 KCA 8.2.7/11
Myriophyllum spicatum *Elodea canadensis*	peak exposure	One 48h peak and two 48h peaks; total test duration 56 d	***Elodea*:** **one peak:** 56-day-EC50 >0.036 14-day-$NOEC_{population}$ 0.004 56-day-$NOEC_{population}$ 0.036 **two peaks:** 56-day-EC50 >0.036 $NOEC_{population}$ 0.004 ***Myriophyllum*:** 56-day-$NOEC_{population}$ > 0.036	[redacted], 2013 M-470995-01-1 KCA 8.2.7/12

* geometric mean of measured concentrations
mm = mean measured

Table 8.2- 2: Toxicity of flurtamone metabolites to aquatic organisms

Test species	Test system	Duration of exposure	Toxicity [mg/L]	Reference
M04 TFMBA (AE C518919)				
Oncorhynchus mykiss (Rainbow trout)	acute, static	96 h	LC_{50} > 76.3	[redacted], 1999; M-243657-01-1
Daphnia magna (Water flea)	acute, static	48 h	EC_{50} > 95.0	[redacted], 1999; M-247910-01-1
Pseudokirchneriella subcapitata (Green alga)	chronic (growth inhibition test), static	72 h	E_rC_{50} > 104.8	[redacted], 1999; M-243659-01-1
Lemna gibba (Duck weed)	chronic, static	7 d	E_rC_{50} 92	[redacted], 2005; M-253816-01-1 KCA 8.2.7/08
M05 TFA (AE C502988)				
Brachydanio rerio (Zebra fish)	acute, static	96 h	LC_{50} > 1200	[redacted] et al., 1992; M-247889-01-1 KCA 8.2.1/02
Brachydanio rerio (Zebra fish)	ELS	144 h	LC_{50} 3000 EC_{50} 700 NOEC 300 (heart rate) NOEC 300 (hatching time)	Ulhaq et al. 2013; M-462660-01-1 KCA 8.2.2.1/02
Daphnia magna (Water flea)	acute, static	48 h	EC_{50} > 1200	[redacted] et al., 1992; M-247890-01-1 KCA 8.2.4.1/03
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
Pseudokirchneriella subcapitata (Green alga)	chronic (growth inhibition test), static	72 h	E_rC_{50} > 1.2 [1]	[redacted], 1993 M-247818-02-1 KCA 8.2.6.1/04
Green algae (various species)	chronic (growth inhibition test), static	72 h	E_rC_{50} >112 to > 2400 [1]	[redacted], 1996 M-247822-01-1 KCA 8.2.6.2/02
Desmodesmus subspicatus (green algae)	chronic (growth inhibition test), static	72 h	E_rC_{50} 120 [1]	[redacted] et al, 1995 M-247825-01-1 KCA 8.2.6.1/05
Lemna gibba (Duck weed)	chronic, static	7 d	$EC_{50,\ frond\ increase}$ 1100	[redacted] et al., 1993; M-247900-01-1 KCA 8.2.7/13
Lemna gibba *Myriophyllum spicatum* *Myriophyllum sibiricum*	chronic, static	7 d 14 d 14 d	EC_{50} 618.3 (wet mass) EC_{50} 312.9 (wet mass) EC_{50} 357 (wet mass)	Hanson & Solomon, 2004 M-455787-01-1 KCA 8.2.7/14
M07 (AE 1083976)				
Cyprinus carpio (Common carp)	acute, static (screening)	96 h	LC_{50} ≥ 36	[redacted], 1997 M-242462-01-1 KCA 8.2.1/07

Daphnia magna (Water flea)	acute, static (screening)	48 h	EC_{50} > 36	[redacted], 1997 M-242461-01-1 KCA 8.2.4.1/06
Pseudokirchneriella subcapitata (Green alga)	chronic (growth inhibition test), static (screening)	72 h	EC_{50} > 0.1	[redacted], 1997 M-242463-01-1 KCA 8.2.6.1/07
Pseudokirchneriella subcapitata (Green algae)	chronic (growth inhibition), static	72 h	ErC_{50} > 100 NOEC 100	[redacted], 2005, M-255213-01-1 KCA 8.2.6.1/07
Lemna gibba (Duck weed)	chronic, static	7 d	E_rC_{50} > 100	[redacted], 2005, M-255206-01-1 KCA 8.2.7/11
M08 (AE 2093305)				
Pseudokirchneriella subcapitata (Green algae)	chronic (growth inhibition test), static	72 h	E_rC_{50} 0.306 NOE_rC 0.0307	[redacted], 2013 M-470662-01-1 KCA 8.2.6.1/06
Lemna gibba (Duck weed)	chronic, static	7 d	E_rC_{50} 0.38	[redacted], 2005; M-255326-01-1 KCA 8.2.7/12
Lemna gibba (Duck weed)	chronic, static	7 d	E_rC_{50} 0.722 NOE_rC 0.0763	[redacted], 2013 M-470493-01-1 KCA 8.2.7/08

[1] test with TFA Na-salt

CA 8.2.1 - Acute toxicity to fish

Report: **KCA 8.2.1/01; [redacted], M.; 2012a**
Title: Fathead minnow (*Pimephales promelas*), acute toxicity test, semi-static conditions
Document No: M-424825-01-1
Guidelines: OECD 203
GLP: Yes (certified laboratory)

Objective:

The purpose of this study was to determine the toxicity of flurtamone technical to fathead minnow (*Pimephales promelas*). The study was conducted according to the OECD Guideline No. 203 as a limit test with one nominal concentration of 100 mg a.s./L under semi-static conditions for 96 hours.

Materials and Methods:

Test item: Flurtamone (tech.), batch AE B107587-01-08; Origin Batch ID: LOT.20500103; Specification No.: 102000002946; TOX No.: 09331-00; purity: 98.3 % w/w.

Ten fish were exposed to a nominal concentration of 100 mg/L flurtamone for 96 hours, while a control group of ten fish were kept in untreated dilution water. A complete change of test media was performed after 48 h. Temperature during the test was in the range of 22.6 to 23.1 °C. The light and dark cycle was 12 to 12 hours. The pH of the water was between 8.2 and 8.5.
The mortality and sublethal effects were assessed after 3, 24, 48, 72 and 96 hours. Chemical analysis was conducted for water samples at the start (0 hours), after 48 and 96 hours.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality of the control	≤ 10%	0%
Constant water quality and environmental conditions during the test	Yes	Yes
Dissolved oxygen concentration throughout the test	≥ 60%	91 - 103 %

All validity criteria for the study were met

Analytical results:

Analyses of test media revealed concentrations of flurtamone between 5.11% and 6.26% of the nominal concentrations for fresh and 5.95% and 9.45% of the nominal concentrations for aged solutions. The geometric mean was calculated to be 6.64 mg a.s./L.

Biological results:

Mortality:

Clinical signs could be observed in one single fish after 24 hr at 100 mg a.s./l (nominal) corresponding to 6.64 mg a.s./L (geometric mean measured). However, no further clinical signs could be observed in the test. Mortality of the fish could not be observed.

Conclusions:

The LC_{50} was determined to be >100 mg a.s./L (nominal) corresponding to 6.64 mg a.s./L (geometric mean).

Report:	**KCA 8.2.1.02 [redacted]. (1992)**
Title:	The Acute Toxicity of Sodium Trifluoroacetate to the Zebra Fish *Brachydanio Rerio*
Guidelines:	OECD Guideline No. 203 (1984)
Document No:	M-247889-01-1
Deviations:	None
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 M05 TFA, 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 $EC_{50\ (96h)}$ 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.

Results:

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:

For the metabolite M05 TFA, as sodium trifluoroacetate the NOEC is 1200mg/L.
Based on the molecular weights, a concentration of 1200 mg sodium trifluoroacetate/1 corresponds to

1000 mg trifluoroacetate anion/L.

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Report:	**KCA 8.2.1/07, [redacted], A.; 1997**
Title:	Acute toxicity (96 hours) to fish under static conditions. Screening test (Phase 1) RPA 203597
Document No:	M-242462-01-1
Deviations:	None
Guidelines:	Not applicable
GLP:	No

Objective:
The study was conducted to determine the toxicity of the metabolite M07 AE 1083976 (RPA 203597) to the Common carp (*Cyprinus carpio*) under static conditions.

Material and methods:
Test item: M07 RPA 203597 (AE 1083976), batch no. 97C01I.
Common carp (7 individuals per concentration) were exposed to nominal concentrations 2.3, 4.5, 9.0, 18.0 and 36.0 mg/L of the test substance for 96 h. Dimethylformamide (DMF) was used as solvent at 0.5 mL/L in each test medium.

Results:
No mortality was observed at any of the test concentrations.

Conclusion:
For metabolite M07 (AE 1083976) the LC50 was > 36 mg/L and the NOEC (96 h) 36 mg/L.

CA 8.2.2 - Long-term and chronic toxicity to fish

CA 8.2.2.1 - Fish early life stage toxicity test

Report:	**KCA 8.2.2.1/01, [redacted], M. 2012b**
Title:	Fathead minnow (*Pimephales promelas*), early life-stage toxicity test, flow through conditions. Test item: flurtamone (tech.).
Document No:	M-443591-01-1
Guidelines:	OECD 210
GLP:	Yes (certified laboratory)

Objective:
The present study was conducted at the Fraunhofer-Institute for Molecular Biology and Applied Ecology to determine the toxicity of flurtamone, technical, to the early life stages of fathead minnow (*Pimephales promelas*). The study was conducted under flow through conditions according to the OECD guideline 210. The obtained data included hatching success and cumulative mortality as well as length and weight of surviving fish.

Materials and Methods:
Test item: flurtamone, technical, chemical name: (5-(methylamino)-2-phenyl-4-[3-(trifluoromethyl)phenyl]furan-3(2H)-one, Batch No.: AE B107587-01-08, purity: 98.3% (CoA).

Freshly fertilised eggs of fathead minnow (*Pimephales promelas*) were exposed to five initial test concentrations of nominal 43.8, 87.5, 175, 350 and 700 µg flurtamone/L, and untreated controls under flow through conditions at 25°C ± 2. Light/dark cycle was 16 h/8 h. Water samples were analysed using LC-MS at an LOQ of 10 µg/L.
Each concentration was performed with 60 fertilised eggs which were distributed evenly between four replicate vessels. Qualitative observations on hatching and survival were made daily. Dead embryos, larvae and juvenile fish were removed as soon as observed. Observations on abnormal appearance of behaviour were made daily, too. After 14, 21, 28 and 35 days larvae/juvenile fish were photographed and the survival rates as well as the lengths (at day 35, only) of the animals were determined using digital image processing (UTHSCSA ImageTool Version 3.0, University of Texas Health Science Center at San Antonio).
At test end, individual wet weight and length was measured. Afterwards, fish of each vessel were pooled and dried overnight at 50°C. The single dry weight per fish was calculated by dividing the group dry weight by the number of surviving fish at test end.
At test start, and weekly thereafter samples of the test media were taken for chemical analysis.

Results:

Validity Criteria	Recommended	Obtained
Dissolved oxygen concentration	60% - 100%	84% - 94%
Water temperature	≤1.5°C between test chambers or between successive days and should be within the range of 25± 2°C	25.0°C to 26.7 °C
Concentration of test substance in solution	±20% of the mean measured values	Mean values 103% - 112 %
Hatching success	> 66	Mean value of 98%
Survival rate (post hatch) in the control	70%	>86%

All validity criteria were met.

Analytical results:
The mean concentrations per treatment were found to be between 103% and 112% of the nominal values and were calculated to be 46.7, 90.4, 188, 393 and 784 µg a.s./L.

Biological results:
Effects on hatching success
A slightly reduced hatching success at 784 µg a.s./L was found, however, no statistical significance could be detected and thus this observation can be considered as trend. The other test concentrations indicated no difference to the control group.
Effects on hatching survival
After day 14 pf (post-fertilisation), post hatch survival was found to be significantly reduced at 393 and 784 µg a.s./L. On day 21 pf, post hatch survival was significantly reduced at 784 µg a.s./L. On day 21 pf, post hatch survival was significantly reduced at 784 µg a.s./L. On day 28 and 35 pf, reduced

post hatch survival could be observed at 393 and 784 µg a.s./L, however, a significant reduction could not be determined.

Effects on wet weight
The mean individual wet weights were significantly elevated at 784 µg a.s./L.
Effects on lengths
The mean fish length was significantly increased at 393 and 784 µg a.s./L.
Effects on dry weight
The single dry weight was found to significantly increased at 393 and 784 µg a.s./L.

Effects of flurtamone on *Pimephales promelas* (based on nominal concentrations of the test item [µg a.s./L])

	Nominal concentration of the test substance [µg a.s./L]					
	Control	**43.8**	**87.5**	**175**	**350**	**700**
Hatching day 7 pf [%]	98 ± 3.3	95 ± 3.3	97 ± 3.8	97 ± 3.8	95 ± 6.4	80 ± 25.5
Post hatch survival day 14 pf [%]	97 ± 3.8	97 ± 4.0	92 ± 6.7	93 ± 8.0	79 ± 11.0 *)	72 ± 13 *)
Post hatch survival day 21 pf[%]	91 ± 3.8	91 ± 10.2	92 ± 6.3	91 ± 6.6	77 ± 12.1	72 ± 13 *)
Post hatch survival day 28 pf [%]	90 ± 4.1	90 ± 12.0	92 ± 6.3	91 ± 6.6	77 ± 12.1	72 ± 13
Post hatch survival day 35 pf [%]	90 ± 4.1	86 ± 16.5	92 ± 6.7	90 ± 8.1	77 ± 12.1	72 ± 13
Mean length, day 35pf [cm]	2.0 ± 0.05	2.0 ± 0.05	2.1 ± 0.08	2.1 ± 0.05	2.2 ± 0.12 #)	2.4 ± 0.14 #)
Mean individual wet weight [g]	0.087 ± 0.009	0.080 ± 0.010	0.099 ± 0.033	0.089 ± 0.007	0.104 ± 0.012	0.136 ± 0.032 #)
Mean individual dry weights [mg]	17.8 ± 1.1	18.3 ± 2.6	18.7 ± 2.6	20.2 ± 1.7	23.7 ± 3.0 #)	32.0 ± 8.3 #)

*) statistical significant reduction compared to control, $p<0.05$, one-sided smaller, Williams test
#) statistical significant increase compared to control, $p<0.05$, one-sided greater, Williams test

Conclusions:
The study determined the toxicity of flurtamone to the most sensitive early life stages of fish using fertilised eggs of fathead minnow (*Pimephales promelas*). Based on the most sensitive endpoint, post hatch survival at day 14 post fertilisation, the NOEC was determined to be 188 µg a.s./L.

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Report:	**KCA 8.2.2.1; Ulhaq, M., Carlsson, G., Örn, S., Norrgren, L., 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 EC_{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 primary 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:	TFAA: Sigma-Aldrich, 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 were within 15 min after collection 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 Carlsson 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 Uhlaq *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	EC_{50} 144 h	LC_{50} 144 h	NOEC/LOEC (mg/L)	
				(mg/L)	(mg/L, 95% C.I.)		Heart rate	Hatching time
TFAA	Trifluoroacetic acid	CF_3COOH	76-05-1	10–3000	700 (460–1000)	>3000	ne	300/1000
PFBA	Perfluorobutyric acid	C_3F_7COOH	375-22-4	10–3000	2200 (1200–22000)	>3000	ne	ne
PFOA	Perfluorooctanoic acid	$C_7F_{15}COOH$	335-67-1	3–1000	350 (290–430)	430 (290–710)	ne	ne
PFNA	Perfluorononanoic acid	$C_8F_{17}COOH$	375-95-1	0.03–10	16 (7.7–450)	>10	ne	ne
PFDA	Perfluorodecanoic acid	$C_9F_{19}COOH$	335-76-2	0.1–30	5.0 (3.8–6.6)	8.4 (5.3–15)	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 (1.1–1.9)	>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.

RESULTS SUMMARY

Under the conditions of this study, the 144 h EC_{50} and LC_{50} of zebrafish embryos was 700 mg TFA/L and 3000 mg TFA/L and the NOEC for heart rate and hatching time was established at 3000 mg TFA/L and 300 mg TFA/L.
In conclusion, comparing the toxic effect levels calculated in this study with measured levels in natural waters, TFA do not indicate a risk for acute toxicity to aquatic organisms.

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

No studies have been conducted and are not required.

CA 8.2.2.3 - Bioconcentration in fish

A study on the bioconcentration of flurtamone in bluegill sunfish was submitted and reviewed for Annex I inclusion ([redacted], 1994, M-162223-01-1). The BCF factor given in the list of end points is BCF = 27-28 on a whole fish basis. No new studies have been conducted.

CA 8.2.3 - Endocrine disrupting properties

The evaluation is based on the definitions stated under Point 8.1.5.

Fish
Population relevant effects of Flurtamone on fish were studied an early life-stage test (ELS) with fathead minnow. The lowest NOEC of 188 µg/L was found for survival with no effects other parameters. Further, in a juvenile growth test with rainbow trout a NOEC of 630 µg/L (growth) was determined. As neither in mammalian nor in avian species specific endocrine effects on reproduction were observed the performance of an ELS is regarded as sufficient to provide information on such behaviour. Also, the NOECs identified in chronic fish tests are orders of magnitude above the regulatory acceptable concentration derived from algal endpoints.

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

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

CA 8.2.4 - Acute toxicity to aquatic invertebrates

CA 8.2.4.1 - Acute toxicity to Daphnia magna

Report:	**KCA 8.2.4.1/01; [redacted], T.; 2011**
Title:	Acute toxicity of flurtamone (tech.) to the waterflea *Daphnia magna* in a static laboratory test system
Document No:	M-420504-01-1
Guidelines:	OECD Guideline No. 202 (2004) U.S. EPA Pesticide Assessment Guidelines, Subdivision E, §72-2 (1982) EC Council Regulation No 440/2008, Method C.2 (2008) OPPTS Guideline 850.1010 Draft (1996), modified JMAFF 12 Nousan No. 8147 (2000)
GLP:	Yes (certified laboratory)

Objective:

The study was performed in order to detect possible effects of flurtamone on mobility of *Daphnia magna* caused by 48 hours of exposure in a static laboratory test system expressed as EC_{50} for immobilisation.

Materials and Methods:

Test item: Flurtamone (tech.), Batch No.: AE B107587-01-08; Origin Batch ID: LOT.20500103; TOX No.: 09331-00; purity: 98.3 % w/w

Neonates of the waterflea *Daphnia magna* (first instars < 24 h old) were exposed in a static test system for 48 hours to nominal concentrations of 0, 3.43, 6.17, 11.1, 20.0 and 36.0 mg a.s./L without feeding. Six vessels (replicates), each provided with five daphnids (equivalent to 10 mL test solution per daphnid), were utilised per treatment group and control (corresponding to 30 animals per study group). The test was conducted in a climate controlled environment (isolated chamber) illuminated in a 16 to 8 hours light-dark cycle, at a light intensity of max. 1300 lux. Temperature was between 18 and 22 °C. After 24 and 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements within approximately 15 seconds after gentle agitation of the test vessel. Additionally all visible features of the test item in water as well as possible signs on sublethal affected daphnids were recorded.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Immobility of the control	≤ 10%	0%
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	9.0 - 9.1 mg/L

All validity criteria for the study were met

Reference test:

For quality control of the breeding stock, an acute non-GLP toxicity test was performed separately in July 2011 using the reference substance $K_2Cr_2O_7$, p.a. grade (test concentrations: 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L (Bayer AG unpublished report ID.: Reference 02/2011). The 24 hour EC_{50} of 0.87 mg/L, as determined in this test, meets the range defined by OECD 202 (0.6 – 2.1 mg/L).

Analytical results:
The actually dissolved and analytically determined amounts of flurtamone in the freshly prepared test solutions at test initiation ranged between 105% and 117% (mean: 109%) of the corresponding nominal concentrations. The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 104% and 113% (mean: 110%) of nominal.
As the measured concentrations ranged well within the recommended range of 80 – 120 % of nominal, all reported results are based on nominal concentrations. No contaminations of flurtamone were detected in samples from untreated water control.

Biological results:
Immobility:
There was no effect on the mobility of *Daphnia magna* at the lowest concentration of 3.43 mg/L while 80% immobility was observed after 48 hours in the highest nominal concentration of 36 mg/L. No immobilities or other effects on behaviour occurred in untreated control within 48 hours of exposure.

Toxicity of flurtamone (tech.) to *Daphnia magna* (based on nominal concentrations)

Nominal test concentration [mg a.s./L]	Exposed daphnids (=100%)	immobilised daphnids			
		24 h:		48 h:	
		n	%	n	%
control	30	0	0.0	0	0.0
solvent control *)	30	0	0.0	0	0.0
3.43	30	0	0.0	0	0.0
6.17	30	0	0.0	1	3.3
11.1	30	0	0.0	5	16.7
20.0	30	1	3.3	5	16.7
36.0	30	14	46.7	24	80.0

* Dimethylformamide (0.1mL / L)

Conclusions:
Based on probit analysis the flurtamone EC_{50} after 24 hours was determined to be 39.6 mg a.s./L (95% confidence interval: 34.1-50.3 mg/L) and the EC_{50} after 48 hours was determined to be 25.1 mg a.s./L (95% confidence interval: 13.8-45.8 mg/L).

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Report: **KCA 8.2.4.1/06; [redacted], A.; 1997**
Title: Acute toxicity (48 h) to daphnids (*Daphnia magna*) under static conditions. Screening test (phase 1) RPA 203597
Document No: M-242461-01-1
Guidelines: Not applicable
GLP: No

Objective:
The study was conducted to determine the toxicity of the metabolite M07 RPA 203597 (AE 1083976) to daphnids under static conditions.

Material and methods:
Test item: M07 RPA 203597 (AE 1083976)

Daphnids (*Daphnia magna*), less than 24 hours old, were exposed to nominal concentrations of 2.3, 4.5, 9.0, 18.0 and 36.0 mg/L test item for 48 h.

Results:

	M07 RPA 203597 % immobilization observed at Test concentrations [mg/L]				
Exposure interval	2.3	4.5	9.0	18.0	36.0
3 hours	5	0	5	0	0
24 h	5	5	15	0	0
48 h	5	15	15	0	0

In view of the absence of immobility at the highest concentrations of 18.0 and 36.0 mg/L, 15% immobilisation observed at the concentrations of 4.5 and 9.0 mg/L was not considered to be significant.

Conclusion:
The metabolite M07 (RPA 203597) was found to have an EC_{50} (48h) > 36 mg/L and a NOEC of 36 mg/L.

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Report: **KCA 8.2.4.1/03; [redacted] (1992)**
Title: The Acute Toxicity of Sodium Trifluoroacetate to *Daphnia magna*
Document No: M-247899-01-1
Guidelines: OECD Guideline 202 (1984)
EPA Guideline 72-2
GLP: Yes (certified laboratory)

Objective:
The study was performed, to detect possible effects of M05 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: M05 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.

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 M05 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 M05 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.

CA 8.2.4.2 - Acute toxicity to Mysid species

No studies have been conducted and none are considered necessary.

CA 8.2.5 - Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 - Reproductive and development toxicity to Daphnia magna

A chronic flow-through study for flurtamone with *Daphnia magna* was evaluated for Annex I inclusion ([redacted] (1992, M-203224-01-1). No new studies have been conducted.

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

No studies on additional invertebrate species have been conducted.

CA 8.2.5.3 - Development and emergence in Chironomus species

A chronic study with Chironomus riparius in spiked water for flurtamone was evaluated for Annex I inclusion([redacted], 1997, M-247873-01-1). No new studies have been conducted.

CA 8.2.5.4 - Sediment dwelling organisms

No further studies have been conducted.

CA 8.2.6 - Effects on algal growth

CA 8.2.6.1 - Effects on growth of green algae

Report:	**KCA 8.2.6.1/01; [redacted] D., 2005**
Title:	*Pseudokirchneriella subcapitata* growth inhibition test with flurtamone
Document No:	M-247782-01-1
Guidelines:	Originally reported under OECD 201 Recalculation is based on OECD 201 (June 1984) "Alga, Growth Inhibition Test" under consideration of the new draft revised proposal for updating OECD 201 (Feb 18, 2004)
GLP:	No

Objective:

The aim of this non-GLP recalculation report was to fulfil the draft revised proposal for updating OECD guideline 201 from February 18, 2004 requirements, which asks for the E_rC_{50} under consideration of additional validity criteria to show that the mean coefficient of variation for section-by-section specific growth rates (days 0-1,1-2 and 2-3) in the control cultures was not exceeding 35%.

Materials and Methods:

Recalculation was done using the commercial program ToxRat Professional.
Since there is a lack of individual cell counts for each replicate in the original report, calculations are based on the means of the treatment groups and control.

Results:

Calculated cell numbers, growth rates and growth rate inhibition (0-72h) of *Pseudokirchneriella subcapitata* exposed to flurtamone (technical)

Mean measured concentration [µg/L]	Cell numbers per ml after 0 h	Cell numbers per ml after 72 h	Average growth rate (0 h →72 h)	% Inhibition of average growth rate (0-72 h)
Control	7,400	1,000,000	1.635	—
6.4	7,400	1,100,000	1.667	-1.9
12	7,400	950,000	1.618	1.0
25	7,400	340,000	1.276	22.0
46	7,400	32,000	0.488	70.2
97	7,400	12,000	0.314	80.8

Sectional growth rates of *Pseudokirchneriella subcapitata* for the control treatment

	Sectional growth rate (µ) (days^{-1})			
	0-24 h	24-48 h	48-72 h	%CV
Control	1.400	2.037	1.470	21.4

Conclusions:

Results based on OECD 201 are (based on mean measured concentrations) for flurtamone : E_rC_{50} (0 - 72 h): 38.0 µg/L (95% Cl: 21.7 - 71.6). The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures was 21.4% and met the validity criteria by not exceeding 35 %.

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Report:	**KCA 8.2.6.1/05; [redacted], K.; 2013**
Title:	*Pseudokirchneriella subcapitata* - growth inhibition test with flurtamone (tech.)
Document No:	M-473178-01-1
Guidelines:	OECD 201 (2006)
GLP:	Yes (certified laboratory)

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

Material and methods:
Test item: Flurtamone (tech.) analysed purity: 97.3 % was tested, specified by origin batch no. CGS 17424-1-1, sample description: TOX10196-00 and specification no. 102000002946.
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 5.00, 10.0, 20.0, 40.0, 80.0 and 160 µg a.s./L in comparison to controls. The pH values ranged from 7.9 to 8.2 in the controls and the incubation temperature ranged from 21.6 °C to 22.4 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6089 lux (mean value).
Quantitative amounts of flurtamone were measured in all treatment groups and in the controls at test start and end (day 3).

Findings:
The study conditions met all validity criteria, requested by the mentioned guideline(s).
The analytical findings of flurtamone in the treatment levels found on day 0 were 91.9 % to 98.4 % of nominal (average 95.8 %). On day 3 analytical findings of 93.8 % to 99.5 % of nominal (average 96.8 %) were found.
Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

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

nominal concentration [µg a.s./L]	cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	inhibition of average specific growth rate [%]
pooled control	770 000	1.447	0.0
5.00	798 000	1.458	-08
10.0	718 000	1.425	1.6
20.0	432 000	1.255*	13.3
40.0	94 000	0.742*	48.7
80.0	38 000	0.442*	69.5
160	34 000	0.410*	71.7

test initiation with 10,000 cells/mL
-% inhibition: increase in growth relative to the control
* significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

Conclusions:
After 72 hours the E_rC_{50} for flurtamone (tech.) is 53.4 µg a.s./L (95 % CI: 45.3 – 63.4 µg a.s./L) and the NOE_rC is 10.0 µg a.s./L.

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Report:	**KCA 8.2.6.1/04; [redacted], K.; 2014**
Title:	*Pseudokirchneriella subcapitata* flow-through growth inhibition test under variable exposure with flurtamone / AE B107587
Document No:	M-474520-01-1
Guidelines:	OECD 201 (2006), OPPTS 850.5400 (1996); adapted for a flow through test design investigating variable exposures
GLP:	Yes (certified laboratory)

Objective:
The purpose of the study was to determine the influence of variable flurtamone concentrations on exponentially growing *Pseudokirchneriella subcapitata* populations under flow through conditions. The study was aimed to represent a generalised multi- peak exposure. For that purpose the growth of the algae until steady state and effects of different peaks and the respective recovery were investigated.

Material and methods:
Test item: Flurtamone / AE B107587 analysed content: 97.3 % w/w was tested, specified by batch ID: CGS 17424-1-1, sample description: FOX 10196-00 and specification no.: 102000002946.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed to 3 peaks of flurtamone using a flow through test system over a period of 38 days. The volume of the two chemostat reactors was 1 L each and the flow through was adjusted to 31 mL medium/h. The respective medium exchange corresponded to ca. 50% reactor volume per day. The test started with a cell density of 60 000 cells/ mL. After 14 days a steady state of around 500 x 10^4 cells/mL was reached. The reactors received nominal flurtamone peaks of 40.0, 20.0, and 35.0 µg a.s./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 and the outflow was determined daily. Orthophosphate und total phosphate was measured. The pH values measured in the sampled test medium at outflow ranged from 7.3 to 7.6 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 constantly added. The reactors were illuminated with a light panel placed directly over the reactor resulting in a light intensity of ca. 8.2 klux (8.1 - 8.3 klux) in both reactors over the entire testing period.

Findings:
The measured concentrations for the three peak exposure events ranged between 99 % and 111 % of nominal values of flurtamone.

The chemical analysis of the first peak (40.0 µg a.s./L) resulted in a measured concentration of 43.1 µg/L for both reactors. The analysis of the second peak (nominal 20.0 µg a.s./L) revealed a measured

concentration of 22.0 µg a.s /L. The accompanying chemical analysis of the third peak (nominal 35.0 µg/L) resulted in 36.6 µg a.s./L.

The first peak was applied after the cell density reached steady state on day 0. After the first peak with 43.1 µg/L the cell density decreased slightly over the two next days. On day 3 the density scaled down to 76.9 % for one day. In the next two days the cell number ranged between 99.9 and 104 % of the steady state (cell density in % compared to cell density of the related steady state).
The second peak (22.0 µg/L) was applied on day 7. This peak had no influence on the cell number. The cell numbers ranged over the next three days between 100 and 103 %.
The last peak, 36.6 µg/L was dispensed on day 14 resulting in a cell density reduction after one day of about 72.9 % followed by a fast recovery of cell density one day later.

The observed results demonstrate the algistatic effect of flurtamone on the green algae *Pseudokirchneriella subcapitata*. A fast recovery of the algae was observed after short term peak exposure concentrations of up to 43.1 µg a.s./L. After three peaks of different heights recovery potential was still observed. The peak exposure pattern used was based on worst case assumption resulting from FOCUS exposure patterns.

After day 24 both reactors reached the steady state therefore the experiment was ended on day 24.

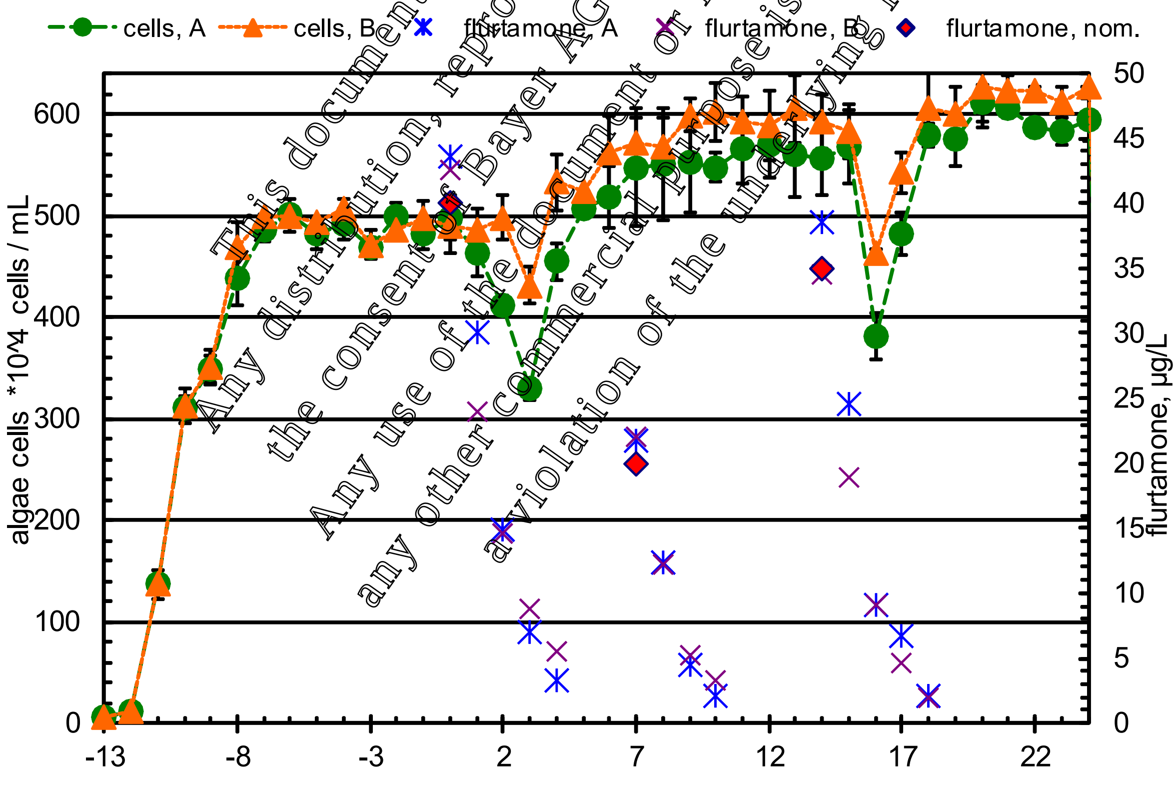

Figure: Population curves over the test duration incl. test substance concentration

Conclusion
A fast recovery of the algae was observed following short term peak exposure concentration of up to 43.1 µg a.s./L (mean measured). This results in a nominal population EC_{50} of > 40 µg a.s./L.

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Effects of metabolites on algal growth

Report:	**KCA 8.2.6.1/03; [redacted].; 1993a**
Title:	The toxicity of sodium trifluoroacetate to the alga *Selenastrum capricornutum* at low concentrations, including Amendment No. 1
Document No.:	M-247818-02-1
Guidelines:	OECD 201 (1984)
GLP/GEP	Yes (certified laboratory)

Objective:
The toxicity of the metabolite M05 TFA Na salt to the alga *Pseudokirchneriella subcapitata* was determined under static conditions.

Material and methods:
Test item: M05 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, 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.

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

Deviations: Initial cell density was 0.64 x 10^4 cells/mL instead of 1.0 x 10^4 cells/mL
The amendment states that two errors were detected in the final report 56635/61/92 (M-247818-02-1 = C047121):

- the mean cell density at day 3 was 0.901 x 10^6 cells/mL instead of 0.911 x 10^6 cells/mL
- the nominal cell density at day 0 was 0.64 x 10^6 cells/mL instead of 1 x 10^6 cells/mL

Conclusion:
The 72 hour growth rate EC_{50} value for M05 Na-TFA to *Pseudokirchneriella subcapitata* was estimated to be greater than 1.20 mg /L, the highest concentration tested.

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Report:	**KCA 8.2.6.1/05; [redacted]. 1995**
Title:	A comparison of the toxicity of sodium trifluoroacetate, sodium difluoroacetate, sodium monofluoroacetate and sodium fluoride to the alga *Scenedesmus supspicatus*
Report No.:	M-247825-01-1
Guidelines:	OECD 201 (1984)
GLP/GEP	Yes

Objective:
The toxicity of the metabolite M05 TFA Na salt and two other acetates to the alga *Desmodesmus subspicatus* (Formerly *Scenedesmus subspicatus*) was determined under static conditions.

Material and methods:
Test item: M05 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: difluoroacetec 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 1.0×10^4 cells/mL. Each day, algal density was determined.

Findings:
Only the results for NaTFA are summarized. 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 (NaTFA)

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

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Report:	**KCA 8.2.6.1/06; [redacted] D., 2005**
Title:	AE 1083976 Alga, growth inhibition test with *Pseudokirchneriella subcapitata*, 72 h
Document No:	M-255213-01-1
Guidelines:	OECD 201 (2004)
GLP:	Yes (certified laboratory)

Objective:

Aim of this study was to determine the toxicity of the metabolite of flurtamone M07 AE 1083976 to the unicellular freshwater green alga *Pseudokirchneriella*. The study was performed as a limit test in accordance to the principles of the OECD Guideline No. 201 (2004). Endpoints calculated were the No Effect Concentration (NOEC) and the Lowest Effect Concentration (LOEC) based on inhibition of the average specific growth rate after 72 h.

Materials and Methods:

The study was designed as a limit test with a nominal concentration of 100 mg/L of M07 AE 1083976 (metabolite of flurtamone), chemical name: 3-(2-methylamino-4-oxo-5-phenyl-4,5-dihydrofuran-3-yl) benzoic acid, Batch No.: PJS809, purity: 96.6 % w/w.
Six replicates were tested for limit concentration and control. Environmental conditions were: Temperature 21 - 24 °C, light intensity: 60 - 120µE m^{-2} s^{-1} and 24 hours of light.
The cell density was measured at the beginning of the test and every 24 h via Chlorophyll-a-fluorescence and the average growth rate as well as the yield (the biomass at the end of the exposure period minus the biomass at the start of the exposure period) was calculated. The concentrations of M07 AE 1083976 were analysed at limit concentration at test start and test end via HPLC.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Increase of the biomass in control cultures	> 16-fold	422-fold
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures	< 35%	3.34 – 8.83%
Coefficient of variation of average specific growth rates during the whole test period in control cultures	< 7%	5.69%

All validity criteria were met

Reference test:

Strain material of defined sensitivity was used, as shown by reference item testing with potassium dichromate. Such reference tests are done periodically. Such work is documented and archived together with strain protocols

Analytical results:

The recovery rate of the test item at the beginning were 99 % and at the end 101 %. All effect values are given based on the nominal concentration of the test item.

Biological results:
Inhibition of average growth rate and yield
There was no statistically significant difference in the average growth rate or in the yield of cultures treated with 100mg/L AE 1083976 compared to the control.

Conclusions:

In this study the effects of the metabolite M07 AE 1083976 on the growth of the freshwater green alga *Pseudokirchneriella subcapitata* were tested. No inhibiting effects on average specific growth rate were found at the nominal concentration level of 100 mg/L.

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Report:	**KCA 8.2.6.1/07; [redacted], A.; 1997**
Title:	Freshwater algal growth inhibition study (72 hours) *Selenastrum capricornutum*, Static screening test RPA 203597
Document No:	M-242463-01-1
Guidelines:	Not applicable
GLP:	No

Objective:
The study was conducted to determine the toxicity of the metabolite M07 RPA 203597 (AE 1083976) to the alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum)* under static conditions.

Material and methods:
Test item: M07 RPA 203597 (AE 1083976), batch no. SC970304, Lot no. PJS809
Algae were exposed under static conditions for 72 h to nominal concentrations of the test item of 6.3, 12.5, 25, 50 and 100 µg/L. Two replicates were performed.

Results and Conclusion:
Based on growth curve area and growth rate, the EC_{50} was > 100 µg/L (NOEC 100 µg/L).

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Report:	**KCA 8.2.6.1/06; [redacted], K.; 2013**
Title:	*Pseudokirchneriella subcapitata* - growth inhibition test with BCS-BT61400
Document No:	M-470662-01-1-01-1
Guidelines:	OECD 201 (2006)
GLP:	Yes (certified laboratory)

Objective:
The aim of the study was to determine the influence of the metabolite M08 AE 2093305 on exponentially growing populations of *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume).

Material and methods:

Test item: M08 AE 2093305, BCS-BT61400 analysed purity: 99.7 % w/w was tested, specified by origin batch no.: SES 12091-7-23, TOX 09838-00 and LIMS no.: 1240764.

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 0.00960, 0.0307, 0.0980, 0.313 and 1.00 mg pure metabolite /L in comparison to controls. The pH values ranged from 8.1 to 8.4 in the controls and the incubation temperature ranged from 21.6 °C to 22.3 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6402 lux (mean value).

Quantitative amounts of BCS-BT61400 were measured in all treatment groups and in the controls at test start and end (day 3).

Findings:

The study conditions met all validity criteria, requested by the mentioned guideline(s).

The analytical findings of BCS-BT61400 in the treatment levels found on day 0 were 113 % to 118 % of nominal (average 116 %). On day 3 analytical findings of 115 % to 118 % of nominal (average 116 %) were found. Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

The static 72 hour algae growth inhibition test provided the following tabulated 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	958 000	1.520	--
solvent control	946 000	1.516	--
pooled controls	952 000	1.518	--
0.00960	1 028 000	1.544	-1.7
0.0307	1 084 000	1.562	-2.9
0.0980	447 000	1.266*	16.6
0.313	52 000	0.548*	63.9
1.00	42 000	0.477*	68.6

test initiation with 10 000 cells/mL
*compared to pooled controls
-% inhibition: increase in growth relative to the control
* significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure

Conclusions:

After 72 hours the E_rC_{50} for the metabolite M08 (AE 2093305) BCS-BT61400 is 0.306 mg p.m./L (95 % CI: 0.230 – 0.415 mg p.m./L) and the NOE_rC is 0.0307 mg p.m./L.

CA 8.2.6.2 - Effects on growth of an additional algal species

Report:	**KCA 8.2.6.2/02; [redacted] A.G. 1996**
Title:	The toxicity of sodium trifluoroacetate to algae
Report No.:	M-247822-01-1
Guidelines:	Not appicable
GLP/GEP	No

This is a review of algal laboratory studies which were conducted with M05 TFA, sodium trifluoroacetate (NaTFA), including M-247818-02-1 , M-247820-01-1 and M-247825-01-1. 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 pelliculisa, Skeletonema costatum, Anabaena flosaquae* and *Microcystis aeruginosa*.
The tests reported for *Pseudokirchneriella subcapitata,* in addition to M-247818-02-1 and M-247820-01-1 did not produce reliable results, because one test was only a preliminary test using 2 erlenmeyers per concentration. In another test the design was also limited to 2 erlenmeyers 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.

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.

CA 8.2.7 - Effects on aquatic macrophytes

Report:	**KCA 8.2.7/01; [redacted] D., 2005**
Title:	*Lemna gibba* G3 - Growth Inhibition Test with Flurtamone (tech.)
Document No:	M-258189-01-1
Guidelines:	Originally reported under US-EPA FIFRA § 122-2 and 123-2 Recent recalculation is based on Revised Proposal for a New Guideline 221 "*Lemna* sp. Growth Inhibition Test", Draft Document (October 2004)
GLP:	No

Objective:
The aim of this non-GLP re-calculation report for flurtamone was to fulfill the OECD 221 requirements, which ask for the EC_{50} for growth rate of both endpoints: frond number and dry weight of plants of *Lemna gibba* exposed to flurtamone.

Materials and Methods:
Results of the study [redacted] (1997, M-244591-01-1, evaluated during the Annex I inclusion) were re-calculated in order to fulfil current requirements. Re-calculation was done using the commercial program ToxRat Professional.

Results:

Table: Frond numbers and dry weights, average growth rates and % inhibition of *Lemna gibba* exposed to flurtamone (technical)

Mean measured concentration [µg a.s./L]	Final frond no. (replicate means, day 14)	Final dry weight of plants (replicate means, day 14) [mg]	%Inhibition*	
			Average growth rate for frond no.	Average growth rate for final dry weight of plants
Control	507	68.5	—	—
Solvent control	528	95.1	—	—
Pooled controls	518	81.8	—	—
0.94	536	85.1	-1.0	-0.5
2.30	535	94.7	-4.0	-4.0
5.80	461	56.4	3.3	10.3
15.0	142	28.4	36.6	40.2
33.0	90	13.1	49.4	51.8
85.0	70	12.1	50.6	53.9

* negative values indicate stimulation of growth

Conclusions:
For flurtamone the endpoints based on OECD 221 are for frond number E_rC_{50} (0 - 14 d): 44.5 µg a.s./L (95% C. L. : 23.2 - 175), LOE_rC: 5.80 µg a.s./L and NOE_rC: 2.30 µg a.s./L. Endpoints for final dry weight of plants are: E_rC_{50} (0-14 d): 42.9 µg a.s./L (95% C. L. : 20.7 - 244), LOE_rC: 15.0 µg a.s./L and NOE_rC: 5.80 µg a.s./L. The (0-14 d)-E_rC_{50} figures are usable as substitutes for (0-7d)-E_rC_{50} figures because of time-independency of such growth rate data.

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Report:	**KCA 8.2.7.1/09; [redacted], IC, 2013**
Title:	*Lemna gibba G3* growth inhibition test with flurtamone (tech) under static conditions
Document No:	M-470528-01-1
Guidelines:	OECD 221 (2006), US EPA OCSPP 850.4400
GLP:	Yes (certified laboratory)

Objective:
The aim of the study was to determine the influence of flurtamone 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.

Material and methods:
Test item: Flurtamone (tech) analysed content: flurtamone: 97.3 % was tested, specified by batch code: AE B107587-01-10, sample description: TOX10196-00 and specification no.: 102000002946.

A total of 4 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 the nominal concentrations of 0.286, 0.916, 2.93, 9.38 and 30 µg a.s./L in comparison to a control and solvent control. The pH values ranged from 7.5 to 8.8 in the control and the incubation temperature ranged from 24.4°C to 24.8°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6726 lux (average of nine measurements).

Quantitative amounts of flurtamone 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 study met all validity criteria, requested by the mentioned guidelines.
The analytical findings of flurtamone found in all freshly prepared test levels on day 0 ranged between 101 and 111 % of nominal. In aged test levels on days 7 the analytical findings ranged between 102 and 110 % of nominal.
All reported results are based on nominal values of flurtamone.

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

nominal test concentration [µg a.s./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means, day 7) [mm^2]	%inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	161	1250		--
solvent control	150	1166	--	--
0.286	151	1131	1.0	1.2
0.916	168	1253	-3.1	-0.3
2.93	140	996	4.0 *	8.4 *
9.38	67.3	447	32.5 *	40.2 *
30.0	33.0	195	60.5 *	70.6 *

-% inhibition: increase in growth relative to the pooled controls
* Results which were significantly different (based on Williams Multiple sequential t-test Procedure) from the control

Observed visual effects on *Lemna gibba*:

nominal test concentration [µg a.s./L]	Observations
control	no visual effects observed
solvent control	no visual effects observed
0.286	no visual effects observed
0.916	no visual effects observed
2.93	no visual effects observed
9.38	white fronds
30.0	white fronds

Observed visual effects on the test item:
On day 7 at 9.38 and 30.0 µg a.s./L the test item precipitated.

Since the analytical measurements showed results of 80.0 – 120 % of nominal the calculated endpoints are based on nominal concentrations of the test item.

end point (0-7 day)	effect on mean growth rate of frond no. [µg/L]	effect on mean growth rate of total frond area of plants [µg/L]
E_rC_{50} (CI 95%)	19.8 (15.2 – 27.7)	14.1 (11.6 – 17.4)
LOE_rC	2.93	2.93
NOE_rC	0.916	0.916

The LOE_rC and NOE_rC determination is based on statistical data analysis.

Conclusions:

For flurtamone the most sensitive response variable in this study was total frond area of plants resulting in a 7 day- E_rC_{50} of 14.1 µg/L. The lowest NOE_rC was 0.916 µg/L.

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Report:	**KCA 8.2.7/10; [redacted], K.; 2014**
Title:	*Lemna gibba* G3 - Growth inhibition test with flurtamone (tech.) under variable exposure conditions
Document No:	M-475376-01-1
Guidelines:	OECD 221
GLP:	Yes (certified laboratory)

Objective:

The aim of the study was to determine the influence of the test item after short term exposure(s) (peak exposure) 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.

Material and methods:

Test item: Flurtamone (tech.) analysed purity 97.3 % was tested, specified by origin batch no.: CGS 17424-1-1, customer order no.: TOX10196-00 and specification no.: 102000002946.
Exponentially growing cultures of *Lemna gibba* were investigated under defined conditions for 2 x 7 days. Plants were exposed in week one for 2 days to concentrations of 10.0, 20.0, 40.0, 80.0 and 160 µg a.s./L followed by a 5 day period without exposure in which the plants were growing in untreated growth media. In the second week half of the replicates of each test concentration were exposed for two days to test concentrations of 10.0, 20.0, 40.0, 80.0 and 160 µg a.s./L, solvent control to nutrient medium containing dimethylformamid, followed again by a 5 day period in untreated medium. The other half of the replicates was again transferred in untreated growth medium. Patterns of medium renewals were identical in the control, solvent controls as in the treatment levels exposed to two peaks. The pH values ranged from 7.5 to 8.8 in the controls and the incubation temperature ranged from 22.6°C to 25.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6630 lux (average of nine measurements).
Quantitative amounts of flurtamone were measured in all freshly prepared test levels on day 0, and day 7 (start of the second peak exposure), in all aged test levels on day 2 and day 9 of the exposure period and additionally in the untreated growth medium on day 2, day 7 and day 9.

Deviation: a slight deviation of pH

Findings:

The study met all validity criteria, requested by the mentioned guidelines.
The analytical findings of flurtamone detected in all freshly prepared test levels on day 0 ranged between 108 and 113 % of nominal. In aged test solutions on day two analytical results ranged between 109 and 114 % of nominal. For the second peak on day seven the analytical findings ranged between 105 and 109 % of nominal peak concentrations. In the aged media on day 9 the chemical analysis revealed recoveries between 106 and 111 %.
In the test concentration level of 10 µg a.s/L (day 7) and 160 µg a.s./L (day 7 and day 14) very small amounts (0 – 1 % of nominal) of the test substance were found. Since all plants were supposed to be in untreated medium from day 2 to 7 and 9 to 14, these contaminations of the test medium are explained by not sufficient washing of the plants before setting them in the new test vessels. Since the measured concentrations were far below the 7 day NOEC for flurtamone, the found concentrations were not considered to result in a negative effect on the plants.
Based on analytical results all results are presented as nominal peak concentrations of the test item.

Effects on *Lemna gibba* such as white, small and necrotic fronds were observed in the study.

single peak [2 days] (day 0-7)	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_{50} (CI 95%)	124 (87.4 – 223)	>61.8 (46.4 – 87.7)
LOE_rC	≤10.0	≤10.0
NOE_rC	<10.0	<10.0

single peak [2 days] (day 7-14)	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_{50} (CI 95%)	>160	>160
LOE_rC	20.0	80.0
NOE_rC	10.0	40.0

two peaks [2 days each] (day 7-14)	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_{50} (CI 95%)	71.9 (61.9 – 85.3)	60.8 (43.9 – 90.8)
LOE_rC	≤10.0	≤10.0
NOE_rC	<10.0	<10.0

Conclusions:

The 7 day E_rC_{50} after one peak over 48 hours was calculated to be 124 µg a.s./L for frond number and 61.8 µg a.s./L for frond area, respectively. The NOE_rC after the first week was determined to be lower than the lowest tested concentration of 10 µg a.s./L. However, within the 5 days of growth in untreated media a recovery of the plants were visible since the E_rC_{50} increased from 91.1 µg a.s./L after 5 days to 124 µg a.s./L after 7 days for frond number and from 46.5 µg a.s./L to 61.8 µg a.s./L for frond area.

Further 7 days of untreated growth further reduced the effects. The E_rC_{50} after 14 days (one peak) was calculated to be higher than the highest test concentration of 160 µg a.s./L for frond number and frond area, respectively. The NOE_rC for frond number was determined to be 10.0 µg a.s./L and 40.0 µg a.s./L for frond area. Altogether no effect > 20 % inhibition was found after 14 days although heavy sublethal effects were observed after the exposure of a single, 48 hours lasting exposure.

The exposure to two peaks over 48 hours led to an E_rC_{50} of 71.9 µg a.s./L for frond number and 60.8 µg a.s./L for frond area at test end (day 14). The corresponding NOE_rC was again lower than the lowest test concentration of 10.0 µg a.s./L. However, as after the first week the observed effects decreased within the five days growth in untreated media after the second peak. The E_rC_{50} increased from 46.8 µg a.s./L to 71.9 µg a.s./L for frond number and from 41.8 µg a.s./L to 60.8 µg a.s./L for frond area.

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Report:	**KCA 8.2.7/04; [redacted]; 2012**
Title:	Toxicity of flurtamone technical to the aquatic macrophyte, *Myriophyllum spicatum*
Document No:	M-431579-01-1
Guidelines:	OECD Guideline No. 221 (2006)
GLP:	Yes (certified laboratory)

Objective:

The objective of this study was to determine the dose-response effect of flurtamone to the rooted aquatic macrophyte, *Myriophyllum spicatum*, over an exposure period of 14 days. Growth in the study is defined as a change (yield) in total shoot length, total plant wet weights and total plant dry weights (shoots and roots). The EC_{50} was estimated for these growth parameters based on growth occurring between study days 0 and 14. The EC_{50} is the approximate concentration of the test substance that inhibits 50% of plant growth as plant total shoot length, wet weight or dry weight relative to the controls.

Materials and Methods:

Test item: flurtamone Technical; Batch Code: AE B107587 00 B99 0001; Origin Batch No.: DP639D; Purity: 99.5%.

The test system consisted of three replicate test vessels per treatment group. Each replicate contained five plants for a total of 15 plants per group. All plants within a replicate were planted into a single 125x65 mm crystallization dish containing 550 g of artificial sediment. Plants were exposed to the test solutions for 14 days. Nominal (mean measured) concentrations were control, 10 (7.1), 20 (15), 40 (32), 80 (60), and 160 (123) µg a.s./L. All test vessels were contained in an environmentally controlled

room with a photoperiod of 16 hours light to 8 hours dark, light intensity 8320 to 12 100 lux (mean = 10 380 lux) and temperature ranging from 19.59 to 20.32 °C.
Following the 14 day exposure period plants were sacrificed and measured. Effects on yield for total shoot length, total plant wet weight and total plant dry weight were determined on a per plant basis, based on the growth of each plant during the 14 day growth intervals.

Results:

Analytical results

Mean measured concentrations are determined based on results of the recoveries from Days 0, 7, and 14 sampling and ranged from 71 to 80% of the nominal concentration. The toxicity values were calculated based on these mean measured concentrations.

Biological results:

Total Shoot Length Yield

Shoot length yield was analyzed at test termination on study day 14. Data analysis indicated a statistically significant difference from the control group in the four highest treatment groups (Dunnett's Test, p = 0.05). Percent inhibitions as compared to the control group were 9.1, 16.9, 20.1, 23.3 and 29.8% for the 7.1, 15, 32, 60 and 123 µg a.s./L test groups, respectively.

Total Plant Wet Weight Yield

Total plant wet weight yield was analyzed at test termination on study day 14. Data analysis indicated a statistically significant difference from the control group in the four highest treatment groups (Dunnett's Test, p = 0.05). Percent inhibitions as compared to the control group were 7.7, 23.3, 22.8, 26.7 and 33.3% for the 7.1, 15, 32, 60 and 123 µg a.s./L test groups, respectively.

Total Plant Dry Weight Yield

Plant dry weight yield was analyzed at test termination on study day 14. Data analysis indicated a statistically significant difference from the control group in the four highest treatment groups (Dunnett's Test, p = 0.05). Percent inhibitions as compared to the control group were 20.0, 36.4, 35.8, 39.7 and 41.9% for the 7.1, 15, 32, 60 and 123 µg a.s./L test groups, respectively.

Plants in the control vessels appeared normal throughout the study. Plants in all treatment groups were observed to have red coloured main and side shoots during the exposure period.

Summary of the effects of flurtamone (tech.) on *Myriophyllum spicatum*

Test Substance	Flurtamone technical		
Test Object	*Myriophyllum spicatum*		
Exposure	14 Day – Static Exposure		
Endpoint Units	(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)	7.1	7.1	7.1
Lowest Concentration With an Effect (LOEC)	15	15	15
E_yC_{50}	**>123**	**>123**	**>123**

Conclusions:

Statistical analysis of the growth data of shoot length, wet weight and dry weight yield indicated a statistical difference from the control group in the four highest treatment levels for all three endpoints. The statistical NOEC, LOEC and E_yC_{50} of flurtamone for all endpoints was 7.1, 15 and >123 µg a.s./L, respectively.

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Report:	**KCA 8.2.7/05; [redacted] I.; 2010**
Title:	Ecological effects of the herbicide flurtamone in outdoor freshwater microcosms
Document No:	M-389526-01-1
Guidelines:	OECD 221 (2006)
GLP:	Yes (certified laboratory)

Objective:

The aim of the study was to assess the initial and longer term ecotoxicological impact of flurtamone (applied as the formulated product Flurtamone SC 600 G, containing 619.7 g flurtamone/L.) to a macrophyte-dominated freshwater community typical for lentic, shallow macrophyte-dominated freshwater ecosystems. Microcosms were incubated with 9 additional macrophyte species in potted plants. Concentration levels of flurtamone relevant to the initial surface water PEC values for a range of agricultural use scenarios were applied.

Materials and Methods:

Test item: flurtamone SC 600 G, Batch Identification: 2009-002504; TOX No.: 08542-00; content of active ingredient: 52.7% w/w.
Flurtamone was applied to the microcosms at intended concentrations of 0, 0.3, 1.0, 3.0, 10, 30, and 100 µg a.s./L. Overlying water samples were taken 0, 2, 7, 14, 28, 42, 56, and 68 days post application for residue analysis. Sediment samples were taken 7, 14, 28, 42, 56, and 68 days post treatment. Temperature, pH, dissolved oxygen and electrical conductivity were measured at regular intervals in each of the microcosms. Approximately 50% of the sediment surface area in the microcosms was reserved for macrophyte bioassays (potted and caged plants), while the remaining area was used for monitoring the growth of 'free-living' so-called volunteer macrophytes. Species used as potted plants were: *Spirodela polyrhiza*, *Salvinia natans*, *Potamogeton natans*, *Sagittaria sagittifolia*, *Eleocharis palustris*, *Myriophyllum spicatum*, *Elodea canadensis*, *Potamogeton crispus*. The additional floating plant *Lemna gibba* was tested in a separate bioassay set-up. The following assessments were made in each effect microcosm:

Species composition and measured endpoints

Macrophytes / Macro algae	Number and type of bioassays per cosm	Endpoints
Myriophyllum spicatum *Elodea canadensis* *Potamogeton crispus*	18 pots 18 pots 18 pots	Shoot length Shoot number Aboveground biomass
		Belowground biomass
Salvinia natans	1 cage (with 8 plants)	Number of leafs/fronds Cumulative leafs harvested Cumulative biomass harvested

		Remaining leafs/biomass
Spirodela polyrhiza	2 cages (with 15 fronds each)	Number of leafs/fronds Cumulative leafs harvested Cumulative biomass harvested Remaining leafs/biomass
Lemna gibba	Glass container (with 30 fronds)	Number of leafs/fronds Cumulative leafs harvested Cumulative biomass harvested
Potamogeton natans *Sagittaria sagittifolia*	3 pots 3 pots	Leaf number Total leaf size
		Aboveground biomass Belowground biomass
Eleocharis palustris	3 pots	Shoot number
		Aboveground biomass Belowground biomass
Free-growing macrophytes	-	Coverage
		Aboveground biomass

Phytoplankton, zooplankton and chlorophyll-a endpoints:
Depth integrated water samples were taken to sample the phytoplankton community and chlorophyll-*a*. Sampling occurred on days -14, -2, 7, 14, 21, 28, 42, 56 and 68. On these days zooplankton was sampled as well except on days 21 and 42; zooplankton was sampled but not identified on day 68. Glass slides (7.6 x 2.6 cm), which were colonised for 14 days, were used as artificial substrates for sampling periphyton chlorophyll-*a*. Substrates were introduced 14 days before the actual sampling of the substrates. Samples were taken at days -7, -1, 7, 14, 21, 28, 42, 56 and 68.
Community metabolism endpoints
Temperature, pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured in the morning on days -14, -2, 7, 14, 28, 42, 56 and 68. In addition, daily maximum values of DO were monitored on days -2, 7 and 21.
Data analysis
Effects on the macrophytes, Chl-*a*, phyto- and zooplankton communities (species composition and abundance), and community metabolism were evaluated using univariate and multivariate statistical techniques to determine the $NOEC_{population}$, $NOEC_{community}$ and $NOEC_{ecosystem}$.

Results:

Analytical results
Based on the mean measured concentrations of flurtamone in the dose solutions, the initial concentrations in the enclosures following treatment and assuming homogeneous mixing throughout the water column was on average 105.3 (± 11.5)% of the intended treatment levels. The overall dissipation rate in the overlying water did not differ between the treatment-levels. After application of the test compound water concentrations gradually declined and at the end of the experimental period 5% (± 0.99%) of the nominal concentration was still present in all treatment levels. In the sediment a maximum of 8.5% of the total dose of flurtamone was measured at day 14 post application. Hereafter a slow decline throughout the remaining experimental period was observed and at day 68 post application 4.3% of the total dose still remained in the sediment.

Biological results:
Macrophyte response
Clear negative effects for almost all plants, with the exception of *Spirodela polyrhiza* were observed at the 100 µg a.s./L treatment level. At 3.0 µ.g a.s./L and below no consistent treatment-related effects

could be observed on aquatic macrophytes. Note that the responses of both *Elodea canadensis* and *Potamogeton crispus* were considered unreliable and could therefore not be used to derive a consistent NOEC value. Based on the most sensitive measurement endpoints NOECs of 3.0 µg a.s./L were observed for the emergent macrophyte *Sagittaria sagittifolia* and the submerged macrophyte *Myriophyllum spicatum.* For *S. sagittifolia* leaf number and leaf length were more sensitive than biomass endpoints, while for *M. spicatum* shoot length and biomass endpoints were equally sensitive.

Phytoplankton response

In total 210 taxa were identified in the phytoplankton samples. Multivariate analysis could not determine a consistent treatment-related response.

Of the individual taxa sampled, consistent treatment-related declines in abundance were identified using univariate analysis for 3 individual phytoplankton taxa (*Chlamydomonas* sp., *Dinobryon divergens* and Pseudoanabaenaceae), 3 main groups (Cyanophyta, Desmidiaceae, and Chrysophyceae), taxa richness, and periphyton Chl-*a*. Corresponding NOEC values for the individual taxa and the remaining endpoints were 3.0 and 30 µg a.s./L respectively. Only *Dinobryon divergens,* Desmidiaceae, and periphyton Chl-*a* showed a consistent, treatment-related increase after flurtamone application.

Zooplankton response

In total, 20 taxa were identified in the zooplankton samples. Multivariate analysis found no consistent treatment-related response of the zooplankton community. Of the individual taxa sampled, a consistent decrease in abundance was detected for the cladocerans *Alonella nana* and *Daphnia* gr. *longispina* in the highest treatment level (100 (µg a.s./L). No treatment-related responses could be observed on the main groups copepoda and rotatoria, while for cladocera a consistent NOEC value of 30 µg a.s./L was determined.

Community metabolism response

Only the endpoints pH and dissolved oxygen showed a treatment-related response to flurtamone application. However, only in the 100 µg a.s./L treatment level consistent effects were observed. Nevertheless, the magnitude of these responses was so limited that they were thought to be of little ecological significance.

Conclusions:

During this study, there was no evidence that flurtamone had any direct treatment-related effect at any treatment level on the phytoplankton communities as a whole. Only some individual taxa and main groups responded to the application of flurtamone to the microcosms. At the treatment level of 10 µg a.s./L only the taxa *Dinobryon divergens* and Pseudoanabena showed clear treatment-related responses, while the taxa *Chlamydomonas* sp. and the main groups Cyanophyta, Desmidiaceae, and Chrysophyceae showed a clear treatment-related response at the 100 µg a.s./L only. In addition, the zooplankton community as a whole also did not show effects of the flurtamone application. Only the individual taxa *Alonella nana* and *Daphnia* gr. *longispina* showed clear treatment-related decreases in abundance at the 100 µg a.s./L treatment level.

Excluding the responses *of Elodea canadensis* and *Potamogeton crispus,* macrophytes were negatively affected at treatment levels of 10 µg a.s./L and higher, with *Myriophyllum spicatum* and *Sagittaria sagittifolia* being the most sensitive of the aquatic vascular plants tested. Sensitivity of *E. canadensis* and *P. crispus* to flurtamone cannot be assessed from the results of the bioassays of this study.

The responses observed for community metabolism endpoints showed effect in the 100 µg a.s./L treatment level but were very limited in magnitude and duration. Consequently, these effects were thought to be ecologically less relevant.

Based on these results the following study specific endpoints have been identified:

- The $NOEC_{population}$ i.e. the highest concentration tested at which no treatment-related effects were observed on any taxa is 3.0 µg a.s./L (based on effects on *Sagittaria sagittifolia, Myriophyllum spicatum,* and Pseudoanabaenaceae)
- The $NOEC_{community}$. i.e., the highest concentration tested at which no treatment-related effects were observed on either the zooplankton, phytoplankton or volunteer macrophyte communities is 10 µg a.s./L (based on a treatment-related increase of volunteer *Chara* sp.).
- The $NOEC_{ecosytem}$, i.e., the highest concentration tested at which no treatment-related effects were observed on any measurement endpoint in the test system was 3.0 µg a.s./L (based on effects on *Sagittaria sagittifolia, Myriophyllum spicatum,* and Pseudoanabaenaceae)

Based on these results the NOEAEC (No Observed Ecologically Adverse Effect Concentration) for flurtamone is 3.0 µg a.s./L since at this treatment level only class 1 effects are observed. If class 3 effects are considered acceptable the NOEAEC will be 3.0 µg a.s./L since class 5B effects are already observed at the 10 µg a.s./L treatment level for *Myriophyllum spicatum*.

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Report:	**KCA 8.2.7/11; [redacted] D., 2013**
Title:	Outdoor potted plant study to the effect of the herbicide Flurtamone on aquatic macrophytes *Elodea canadensis* and *Potamogeton crispus.*
Document No:	M-469643-01-1
Guidelines:	HARAP (Campbell, Arnold et al. 1999) CLASSIC guidance document (Giddings, Brock et al. 2002) SANCO (SANCO/3268/2001 rev4 (final) 2002)
GLP:	Yes (certified laboratory)

Objective:

The aim of the study was to assess the ecotoxicological impact of flurtamone (applied as the formulated product Flurtamone SC 600 G, containing 619.7 g flurtamone/L) as perceived on two macrophyte species, *Elodea canadensis* and *Potamogeton crispus* in potted plants at concentration levels relevant to the initial surface water PEC values for a range of agricultural use scenarios.

Material and methods:

Test item: Flurtamone SC 600 G; Batch ID: 2009-002504; Sample description: TOX 08542-00; content of a.s.: 52.7%.

The potted plants experiment was performed in 16 microcosms , four microcosms served as controls and the remaining 12 microcosms were treated with four different concentrations of flurtamone (0.3, 1, 3, 10 µg a.s./L.), each in triplicate. The test lasted for 42 days.
The following assessments were made in each effect microcosm:
Using macrophyte bioassays with potted plants the impact of flurtamone on *Elodea canadensis* and *Potamogeton crispus* was investigated. Macrophyte endpoints were nominally assessed on days -1 , 28 and 42.

Endpoints measured were:

Macrophytes	Number and type of macrophyte bioassays per cosm	Endpoints
Elodea canadensis	12 pots	Shoot length Shoot number Aboveground biomass Belowground biomass
Potamogeton crispus	12 pots	Shoot length Shoot number Aboveground biomass Belowground biomass

(note: 3 pots per species per sampling were harvested)

Temperature, pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured in the morning on days 0, 7, 14, 21, 28, 35 and 42.
Overlying water samples were taken -1 d, 2 h, 28, and 42 days post application for residue analysis.

Findings:

Analytical Findings

Based on the mean measured concentrations of flurtamone in the dose solutions, the initial concentrations in the enclosures following treatment and assuming homogeneous mixing throughout the water column was on average 98.3 ± 5.0% of the intended treatment levels.
The overall dissipation rate in the overlying water did not differ between the treatment-levels. After application of the test compound water concentrations gradually declined and at the end of the experimental period 4.6 ± 1.2% of the nominal concentration was still present in all treatment levels.

Macrophyte response

From the macrophyte responses it can be concluded that for *Elodea canadensis* clear negative effects were observed at the 3 and 10 µg a.s./L treatment levels. At 1 µg a.s./L and below no consistent treatment-related effects could be observed on the *Elodea canadensis* macrophyte bioassays present in the test systems. For *Potamogeton crispus* treatment-related effects were observed at the highest treatment level of 10 µg a.s./L, only.

Macrophyte bioassays	Sample day NOEC (µa.s./L)				Consistent NOEC (µg a.s./L)
	1	14	28	42	
E. canadensis					
Number of main shoots	-	-	-	-	
Total length shoots	-	-	-	1(↓)	
Above ground biomass	-	-	-	1(↓)	
Below ground biomass	-	-	-	1(↓)	
P. crispus					
Number of main shoots					
Total length shoots	3	-	-	3*	3*
Above ground end biomass	-	-	-	-	-
Below ground end biomass	-	-	-	-	-

*Based on visual inspection of the data
↓ = treatment-related decrease

Based on the most sensitive measurement endpoints NOEC values of 1.0 µg a.s./L were observed for *Elodea canadensis*. Shoot length and biomass endpoints were more sensitive than number of main shoots while for *Potamogeton crispus* only the total shoot length was a sensitive endpoint.

Community metabolism response

Although one test system of the 10 µg a.s./L treatment displayed a deviating electrical conductivity (EC) response this originated from the pre-treatment period and was not treatment-related. Also on pH and dissolved oxygen no treatment-related responses could be observed.

Using the effect class methodology this results in an effect class 1 for treatments up to and including the 1.0 µg a.s./L treatment level and 4-5B for the 3.0 and 10 µg a.s./L treatment levels.

	Intended concentration (µg a.s./L)			
	0.3	**1.0**	**3.0**	**10**
Macrophytes (bioassays)				
Elodea canadensis	1	1	4-5B(↓)	4-5B(↓)
Potamogeton crispus	1	1	1	4-5B(↓)
Community metabolism				
pH	1	1	1	1
DO production	1	1	1	1
Electric conductivity	1	1	1	1

↓ = treatment-related decrease

Conclusions:

The test systems received their intended doses and during the exposure period flurtamone water concentrations decreased to approximately 4.6±1.2% of nominal concentrations

E. canadensis was negatively impacted at treatment levels of 3.0 and 10 µg a.s./L. *P. crispus* was negatively impacted at the highest treatment level of 10 µg a.s./L only. Although treatment-related effects were observed both macrophytes still showed growth in the affected treatment levels. No treatment-related responses could be observed on the community metabolism endpoints pH, Dissolved Oxygen, and Electric Conductivity. Based on these results the $NOEC_{population}$ i.e. the highest concentration tested at which no treatment-related effects were observed on any taxa is 1.0 µg a.s./L (based on effects on *Elodea canadensis*).

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Report:	**KCA 8.2.7/12; [redacted] E.; 2013**
Title:	Ecological effects of the herbicide flurtamone in outdoor freshwater microcosms
Document No:	M-470995-01-1
Guidelines:	OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)" (2006) Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, 1991) Community-Level Aquatic System Studies – Interpretation Criteria (Guidance Document from the CLASSIC Workshop, SETAC, 2002) Linking aquatic exposure and effects: risk assessment of pesticides. (ELINK, 2010)
GLP:	Yes (certified laboratory)

Objective:

The aim of the study was to assess the initial and longer term ecotoxicological impact of short term (peak) exposures with flurtamone to two macrophyte species, *Elodea canadensis* and *Myriophyllum spicatum* as potted plants. Concentration levels and exposure durations relevant to the initial surface water PEC values for a range of agricultural use scenarios were investigated. The study was performed in a mesocosm test device consisting of 15 ponds.

Material and methods:

Test item :Flurtamone (tech.); Batch ID: CGS 17424-1-1; Sample description: TOX10196-00; Specification No.: 102000002946; Purity: 97.3%

Fifteen test tanks (6 m^3 water, 1 m water depth) which were used in this study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 14 cm in height) eight months prior to the study start. The water was local water. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before study start. In general, the artificial mesocosms are representative of a small stagnant water body.
The two bioassay macrophyte species were incubated in the mesocosms about three weeks before the intended first peak. Plants were planted in plastic pots containing well-mixed sediment substrate (approximately 0.8 L volume). In each pot only one species was planted (three shoots of 12 cm length, without roots and side shoots/pot). The pots were distributed over the 15 mesocosms for acclimatization to the test conditions. The planting of the macrophytes was excluded from the GLP claim.
Three of the fifteen mesocosms were designed as exposure ponds for the peak exposure at three different concentrations (4.0, 12.0 and 36 µg/L). After 48 hours of exposure to the test concentration the pots were removed from the exposure ponds, dipped into untreated water and transferred to the corresponding untreated mesocosm. The control pots were handled in the same way but without exposure. One half of the potted plants received one single peak. The other half of the potted plants were exposed towards two peaks. The two peaks were of the same nominal concentration. The duration of each peak was 48 hours. The interval between the two peaks was 7 days.
The test substance was applied in the three exposure mesocosms on July 02, (= 1. peak) and on July 9, (= 2. peak). The following test concentrations were applied per peak: 4.0, 12.0 and 36 µg a.s./L. The amount of test item which was applied for the second peak depended on the residues which existed in the exposure mesocosms on day 7 as a result from the first peak.
Several times during the exposure period water samples were taken and analysed to investigate the initial concentrations and the fate of the test substance in water.

Assessments of macrophyte related endpoints were made on days -1, 14, 28, 42 and 56. Six pots of each species were selected at random from each mesocosm (3 pots treated with one peak, 3 pots treated with two peaks each) for assessment of shoot number, total shoot length and aboveground biomass at each measurement point. In addition the physico-chemical water parameters were evaluated, as well as the coverage of the sediment with macrophytes and filamentous algae.

Findings:

Analytical Findings

All analytical results correspond very well to each other (average over all concentrations at days 0/+4h and 7/+4h: 104.7% of nominal). The results demonstrate that the nominal concentrations had been correctly applied for both peaks. At the end of the 48 h exposure periods 99% of nominal were measured on average in the three exposure ponds. The analytical results of the water of the untreated mesocosms were always below the limit of quantification. All reported results are based on nominal values of the test item.

Biological results:

The following tables provide the results of the statistical determination of the measured endpoints: shoot number, total shoot length and aboveground biomass for *Elodea candensis* and *Myriophyllum spicatum*. The results for *Elodea canadensis* were calculated and evaluated in comparison to the pooled controls (one and two peaks) to increase the statistical power. In case of *Myriophyllum spicatum* this was not necessary as no major effects occurred at all. For *Myriophyllum spicatum* no EC_x calculation was performed due to the absence of a dose response relationship.

From day 14 onwards a separation of the three originally inserted plants of *Elodea candensis* was not possible anymore, thus all shoots per pot were determined in total for number and length of shoots. The single plants of *Myriophyllum spicatum* could be separated during the whole study period. The biomass was determined per pot for both species.

NOEC calculations ($p \leq 0.05$, one-sided smaller) were carried out using the Williams test. The EC_{10} values were calculated by Probit analysis using linear max. likelihood regression.

Testconc. (µg/L)	*Elodea canadensis* – 1 peak: No. of shoots/pot (comparison to pooled control)											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	17.9	-14.4		5.4	-26.1		0.7	-29.5		0.2	-28.8	
12	28.3	-15.0	< 4.0	0.7	-27.1	≥36	3.4	-30.5	≥36	30.8	-29.9	≥36
36	29.7	-15.1		1.2	-27.4		18.6	-30.9		-8.2	-30.2	
EC_{10} (µg/L) (c.l. 95%)			n.a.			n.a.			22.7 (6.5-29)			n.a.
EC_{50} (µg/L)			>36			>36			>36			>36

Testconc. (µg/L)	*Elodea canadensis* – 1 peak: length of shoots/pot (comparison to pooled control)											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	-1.2	-16.4		5.9	-25.3		-11.1	-33.6		-7.3	-32.4	
12	18.9	-17.0	4.0	1.1	-26.2	≥36	-6.9	-34.8	≥36	25.4	-33.6	≥36
36	30.5	-17.2		8.8	-26.5		24.6	-35.2		-16.2	-34.0	
EC_{10} (µg/L) (c.l. 95%)			7.95 (n.a.)			n.a.			29.1 (n.a.)			n.a.
EC_{50} (µg/L)			>36			>36			>36			>36

Testconc. (µg/L)	*Elodea canadensis* – 1 peak: biomass aboveground part/pot (comparison to pooled control)											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	6.9	-18.2	4.0	18.5	-23.1	≥36	4.2	-29.1	≥36	-16.1	-28.1	≥36
12	27.5	-18.8		15.1	-23.9		7.6	-30.2		27.5	-29.1	
36	36.8	-19.0		21.4	-24.2		26.2	-30.5		-17.2	-29.5	
EC_{10} (µg/L) (c.l. 95%)			3.54 (n.a.)			n.a.			n.a.			n.a.
EC_{50} (µg/L)			>36			>36			>36			>36

n.a. = not applicable

Testconc. (µg/L)	*Elodea canadensis* – 2 peaks: No. of shoots/pot (comparison to pooled control)											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	35.7	-12.4	< 4.0	0.0	-21.9	12	4.3	-30.4	12	17.6	-32.5	12
12	32.9	-12.9		16.9	-22.7		1.1	-31.5		12.6	-33.7	
36	49.1	-13.0		43.8	-22.9		39.1	-31.8		40.5	-34.0	
EC_{10} (µg/L) (c.l. 95%)			n.a.			8.68 (n.a.)			8.16 (4.3-12)			n.a.
EC_{50} (µg/L) (c.l. 95%)			>36			>36			54.5 (42-89)			>36

Testconc. (µg/L)	*Elodea canadensis* – 2 peaks: length of shoots/pot (comparison to pooled control)											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	26.5	-12.5	< 4.0	-6.1	-20.7	12	-3.6	-30.6	12	31.1	-29.2	12
12	24.1	-13.0		16.8	-21.4		7.8	-31.1		15.3	-30.2	
36	53.6	-13.1		39.1	-21.7		41.3	-31.5		41.4	-30.6	
EC_{10} (µg/L) (c.l. 95%)			n.a.			8.7 (n.a.)			13.6 (8.7-17)			n.a.
EC_{50} (µg/L) (c.l. 95%)			>36			>36			43.9 (38-55)			>36

Testconc. (µg/L)	*Elodea canadensis* – 2 peaks: biomass aboveground part/pot (comparison to pooled control)											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	26.5	-16.1	< 4.0	2.3	-18.9	4.0	8.0	-28.9	4.0	31.0	-27.2	< 4.0
12	28.0	-16.7		27.4	-19.6		34.8	-30.0		30.2	-28.2	
36	59.1	-16.8		51.7	-19.8		40.0	-31.5		35.2	-28.5	
EC_{10} (µg/L) (c.l. 95%)			n.a.			5.43 (n.a.)			2.46 (n.a.)			n.a.
EC_{50} (µg/L) (c.l. 95%)			26.8 (n.a.)			32.3 (n.a.)			>36			>36

Testconc. (µg/L)	*Myriophyllum spicatum* – 1 peak: No. of shoots/plant											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	6.6	-17.9	≥36	-32.4	-25.2	≥36	-25.2	-24.2	≥36	-46.1	-31.5	≥36
12	12.5	-18.1		-28.6	-26.8		-17.2	-25.3		-41.4	-32.9	
36	-5.2	-18.3		-48.9	-26.6		-58.3	-25.6		-108	-33.4	

Testconc. (µg/L)	*Myriophyllum spicatum* – 1 peak: No. of shoots/pot											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	6.6	-16.3	≥36	-32.4	-24.9	≥36	-25.2	-24.2	≥36	-46.1	-32.3	≥36
12	12.5	-16.6		-19.1	-26.0		-17.2	-25.3		-41.4	-33.9	
36	-5.2	-16.8		-48.9	-26.4		-58.3	-25.6		-101	-34.4	

Testconc. (µg/L)	*Myriophyllum spicatum* – 1 peak: total length of shoots/plant											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	21.7	-16.5	≥36	-5.3	-21.0	≥36	-13.0	-24.7	≥36	-19.7	-27.4	≥36
12	20.6	-16.7		-8.7	-22.3		-15.9	-25.8		-16.6	-28.6	
36	1.6	-16.9		-42.1	-22.2		-55.4	-26.1		-99.4	-29.3	

Testconc. (µg/L)	*Myriophyllum spicatum* – 1 peak: total length of shoots/pot											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	21.7	-16.8	≥36	-5.3	-19.6	≥36	-13.0	-24.2	≥36	-19.7	-28.2	≥36
12	20.6	-17.0		-0.6	-20.4		-15.9	-28.5		-16.6	-29.5	
36	1.6	-17.3		-42.1	-20.7		-55.4	-28.9		-92.0	-30.0	

Testconc. (µg/L)	*Myriophyllum spicatum* – 1 peak: biomass aboveground part/pot											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	20.1	-22.0	≥36	9.7	-21.6	≥36	-3.8	-26.5	≥36	-11.1	-24.9	≥36
12	20.7	-22.4		22.9	-22.6		5.4	-27.7		12.4	-26.1	
36	16.4	-22.7		-14	-22.9		-19.5	-28.1		-58.5	-26.5	

Testconc. (µg/L)	*Myriophyllum spicatum* – 2 peaks: No. of shoots/plant											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	6.2	-20.3	≥36	-31.0	-32.2	≥36	-31.9	-27.9	≥36	-51.1	-33.9	≥36
12	2.8	-21.2		-35.4	-33.3		-28.3	-29.1		-59.3	-35.1	
36	-20.8	-20.9		-93.7	-34.4		-88.3	-29.5		-107	-35.5	

Testconc. (µg/L)	*Myriophyllum spicatum* – 2 peaks: No. of shoots/pot											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	6.3	-22.8	≥36	-33.8	-33.1	≥36	-31.9	-30.3	≥36	-51.1	-35.7	≥36
12	-1.9	-23.1		-35.4	-34.7		-28.3	-31.7		-65.4	-37.3	
36	-20.8	-23.4		-86.6	-35.1		-88.3	-32.1		-115	-37.8	

Testconc. (µg/L)	*Myriophyllum spicatum* – 2 peaks: total length of shoots/plant											
	Day 14			Day 28			Day 42			Day 56		
	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)	% decrease	%MDD	NOEC (µg/L)
4.0	-7.0	-20.0	≥36	-7.1	-20.4	≥36	-25.4	-25.8	≥36	-33.3	-30.1	≥36
12	6.8	-20.8		2.8	-21.1		-26.4	-27.0		-30.8	-31.1	
36	-13.2	-20.5		-48.1	-21.5		-86.9	-27.3		-95.2	-31.6	

Testconc. (µg/L)	*Myriophyllum spicatum* – 2 peaks: total length of shoots/pot											
	Day 14			Day 28			Day 42			Day 56		
	% de-crease	%MDD	NOEC (µg/L)	% de-crease	%MDD	NOEC (µg/L)	% de-crease	%MDD	NOEC (µg/L)	% de-crease	%MDD	NOEC (µg/L)
4.0	-7.0	-17.8	≥36	-3.1	-19.2	≥36	-25.4	-28.6	≥36	-33.3	-28.8	≥36
12	2.0	-18.0		2.8	-20.1		-26.4	-29.9		-35.9	-30.1	
36	-13.2	-18.3		-42.6	-20.4		-86.9	-30.3		-103	-30.5	

Testconc. (µg/L)	*Myriophyllum spicatum* – 2 peaks: biomass aboveground part/pot											
	Day 14			Day 28			Day 42			Day 56		
	% de-crease	%MDD	NOEC (µg/L)	% de-crease	%MDD	NOEC (µg/L)	% de-crease	%MDD	NOEC (µg/L)	% de-crease	%MDD	NOEC (µg/L)
4.0	-6.7	-17.9	4.0	-0.9	-18.4	≥36	-14.8	-22.5	≥36	-20.0	-24.1	≥36
12	25.7	-18.2		25.2	-19.3		-28.8	-23.6		-1.6	-25.3	
36	43.1	-18.4		8.1	-19.5		-34.6	-23.9		-39.3	-25.1	

In addition to the presented NOEC and ECx values the results are presented as effect classes. The effect classes allow to compare the results directly to the existing mesocosm with flurtamone. The effect classes were chosen according to the categorization by De Jong et. al. (2008).

Effect class table for a single pulse exposure of flurtamone towards *Elodea canadensis* and *Myriophyllum spicatum*

	Intended concentration [µg a.s./L]		
	4	**12**	**36**
Macrophytes (bioassays)			
Elodea canadensis # of shoots/pot	1-2	2	1-2
Elodea canadensis length of shoots/pot	1	1	1-2
Elodea canadensis biomass above ground	1	1	1-2
Myriophyllum spicatum # of shoots/pot	1	1	1
Myriophyllum spicatum length of shoots/pot	1	1	1
Myriophyllum spicatum biomass above ground	1	1	1

Effect class table for 2 pulsed exposures of flurtamone towards *Elodea canadensis* and *Myriophyllum spicatum*

	Intended concentration [µg a.s./L]		
	4	**12**	**36**
Macrophytes (bioassays)			
Elodea canadensis # of shoots/pot	1-2	2-3	4-5B
Elodea canadensis length of shoots/pot	1-2	2-3	4-5B
Elodea canadensis biomass above ground	1-2	2-3	4-5B
Myriophyllum spicatum # of shoots/pot	1	1	1
Myriophyllum spicatum length of shoots/pot	1	1	1
Myriophyllum spicatum biomass above ground	1	2	1 - 2

Conclusions:

The test plants received the intended peak concentration for one respectively two times 48 hours. In case of *Myriophyllum spicatum* only minor effects were observed. *Myriophyllum spicatum* was clearly less sensitive compared to *Elodea canadensis*.
Elodea canadensis was negatively impacted at the two highest test item concentrations of 12 and 36 µg/L after one and two short term exposures.

At 4 µg/L only slight effects were observed after exposure to 2 peaks. After exposure to one single peak the NOEC(day 14) was, with one single exception (for the parameter number of shoots per pot on sampling) 4 µg/L. After 28, 42 and 56 days a NOEC of ≥ 36 µg/L was observed.

After two short term exposures the effects were more pronounced. After 14 days the NOEC was < 4 µg /L. For the following measurement points (28, 42 and 56 days) the most sensitive endpoints was above ground biomass. For day 28 and day 42 a NOEC of 4 µg/L was observed. For the endpoints number of shoots per pot and total length of shoots per pot the NOEC determined for 28, 42 and 56 days was 12 µg/L. Only for the biomass above ground a NOEC of <4 µg/L was observed after 56 days. The free growing macrophytes observed in the exposure pond used for the concentration 4 µg/L demonstrated that *Elodea canadensis* was growing again, even under long lasting exposure.

Although treatment related effects were observed after exposure towards 2 short term peaks with flurtamone *Elodea canadensis* still showed growth in the affected treatment levels.
No treatment-related responses were observed in the community metabolism endpoints.

Based on these results the $NOEC_{population}$-56 days for *Elodea canadensis* after a single short term exposure towards Flurtamone is ≥ 36 µg/L. After 14 days the respective NOEC is 4 µg/L.

For the exposure to 2 short term exposures with flurtamone the NOEC population after 56 days for *Elodea canadensis* is 4 µg/L. This value represents a threshold level as for the endpoint biomass above ground a single NOEC of ≥ 4 µg/L has been determined for the measurement day 56. Based on the overall findings as well as on the observations made in the exposure ponds it seems to be reasonable to set the overall NOEC after two short term exposures with flurtamone for *Elodea canadensis* to 4 µg/L.

For *Myriophyllum spicatum* the overall $NOEC_{population}$(56 days) after 1 and 2 peak exposures was ≥ 36 µg/L.

Effects of metabolites on aquatic macrophytes

Report:	**KIIA 8.2.7/08; [redacted] J., 2005**
Title:	TFMBA (3-trifluoromethyl benzoic acid): *Lemna gibba* growth inhibition test
Document No:	M-253816-01-1
Guidelines:	OECD 221 (2004)
GLP:	Yes (certified laboratory)

Objective:

The aim of this study was to assess the effect of M04 TFMBA, 3-trifluoromethyl benzoic acid (metabolite of flurtamone) on the growth of the freshwater plant *Lemna gibba* over a 7-day period. The method followed that described in the OECD Guideline No. 221 (2004). Calculated endpoints were the E_rC_{50} and the NOEC, both based on frond number as well as on dry weight.

Materials and Methods:

Test item: M04 TFMBA, 3-trifluoromethyl benzoic acid (metabolite of flurtamone), Batch No.: 56469/2, purity: 98.6% w/w.

Lemna gibba was exposed to an aqueous solution of M04 TFMBA under semi-static conditions for a period of 7 days. Concentrations used were 0.10, 0.32, 1.0, 3.2, 10, 32 and 100 mg/L. Three replicate flasks per concentration were placed under constant illumination (light intensity approximately 7000 lux) at a temperature of 24 ± 1 °C. The test solutions were renewed on days 2 and 4. Each control and test flask was inoculated with 3 colonies of *Lemna gibba* (total 9 fronds).

Water samples were taken from the control and each test group on days 0 (fresh media) and on days 2, 4 and 7 (old media) for quantitative analysis. The number of fronds present in each test and control culture was recorded on days 0, 2, 4 and 7 along with observations on frond size, appearance, root length and number of colonies present.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Doubling time of control cultures	< 2.5 days	1.95 days

All validity criteria were met

Reference test:

A positive control conducted every 6 months used 3,5-dichlorophenol as the reference material in order to confirm the sensitivity of the *Lemna* cultures..

Analytical results:

Chemical analysis of the test solutions on Day 0 (fresh media) and days 2, 4 and 7 (old media) showed the measured concentrations to range from 90-114% of nominal values with the exception of the 0.10 mg/L test concentration on Day 2 which gave a measured concentration of 138% Given that all other measured concentrations were within the required range of 80 - 120% of nominal values and that the corresponding Day 0 fresh media measured concentration for the 0.10 mg/L test group was 90% of nominal it was considered that the measured concentration of 138% of nominal was an erroneous result. As the test concentration of 0.10 mg/L was below the No Observed Effect Concentration it was considered unnecessary to conduct any analysis of duplicate frozen test samples. Given that the

majority of measured test concentrations were within the required range of 80 - 120% of nominal values it was considered justifiable to express the results of the study in terms of nominal test concentrations only.

Biological results:

Effects on frond growth

In terms of frond numbers, there were no statistically significant differences between the control and 0.10, 0.32 and 1.0 mg/L test concentrations ($p \geq 0.05$, t-test), however all other test concentrations were significantly different ($p < 0.05$, t-test) and, therefore the NOEC in terms of inhibition of average specific growth rates calculated from frond numbers was 1.0 mg/L.

Effects on dry weight:

In terms of dry weight there were no statistically significant differences between the control and 0.10, 0.32, 1.0, 3.2 and 10 mg/L test concentrations ($p > 0.05$, t-test), however all other test concentrations were significantly different ($p < 0.05$, t-test) and, therefore the statistically NOEC in terms of inhibition of average specific growth rates calculated from dry weight was 10 mg/L. It is clear that the statistically determined NOEC for dry weight was higher than the E_rC_{50} value. This effect was considered to be due to the variability observed within the dry weights for the replicate control and test material cultures masking the true NOEC. Visual inspection of the dry weight data indicated that the true NOEC in terms of inhibition of average specific growth rates calculated from dry weight was 3.2 mg/L.

Inhibition of Average Specific Growth Rate based on frond numbers and dry weight of *Lemna gibba* exposed to M04 TFMBA

Nominal test concentrations [mg/L]		Frond numbers (0 – 7 Day)	Frond numbers %Inhibition	Dry weight (0 – 7 Day)	Dry weight %Inhibition
Control	Mean	0.285	-	0.208	-
	SD	0.019		0.022	
0.10	Mean	0.294	[3]	0.130	38
	SD	0.027		0.148	
0.32	Mean	0.306	[7]	0.236	[13]
	SD	0.018		0.026	
1.0	Mean	0.299	[5]	0.229	[10]
	SD	0.012		0.063	
3.2	Mean	0.271	5	0.198	5
	SD	0.020		0.010	
10	Mean	0.254	11	0.101	51
	SD	0.019		0.114	
32	Mean	0.235	18	-0.010	105
	SD	0.005		0.051	
100	Mean	0.213	25	0.052	75
	SD	0.022		0.124	

[Increase in growth as compared to control]

Conclusions:

Based on frond number the E_rC_{50} for M04 TFMBA was calculated to be > 100 mg/L and the NOEC 1.0 mg/L. Based on dry weight the E_rC_{50} was calculated to be 9.2 mg/L and the NOEC 3.2 mg/L. It was not possible to calculate the 95% confidence limits.

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Report:	**KCA 8.2.7/13; [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 M05 TFA - 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: M05 Sodium trifluoroacetate analyzed purity: 99.7%. The sample of the test material was assigned to 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 plc, 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.

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:
Fot the metabolite M05 TFA conducted as the sodium salt 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.

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Report:	**KCA 8.2.7/14; Hanson, Mark L.; Solomon, Keith R., 2004**
Title:	Haloacetic acids in the aquatic environment - Part I: macrophyte toxicity
Source:	Environmental Pollution 130(3), 371-383
DOI No:	doi:10.1016/j.envpol.2003.12.016
Document No:	M-455787-01-1

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 M05 (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 M05 (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:	TFA: Aldrich Chemicals (Milwaukee,WS, 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 unsolved 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 Hanson & Solomon 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)	492.2 (121.1, 633.3)	1583.6 (897.5, 2269.7)	Logistic	$t=17.876$ $x=1583.553$ $b=0.787$	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, 810.70	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	$t=436.060$ $x=356.991$ $b=0.961$	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 Hanson & Solomon, 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	$t=18.201$ $x=947.874$ $b=0.766$	0.87
Root number	88.5 (7.9, 169.1)	243.2 (97.9, 388.4)	668.0 (404.5, 931.6)	Logistic	$t=7.442$ $x=668.052$ $b=1.087$	0.87
Root length	37.9 (15.8, 59.9)	91.7 (56.8, 126.7)	222.1 (166.1, 278.0)	Logistic	$t=57.407$ $x=222.157$ $b=1.[illegible]$	0.95
Longest root length	52.4 (23.8, 81.0)	129.3 (83.0, 175.5)	318.8 (242.4, 395.2)	Logistic	$t=57.731$ $x=318.790$ $b=1.[illegible]$	0.95
Wet mass	41.8 (8.8, 74.8)	114.4 (55.0, 173.8)	312.9 (205.0, 420.8)	Logistic	$t=277.373$ $x=312.908$ $b=1.092$	0.90
Dry mass	46.3 (0, 95.4)	144.5 (51.6, 237.3)	450.3 (265.5, 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)	37,965.4 (28,170.0, 73053.7)	Logistic	$t=0.096$ $x=37965.400$ $b=0.545$	0.68
Chlorophyll *b*	>10,000	>10,000	>10,000	nc[a]	nc	nc
Carotenoids	>10,000	>10,000	>10,000	nc[a]	nc	nc

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

Table 3 (taken from Hanson & Solomon, 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)	883.0 (654.3, 1113.6)	Hormetic	$t=39.415$ $h=0.011$ $x=883.961$ $b=0.829$	0.94
Colony number	541.1 (407.2, 675.0)	693.2 (516.3, 870.1)	1140.4 (767.5, 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 (161.0, 406.0)	618.3 (421.1, 815.5)	Hormetic	$t=265.02$ $h=0.009$ $x=618.269$ $b=0.662$	0.91
Frond mass	11.2 (0, 44.2)	506.6 (0, 1189.8)	22965.3 (0, 70230.3)	Logistic	$t=3.[illegible]$ $x=22965.257$ $b=0.288$	0.71
Growth rate	445.2 (342.8, 547.6)	790.1 (638.5, 941.8)	2505.2 (1761.1, 3249.3)	Hormetic	$t=41.445$ $h=0.017$ $x=2505.208$ $b=0.361$	0.95
Chlorophyll *a*	>3000	>3000	>3000	nc	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 Hanson & Solomon, 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 (−4)	10 (−7)	10 (+1)	100 (−6)	30 (−7)
Node number	10 (−38)	10 (−3)	10 (−4)	100 (+1)	30 (−5)
Root mumber	5 (−32)[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)[a]	30 (−19)	100 (−3)	300 (−45)[a]
Wet mass	2.5 (−4)	10 (−9)	3 (+7)	100 (−10)[b]	10 (+4)
Dry mass	5 (−[illegible])	10 (−11)	10 (−9)	100 (−7)	10 (+2)
Chlorophyll *a*	10 (−5[illegible])[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 Hanson & Solomon, 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)	30 (+1)
Node number	5 (−6)	10 (−7)	3 (0)	10 (−2)	30 (−5)
Root number	2.5 (−12)	10 (−23)[a]	10 (−3)	10 (−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)	30 (−43)	30 (−11)	300 (−49)[a]
Wet mass	5 (−17)[b]	3 (−1)	30 (−12)	30 (−3)	10 (0)
Dry mass	10 (−45)[a]	3 (+4)	10 (−41)	10 (−16)	10 (−5)
Chlorophyll *a*	10 (−31)[a]	300 (−18)	30 (−15)	30 (−5)	300 (−13)
Chlorophyll *b*	10 (−30)[a]	300 (−4)	300 (−20)	>10000 (0)	3000 (−10)
Carotenoids	10 (−32)[a]	300 (−8)	300 (−20)	>10000 (+1)	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 Hanson & Solomon, 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)	30 (+9)	30 (+8)	300 (+5)	30 (+1)
Colony number	10 (−3)	100 (−21)	100 (−15)	<1000	100 (+2)[c]
Wet mass	3 (−9)	50 (−16)	100 (−6)	100 (+6)	30 (0)[c]
Frond mass	3 (−12)	25 (0)	>800 (+19)[b]	30 (−11)	100 (−11)
Growth rate	10 (−3)	50 (−4)	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

For the metabolite M05 TFA, under the conditions of this study, the overall lowest 14 day EC_{50} of *Myriophyyllum* 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 *Myriophyyllum* 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.

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Report:	**KCA 8.2.7/11; [redacted] D., 2005**
Title:	AE 1083976 Aquatic plant toxicity test, *Lemna gibba*, static, 7 d
Document No:	M-255206-01-1
Guidelines:	OECD Guideline No. 221 (2004)
GLP:	Yes (certified laboratory)

Objective:

The aim of this study was to assess the effect of M07 AE 1083976 (aqueous photolysis metabolite of flurtamone) on the growth of the freshwater plant *Lemna gibba* over a 7-day period in a static test. The method followed that described in the OECD Guideline No. 221 (2004). Calculated endpoints were the E_rC_{50} and the NOEC, both based on frond number as well as on dry weight.

Materials and Methods:

Test item: M07 AE 1083976, chemical name: 3-(2-methylamino-4-oxo-5-phenyl-4,5-dihydrofuran-3-yl) benzoic acid, Batch No.: PJS899, purity: 96.6 % w/w.
Five nominal concentration levels were chosen in a geometrical series with a dilution factor of 3.2: 1 - 3.2 -10 - 32 -100 mg/L. The experiment was carried out as a static exposure test over seven days. Three replicates were investigated for the test concentrations and six for the control. Environmental conditions were 24 hours of light (light intensity: 6500 - 10000 lux) and a temperature of 24 ± 2 °C. Frond numbers were assessed on days 0, 2, 5 and 7. The amounts of plants were determined on start and end of the test and days 2 and 5. After 7 days determination of dry weight was carried out. Colonies from each test vessel were collected, rinsed with deionised water and then dried at 60 °C to a constant weight. The starting biomass dry weight was determined based on a sample of fronds (same number as in the test vessels) taken from the same batch used to inoculate the test vessels. Based on frond number and biomass dry weight inhibition of average specific growth rate and yield biomass (biomass at the end of the test minus starting biomass) were calculated for each replicate. The concentrations of AE 1083976 were analysed via HPLC on days 0 (freshly prepared solution) and 7 (old solution).

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Doubling time of control cultures	< 2.5 days	1.85 days

All validity criteria were met

Reference test:

Strain material of defined sensitivity was used, as shown by reference item testing with zinc chloride. Such reference tests are done periodically. Such work is documented and archived together with strain protocols.

Analytical results:
The recovery rates of AE 1083976 were 97 - 101 % in the fresh solutions (day 0) and 91 - 99 % (day 7) in the old solutions. All effect levels are given based on nominal test item concentrations.

Biological results:
Effects on frond growth
There was no statistically significant difference between any of the treatment groups and the control group (Dunnett's test).

Effects on dry weight:
There was a statistically significant reduction in average growth rate and biomass yield for the treatment group with 100 mg/L compared to the control group ($p < 0.05$, Dunnett's test).

Effects of M07 AE 1083976 *on Lemna gibba* (based on nominal concentrations of the test item [mg/L])

	2 d	5 d	7 d
	Inhibition of average specific growth rate (frond number)		
E_rC_{50} value	> 100	> 100	> 100
95% confidence interval	n. a.	n. a.	n. a.
LOEC	> 100	> 100	> 100
NOEC	100	100	100
	Inhibition of average specific growth rate (dry weight)		
$E_{r\,dw}C_{50}$ value	n. a.	n. a.	> 100
95% confidence interval	n. a.	n. a.	n. a.
LOEC	n. a.	n. a.	100
NOEC	n. a.	n. a.	32

n.a. = not applicable

Conclusions:

In this study M07 AE 1083976 was found to inhibit the growth of the monocotyledon *Lemna gibba* at nominal concentrations of ≥ 100 mg/L (E_rC_{50} for frond number and dry weight). The NOEC based onfrond number was found to be 100 mg/L and 32 mg/L based on dry weight.

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Report: **KCA 8.2.7/12; [redacted] D., 2005**
Title: AE 2093305 Aquatic Plant Toxicity Test, Lemna gibba, Static, 7 d
Document No: M-255526-01-1
Guidelines: OECD 221 (2004)
GLP: Yes (certified laboratory)

Objective:
The aim of this study was to assess the effect of M08 AE 2093305 (a water sediment metabolite of flurtamone) on the growth of the freshwater plant *Lemna gibba* over a 7-day period in a static test. The method followed that described in the OECD Guideline No. 221 (2004). Calculated endpoints were the E_rC_{50} and the NOEC, both based on frond number as well as on dry weight.

Materials and Methods:

The test item used in this study was M08 AE 2093305, a metabolite of flurtamone, chemical name 5-methylamino-4-(alpha,alpha,alpha-trifluoro-m-tolyl)furan-3(2H)-one, Batch No.: LCTI48-2-2, purity: 97.9 % w/w.
Five nominal concentration levels were chosen in a geometrical series with a dilution factor of 3.2: 0.01 - 0.032 - 0.10 - 0.32 - 1 mg/L. The experiment was carried out as a static exposure test over seven days. Three replicates were investigated for the test concentrations and six for the control.
Environmental conditions were 24 hours of light (light intensity 6500 - 10000 lux) and a temperature of 24 ± 2 °C.
The concentrations of M08 AE 2093305 were analysed via HPLC on days 0 (freshly prepared solution) and 7 (old solution). Frond numbers were assessed on days 0, 2, 5 and 7. The amounts of plants were determined on start and end of the test and days 2 and 5. After 7 days determination of dry weight was carried out. Colonies from each test vessel were collected, rinsed with deionised water and then dried at 60 °C to a constant weight. The starting biomass dry weight was determined based on a sample of fronds (same number as in the test vessels) taken from the same batch used to inoculate the test vessels. Based on frond number and biomass dry weight inhibition of average specific growth rate and yield biomass (biomass at the end of the test minus starting biomass) were calculated for each replicate.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Doubling time of control cultures	2.5 days	1.85 days

All validity criteria were met

Reference test:
Strain material of defined sensitivity was used, as shown by reference item testing with zinc chloride. Such reference tests are done periodically. Such work is documented and archived together with strain protocols.
Analytical results:
The recovery rates were 103-107 % in the fresh solutions and 100-106 % in the old solutions. All effect levels are given based on nominal test item concentrations.

Biological results:
Effects on frond growth
There was a statistically significant difference between the treatment groups > 32mg/L and the control groups from day 2 of the experiment on. However, a recovery of the inhibition of the growth rate was observed for the concentration 0.32 mg/L from day 2. Therefore the E_rC_{50} for day 2-5 and 2-7 was additionally calculated as 0.45 (0.351 - 0572, 95% confidence interval.) and 0.566 (0.476 - 0.673, 95% confidence interval), respectively.

Effects on dry weight:
There was a statistically significant reduction in average growth rate and biomass yield for the treatment group > 0.32 mg/L compared to the control group.

Effects of M08 AE 2093305 on *Lemna gibba* (based on nominal concentrations of the test item [mg/L])

	2 d	5 d	7 d
	Inhibition of average specific growth rate (frond number)		
E_rC_{50} value	0.18	0.33	0.38
95% confidence interval	0.150 - 0.214	0.245 - 0.434	0.287 - 0.514
LOEC	0.32	0.32	0.32
NOEC	0.1	0.1	0.1
	Inhibition of average specific growth rate (dry weight)		
$E_{r\,dw}C_{50}$ value	n. a.	n. a.	1.00
95% confidence interval	n. a.	n. a.	0.175 - > 1*
LOEC	n. a.	n. a.	0.32
NOEC	n. a.	n. a.	0.1

*highest concentration level = 1 mg/L
n.a. = not applicable

Conclusions:

In this study M08 AE 2093305 was found to inhibit the growth of the monocotyledon *Lemna gibba* at nominal concentrations of > 0.32 mg/L. The E_rC_{50} value based on frond number is 0.38 mg/L and the E_rC_{50} based on dry weight is 1 mg/L. The NOEC was found to be 0.1 mg/L based on frond number as well as on dry weight.

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Report:	**KCA 8.2.7/08; [redacted], K.; 2013**
Title:	*Lemna gibba* G3 - Growth inhibition test with BCS-BT61400 under static conditions
Document No:	M-470493-01-1
Guidelines:	OECD Guideline No. 221 (2006)
GLP:	Yes (certified laboratory)

Objective:

The aim of the study was to determine the influence of the metabolite M08 AE 2093305 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.

Material and methods:

Test item: M08 AE 2093305 (BCS-BT61400) analysed content: 99.8 % w/w was tested, specified by batch code: AE 2093305-01-01, sample description: TOX09838-01 and origin batch no.: SES12091-7-23.

A total of 3 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 the nominal concentrations of 0.0763,

0.244, 0.781, 2.50 and 8.00 mg p.m./L in comparison to a control and a solvent control. The pH values ranged from 7.5 to 8.9 in the controls and the incubation temperature ranged from 23.8°C to 24.4°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6776 lux (average of nine measurements).

Quantitative amounts of M08 BCS-BT61400 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 doubling time of frond number in the control was 1.8 corresponding to a 16 fold increase. Therefore the study met all validity criteria, requested by the mentioned guidelines.

The analytical findings of M08 BCS-BT61400 found in all freshly prepared test levels on day 0 ranged between 109 and 110 % of nominal. In aged test levels on days 7 analytical findings ranged between 110 and 115 % of nominal. All reported results are based on nominal values of the test item.

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

nominal test concentration [mg p.m./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means, day 7) [mm²]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	192	1647	-	-
solvent	198	1679	-	-
0.0763	185	1484	1.8	0.9
0.244	170	1294	4.7	5.0 ●
0.781	56.7	314	47.6 ●	58.4 ●
2.50	22.3	197	77.9 ●	85.9 ●
8.00	22.7	154	77.6 ●	82.8 ●

● Results which were significantly different (based on Williams Multiple sequential t-test Procedure) from the pooled controls

Observed visual effects on *Lemna gibba*:

nominal test concentration [mg p.m./L]	Observations
Control	no visual effects observed
solvent control	no visual effects observed
0.0763	no visual effects observed
0.244	no visual effects observed
0.781	light green fronds on day 2, white fronds on day 5 and 7
2.50	light green fronds on day 2, white fronds on day 5 and 7
8.00	light green fronds on day 2, white fronds on day 5 and 7

Since the analytical measurements showed results of 80.0 – 120 % of nominal the calculated endpoints are based on nominal concentrations of the test item.

endpoint (0-7 day)	effect on mean growth rate of frond no. [mg p.m./L]	effect on mean growth rate of total frond area of plants [mg p.m./L]
E_rC_{50} (CI 95%)	0.953 (0.447 – 2.17)	0.722 (0.0740 – 5.82)
LOE_rC	0.244	0.244
NOE_rC	0.0763	0.0763

The LOE_rC and NOE_rC determination is based on statistical data analysis.
The highest concentration of 8.00 mg p.m./L was excluded from the statistical evaluation since no increase of the effects was observed.

Conclusions:
The results showed that for M08 AE 2093305 (BCS-BT61400) the most sensitive response variable in this study was total frond area of plants resulting in a 7 day E_rC_{50} of 0.722 mg p.m./L. The NOE_rC was 0.0763 mg p.m./L and was based on statistical data analysis of the total frond area of plants and frond number.

CA 8.2.8 - Further testing on aquatic organisms

Report:	**KCA 8.2.8/02; [redacted], 2013**
Title:	Acute Toxicity of Flurtamone Technical to the African Clawed Frog (*Xenopus laevis*) under Static Conditions
Document No:	M-475146-01-1
Guidelines:	OCSPP Guideline 850.1075, USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1 and OECD Guideline 203
GLP:	Yes (certified laboratory)

Objective:
This study was conducted to determine the acute toxicity of flurtamone technical to *Xenopus laevis* under static conditions.

Material and methods:
Test item: Flurtamone (tech.), CAS no. 96525-23-4, batch no. DP639D, purity 99.5 % w/w

Experimental system:

Test System:	Static
Replicates:	3
Randomization:	Test vessels randomly positioned within a walk-in chamber]
Test Vessel:	4-L glass beaker
Test Vessel Fill Volume:	1.5-L
Test System and Temperature Control:	Temperature controlled walk-in chamber
Photoperiod:	16 hours light, 8 hours dark
Light/Dark Transition Period:	30 minute dawn/dusk transition period
Light Intensity:	600 to 920 lux (mean: 759 lux)
Light Source:	Cool white fluorescents

Temperature (datalogger):	21.4 to 22.1 °C (mean: 21.7°C)

Biological procedure:
Xenopus laevis tadpoles were exposed under static conditions to determine the 48-hour LC_{50}. Test levels were set up to the functional limit of solubility of flurtamone in this this test system. The following nominal (mean measured) concentrations were included in the study: Control (<0.10), Solvent Control (<0.010), 1.25 (1.11), 2.50 (2.19), 5.00 (4.37), 10.0 (8.31), and 20.0 (16.3) mg a.s./L. There were three replicates of 10 tadpoles each in the controls and toxicant levels.

Findings:
The mean measured recovery of solutions analyzed on day 0 and day 2 ranged from 81 to 89% 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.

Test Substance	Flurtamone
Test Object	*Xenopus laevis*
Exposure	48-Hour Static
LC_{50} 48 hours	> 20.0 mg a.s./L
Lowest Concentration With an Effect (LOEC)	10.0 mg a.s./L
Highest Concentration Without Toxic Effect (NOEC)	5.00 mg a.s./L

Observations:

Nominal Concentration (mg a.s./L)	Hour 6		24 Hour		48 Hour	
	Dead	Obs	Dead	Obs	Dead	Obs
Control	0	30 N	0	30 N	0	30 N
Solvent Control	0	30 N	0	30 N	0	30 N
1.25	0	30 N	1	29 N	1	29 N
2.50	1	29 N	1	29 N	1	29 N
5.00	0	30 N	0	30 N	0	30 N
10.0	0	30 N	0	30 N	0	22 N, 8 LE
20.0	0	13 N, 17 Q	0	23 N, 7 LE	1	22 N, 7 LE

Dead = Cumulative number of dead
Obs = Observations (number of individuals observed alive plus observation)
N = Normal
Q = Quiescent
LE = Loss of equilibrium

There were 30 organisms (10 per replicate) present in each test concentration at the start of the test. There were no mortalities or sublethal effects observed in the control and solvent control replicates. Mortalities were observed in the two lowest treatment levels as well as the highest treatment level. As these mortalities do not follow a dose response trend, they are not considered treatment related. Sublethal effects were observed in the two highest treatment levels.

Conclusions:

The final results for the test are based on the nominal concentrations of Flurtamone in the test system. Based on mortalities and sublethal effects:

48 Hour LC_{50} (95% C.I.)	> 20.0 mg a.s./L (not applicable)
48 Hour LOEC	10.0 mg a.s./L
48 Hour NOEC	5.00 mg a.s./L

Due to the nature of the data the slope of the toxicity curve could not be calculated.

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 flurtamone, 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 flurtamone, a further laboratory study on acute oral and contact toxicity to honey bees has been performed with technical flurtamone 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 flurtamone (KCA 8.3.1.2/01) as well as bee brood feeding test in order to investigate potential side effects of flurtamone on immature honey bee life stages (KCA 8.3.1.3/01). The respective study summaries are presented below.

Table 8.3.1- 1: Acute and chronic toxicity to bees

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity (laboratory) in honey bees			
Flurtamone, tech.	LD50 oral 48 h	> 304 µg a.s./bee	[redacted], 1995; M-170680-01-1
Flurtamone, tech.	LD50-contact 48 h	> 100 µg a.s./bee	[redacted], 1989; M-160668-01-1
Flurtamone, tech.	LD50-oral, 48 h LD50-contact, 48 h	> 105.1 µg a.s./bee > 100 µg a.s./bee	[redacted], 2011; M-421682-01-1 KCA 8.3.1.1.1/01
Acute contact toxicity (laboratory) in bumble bees			
Flurtamone, tech.	LD50-contact, 48 h	LD50 > 100 µg a.s./bee	[redacted], 2014; M-478122-01-1 KCA 8.3.1.1.2/03
Chronic toxicity in adult honey bees (laboratory)			
Flurtamone SC 350	10 d chronic adult feeding study	LC50 > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted], 2014; M-477293-01-1 KCA 8.3.1.2/01
Bee brood feeding test			
Flurtamone SC 350	Honey bee brood feeding (Oomen 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 at a concentration typically present in the spray tank (313 ppm)	[redacted], 2013 M-462016-01-1 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 8.3.1.1.1/01; [redacted] S.; 2011**
Title:	Effects of flurtamone tech. (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Document No:	M-421682-01-1
Guidelines:	OECD 213 and 214 (1998)
GLP:	Yes (certified laboratory)

Objective:

The purpose of this study was to determine the acute contact and oral toxicity of flurtamone technical to the honey bee (*Apis mellifera* L.). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Materials and Methods:

Test item: Flurtamone tech.: Origin Batch No. LOT.20500103; Customer Order No.: TOX 09331-00; Specification No.: 102000002946; LIMS No. 1107057; Article No. 05933218; purity: 98.3 % w/w (analytical). As a toxic reference Perfekthion (BAS 152 10 I, a.s. dimethoate, 400 g/L nominal) was used.

A total of 50 worker bees of *Apis mellifera* were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees to a single dose of 105.1 µg a.s. per bee by feeding (oral limit test, value based on the actual intake of the test item).

For the contact test a single 5 µL droplet of flurtamone tech. in an appropriate carrier (acetone) was placed on the dorsal bee thorax. For the controls, one 5 µL droplet of a) tap water containing 0.5 % wetting agent and b) pure acetone was used. The reference item was also applied in a 5 µL droplet (dimethoate made up in acetone).

For the oral test appropriate amounts of flurtamone tech. dilutions in acetone were mixed with syrup (30 % sucrose, 31 % glucose, 39 % fructose) in order to achieve the required test concentrations in a final dilution of 95 % syrup solution (95 % syrup and 5 % acetone (w/w)). For the solvent control and the reference item, a final dilution of 50 % syrup solution (45 % water, 50 % syrup and 5 % acetone (w/w)) was used whereas the water control consisted of a 50 % aqueous syrup solution (50 % water and 50 % syrup (w/w)). The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 1 hour 35 minutes for the test item treatments). After a maximum of 1 hour 35 minutes, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food. The number of dead bees and behavioural abnormities were assessed after 4 hours the first day and then after 24 and 48 hours.

Results:

Validity criteria:

Validity Criteria		Recommended	Obtained
Control Mortality	Contact Test		
	CO_2/water control	< 10%	2.0 %
	CO_2/acetone control		0.0 %
	Oral Test		
	water/sugar control	< 10%	0.0 %
	acetone/sugar control		0.0 %
LD_{50} of Reference Item (24 h)	Contact Test		
		0.10 - 0.30 µg a.s./bee	0.18 µg a.s./bee
	Oral Test		
		0.10 - 0.30 µg a.s./bee	0.13 µg a.s./bee

All validity criteria for the study were met

Biological results:

Contact Test:

At the end of the contact toxicity test (48 hours after application) there was no mortality at 100.0 µg a.s./bee. 2.0 % mortality occurred in the water control group and there was no mortality in the solvent control group.

Oral Test:

In the oral toxicity test, the maximum nominal test level of flurtamone tech. (i.e. 100 µg a.s./bee) corresponded to an actual intake of 105.1 µg a.s./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the solvent control group and in the water control group, respectively.

No test item induced behavioural effects were observed at any time in both toxicity tests.

Effects of flurtamone (tech.) on honeybees (contact, oral)

Test Item	Flurtamone tech.	
Test Object	*Apis mellifera*	
Exposure	contact (solution in acetone)	oral (sugar/acetone solution)
Application rate µg a.s./bee	100.0	105.1
LD_{50} µg a.s./bee	> 100.0	> 105.1
LD_{20} µg a.s./bee	> 100.0	> 105.1
LD_{10} µg a.s./bee	> 100.0	> 105.1
NOED µg a.s./bee*	≥ 100.0	≥ 105.1

* The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Conclusions:

For flurtamone the contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 105.1 µg a.s./bee.

CA 8.3.1.1.2 - Acute contact toxicity

Report:	**KCA 8.3.1.1.2/03; [redacted], E; 2014**
Title:	Flurtamone (tech.): Acute contact toxicity to the bumble bees, *Bombus terrestris* L. under laboratory conditions
Document No:	M-478122-01-1
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)

Objective:

The purpose of this study was to determine the acute contact toxicity of flurtamone technical to the bumble bee (*Bombus terrestris* L.). The contact toxicity test was carried out as a limit test with one dose of flurtamone (tech.), one dose of the reference item, as well as an acetone and water control.

Materials and Methods:

Test item: Flurtamone tech.: Origin Batch No.: LOT 20500103, Customer Order No.: TOX 09331; purity: 98.3 % w/w (analysed). As a toxic reference Perfekthion (BAS 152 11 I, a.s. dimethoate, 400 g/L nominal) was used.

The contact toxicity of flurtamone (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, the bumble bees were exposed to 100 µg flurtamone 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.

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.

Table: LD_{50} values in the contact toxicity test with the test item flurtamone (tech.)

Flurtamone (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 flurtamone (tech.) on bumble bees at the treatment level of 100 µg flurtamone a.s./bumble bee, caused no adverse effects regarding mortality, sub-lethal effects and behaviour.

Conclusion:

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

CA 8.3.1.2 - Chronic toxicity to bees

Report:	**KCA 8.3.1.2/01; [redacted], A.; 2014**
Title:	Flurtamone SC 350 G - Assessment of Chronic Effects to the Honeybee, *Apis mellifera* L., in a 10 Days Continuous Laboratory Feeding Limit Test
Document No:	M-477293-01-1
Guidelines:	No agreed and ring tested guideline available
GLP:	Yes (certified laboratory)

Objective

The objective of this study was to determine the chronic effects of Flurtamone SC 350 G on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory. The NOEC (no observed effect concentration, respectively) was determined at the end of the test period.

Materials and Methods:

Test item: Flurtamone (AE B107587) SC 350 G; TOX-No. 09723-00; Batch-No. 2012-001528; Specification No. 102000027038, content of active substance (a.s.) 31.0 % w/w (343.2 g/L) (analysed).
The chronic effects of the test item Flurtamone SC 350 G on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test 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 Flurtamone SC 350 G by continuous and *ad libitum* feeding. 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. Mortality, sub-lethal effects and behavioural observations were assessed every day throughout the 10 days continuous exposure period. Furthermore, the daily food uptake was determined.

Findings

After 10 days of continuous exposure, mortality at the test item treatment level of 120 mg a.s./kg of Flurtamone SC 350 G was not statistically significantly different when compared to the control group.

The cumulative control mortality was 1.0 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg Flurtamone SC 350 G was 1.0 % (corrected 0.0 %) at the final assessment.

At 120 mg a.s./kg Flurtamone SC 350 G, no 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 Flurtamone SC 350 G at the treatment level of 120 mg a.s./kg was 47.28 µg a.s./bee, the corresponding average daily dose was therefore 4.7 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 when compared to the untreated control group (39.4 mg/bee at 120 mg a.s./kg, compared to 37.4 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).

Mean consumption of application (feeding) 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]	Flurtamone SC 350 G at 120 mg a.s./kg (nominal) [2]
Cumulative mortality after ten days of continuous exposure [%]	1.0	1.0
Corrected cumulative mortality after ten days of continuous exposure [%]		0.0
Overall mean daily consumption of application (feeding) solution [mg/bee] [3]	37.4	39.4
Mean nominal intake accumulated over ten test days [µg a.s./bee/10 d]		47.28
Average daily dose (nominal) throughout ten days of continuous exposure [µg a.s./bee/d]	-	4.7
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

[2] Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing Flurtamone SC 350 G

[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-Holm corrected, one-sided, $p \leq 0.05$)

a.s. = active substance

Analytical Results

The actual concentration of flurtamone in the application (feeding) solutions, determined for each preparation day, was in the range from 87 to 110% of the nominal concentration. No residues of flurtamone above the LOQ (10 mg/kg) were found in any of the control samples. The average actual concentration of flurtamone over a period of 10 consecutive days accounted to 96% of nominal.

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 Flurtamone SC 350 G 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 the application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different when 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.

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} 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.; 2013**
Title:	Study on the Effects of Flurtamone SC 350 G on Honey Bee Brood (*Apis mellifera* L.) - Brood Feeding Test
Document No:	M-462016-01-1
Guidelines:	Oomen P.A., de Ruijter, A. & van der Steen, J., 1992
GLP:	Yes (certified laboratory)

Objective

The objective of this study
A bee brood test was conducted in order to assess the effect of Flurtamone SC 350 G to the honey bee colonies and bee brood brood development.

Material and methods

Test item: Flurtamone SC 350 G: flurtamone (AE B107587): 31.0% w/w (343.2 g/L)(analytical); Batch ID.: 2012-001528; Sample Description: YOX09723-00; Specification No.: 102000027038; Density: 1.107 g/mL (20 °C).
Test species: Honey bees (*Apis mellifera* L.); honey bee colonies were maintained according to normal beekeeping practice, containing two magazines with 11 combs, each. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply of nectar and pollen. The mean strength of the colonies per treatment group, two days before application, was similar and ranged between 15180 and 19485 adult bees. Colonies were free flying, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops or flowering weeds in the surrounding area.
Test design: A bee brood test was conducted, in order to assess the effect of Flurtamone SC 350 G to the honey bee brood. An untreated control and a toxic reference were included in the study. Three bee colonies were used per treatment group. The test item and reference item solutions were mixed with ready-to-use sugar syrup (Apiinvert) and applied to the bee colonies via a feeding trough, which was put directly into the colony on top of the second magazine. Pure sugar syrup (Apiinvert) was used for the controls. Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a period of 22 days following the application for each treatment group and colony. This was assessed one day before the application, by selecting one (or several) brood comb(s) from of each colony and by taking a digital photo of this (these) brood comb(s). After saving the photo-file on a computer, eggs, young- and old larvae were marked at this first Brood area Fixing Day (BFD0). For each subsequent brood assessment (BFDn), again, the same comb(s) was (were) selected from the respective colony and another digital photo was taken, in order to investigate the progress of brood development. Ontogenesis of the bee brood was observed for a period of 22 days after application (*i.e.* 23 days following BFD0). Mortality of adult bees and pupae was also assessed.

Endpoints:

- Mortality of adult bees as well as pupae or larvae: between 3 days before to 21 days after application (= end of the trial);
- Bee brood development (eggs, young- and old larvae): one day before (= BFD0) and 4 (= BFD 5), 8 (= BFD 9), 15 (= BFD 16), 22 (= BFD 23) days after the application.

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

Test Item: 1.01 g test item (Flurtamone SC 350 G) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to an active substance concentration of 0.313 g flurtamone a.s./L.
Reference Item: 3.0 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 0.75 g fenoxycarb a.s./L.

Test conditions: Natural field conditions. Temperature, relative humidity and rain were recorded during the experimental time.
Statistics: Statistical evaluation was done for mortality and the brood termination rates using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student`s t-test (pairwise). Software: ToxRat Professional, Version 3.10.05, © ToxRat Solutions GmbH.

Findings

Effects of Flurtamone SC 350 G on honey bee brood

Test item	Flurtamone SC 350 G		
Test species	Honey bees (*Apis mellifera* L.) (complete colonies)		
Exposure	*via* treated sugar solution		
Treatment	Untreated control	Flurtamone SC 350 G	Reference Item (Insegar, a.i. = fenoxycarb)
Rate per L sugar solution [product][1)]	-	1.01 g/L	3.0 g/L
Rate per L sugar solution [a.s.][1)]	-	0.313 g a.s./L	0.75 g a.s./L
Termination rate of the eggs [%] [2)]	16.0 %	30.4 % (n.s.)	97.4 % (*)
Termination rate of the young larvae [%] [2)]	18.9 %	8.0 % (n.s.)	83.1 % (*)
Termination rate of the old larvae [%] [2)]	7.7 %	4 % (n.s.)	16.2 % (*)
Mean brood termination rate over all stages	12.5 %	13.6 % (n.s.)	65.6 % (*)
Mean mortality of worker bees/colony/day during pre-application phase [3)] during the entire post-application phase [3)]	13.3 22.5	14.2 (n.s.) 18.7 (n.s.)	12.7 (n.s.) 54.7 (*)
Mean mortality of pupae or larvae/colony/day during pre-application phase [4)] during the entire post-application phase [4)]	0.1 3.4	0.1 (n.s.) 2.0 (n.s.)	0.6 (n.s.) 11.9 (*)
Mean number of bees before application	18165	15180	19485

1) test and reference item was mixed in sugar solution
2) mean termination rate of 3 colonies per treatment group
3) mean number of dead honey bees per day and colony found in dead bee traps
4) mean number of dead pupae/larvae per day and colony found in dead bee traps
Statistics: n.s. = not statistically significantly different compared to the control;* = statistically significantly different compared to the control; Student t-test, $\alpha = 0.05$, pairwise comparison, two-sided (before application), one-sided greater (after application)

Conclusions:

Although the mean termination rate of eggs was slightly higher in the test item treatment group (30.4 %) when compared to the values of the control group (16.0 %), there was no statistically significant difference.

No effect on the development of young larvae was observed after consumption of the test item. The mean termination rate of young larvae in the test item treatment group was lower with a mean of 8.0 % compared to 18.9 % in the control group. Accordingly, this was not statistically significant compared to the control group.

There was also no effect on the development of old larvae after consumption of the test item. The mean termination rate of old larvae in the test item treatment group was 2.4 % compared to 2.7 % in the control group. Accordingly, this was not statistically significant compared to the control group. Adult bee mortality in the test item treatment group was lower and thus not statistically significant different when compared to the control group.

No effects of the test item on honey bee pupae and larvae were observed.

The reference item treatment (Insegar, a.i. = fenoxycarb) resulted in a statistically significantly increase of unsuccessful egg-, young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Overall, it can be concluded according to the results of this study that Flurtamone SC 350 G does neither adversely affect honey bee colonies nor bee brood development.

CA 8.3.1.4 - Sub-lethal effects

No further studies have been conducted. 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

A number of laboratory studies were conducted to assess the toxicity of flurtamone (formulated as FLT + DFF SC 350) to a number of representative non-target arthropod taxa (*Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Poecilus cupreus* and *Pardosa spp.,*). The overall assessment indicated a low risk for non-target terrestrial arthropods. New studies, following the ESCORT 2 guidance[3], on *A. rhopalosiphi* and *T. pyri* (Waltersdorfer, 2005a, b) are summarised below.

Table 8.3.2- 1: FLT + DFF SC 350: Effects on non-target terrestrial arthropods

Test species, Reference	Tested Formulation, study type, Duration, exposure	Ecotoxicological Endpoint	
Flurtamone + Diflufenican SC 350			
Aphidius rhopalosiphi M-170701-01-1 Rep.Nr R005248 [redacted], M. P (1995)	SC (250 + 100) Lab. Glass plates, 24h 1L product/ha	Corr. Mortality [%] 13.3	Effect on Reproduction[%] 43.6
Typhlodromus pyri M-170715-01-1 Rep.Nr R005248 [redacted], M. P (1995)	SC (250 + 100) Lab. Glass plates, 14d 1L product/ha	Corr. Mortality [%] 0	Effect on Reproduction [%] 8.2%
Poecilus cupreus M-170719-01-1 Rep.Nr R005252 [redacted], P (1995)	SC (250 + 100) Laboratory, spray deposits on sand, exposure (15d). 1L product/ha	Corr. Mortality [%] 0	Effect on Feeding Rate [%] 26.1
Pardosa sp M-170885-01-1 Rep.Nr: R005402 [redacted], M. D. (1995)	SC (250 + 100) Laboratory, spray deposits on quartz sand, exposure (14d). 1L product/ha	LR50 [L a.s./ha] value Corr. Mortality [%] 0	Effect on Feeding Rate [%] 4
Aphidius rhopalosiphi M-248106-01-1 Rep.Nr CW04/051 [redacted] A (2005a)	SC (250 + 100) Lab. glass plates 48h 0.100 L product/ha 0.125 L product/ha 0.464 L product/ha 1 L product/ha	LD_{50} > 1 L product/ha Corr. Mortality [%] -5.5[B] -5.5 [B] -9.1 [B] -9.1 [B]	Effect on Reprod. [%] not detected -27.2[A] - 0.6 [A] 11.4
Typhlodromus pyri M-248338-01-1 Rep.Nr CW04/054 [redacted], A (2005b)	SC (250 + 100) Lab. Glass plates, 14d 0.100 L product/ha 0.125 L product/ha 0.464 L product/ha 1 L product/ha	LR_{50}> 1 L product/ha Corr. Mortality [%] 0 6.1 0 5.1	Effect on Reproduction [%] -39.0[A] -23 [A] -33.0 [A] -2.4 [A]

[A]: A negative value indicates a higher reproduction rate in the treatment than in the control.
[B]: A negative value indicates a lower mortality in the treatment than in the control

[3] [redacted], M. P. *et al.*, (2001): Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop. SETAC, Pensacola, 46 p.

CA 8.3.2.1 - Effects on Aphidius rhopalosiphi

Report:	**KCA 8.3.2.1 /01; [redacted] A.; 2005a**
Title:	Toxicity to the parasitoid wasp *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae) in the laboratory; Flurtamone + Diflufenican Suspension concentrate 250 + 100g/l
Document No:	M-248106-01-1
Guidelines:	IOBC (Mead-Briggs et al. 2000)
GLP:	Yes (certified laboratory)

Objective:
The objective of this laboratory study was to investigate the lethal and sublethal toxicity of Flurtamone + Diflufenican 250 + 100 (AE F088657 01 SC31 A202) on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a glass surface.

Materials and Methods:
Test item: Flurtamone + Diflufenican 250 + 100 SC; Batch No.: V355010344; LOX No.: 06668-00; Product Code: AE F088657 01 SC31 A202; content of active ingredients: flurtamone: 23.2% w/w + diflufenican: 9.18 % w/w.
The test item was applied at nominal rates of 0.100; 0.215; 0.464 and 1 L product/ha and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 0.0002 L prod./ha, and a water treated control. Mortality of 60 adults was assessed 24 and 48 hours after exposure. From the water control and the dose rates 0.215; 0.464 and 1 L test item, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated cereal plants infested with *Rhopalosiphum padi* for a period of 24 hours. The number of mummies was assessed 11 days later.
The experiment was performed in a controlled environment room. The light / dark cycle was 16 hours light 8 hours dark. Relative humidity was between 60 and 90%, temperature between18 and 22°C. Light intensity ranged from 955 to 1395 Lux during the mortality phase and from 11 500 to 18 00 Lux during the reproduction phase.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality in water control	≤ 13%	8.3%
Mean reproduction per female in water control	≥ 5	10.5
No more than 2 wasps producing zero reproduction in water control	< 2	1

All validity criteria for the study were met

Biological results:
Mortality:
After 48 hours of the test 91.67% of the wasps were found alive in the control group. In the group treated with 1 L and the 0.464 L test item/ha rate 100% recovered alive. In the 0.215 L and the 0.100 L prod./ha rate 96.67% of the wasps survived. In the reference item group, 100% of the wasps were dead after 48 hours of exposure.

Reproduction:
The mean number of mummies per female in the control group was 10.5. This compared to 9.3 mummies/female in the 1 L product/ha rate of the test item, 10.6 mummies/female in the 0.464 L prod./ha rate and 13.4 mummies/female in the 0.215 L prod./ha rate.

Summary of the effects of Flurtamone + Diflufenican 250 + 100 SC on *Aphidius rhopalosiphi*

		Mortality [%]		Reproduction	
Treatment	**L prod. /ha**	**Uncorr.**	**Abbott[1]**	**Rate**	**Red. Rel. to Control [%]**
Control (deionised water)	-	8.3		10.5	
FLT + DFF 250 + 100	0.100	3.3	-5.5	not detected	
FLT + DFF 250 + 100	0.215	3.3	-5.5	13.4	-27.2
FLT + DFF 250 + 100	0.464	0	-9.1	10.6	-0.6
FLT + DFF 250 + 100	1	0	-9.1	9.3	11.4
Dimethoate	0.0002	100	100	not detected	
LD50: > 1 L product /ha					

[1]corrected mortality according to the formula of Abbott (1925).

Conclusions:

In the highest dose rates of 0.464 and 1 L product/ha, 0% of mortality was observed. At the lower rates of 0.215 and 0.100 L prod./ha 3.3% mortality was detected. The reduction in reproductive success relative to the control at the 1 L prod./ha rate was 11.4%. At the lower rates tested no influence on reproduction was observed. The LD_{50} was estimated to be > 1 L product/ha.

CA 8.3.2.2 - Effects on *Typhlodromus pyri*

Report: **KCA 8.3.2.2/01; [redacted], A., 2005b**
Title: Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) in the laboratory Flurtamone & Diflufenican Suspension concentrate 250 + 100 g/L
Document No: M-288338-01-1
Guidelines: IOBC (Blümel et al. 2000)
GLP: Yes (certified laboratory)

Objective:
The objective of this laboratory study was to investigate the lethal and sublethal toxicity of Flurtamone + Diflufenican 250 + 100 SC (AE F088657 01 SC31 A202) to the predatory mite *Typhlodromus pyri* when exposed on a glass surface.

Materials and Methods:
Test item: Flurtamone + Diflufenican 250 + 100 SC (AE F088657 01 SC31 A202); Batch No.: V355010344; content of active ingredients: Flurtamone: 23.2%w/w, diflufenican: 9.18%w/w.
The test item was applied at nominal rates of 100; 215; 464 and 1000 mL product/ha and the effects were compared to a toxic reference (a.s.: dimethoate) applied at 10.1 mL product/ ha, and a water treated control.
The experiment was performed in a controlled environment room at a temperature of 23.0 -25.58°C and a relative humidity of 70 - 80%. The light / dark cycle was 16:8 hours. The light intensity was 944 - 1286 Lux.

Mortality of 100 protonymphs was assessed 1, 3, 7, 10, 12 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed. The reproduction rate of surviving mites was then evaluated over the period of 7-14 days after treatment by counting the total number of offspring (eggs and larvae) produced.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality/Escaped value in the control	≤ 20%	2%
Average mortality for the reference item	> 50%	79%
Average number of eggs/female	> 4	5.86

All validity criteria for the study were met

Deviations: Very short decline in temperature, and rel. humidity

Biological results:

Mortality:

Up to day 7 of the test 98% of the mites were found alive in the control group. This compared with 98% recovered alive in the group treated with 100 mL test item/ha, with 2% of mites dead. In the 215 mL/ha rate 92% survived, 4% of mites were dead and 4% were missing. A total of 98% of the mites in the 464 mL/ha rate recovered alive, 1% were dead and 1% missing. In the highest dose rate of 1000 mL test item/ha 93% of the mites alive, 3% were found dead and 4% were missing. In the reference item group, 52% of the mites were dead and 27% escaped and 21% were alive on day 7 of the study.

Reproduction:

The mean number of offspring produced per female in the control group was 5.86. This compared to 8.14 eggs/female in the 100 mL/ha rate of the test item, 7.20 eggs/female in the 215 mL/ha rate, 7.79 eggs/female in the 464 mL/ha rate and 6.00 eggs/female in the 1000 mL/ha rate.

Summary of the effects of Flurtamone + Diflufenican 250 + 100 on *Typhlodromus pyri*

		Mortality [%]		Reproduction	
Treatment	**mL prod./ ha**	**Uncorr.**	**Abbott[1]**	**Rate**	**Rel. to Control [%]**
Control (deionised water)	0	2	-	5.86	-
Test item	100	2	0	8.14	-39.03
Test item	215	8	6.1	7.20	-22.98
Test item	464	2	0	7.79	-33.00
Test item	1000	7	5.1	6.0	-2.42
Reference item	10.1	79	78.6	not detected	-
LD_{50}: > 1000 mL product/ ha					

[1]corrected mortality according to the formula of Abbott (1925).

Conclusions:

In the highest dose rate of 1000 mL/ha test item there was 5.1% corr. mortality. The reduction in reproductive success relative to the control at this rate was -2.42%. At the lower rates of 464; 215 and 100 mL/ha test item 0; 6.1 and 0 % corr. mortality were found and the reduction of reproduction was -33; -23 and -39 %. The LD_{50} was estimated to be > 1000 mL product/ha.

CA 8.4 - Effects on non-target soil meso- and macrofauna

Effects on Earthworms

Originally included in the Monograph for Annex I was an acute toxicity study with flurtamone with an LC_{50} value of > 1800 mg a.s./kg dws (Roberts 1992). The study was performed with an organic matter content of 10% peat. New studies, conducted after the Annex I inclusion for flurtamone and for the metabolites M04 TFMBA and M05 TFA as presented in Table 8.4-1, in addition new chronic studies to meet the new requirements.

Table 8.4- 1: Effects of flurtamone on soil macro-organisms - earthworms

Test species	Test substance	Test design	Ecotoxicological endpoint	Reference
Eisenia fetida	Flurtamone	acute, 14 d (10% peat in test soil)	LC_{50} > 900 * mg as/kg dws	[redacted], 1992; M-203282-01-1
		chronic, 56 d (5% peat in test soil)	NOEC 47.5* mg as/kg dws	[redacted], 2011 M-419904-01-1 KCA 8.4.1/01
Eisenia fetida	M04 TFMBA	acute, 14 d (10% peat in test soil)	LC_{50} 123.2 mg pm/kg dws	[redacted], 2005 M-252227-01-1
		chronic, 56 d (10% peat in test soil)	NOEC ≥ 100 mg pm/kg dws	[redacted], 2013 M-444573-01-1 KCA 8.4.1/02
Eisenia fetida	M05 TFA	chronic, 56 d (10% peat in test soil)	LC_{50} 1000 mg pm/kg dws NOEC 320 [1)] mg pm/kg dws	[redacted], 2005; M-251328-01-1 KCA 8.4.1/03

* endpoints corrected to account for logPow
dws = dry weight soil, pm = pure metabolite
[1)] NOEC reduced to 320 mg/kg based on effects on the body weight in the concentration 1000 mg/kg.

Effects on other soil non-target macro-organisms

Studies on other soil organisms (*Folsomia candida* and *Hypoaspis aculeifer*) were not required for the Annex I inclusion. All studies listed below are new studies conducted to fulfill current regulatory requirements.

Table 8.4- 2: Effects on other soil non-target macro-organisms

Test species	Test design	Ecotoxicological endpoint	Reference
Flurtamone			
Folsomia candida	chronic 28 d (5% peat in test soil)	NOEC ≥ 500* mg as/kg dws	[redacted], 2012; M-438621-01-1 KCA 8.4.2/01
Hypoaspis aculeifer	chronic 14 d (5% peat in test soil)	NOEC 89* mg as/kg dws	[redacted], 2012; M-439623-01-1 KCA 8.4.2/02
M04 TFMBA			
Folsomia candida	chronic 28 d (5% peat in test soil)	NOEC 52 mg pm/kg dws	[redacted], 2013; M-444231-02-1 KCA 8.4.2/03
Hypoaspis aculeifer	chronic 14 d (5% peat in test soil)	NOEC ≥ 100 mg pm/kg dws	[redacted], 2012; M-443198-01-1

Test species	Test design	Ecotoxicological endpoint	Reference
			KCA 8.4.2/04
M05 TFA Na-salt			
Folsomia candida	chronic 28 d (10% peat in test soil)	NOEC ≥ 100 mg pm/kg dws	[redacted], 2012; M-436127-01-1 KCA 8.4.2/05
Hypoaspis aculeifer	chronic 14 d (5% peat in test soil)	NOEC ≥ 100 mg pm/kg dws	[redacted], 2012 ; M-436326-01-1 KCA 8.4.2/06

* endpoints corrected to account for logPow > 2
dws = dry weight soil, pm = pure metabolite

CA 8.4.1 - Earthworm, sub-lethal effects

Report:	**KCA 8.4.1/01; [redacted] U.; 2011**
Title:	Flurtamone: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Document No:	M-415904-01-1
Guidelines:	OECD 222 (2004) ISO 11268-2 (1998)
GLP:	Yes (certified laboratory)

Objective:
The purpose of this study was to investigate the effects of flurtamone on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida* by dermal and alimentary uptake during an exposure of 56 days in an artificial soil. The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998).

Materials and Methods:
Test item: Flurtamone technical, Customer Order No./Tox-No: 09331-00, Batch Code: AE B107587-01-08, Origin Batch No.:LOT.20500103, Purity: 98.3% w/w.

Adult earthworms (10 to 11 months old) were exposed to concentrations of flurtamone mixed into the soil at nominal concentrations of 100, 178, 316, 562 and 1000 mg test item/kg artificial soil (containing 74.8 % fine quartz-sand, 20 % Kaolin clay, 5.0 % Sphagnum-peat and 0.2 % $CaCO_3$). A second experiment was performed by mixing the test item into the soil at concentrations of 53 and 95 mg test item/kg artificial soil. In both experiments earthworms *Eisenia fetida* (80 worms per control, 40 worms per test item group) were exposed at temperatures within the range of 18 to 22 °C, light within the range of 400 to 800 lux, 16 h light to 8 h dark and were fed weekly with dried cattle manure.
Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality of adults in the control:	≤10 %	0 % (both experiments)
Reproduction per replicate in the control:	≥30	260 to 317 (1st experiment) 198 to 399 (2nd experiment)
Coefficient of variation of reproduction in control:	≤30 %	20.1 % (1st experiment) 18.6 % (2nd experiment)

All validity criteria for the study were met

Reference test:
In the most recent test with the reference item Luxan Carbendazim 500 FC, there were statistically significant effects on reproduction at a concentration of 1.0 mg carbendazim/kg soil and higher, the EC_{50} for reproduction was calculated as 1.21 mg carbendazim/kg soil which is within the guideline value.

Deviations: A minor deviation occurred as the water content at experimental end was > 60% of the maximum water holding capacity in both experiments.

Biological results:
Mortality:
No mortality was observed in any treatment group. Food was consumed in all treatment groups. The results show that the turnover of biomass of those earthworms exposed to the different rates of the test item was comparable to the control. No behavioural abnormalities were observed and all worms did burrow into the soil within 15 min after introduction.
Growth and Reproduction:
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 562 mg test item/kg artificial soil dry weight. At the concentration of 1000 mg test item/kg artificial soil the body weight increase was statistically significantly lower compared to the control (Williams' t-test, $\alpha = 0.05$, two-sided).
The reproduction rates were not significantly different compared to the control up to and including the concentration of 95 mg test item/kg artificial soil dry weight. At the concentration of 100 mg test item/kg artificial soil reproduction was statistically significantly lower compared to the control (Williams' t-test, $\alpha = 0.05$, one-sided smaller).

Effects of flurtamone on adult earthworms (mortality, growth and reproduction)

1st Experiment						
Flurtamone [mg test item/kg soil dry weight]	**Control**	**100**	**178**	**316**	**562**	**1000**
Mortality (day 28) [%]	0	0	0	0	0	0
Weight change (day 28) [%]	59.9	60.2 n.s.	57.6 n.s.	62.1 n.s.	61.8 n.s.	45.3 *
No. of juveniles (day 56)	388	291 *	318 *	303 *	265 *	261 *
Reproduction in [%] of control (day 56)	-	74.8	81.9	78.1	68.1	67.3
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0

2nd Experiment			
Flurtamone [mg test item/kg soil dry weight]	**Control**	**53**	**95**
Mortality (day 28) [%]	0	0	0
Weight change (day 28) [%]	41.2	35.8 n.s.	30.3 n.s.
No. of juveniles (day 56)	323	289 n.s.	307 n.s.
Reproduction in [%] of control (day 56)	-	89.3	95.0
Food consumption [g]	25.0	25.0	25.0

Endpoints [mg/kg soil dry weight]	
NOEC (day 28 mortality)	≥1000
NOEC (day 28 weight)	562
LOEC (day 28 weight)	1000
NOEC (day 56 reproduction)	95
LOEC (day 56 reproduction)	100

n.s. = not significantly different compared to the control
* = significantly different compared to the control
1) Williams t-test, $\alpha = 0.05$, two-sided for weight and one-sided smaller for reproduction

Conclusions:

In this study the no-observed-effect-concentration (NOEC) of flurtamone for mortality of the earthworm *Eisenia fetida* was 1000 mg test item/kg artificial soil, *i.e.* the highest concentration tested. For weight changes the NOEC was 562 mg test item/kg artificial soil and the lowest observed effect concentration (LOEC) was 1000 mg test item/kg artificial soil.
For reproduction the NOEC was 95 mg test item/kg artificial soil and the lowest observed effect concentration (LOEC) was 100 mg test item/kg artificial soil.

Report: **KCA 8.4.1/02; [redacted], S., 2013**
Title: [redacted], S. (2013) TFMBA [BCS-AA52670]: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Document No: M-444573-01-1
Guidelines: ISO 11268 -2 (1998), BBA VI2-2, 1994
GLP: Yes (certified laboratory)

Objective:
The purpose of this study was to determine the sublethal effects of the metabolite M04 TFMBA on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil.
The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998) as a limit test.

Materials and Methods:

Test item: M04-TFMBA (BCS-AA52670), Substance code: AE C518919, Batch code: AE C518919-01-01, Origin Batch No.: GSE 57203-1-1, CAS No.: 454-92-2, LIMS No.: 1229361, Custumer order No.: TOX 09839-00, analysed purity: 99.8 % w/w.

Adult earthworms (*Eisenia fetida,* about 3 months old) were exposed to 100 mg test item/kg soil dry weight (d.w.) mixed into soil, containing 68.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat and 0.5 % $CaCO_3$, at 18.0 – 21.0 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.
Toxic standard: 5 and 10 mg Nutdazim 50 FLOW/kg soil d.w.; control: quartz sand, solvent control: none.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality of adults in the control:	≤10 %	0 %
Reproduction per replicate in the control:	≥30	128, 91, 136, 142, 79, 116, 124 and 109
Coefficient of variation of reproduction in control:	≤30 %	18.6 %

All validity criteria for the study were met.

Reference test:
In a reference test, the number of juveniles was reduced by 47.7 and 100 % by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

Biological results:
Mortality:
Mortality of 0% was observed at the concentrations of 100 mg test item/kg soil dry weight (d.w.). The mortality in the test item treated group was not different compared to the control where 0% of the worms were dead.

Growth and Reproduction:
Body weight changes of the earthworms exposed to M04 TFMBA (BCS-AA52670) were not statistically significantly different compared to the control (change in fresh weight after 4 weeks relative to initial fresh weight), i.e. a mean weight increase of 38.7% was recorded in the control group and 37.6% at 100 mg test item/kg soil d.w.

No statistically significant effect on the number of juveniles compared to the control group was recorded at 100 mg test item/soil d.w.

Effects of M04 TFMBA on adult earthworms (mortality, growth and reproduction)

Test item Test object Exposure	**M04 TFMBA (BCS-AA52670)** ***Eisenia fetida*** **Artificial soil**		
	Mortality	Biomass change	Reproduction
	[mg test item/kg d.w.]		
NOEC	≥ 100	≥ 100	≥ 100
LOEC	> 100	> 100	> 100
LC_{50}/EC_{50}	> 100	> 100	> 100
95 % confidence limit	-	-	-

Observations:

M04 TFMBA (BCS-AA52670) [mg test item/kg d.w.]		
	Control	100
Mortality of adult worms after 4 weeks		
Mortality (%)	0	0
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)		
Mean (mg)	141.0	137.4
Mean (%)	38.7	37.6
Number of juveniles per surviving adult worm after 8 weeks		
Mean	10.6	9.9
Number of juveniles per replicate after 8 weeks		
Mean	115.6	99.1
Reproduction per treatment (%)		
% of control	100	85.7

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Student-t-test, p □ 0.05, one-sided smaller)

Conclusions:

M04 TFMBA (BCS-AA52670) showed no statistically significantly adverse effects on mortality, growth and reproduction of the earthworm *Eisenia fetida* in artificial soil at 100 mg test item/kg soil dry weight.

Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil d.w.

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Report: **KCA 8.4.1 /03; [redacted] U.; 2005**
Title: Effects of AE C502988 00 1B99 0001 on reproduction and growth of earthworm *Eisenia fetida* in artificial soil
Document No: M-251328-01-1
Guidelines: ISO 11268 -2 (1998), BBA VI2-2, 1994
GLP: Yes (certified laboratory)

Objective:

The purpose of this study was to investigate the effects of the metabolite trifluoroacetic acid M05 TFA, coded AE C502988 00 1B99 0001 on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida* by dermal and alimentary uptake during an exposure of 56 days in an artificial soil. The test was performed according to BBA VI2-2, (1994) and the International Standard ISO 11268-2 (1998).

Materials and Methods:

Test item: AE C502988 00 1B99 0001, Chemical Name: trifluoroacetic acid, Batch No.: 18921, Purity: 98.8% w/w

M05 TFA was mixed into artificial soil (containing 69.5 % fine quartz-sand, 20 % Kaolin clay, 10 % Sphagnum-peat and 0.5 % $CaCO_3$) at 10, 32, 100, 320 and 1000 mg test item/kg soil dry weight to which earthworms *Eisenia fetida* (40 worms per treatment group) were exposed under the following conditions: temperature 19-21 °C, light intensity 480 - 790 lux, photoperiod 16 h light : 8 h dark, soil water content 32.3% - 34.6% at start, 32.9% to 35.7% at experimental termination, pH 5.7 to 6.0 at start, pH 5.6 - 5.8 at experimental termination. Endpoints were mortality, body weight change, feeding activity and reproduction.
Toxic standard: Brabant Carbendazim Flowable (500 g/L), active ingredient carbendazim is tested at least once a year in a dose response study; control: untreated

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality of adults in the control:	≤10 %	5 %
Reproduction per replicate in the control:	≥30	246 to 375
Coefficient of variation of reproduction in control	≤30 %	19.8 %

All validity criteria for the study were met

Reference test:

In the most recent test showed statistically significant effects on reproduction at a concentration of 1.1 mg carbendazim/kg soil and higher; the EC_{50} for reproduction was calculated as 1.25 mg carbendazim/kg soil.

Biological results:

Mortality:

Slight mortalities of 2.5% and 5% were observed at the concentrations of 32 and 100 mg test item/kg soil. The mortality in the test item treated group was not significantly different compared to the control where 5% of the worms were dead (Fisher exact test, a = 0.05) and was not considered to be treatment related since at the two highest concentrations no mortality was observed.

Growth and Reproduction:

Body weight changes of the earthworms exposed to M05 TFA were not statistically significantly different compared to the control up to and including the concentration of 320 mg test item/kg soil (Dunnett test, a = 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).
Statistically significant effects on reproduction could not be observed up to and including the concentration of 1000 mg test item/kg soil (Dunnett test, a = 0.05, one-sided smaller) and no behavioural abnormalities were observed in any of the treatment groups. The feeding activity was comparable to the control in all test item treated groups

Effects of M05 TFA on adult earthworms (mortality, growth and reproduction)

Test item: Test species: Exposure: Test duration:	M05 TFA *Eisenia fetida* test item mixed into soil 56 days					
Test item [mg test item/kg soil dry weight]	Control	10	32	100	320	1000
Mortality (day 28) [%]	5.0 ±5.8	0.0 ±0.0	2.5 n.s.[2] ±5.0	5.0 n.s.[2] ±5.8	0.0 ±0.0	0.0 ±0.0
Weight change (day 28) [%]	42.1 ±4.5	36.3 n.s.[3] ±4.5	39.0 n.s.[3] ±4.5	34.2 n.s.[3] ±4.5	35.9 n.s.[3] ±4.5	28.4 *[3] ±4.5
No. of juveniles (day 56)	291 ±4.5	307 n.s.[3] ±4.5	377 *[3] ±4.5	304 n.s.[3] ±4.5	322 n.s.[3] ±4.5	309 n.s.[3] ±4.5
Reproduction in [%] of control (day 56)	-	105.7	129.7	104.6	110.7	106.2
Food consumption [g]	25.0 ±0.0	25.0 ±0.0	25.0 ±0.0	25.0 ±0.0	25.0 ±0.0	25.0 ±0.0

[1] mean ± standard deviation of 4 replicates; the results represent rounded values calculated on the exact raw data
- = not relevant
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[2] = Fisher-exact test, $\alpha = 0.05$
3 = Dunnett test, a = 0.05, (two sided for weight changes, one-sided smaller for reproduction)

Conclusions:

M05 TFA did not show effects on mortality, reproduction and feeding activity of the earthworm *Eisenia fetida* if applied up to 1000 mg test item/kg soil dry weight. The earthworm weight changes were statistically significantly lower at the concentration of 1000 mg test item/kg soil. However, since the body weight at this concentration still showed a considerable increase and reproduction is considered to be the biologically more relevant parameter, the No Observed Effect Concentration (NOEC) is proposed to be 1000 mg test item/kg soil dry weight, *i.e.* the highest concentration tested.

CA 8.4.2 - Effects on non-target soil meso- and macrofauna (other than earthworms)

Report:	**KCA 8.4.2/01; [redacted], S.; 2012**
Title:	Flurtamone a.s.: Effects on the reproduction of the collembolans *Folsomia candida*
Document No:	M-438621-01-1
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP	Yes (certified laboratory)

Objective

The purpose of this study was to determine potential effects of different concentrations of the test item flurtamone a.s. on the reproductive output of the collembolan *Folsomia candida* during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted. The NOEC and LOEC were determined. The test was performed in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).

Material & Methods

Test item: flurtamone a.s. [AE B107587, shortname: FLT a.s.], Batch code: AE B107587-01-08, Origin Batch No.: LOT.20500103, Specification No.: 102000002916, LIMS No.: 1107057, Customer order No.: TOX-No.: 09331-00, analysed purity: 98.3 % w/w.

Ten *Collembola* (9-12 days old) were exposed to 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight (d.w.) containing 74.7% quartz sand, 20% Kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$ (4 replicates per treatment). Temperature was between 18.0 – 21.5°C and a light/dark cycle of 16 h/8 h (750 lx) was adjusted. The collembolans were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.
As toxic standard was used boric acid (100% analysed) in concentrations of 44, 67, 100, 150 and 225 mg/kg soil d.w. The control substrate was left untreated, i.e. was prepared with deionised water only (8 replicates).

Results

Validity criteria

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	6.3%
Mean number of juveniles per test vessel	≥ 100	average of 941/vessel
Coefficient of variation for the mean	< 30%	8.9%
Precision of counting method	Average Error < 10%	Average Error 3.6%

Reference item

In a separate study the EC_{50} (reproduction) of the reference item boric acid was calculated to be 104 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

Biological results

Effects on mortality:

There were no statistical significant differences between the treated groups and the control (Fisher`s Exact Binominal Test with Bonferroni Correction; $\alpha = 0.05$, one-sided greater).

Effects on reproduction:
No statistically significant differences compared to the control were calculated for reproduction (Williams t-test; $\alpha = 0.05$, one-sided smaller).

Effects of flurtamone a.s. on *Folsomia candida* (concentrations of the test item [mg/kg soil d.w.])

Test item Test object Exposure	**Flurtamone a.s.** *Folsomia candida* Artificial soil		
mg test item/kg soil d.w.	Mean mortality of parental collembolans after 4 weeks (%)	Mean number of juveniles after 4 weeks	Reduction of reproduction compared to control (%)
Control	6.3	941	-
100	7.5	948	-1
178	0.0	963	-2
316	7.5	937	0
562	5.0	1018	-8
1000	2.5	976	-4
	Adult mortality	Reproduction	
	mg test item/kg soil d.w.		
NOEC	≥ 1000	≥ 1000	
LOEC	> 1000	> 1000	

Conclusion
The test item flurtamone a.s. showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil up to and including 1000 mg test item/kg soil d.w. Therefore, the NOEC was determined to be ≥ 1000 mg test item/kg soil d.w., and the LOEC was determined to be > 1000 mg test item/kg soil d.w.

Report: **KCA 8.4.2/02, [redacted], L.; 2012**
Title: Flurtamone a.s.: Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Document No: M-439623-01-1
Guidelines: OECD Guidelines for testing of chemicals No. 226 (adopted 3 October 2008)
GLP Yes (certified laboratory)

Objective
The purpose of this study was to determine potential effects of the test item on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro-arthropods during a test period of 14 days. The test was performed according to the OECD guideline 226 (2008).

Material & Methods

Test item: Flurtamone a.s. [AE B107587, short name: FLT a.s.], Batch code: AE B107587-01-08, Origin Batch No.: LOT.20500103, Specification No.: 102000002946, LIMS No.: 1107057, Customer order No.: TOX-No.: 09331-00, analysed purity: 98.3 % w/w.

Ten adult soil mites (females) were exposed to 100, 178, 316, 562 and 1000 mg test item/kg dry weight (d.w.) of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$, at 19.5 - 21.4°C. The photoperiod was light : dark = 16 h : 8 h (630 lx). Furthermore, the mites were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK) (4 replicates). Mortality and reproduction were determined after 14 days of exposure.
A toxic standard (Dimethoate EC 400) was used in five concentrations (4.00, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg soil dws). The control substrate was prepared with quartz sand only.

Results

Validity *Criteria*

Validity Criteria	Recommended	Obtained
Mean mortality of adult females	≤ 20%	7.5%
Mean number of juvenile per replicate	≥ 50%	315.0
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	14.7

Reference test

In a separate study the EC_{50} (reproduction) of the reference item Dimethoate EC 400 was calculated to be 6.87 mg a.s./ kg dws. The results of the reference test demonstrate the sensitivity of the test system.

Biological results:

Effects on mortality

The mortality showed no statistically significant difference to the control (Fisher`s Exact Binomial Test, $\alpha = 0.05$, one side greater).

Effects on reproduction

The reproduction was at 316, 562 and 1000 mg test item/kg soil d.w. statistically significant different compared to the control (William's t-test $\alpha = 0.05$ one sided smaller).

Effects of flurtamone a.s on *Hypoaspis aculeifer* (concentrations of the test item [mg test item/ kg soil d.w.])

Test item Test object Exposure	**Flurtamone a.s** *Hypoaspis aculeifer* Artificial soil		
	Mean mortality of soil mites after 14 days (%)	Mean number of juveniles after 14 days	Reproduction (% to control)
Control	7.5	315.0	-
100	10.0	288.0	91.4
178	10.0	269.8	85.6
316	5.0	232.3*	73.7
562	5.0	226.0*	71.7
1000	12.5	219.3*	69.6
	Adult mortality	Reproduction	

	mg test item/kg soil d.w.	
NOEC	≥ 1000	178
LOEC	> 1000	316
LC_{50}/EC_{50}	> 1000	> 1000

* statistically significant different compared to the control (Williams' t-test for reproduction; α = 0.05, one-sided smaller)

Conclusion

The test item flurtamone a.s. showed no statistically significantly adverse effects on adult mortality of the predatory mite *Hypoaspis aculeifer* in artificial soil at all tested concentration. Furthermore, the test item showed no statistically significantly adverse effects on reproduction of *Hypoaspis aculeifer* in artificial soil up to and including a test concentration of 178 mg test item/kg soil d.w. However, at concentrations of 316, 562 and 1000 mg test item/kg soil d.w. statistically significant effects on reproduction could be observed.

Therefore, the overall No-Observed-Effect-Concentration (NOEC) for mortality and reproduction was determined to be ≥ 1000 mg test item and 178 mg test item/kg soil d.w., respectively. The Lowest-Observed-Effect-Concentration (LOEC) for mortality and reproduction was determined to be > 1000 mg test item and 316 mg test item/kg soil d.w., respectively.

Report: **KCA 8.4.2/03 [redacted], S.; 2013**
Title: Flurtamone (TFMBA (BCS-AA52670): Effects on reproduction of the collembolan *Folsomia candida*
Document No: M-444231-02-1
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP Yes (certified laboratory)

Objective

Aim of this study was to assess the effect of M04 TFMBA on the reproductive output of the collembolan *Folsomia candida* during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted. The NOEC and LOEC were determined. The test was performed in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).

Material & Methods

Test item: M04 TFMBA (BCS-AA52670), , Substance code: AE C518919, Batch code: AE C518919-01-01, Origin Batch No.: GSE 57203-1-1, CAS No.: 454-92-2, LIMS No.: 1229361, Customer order No.: TOX 09839-00, analysed purity: 99.8 % w/w.

A limit test (1st test run) and a dose response test (2nd test run) were conducted:
1st test run: 10 *Collembola* (9-12 days old) were exposed to 100 mg test item/kg soil dry weight (d.w.) of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$ (8 replicates per treatment). Temperature was between 18.7 – 21.5°C and a light/dark cycle of 16 h/8 h

(680 lx) was adjusted. The collembolans were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

2nd test run: 10 *Collembola* (9-12 days old) were exposed to 10, 17, 30, 52 and 90 mg test item/kg soil d.w. of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$ (4 replicates per treatment). Temperature was between 18.0 – 20.7°C and a light/dark cycle of 16 h/8 h (570 lx) was adjusted. The collembolans were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

As toxic standard was used boric acid (100% analysed) in concentrations of 44, 67, 100, 150 and 225 mg/kg soil d.w. The control substrate was left untreated, i.e. was prepared with deionised water only (8 replicates)

Results

Validity Criteria

Validity Criteria	Recommended	Obtained 1st test	Obtained 2nd test
Mean adult mortality	≤ 20%	2.5%	0%
Mean number of juveniles per test vessel	≥ 100	average of 972/vessel	average of 786/vessel
Coefficient of variation for the mean	≤ 30%	14.7%	13.3%

Reference item

In the most recent study the EC_{50} was determined to be 104 mg/kg soil dry weight. The LC_{50} was determined to be 199 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg/kg soil dry weight, respectively.

Biological results

1st test run

Effects on mortality

No statistically significant differences compared to the control were calculated (Fisher's Exact Binomial Test, $\alpha = 0.05$, one-sided greater).

Effects on reproduction

The reproduction of *Folsomia candida* indicated a statistical significant difference compared to the control (Student-t-test for reproduction, $\alpha = 0.05$, one-sided smaller).

2nd test run

Effects on mortality

There were no statistical significant differences between the treated groups and the control (Fisher`s Exact Binominal Test with Bonferroni Correction; $\alpha = 0.05$, one-sided greater).

Effects on reproduction

Statistical significant differences compared to the control were only estimated in the concentration 90 mg test item/soil d.w. (Williams t-test; $\alpha = 0.05$, one-sided smaller). The other concentration showed no statistical significant differences compared to the control.

Effects of on M04 TFMBA on *Folsomia candida* (concentrations of the test item [mg/kg soil d.w.])

Test item Test object Exposure	**TFMBA** *Folsomia candida* Artificial soil		
1st test run			
mg test item/kg soil d.w.	Mean mortality of parental collembolans after 4 weeks (%)	Mean number of juveniles after 4 weeks	Reproduction compared to control (%)
Control	2.5	972	-
100	3.8	194*	20
2nd test run			
mg test item/kg soil d.w.	Mean mortality of parental collembolans after 4 weeks (%)	Mean number of juveniles after 4 weeks	Reproduction compared to control (%)
Control	0.0	786	
10	0.0	788	100
17	2.5	766	97
30	0.0	757	96
52	2.5	730	93
90	5.0	293*)	37
	Adult mortality	Reproduction	
	mg test item/kg soil d.w.		
NOEC	100	52	
LOEC	100	90	
95% confidence limit	-	(76 – 87)	
EC_{10}	-	55	
EC_{20}	-	63	
LC_{50}/EC_{50}	>100	81	

* statistically significantly different from control (Student t-test; one-sided smaller, $\alpha = 0.05$)
*) statistically significantly different from control (Williams t-test for reproduction; $\alpha = 0.05$, one-sided smaller)

Conclusion

The test item M04 TFMBA (BCS-AA52670) showed no statistically significantly adverse effects on adult mortality of the collembolan *Folsomia candida* in artificial soil at 100 mg test item/kg d.w. The test item caused a significant reduction of reproduction of the collembolan *Folsomia candida* in artificial soil at 100 and 90 mg test item/kg soil d.w. Therefore, the overall NOEC was determined to be 52 mg test item/kg soil d.w., and the LOEC was determined to be 90 mg test item/kg soil d.w. The LC_{50} was estimated to be higher than 100 mg test item/kg soil d.w. The EC_{50} for number of juveniles was calculated to be 81 mg test item/kg soil d.w. with 95 % confidence limits ranging from 76 to 87 mg test item/kg soil d.w.

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Report:	**KCA 8.4.2/04 [redacted], L.; 2012**
Title:	Flurtamone-TFMBA (BCS-AA52670): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Document No:	M-443198-01-1
Guidelines:	OECD 226 (2008)
GLP	Yes (certified laboratory)

Objective

The purpose of this study was to determine potential effects of M04 TFMBA on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* as a representative of soil micro-arthropods during a test period of 14 days. The test was performed as limit test according to the OECD guideline 226 (2008).

Material & Methods

Test item: M04 TFMBA, Substance code: AE C518919, Batch code: AE C518919-01-01, Origin Batch No.: GSE 57203-1-1, CAS No.: 454-92-2, LIMS No.: 1229361, Customer order No.: TOX 09839-00, analysed purity: 99.8 % w/w.

Ten adult soil mites (females) were exposed to 100 mg test item/kg dry weight (d.w.) of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$, at 19.1 - 20.2°C and a photoperiod: light : dark = 16 h : 8 h (473 lx) and were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure. Eight replicates were performed.
As toxic standard was used dimethoate EC 400 in five concentrations (4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg soil d.w). The control contained quartz sand only.

Results

Validity *Criteria*

Validity Criteria	Recommended	Obtained
Mean mortality of adult females	≤ 20%	7.5
Mean number of juveniles per replicate	≥ 50%	243.3
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	9.7

Reference test

In a separate study the EC_{50} (reproduction) of the reference item dimethoate EC 400 was calculated to be 6.87 mg a.s./ kg soil d.w.
The results of the reference test demonstrate the sensitivity of the test system.

Biological results:
Effects on mortality
For the mortality were observed no statistically significant difference between the treatment and the control (Chi^2 2x2 test. $\alpha = 0.05$).
Effects on reproduction
No statistically significant differences were calculated between the treatment and the control (Student t-test $\alpha = 0.05$).

Effects of M04 TFMBA on *Hypoaspis aculeifer* (concentrations of the test item [mg test item/ kg soil d.w.])

Test item Test object Exposure	**M04 TFMBA** *Hypoaspis aculeifer* Artificial soil		
	Mean mortality of soil mites after 14 days (%)	Mean number of juveniles after 14 days	Reproduction (% to control)
Control	7.5	243.3	-
100	2.5	227.4	93.5
	Adult mortality	Reproduction	
	mg test item/kg soil d.w.		
NOEC	≥ 100	≥ 100	
LOEC	> 100	> 100	

Conclusion

The test item M04 TFMBA (BCS-AA52670) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil d.w.

Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil d.w.

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Report: **KCA 8.4.2/05, [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-436137-01-1

Guidelines: OECD 232 (2009)

GLP: Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of M05 trifluoroacetic acid Na-salt (BCS-AZ56567) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted. The NOEC and LOEC were determined. The test was performed in accordance with the OECD Guideline 232 (2009).

Materials and Methods:

Test item: M05 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.

In a limit test, 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 (containing 74.8% quartz sand, 20% Kaolin clay, 5% Sphagnum peat and 0.2% $CaCO_3$) 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.
As toxic reference was used the most recent non-GLP-test with the reference item Boric acid at test concentrations 44, 67, 100, 150 and 225 mg Boric acid/kg artificial soil dry weight.

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 with thereference 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% 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, $\alpha = 0.05$) 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 M05 TFA

Test item Test object Exposure	M05 Trifluoroacetic acid Na-salt (BCS-AZ56567) *Folsomia candida* 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, $\alpha = 0.05$)

Conclusions:

For M05 TFA:

$NOEC_{reproduction}$: ≥ 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 8.4.2/06, [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 (Oct 03, 2008)
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: M05 TFA Trifluoroacetic acid Na-salt (BCS-AZ56567); (Batch code: AE 1046319-01-01; Origin Batch No: SES 11755-1-1; Material: AE 1046319; Certificate No.: MZ00513; Customer order No.: TOX 09476-01; purity: 95.1 %w/w).

In a limit test, 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. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). 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.

Reference test:
The most recent non-GLP-test with the reference item dimethoate showed that the test organisms are sufficiently sensitive according to the guideline.

Biological results:

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, α = 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.

Effect of M05 trifluoroacetic acid Na-salt on soil mite species *Hypoaspis aculeifer* in a 14-day reproduction study

Test item	M05 Trifluoroacetic acid Na-salt (BCS-AZ56567)		
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	0.0	372.1 ± 19.1	107.4
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, α = 0.05) was found.

Conclusions:

For M05 TFA:

NOEC: ≥ 100 mg test item/kg dry weight artificial soil.

LOEC: > 100 mg test item/kg dry weight artificial soil.

CA 8.4.2.1 - Species level testing

Refer to Point 8.4.2.

CA 8.5 - Effects on soil nitrogen transformation

A study on the assessment of soil microflora respiration and nitrogen-turnover of flurtamone was reported in the Monograph and list of end points ([redacted], 1991, M-201663-01-1). Significant stimulation of biomass carbon (or soil respiration) was found in both soils on day 28 but differences were within 15 % of the respective control treatment values at 0.375 kg a.s./ha and 1.875 kg a.s./ha. Since this old study has some deficiencies, new N-cycle studies were conducted with flurtamone and additionally with M04 TFMBA and M05 TFA.

Table 8.5- 1: Effects on soil nitrogen transformation

Test	Test item	Test design	Ecotoxicological endpoint		Reference
N-cycle	Flurtamone	28 d	no influence	≥0.625 kg as/ha ≥0.83 mg as/kg dws	[redacted], 2012; M-441247-01-1 KCA 8.5/01
N-Cycle	M04 TFMBA	28 d	no influence	≥0.357 kg pm/ha ≥0.48 mg pm/kg dws	[redacted], 2013; M-444428-01-1 KCA 8.5/02
N-Cycle	M05 TFA	28 d	no influence	≥1.20 kg pm/ha ≥1.60 mg pm/kg dws	[redacted], 2013; M-444423-01-1 KCA 8.5/03

Study summaries (N-cycle)

Report: **KCA 8.5/01; [redacted], L.; 2012**
Title: Flurtamone a.s.: Effects on the activity of soil microflora (Nitrogen transformation test)
Document No: M-441247-01-1 (EBFTN003)
Guidelines: OECD 216 (2000)
GLP: Yes (certified laboratory)

Objective:
The purpose of this study was to determine the effects of flurtamone 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: Flurtamone a.s. (Flurtamone (AE B107587)), Specification No.: 102000002946, Batch code: AE B107587-01-08, Customer order No.: TOX-No.: 09331-00, LIMS No.: 1107057, Origin Batch No.: LOT.20500103), analysed purity: 98.2 % w/w.
A loamy sand soil (DIN 4220) was exposed for 42 days to 0.17 and 0.83 mg test item/kg soil dry weight. Application rates were equivalent to 0.125 and 0.625 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 at different sampling intervals (0, 7, 14, 28 and 42 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 dinoterb was used 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%	1.8%

All validity criteria were met.

Reference test:
In the most recent test, , 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:
The test item flurtamone a.s. caused temporary inhibitions of thenitrate transformation rate at the tested concentrations of 0.17 mg/kg and 0.83 mg/kg soil dry weight in the time intervals 7-14 days and 14-28 days after application, respectively. However, no adverse effects could be observed in the time intervals 14-28 days and 28-42 days after application, at the tested concentrations of 0.17 mg/kg and 0.83 mg/kg soil respectively.
Differences from the control of +23.1% (test concentration 0.17 mg test item/kg dry soil) and -6.6% (test concentration 0.83 mg/kg dry soil) were measured at the end of the 28-day (time interval 14-28 days after application) and 42-day incubation period (time interval 28-42 days after application), respectively.

Effects of flurtamone a.s. on nitrogen transformation in soil (based concentrations of the test item [mg test item/kg soil dry weight])

Time Interval (days)	Control			0.17 mg test item/kg soil dry weight equivalent to 1 kg flurtamone/ha				0.83 mg/kg dry weight soil equivalent to 5 kg flurtamone/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	1.29	±	0.02	1.40	±	0.08	**+8.1** n.s.	2.32	±	0.12	**+2.2** n.w.
7-14	0.94	±	0.03	0.67	±	0.05	**-29.3** *s.	0.75	±	0.08	**-20.7** *s.
14-28	0.59	±	0.02	0.72	±	0.02	**+23.1** n.s.	0.76	±	0.04	**-30.0** *s.
28-42	0.62	±	0.05	-[2)]	±	-[2)]	-[2)]	0.58	±	0.14	**-6.6** n.s.

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
2) Since in this application rate the deviation from the control was below ±25% on day 28, no further evaluations were performed
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous 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:
Flurtamone a.s. caused no adverse effects on the soil nitrogen transformations (measured as NO_3-N production) at the end of the 42-day incubation period.

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Report:	**KCA 8.5/02; [redacted], L.; 2013b**
Title:	Flurtamone-TFMBA (BCS-AA52670): Effects on the activity of soil microflora (Nitrogen transformation test)
Document No:	M-444428-01-1 (EBFTX037)
Guidelines:	OECD 216 (2000)
GLP:	Yes (certified laboratory)

Objective:
The aim of this study was to investigate the effects of M04 TFMBA 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: M04 TFMBA, Substance code: AE C518919, BCS-code BCS-AA52670, Batch code: AE C518919-01-01, Origin Batch No.: GSE 57203-1-1, CAS No.: 454-92-2, LIMS No.: 1229761, Customer order No.: TOX 09839-00, analysed purity: 99.8 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.09 and 0.48 mg TFMBA/kg soil dry weight. Application rates were equivalent to 0.071 and 0.357 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 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 (0.80, 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%	5.3

All validity criteria were met.

Reference test:
In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +40.4%, +68.1% and +83.5% at 0.80 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 M04 TFMBA on nitrogen transformation in soil could be observed in both test concentrations (0.09 mg/kg dry soil and 0.48 mg/kg dry soil) after 28 days. Differences from the control of -12.3% (test concentration 0.09 mg/kg dry soil) and +10.4% (test concentration 0.48 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Effects of M04 TFMBA on nitrogen transformation in soil (based concentrations of the test item [mg test item/kg soil dry weight])

Time Interval (days)	Control			0.09 mg test item/kg soil dry weight equivalent to 0.071 kg test item/ha				0.48 mg test item/kg soil dry weight equivalent to 0.357 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.55	±	0.36	3.74	±	0.29	**+5.2** n.s.	4.08	±	0.35	**+14.9** n.s.
7-14	1.27	±	0.36	1.49	±	0.39	**+17.3** n.s.	1.24	±	0.19	**-2.3** n.s.
14-28	0.76	±	0.19	0.66	±	0.11	**-12.3** n.s.	0.84	±	0.15	**+10.4** n.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, $p \leq 0.05$)

Conclusion:
M04 TFMBA caused no adverse effects on the soil nitrogen transformations (measured as NO_3-N production) at the end of the 28-day incubation period.

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Report: **KCA 8.5/03; [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 (SBFOP033)
Guidelines: OECD 216 (2000)
GLP: Yes (certified laboratory)

Objective:
The purpose of this study was to determine the effects of M05 TFA 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: M05 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 TFA Na salt/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 114ucerne meal (concentration in soil 0.5%). NH_4-N, NO_3- and NO_2-N were determined using the Autoanalyzer 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, the toxic standard dinoterb caused an effect of +40.4%, +68.1% and +83.5% (required ≥ 25%) on the nitrogen transformations 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 transformations 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 M05 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:
M05Trifluoroacetic acid Na-salt caused no adverse effects on the soil nitrogen transformation at the end of the 28-day incubation period.

CA 8.6 – Effects on terrestrial non-target higher plants

Studies on non-target plants were not submitted for Annex I listing. New studies with the formulation FLT + DFF SC 350 were conducted according to OECD 208 (2000 draft) by Pallett & Gosch (2005a, b) and these have been submitted at the national level.

Table 8.6- 1: Effects on non-target plant tests performed with FLT + DFF SC 350

Test organism	Study type	Test duration	lowest EC_{50} (mL prod/ha)	most sensitive species	References
FLT + DFF SC 350					
terrestrial non-target plants; 10 species	vegetative vigour; Tier 2 dose response	21 days	192.6 (shoot dry weight)	sugar beet	[redacted] 2005a; M-251319-01-1 KCA 8.6.2/01
terrestrial non-target plants; 10 species	seedling emergence; Tier 2 dose response	14 days after 65% emergence in the controls	25.2 (survival) 36.3 (shoot dry weight)	sugar beet	[redacted], 2005b; M-251318-01-1 KCA 8.6.2/02

Test organism	Study type	Lowest endpoint		most sensitive species	References
Flurtamone					
terrestrial non-target plants; 9 species	Bioassay in soil	NOED (ED_{10})	15.3 µg as/kg soil	oilseed radish	[redacted], 1995 M-247804-01-2 KCA 8.6.2/05

CA 8.6.1 - Summary of screening data

Not relevant.

CA 8.6.2 - Testing on non-target plants

Report: **KCA 8.6.2/01; [redacted].; 2005**
Title: Diflufenican and flurtamone (AE F088657 01 SC31 A202)
Effects on ten species of non-target terrestrial plants: vegetative vigour test (Tier 2)
Document No: M-251319-01-1
Guidelines: OECD 208 B (July 2000, draft)
GLP: Yes (certified laboratory)

Objective:

The purpose of this study was to evaluate the effect of Flurtamone + Diflufenican 250 + 100 SC (AE F088657 01 SC31 A202) on the vegetative vigour of ten plant species representing a broad range of both dicotyledonous and monocotyledonous plant families. Statistical analysis of data was performed to obtain NOEC and EC_{50} values for survival and biomass (shoot dry weight).

Materials and Methods:

Test item: Flurtamone + Diflufenican 250 + 100 SC (AE F088657 01 SC31 A202); Batch No. V355010344; content of active ingredients: flurtamone 23.3% w/w, diflufenican 9.18% w/w.

Plants from ten species; corn (*Zea mays*), cucumber (*Cucumis sativus*), oats (*Avena sativa*), oilseed rape (*Brassica napus*), onion (*Allium cepa*), radish (*Raphanus sativus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus*) and tomato (*Lycopersicon esculentum*) were sprayed with the test item at the 2-4 leaf stage.

Solutions of the product and serial dilutions were sprayed with doses of the product ranging from 1000 mL product/ha down to 31.3 mL product/ha using a laboratory track sprayer. There were five or six dose rates that differed with each species. For corn, oats, oilseed rape, onion and tomato these were 1000, 500, 250, 125 and 62.5 mL/product/ha. For cucumber, radish, soybean, sugar beet and sunflower an additional rate of 31.3 mL product/ha was sprayed. Plants were grown and maintained under glasshouse conditions with a temperature control set at 23 ± 5°C during day and 18 ± 5°C at night. The photoperiod was 16 h of light and 8 h dark.

Assessments were made 7, 14 and 21 days after application against the untreated controls. Plants were checked for phytotoxic symptoms (e.g. wilting, chlorosis, bleaching, necrosis) and the dry weight was was determined at the final assessment.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Survival of untreated controls	> 90%	100%
Visible phytotoxic symptoms in the control plants	0	0

All validity criteria for the study were met

Biological results:

Phytotoxicity

All species showed the relevant phytotoxic symptoms visible as bleaching, necrosis and stunting.

Survival:

Phytotoxicity due to Flurtamone + Diflufenican 250 + 100 SC resulted in a suppression of growth as measured by growth stage in oats, oilseed rape, sugar beet and tomato. In the other species the visible phytotoxicity did not lead to an adverse impact on growth stage development.

Plant biomass:

Mortality was seen at the highest application rates tested for oilseed rape, onion and sugar beet. No mortality was apparent in the other seven species. Sugar beet was the most sensitive species to Flurtamone + Diflufenican 250 + 100 SC, biomass measured as shoot dry weight was the most sensitive endpoint.

Summary of the NOEC, EC_{25} and EC_{50} for survival and shoot dry weight for ten plant species exposed to Flurtamone + Diflufenican 250 + 100 SC

	Survival			Shoot dry weight		
Species	**NOEC**	**EC_{25}**	**EC_{50}**	**NOEC**	**EC_{25}**	**EC_{50}**
Corn	1000	>1000	>1000	250	>1000	>1000
Cucumber	1000	>1000	>1000	<31.1	1000	>1000
Oats	1000	>1000	>1000	125	262.5	969.6
Oilseed rape	1000	906.1	>1000	62.5	254.3	764.2

Onion	1000	>1000	>1000	62.5	>1000	>1000
Radish	1000	>1000	>1000	<31.3	149.4	>1000
Soybean	1000	>1000	>1000	62.5	474.1	>1000
Sugar beet	250	406.7	637.9	<31.3	28.5	192.6
Sunflower	1000	>1000	>1000	<31.3	128.5	914.4
Tomato	1000	>1000	>1000	<62.5	140.5	425.1

Conclusions:

Based on the results of this study in which the effect of Flurtamone + Diflufenican 250 + 100 SC to ten plant species was tested under glasshouse conditions the most sensitive species was sugar beet with the lowest EC_{50} of 192.5 mL product/ha for shoot dry weight.

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Report:	**KCA 8.6.2/02; [redacted].; 2005**
Title:	Diflufenican and flurtamone (AE F088657 01 SC31 A202): Effects on ten species of non-target terrestrial plants: seedling emergence and growth test (Tier 2)
Document No:	M-251318-01-1
Guidelines:	OECD 208 a (July 2000, draft)
GLP:	Yes (certified laboratory)

Objective:

The purpose of this specific study was to evaluate the effect of Flurtamone + Diflufenican 250 + 100 SC (AE F088657 01 SC31 A202) on the seedling emergence and growth of ten plant species representing a broad range of both dicotyledonous and monocotyledonous plant families. Analysis of data was performed to obtain NOEC, EC_{25} and EC_{50} values for emergence, survival and biomass (shoot dry weight).

Materials and Methods:

Test item: Flurtamone + Diflufenican 250 + 100 SC (AE F088657 01 SC31 A202), Batch No. V355010344 (content of active ingredients: Flurtamone 23.3% w/w + diflufenican 9.18% w/w). Seeds of ten species; corn (*Zea mays*), cucumber (*Cucumis sativus*) oats (*Avena sativa*), oilseed rape (*Brassica napus*), perennial ryegrass (*Lolium perenne*), radish (*Raphanus sativus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus*) and tomato (*Lycopersicon esculentum*) were sprayed with the test item. Solutions of the product and serial dilutions were sprayed with doses of the product ranging from 1000 mL product/ha down to 7.8 mL product/ha using a laboratory track sprayer. There were six dose rates that differed with each species. For radish, sunflower, cucumber, soybean, oats, perennial ryegrass and corn these were 1000, 500, 250, 125, 62.5 and 31.3 mL product/ha. For sugar beet, oilseed rape and tomato these were 250, 125, 62.5, 31.3, 15.7 and 7.8 mL product/ha. Plants were grown and maintained under glasshouse conditions with a temperature control set at 23 ± 5°C during day and 18 ± 5°C at night. The photoperiod was 16 h of light and 8 h dark. Assessments were made daily until 65% emergence of control seedlings, then 7 and 14 days after this time were evaluated against the untreated controls for emergence, survival, biomass (shoot dry weight) growth and phytotoxicity.

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Germination rates of control plants	≥ 65%	65-100%
Survival of the control plants	> 90%	91-100%
Visible phytotoxic symptoms in the control plants	0	0

All validity criteria for the study were met

Biological results:

Phytotoxicity

All species showed the relevant phytotoxic symptoms visible as bleaching, necrosis and stunting.

Survival:

There was no effect on survival for corn, while all other plants showed a significant reduction of biomass at different application rates, with sugar beet being the most sensitive species.

Emergence:

No adverse effects on seedling emergence were recorded for: corn, cucumber, oats, radish, ryegrass, soy bean, sunflower and tomato. There was a significant reduction of seedling emergence for oilseed rape and sugar beet.

Plant biomass

There was no effect on biomass (shoot dry weight) for sunflower, while all other plants showed a significant reduction of biomass at different application rates.

Summary of the effects on emergence, survival and shoot dry weight for ten plant species exposed to Flurtamone + Diflufenican 250 + 100 SC

	Emergence			Survival			Shoot dry weight		
Species	**NOEC**	**EC_{25}**	**EC_{50}**	**NOEC**	**EC_{25}**	**EC_{50}**	**NOEC**	**EC_{25}**	**EC_{50}**
Corn	1000	>1000	>1000	1000	>1000	>1000	250	572.8	>1000
Cucumber	1000	>1000	>1000	125	171.1	369.4	31.3	29.4	112.9
Oats	1000	>1000	>1000	500	529.2	701.1	125	204.8	681.2
Oilseed rape	250	>250	>250	31.3	56.7	73.7	31.3	40.5	63.5
Radish	1000	>1000	>1000	125	194.3	345.6	31.3	52.1	144.7
Ryegrass	1000	>1000	>1000	<31.3	86.4	156.8	31.3	41.0	63.2
Soybean	500	>1000	>1000	500	>1000	>1000	62.5	212.0	569.5
Sugar beet	125	117.1	>250	7.8	18.6	25.2	31.3	26.5	36.3
Sunflower	1000	>1000	>1000	500	>1000	>1000	1000	936.3	>1000
Tomato	62.5	81.5	296.3	62.5	69.0	88.1	62.5	79.3	90.7

Conclusions:

Based on the results of this study in which the effect of Flurtamone + Diflufenican 250 + 100 SC to seedling emergence, survival and shoot biomass of ten plant species was tested under glasshouse conditions the most sensitive species was sugar beet with the lowest EC_{50} of 25.2 mL product/ha for survival.

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Report: **KCA 8.6.2/05; [redacted], W.; 1995**
Title: Bioassays to determine the no-observed-effect level (ED_{10}) for the active ingredient flurtamone and selected succeeding crops in soil
Document No: M-247804-01-2
Guidelines: None
GLP: No

Objective

The objective was to determine the no-observed-effect level (NOEL) of the active ingredient flurtamone for selected crop plants. A range-finding test was performed initially to determine the effective range of concentrations. Dose-effect relationships were determined in a light, low-humus sandy soil.

Material and methods

Test item: Flurtamone (formulated as RPA 31248 H at a concentration of 600 g/L).
Test substrate: low-humus, silty sand (Su4) with an organic carbon content of 0.9% and a pH (0.02N $CaCl_2$) of 7.5. The mineral fraction was made up of 9.6% clay, 38.2% silt and 52.2% sand. The maximum water capacity (WC_{max}) was 22.8 ml/100 g dry mass (DM).
Test concentrations: 0-25, 50-100 ans 150-200 µg flurtamone/kg dws

Findings

The reduction in fresh weight as compared to the control was measured as follows:

Test plant	NOEL values (ED_{10} values) determined from dose effect curves in µg active substance/kg soil [% max. application rate = 400 g/ha]		
	NOEL (ED_{10})	ED_{30}	ED_{50}
Oilseed rape	23.7 [5.9]	29.7 [7.4]	34.3 [8.6]
Sugar beet	39.8 [10.0]	51.6 [12.9]	60.7 [15.2]
Oilseed radish	**15.3** [3.8]	40.8 [10.2]	75.6 [18.9]
Maize	100 [25]	n.c.	n.c.
Sunflower	> 200 [50]	n.c.	n.c.
Red clover	46.5 [11.6]	65.6 [16.4]	82.1 [20.5]
Oat	29.4 [7.4]	60.3 [15.1]	95.2 [23.8]
Pea	131.9 [33]	1,691.0 [> 100]	8,492.7 [> 100]
Potato	> 200 [50]	n.c.	n.c.

n.c. = Not calculated or outside the confidence interval

For the tested plants, the NOEL (ED_{10}) values for flurtamone were between 15.3 µg (oilseed radish) and > 200 µg/kg (potatoes, sunflower), corresponding to 3.8 and > 50% respectively of the maximum application rate in the present test system (calculated on the basis of a soil depth of 0-10 cm).

Conclusion

The results simulate a worst-case scenario and thus give a considerable safety margin because the optimal absorption conditions resulting in greater sensitivity prevailing in the bioassay are not representative of field conditions. The lowest ED_{10} was found for oilseed radish with 15.3 µg a.s./kg.

CA 8.7 - Effects on other terrestrial organisms (flora and fauna)

Report:	**KCA 8.7/03; Benesch, J.A., Gustin, M.S., Cramer, G.R., Cahill, 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 M05 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:	Sigma Chemical, St. Louis, MO, 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*.
Cultivar:	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, Carpenteria, 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 mmol/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.

<u>Plants:</u>

(1) TFA uptake via roots: Two hundred plants of *Deschampsia*, *Lasthenia*, and *Oryza* were germinated under 60 µmol/m^2/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/m^2/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/m^2/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 0.5 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, 50, 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 Cahill 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.

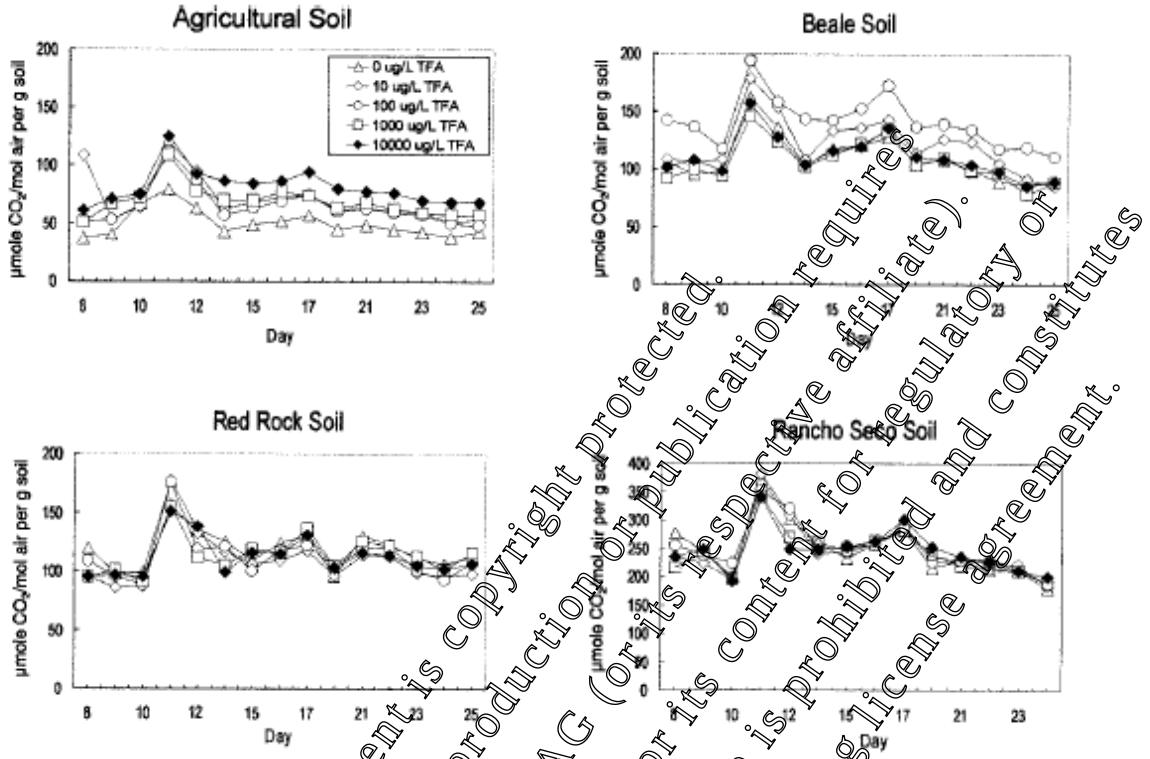

Figure 1 (taken from Benesch et al., 2002): Microbial respiration (µmol CO_2/mol air/g soil) in microcosms amended with TFA as a function of time, starting with day 8, when respiration had stabilized. Each point represents a mean of three CO_2 measurements for three replicate microcosms.

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	53 ± 10 *n* = 9*		58 ± 9 *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	75 ± 19 *n* = 17*				159 ± 33 *n* = 9	295 ± 50 *n* = 9*			
Lasthenia flowers	12 ± 2 *n* = 3	20 ± 7 *n* = 3				81 ± 27 *n* = 3	108 ± 32 *n* = 3			
Lasthenia seeds			22 ± 3 *n* = 9					17 ± 2 *n* = 9		
Deschanmpsia leaves	27 ± 7 *n* = 9	39 ± 5 *n* = 9*		30 ± 7 *n* = 18*		210 ± 80 *n* = 9	171 ± 52 *n* = 9		248 ± 50 *n* = 9*	

Table 1 (taken from Benesch 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 ± 3 µg/g for the 1000-µg/L (controls contained < 0.07 µg/g). *Lasthenia* seeds had TFA concentra-tions of 2 ± 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 0.30 ± 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 M05 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 M05 TFA concentrations.

Comments by the Notifier:
This study confirms the results from 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; Smit, M.F., van Heerden, P.D.R., Pienaar, J.J., Weissflog, L., Strasser, R.J., Krüger, 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
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 %, 18 %, 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 to 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.

Report:	**KCA 8.7/01; Oehrle, N.W.Green, L.S., Karr, D.B., Emerich, D.W.**
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) 336-342
DOI No: Document No:	doi:10.1016/j.soilbio.2003.10.00 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:	E.I. DuPont de Nemours & Co., Inc. in conjunction with the AFEAS. Additional TFA from Fisher Scientific
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)
Source of test 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 1 mM 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. Chi² 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).

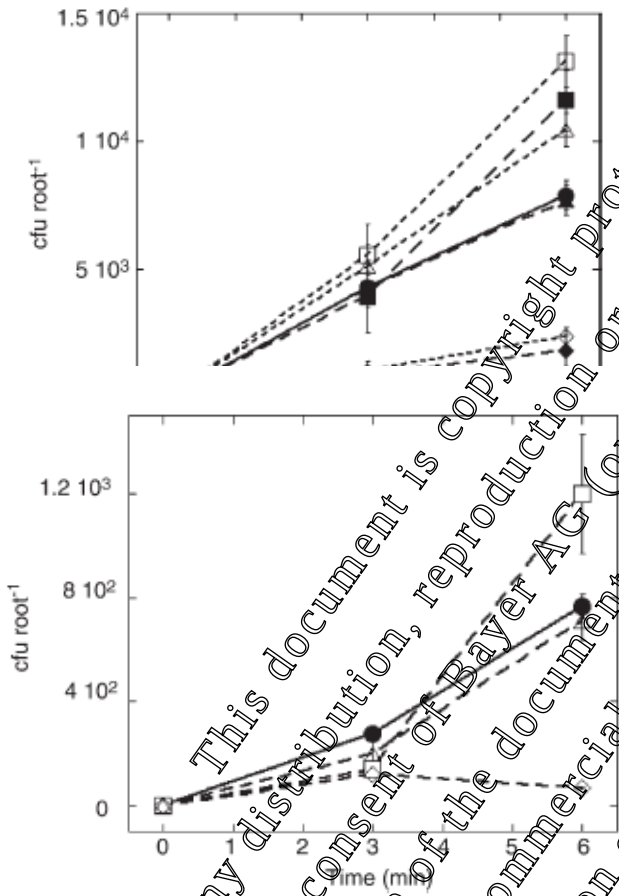

Fig. 2. Attachment (in cfu root^{-1}) of *B. japonicum* strain 2143 grown on T. acetate (●), and T. acetate plus 10 µM TFA (□), 100 µM TFA (△) or 1 mM TFA (◇). Data point error is reported as SEM.

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.

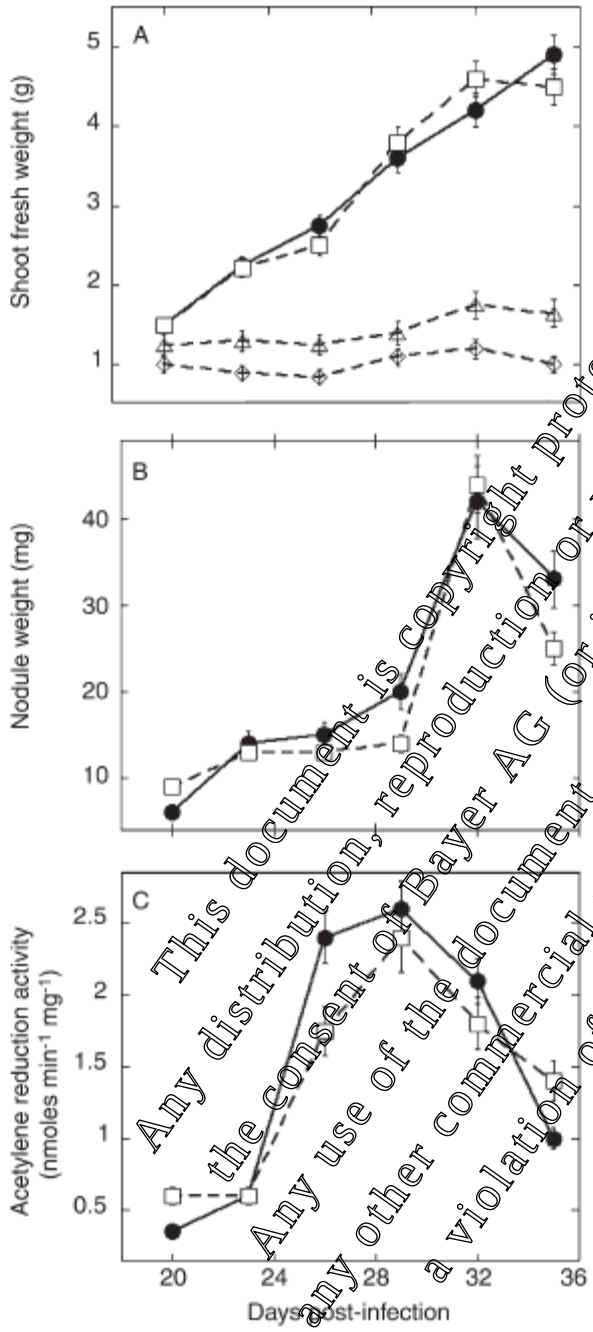

Fig. 3. (A) Shoot weights of soybean when both plant and bacteria were pre-incubated with TFA for 1 h before inoculating and planting in soil containing either no TFA (●), 0.674 µl TFA kg^{-1} soil (□), 6.74 µl TFA kg^{-1} (△) and 67.4 µl TFA kg^{-1} (◇). (B) The nodule weight of those plants in (A) 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.

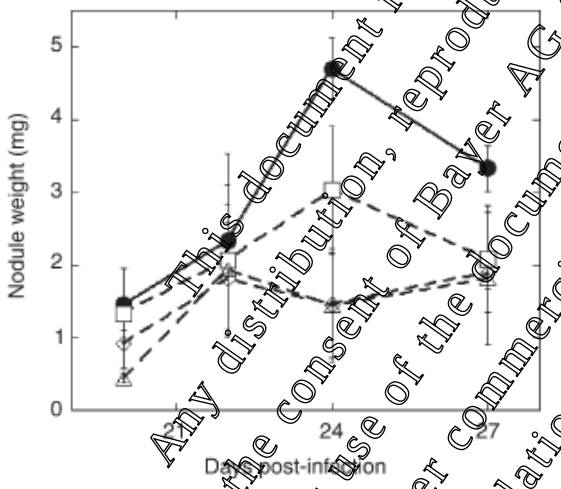

Fig. 4. Nodule weight of soybean seedlings grown hydroponically after inoculation with *B.japonicum* strain 110. Seedlings were grown with no TFA (●), 0.003 µl TFA l^{-1} nutrient solution (□), 0.031 µl TFA l^{-1} (△) and 0.314 µl TFA l^{-1} (◇). Data point errors are reported as SEM.

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.

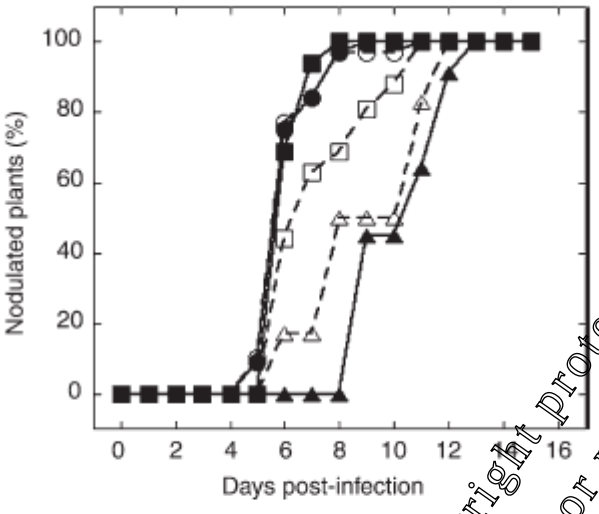

Fig. 5. Nodulation kinetics of *B. japonicum* strain 110 (●), 2143 (■) and 184 (▲) in the absence of TFA on hydroponically grown soybean seedlings. In the presence of 0.003 µl TFA l^{-1} nutrient solution, strain 110 (○) was unaffected, strain 2143 (□) had reduced nodulation and mutant strain 184 (△) 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

Report:	**KCA 8.7 /01; [redacted]., 2005**
Title:	Toxicity of AE B107587 00 1B99 0001 to activated sludge in a respiration inhibition test
Report No.:	M-249637-01-1
Guidelines:	OECD 209 (1984)

	EEC Commission Directive 87/302/EEC
GLP/GEP	Yes

Material and Methods:
The test substance was Flurtamone, technical (code AE B107587 00 1B99 0001) with the purity of 99.5% w/w. Batch No: DP639D.
Activated sewage sludge was exposed to the test material at concentrations of 10, 32, 100, 320 and 1000 mg/L for a period of 3 hours at 18-20 °C.
The rate of respiration was determined after 3 hours contact time and compared to data for the control and a reference material, 3,5-dichlorophenol.
Observations were made on the test preparations throughout the study period, and the pH of the control, reference material and test material preparations was measured at the end of the exposure period prior to measurement of the oxygen consumption rate.

Findings:
At the end of the test a pH value of 8.3 was measured in all flasks.

Treatment group	O_2 consumption rates (mg O_2/L/min) after 3 hours	% inhibition after 3 hours
Control		
Replicate 1	0.500	-
Replicate 2	0.514	-
Flurtamone		
10 mg/L	0.476	7.9
32 mg/L	0.507	0
100 mg/L	0.500	1.4
320 mg/L	0.538	-6.1
1000 mg/L	0.453	10.7
3.5-Dichlorophenol		
3.2 mg/L	0.390	23.1
10 mg/L	0.180	64.5
32 mg/L	0.080	84.2

No clear dose-response relationship could be observed and it was not possible to calculate the EC_{20}/EC_{50} values.

Conclusion:
The effects of flurtamone on the respiration of activated sewage sludge micro-organisms gave a 3-hour EC_{50} of greater than 1000 mg/L. The No Observed Effect Concentration (NOEC) after 3 hours exposure was 1000 mg/L.

CA 8.9 - Monitoring data

No monitoring data for ecotoxicological effects are available.