Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Foramsulfuron

PUBLIC VERSION

Data Requirements

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

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

Date

29th November 2013

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority.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authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

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

Version history

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

[1] Changes will be presented according to the approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an assessment report.

Table of Contents

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

This document contains only summaries of ecotoxicological studies on the active substance, foramsulfuron (AE F130630), and its metabolites, which were not available at the time of the first Annex I inclusion of foramsulfuron and were therefore not evaluated during the first EU review of this compound. In order to facilitate discrimination between new and original information, the old information is written in grey letters. All studies, which were already submitted by Bayer CropScience for the first Annex I inclusion are contained in the Monograph, its Addenda and are included in the original (baseline) dossier provided by Bayer CropScience and are not summarised. For all new studies a detailed study summary is provided.

For a better overview, study endpoints resulting from the evaluation process of Annex I inclusion are presented in this document, together with the information whether or not this endpoint was listed in the List of Endpoints in the Review Report (SANCO/10324/2002-Final).

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

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

Compartment	Compound / Code
Soil	Foramsulfuron AE F092944 AE F130619 AE F153745
Groundwater	Foramsulfuron AE F092944 AE F130619 AE F153745
Surface water	Foramsulfuron AE F092944 AE F130619 AE F153745 AE 0338795 AE F099095 4-Amino-N-methylbenzamide 4-Formamido-N-methylbenzamide Foramsulfuron sulfamic acid
Plant material	Foramsulfuron

*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.

Metabolite testing for aquatic organisms

Data of the parent compound show unambiguously that the aquatic macrophyte species *Lemna gibba* is by far the most sensitive organism in the aquatic environment (the next sensitive organism, the blue-green alga *Anabaena flos-aqua*, is by a factor of about 8000 less sensitive to foramsulfuron than *Lemna*). The sensitivity of this macrophyte species is clearly driving the risk assessment for foramsulfuron.

The risk that one of the metabolites would be toxic to fish, Daphnia or algae to an extent that this could actually impact the risk assessment seems to be negligibly low. Therefore, it is considered justified that the testing for metabolites potentially reaching aquatic systems should be limited to this most sensitive species, *Lemna*.

The appropriateness of this strategy was confirmed by singular tests on fish, Daphnia and algae with the metabolites AE F095944 and AE 099095. Both metabolites turned out to be completely non-toxic to these species at relevant exposure levels, with all EC$_{50}$ values above the highest tested dose levels.

Metabolite testing for soil organisms

The sensitivity of soil macro- and microorganisms to foramsulfuron is generally low. The No Observed Effect Concentrations were above the highest tested concentration for earthworms, soil mites and N-transformation. The NOEC for *Folsomia candida* was also high with 178 mg a.s./kg dws. Consequently, the risk assessment in soil is not driven by one single species. Therefore, for all soil metabolites, all species (earthworm, *Hypoaspis aculeifer*, *Folsomia candida*, and N-transformation in soil) were tested.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

CA 8.1.1.1 Acute oral toxicity to birds

Two acute studies on non-related bird species, bobwhite quail and mallard duck, were performed. The highest tested dose level in both studies was 2000 mg/kg bw. No mortality occurred. Details of the studies are provided in the following table.

Table 8.1.1.1- 1: Avian acute oral toxicity data of foramsulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	acute, oral	LD_{50} > 2000 [1)] $LD_{50\ extrapol}$ 3776 [2)] mg as/kg bw	[redacted], 1998 M-143541-01-1 KCA 8.1.1.1/01
Mallard duck	acute, oral	LD_{50} > 2000 [1)] $LD_{50\ extrapol}$ 3776 [2)] mg as/kg bw	[redacted]1997 M-142752-01-1 KCA 8.1.1.1 /02

Bold letters: Values considered relevant for risk assessment in the MCP document
[1)] 10 birds per group
[2)] LD_{50} extrapolated according to EFSA GD Birds & Mammals (2009) by applying a factor of k 888 to the top dose in case 10 animals have been tested and no mortality occurred

Report:	K[redacted]ü;[redacted];;1998;M-143541-01
Title:	Code: Hoe 130360 00 ZC98 0002 - Bobwhite quail acute oral toxicity study
Report No:	A59882
Document No(s):	Report includes Trial Nos.: 96-0762 FOX96116 M-143541-01-1
Guidelines:	**USEPA (=EPA): 71-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
LC_{50} > 2000 mg as/kg bw

Report:	K[redacted]f ;[redacted];1997;M-142752-01
Title:	Hoe 130360 (AE F130360) Code: Hoe 130360 00 ZC98 0001 - Mallard duck acute oral toxicity study
Report No:	A59043
Document No(s):	Report includes Trial Nos.: 96-0763 FOX96280 M-142752-01-1
Guidelines:	**USEPA (=EPA): F 71-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
LC_{50} > 2000 mg as/kg bw

CA 8.1.1.2 Short-term dietary toxicity to birds

Two short-term dietary studies on non-related bird species, bobwhite quail and mallard duck were performed. The lowest LC_{50} was determined to be > 5000 ppm corresponding to an LDD_{50} of > 985 mg a.s./kg bw/d. Details of the studies are provided in the following table.

Table 8.1.1.2-1: Avian short-term dietary toxicity data of foramsulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	5-day dietary	LC_{50} > 5000 [1] ppm ≡ LDD_{50} > 985 mg as/kg bw/d	[redacted]1998 M-147825-01-1 KCA 8.1.1.2 /01
Mallard duck	5-day dietary	LC_{50} > 5000 [1] ppm ≡ LDD_{50} > 1792 mg as/kg bw/d	[redacted]1998 M-147826-01-1 KCA 8.1.1.2 /02

1) 10 birds per group

Report:	K[redacted]g[redacted];;1998;M-147825-01
Title:	Bobwhite quail dietary LC50 study Code: AE F130360 00 1C98 0002
Report No:	A67441
Document No(s):	Report includes Trial Nos.: 96.0781 Tox96117 M-147825-01-1
Guidelines:	**OECD: 205; USEPA (=FIFRA): E 71-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
LC_{50} > 5000 ppm

Report:	K[redacted]; [redacted];1998;M-147826-01
Title:	Mallard duck dietary LC50 study Code: AE F130360 00 1C98 0001
Report No:	A67442
Document No(s):	Report includes Trial Nos.: 96.0780 Tox96118 M-147826-01-1
Guidelines:	**OECD: 205; USEPA (=FIFRA): E 71-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
LC_{50} > 5000 ppm

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Two reproductive studies on non-related bird species, bobwhite quail and mallard duck were performed. The lowest NOEL was determined to be ≥ 104 mg a.s./kg bw/d. Details of the studies are provided in the following table.

Table 8.1.1.3-1: Avian reproductive toxicity data of foramsulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Bobwhite quail	21-weeks feeding chronic, reproduction	NOEC ≡ NOEL	≥ 1000 **≥ 104***	ppm mg as/kg bw/d	[redacted] 1999 M-194248-01-1 KCA 8.1.1.3/01
Mallard duck	21-weeks feeding chronic, reproduction	NOEC ≡ NOEL	≥ 1000 ≥ 132	ppm mg as/kg bw/d	[redacted] 1999 M-194250-01-1 KCA 8.1.1.3/02

Bold letters: Values considered relevant for risk assessment in the MCP document
* Calculated test substance intake is presented in the study report (M-194248-01-1)

Report:	K[redacted]7;[redacted];;1999;M-194248-01
Title:	Northern Bobwhite quail dietary reproduction study AE F130360 Code: AE F130360 00 1C97 0002
Report No:	C006593
Document No(s):	Report includes Trial Nos.: TOX9612 M-194248-01-1
Guidelines:	**OECD: 206; USEPA: EPA; FIFRA 71-4; Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
NOEC* = 1000 ppm

* Mistakenly presented in the Review Report for foramsulfuron (SANCO/10324/2002-Final) as NOEL.

Report:	K[redacted];[redacted];;1999;M-194250-01
Title:	Mallard duck dietary reproduction study AE F130360 Code: AE F130360 00 1C97 0002
Report No:	C006594
Document No(s):	Report includes Trial Nos.: TOX9613 M-194250-01-1
Guidelines:	**OECD: 206; USEPA: EPA; FIFRA 71-4; Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
NOEC* = 1000 ppm

* Mistakenly presented in the Review Report for foramsulfuron (SANCO/10324/2002-Final) as NOEL.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

An acute study on male and female rats was performed. The LD_{50} was greater than 5000 mg/kg bodyweight. Details of the study are provided in the following table.

Table 8.1.2.1-1: Mammalian acute oral toxicity data of foramsulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Rat	acute, oral	LD_{50}	**> 5000** [1)]	mg as/kg bw	XXXXX 1987 M-141959-01-1 KCA 5.2.1 /01

Bold letters: Values considered relevant for risk assessment in the MCP document
[1)] 10 rats per group, no mortality occurred

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):

LD_{50} > 5000 mg/kg bw

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

A two-generation reproductive toxicity study on male and female rats was performed. The NOAEC and NOEC was determined to be 15 000 ppm. Details of the studies are provided in the following table.

Table 8.1.2.2-1: Mammalian reproductive toxicity data of foramsulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Rat	reproductive, 2 generations	NOAEC	≥ 15000	ppm	XXXXX 1999 M-287748-01-1 KCA 5.6.1 /01
		≡ $NOAEL_{male}$	≥ 1038	mg as/kg bw/d	
		$NOAEL_{female}$	1430	mg as/kg bw/d	
		$NOAEL_{geomean}$	**≥ 1218** [1)]	mg as/kg bw/d	

Bold letters: Values considered relevant for risk assessment in the MCP document
[1)] Geometric mean of male and female

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):

NOEC/NOAEC = 15 000 ppm

(Mistakenly this endpoint was presented as NOEL/NOAEL in the Review Report for foramsulfuron (SANCO/10324/2002-Final).)

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

As the log P_{ow} of the active substance foramsulfuron and its metabolites is below the trigger (< 3), no evaluation of secondary poisoning is needed.

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

Since foramsulfuron is of low toxicity to birds and laboratory rodents, no risk for reptiles and amphibians is to be expected.

CA 8.1.5 Endocrine disrupting properties

Following EU regulation 1107/2009, an assessment has to be provided concerning potential endocrine disrupting properties of the active substance concerned.

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, 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 foramsulfuron to wildlife presented below.

Wild Mammals:
Potential endocrine activity and potential population relevant effects of foramsulfuron on mammals were studied in 90-d, chronic, and multi-generation studies in rats, 90-d and chronic studies in mice, 90-d and 1-year studies in dogs, and in teratology studies in rats and rabbits. In none of these studies any observations of effects were noticed that could be related to primary endocrine activity.
Based on the absence of any indication of relevant effects it can be concluded that foramsulfuron is not an endocrine disrupter.

Birds:
The population relevant effects of foramsulfuron on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. For both species there were no effects on reproductive parameters up to and including the highest tested dietary concentration of 1000 ppm a.s.
As reproduction was not affected in either species it is concluded that there are no population relevant adverse effects of foramsulfuron. No additional studies seem necessary.

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 exist, this test was developed to evaluate to potential effect 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.

Conclusion:

Neither in mammals, nor birds were any indications for adverse endocrine activity observed. Therefore further special testing for endocrine disrupting behaviour is not warranted.

CA 8.2 Effects on aquatic organisms

Aquatic organisms have been tested with the active ingredient and the metabolites included in the residue definition for aquatic risk assessment (see MCA Section CA 7.4.1).
Due to the fact that *Lemna* is by far the most sensitive standard aquatic organism to the parent compound, metabolite testing was confined to this species in most cases, with two exceptions: AE F092944 and AE F099095. These are common metabolites with one or more sulfonyl urea

[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.

herbicides. Tests with further aquatic species have been performed in context of risk assessments for other parent compounds. Although for the risk assessment of foramsulfuron these studies on further species are not considered essential, they are provided here for sake of completeness.

CA 8.2.1 Acute toxicity to fish

For foramsulfuron three acute toxicity studies on three different fish species were performed. The tested dose level in all studies was 100 mg a.s./L. No sublethal effects and only random mortality (in one study only) were observed in the treatment, resulting in an LC_{50} of >100 mg a.s./L.
For the metabolite AE F092944 one acute study on rainbow trout was conducted with test doses ranging from 18 to 1000 mg/L. The 96-hour-LC_{50} was 254 mg/L.
Details of all studies are provided in the following table.

Table 8.2.1-1: Acute toxicity data of foramsulfuron and metabolite to fish presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Foramsulfuron-sodium				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} **>100**	XXXXX [redacted] 1997 A57725 XXXXX [redacted], 1997 A57751 (Amendment) M-141405-02-1 KCA 8.2.1 /01
Lepomis macrochirus (bluegill sunfish)	static acute	96 h	LC_{50} **>100**	XXXXX [redacted] 1997 A57726 [redacted], 1997 A57752 (Amendment) M-141406-02-1 KCA 8.2.1 /02
Cyprinodon variegatus (sheephead minnow)	static acute	96 h	LC_{50} > 100	XXXXX [redacted] 1998 A59901 M-143551-01-1 KCA 8.2.1 /03
AE F092944				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} **254**	XXXXX [redacted] 1993 A50396 M-131422-01-1 KCA 8.2.1 /04

Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on Foramsulfuron

Report:	K[redacted];[redacted];;1997;M-141405-02; Amended: 1997-06-05
Title:	96-hour acute toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a static renewal system AE F130360 technical 98.6 % w/w Code: AE F130360 00 1C98 0001
Report No:	A57725
Document No:	M-141405-02-1
Guidelines:	**OECD: 203; USEPA (=EPA): E 72-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):

EC_{50} > 100 mg/L

Report:	K[redacted]7; [redacted];;1997;M-141406-02; Amended: 1997-06-05
Title:	AE F130360; technical 98.6 percent w/w; Code: AE F130360 00 1C98 0001 - 96 hour acute toxicity to the bluegill sunfish, *Lepomis macrochirus*, in a static renewal system
Report No:	A57726
Document No:	M-141406-02-1
Guidelines:	**OECD: 203; USEPA (=EPA): E 72-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final)
EC_{50} > 100 mg/L

Report:	K[redacted]k; [redacted];1998;M-143551-01
Title:	96 hour acute toxicity to the Sheepshead minnow (*Cyprinodon variegatus*) in a static system AE F130360 technical 94.2% w/w Code: AE F130360 00 1C94 0001
Report No:	A59901
Document No:	M-143551-01-1
Guidelines:	**OECD: 203; USEPA (=EPA): E 72-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Studies on the metabolites of foramsulfuron

AE F092944

Report:	K[redacted];[redacted];;1993;M-131422-01
Title:	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to *Oncorhynchus mykiss* (Rainbow trout) in a Static-Acute Toxicity Test (method OECD)
Report No:	A50396
Document No:	M-131422-01-1
Guidelines:	**OECD: 203 (1984);Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the acute effects of metabolite AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE F092944 00 ZD99 0001; purity >99.0%) to rainbow trout (*Oncorhynchus mykiss*).
Oncorhynchus mykiss (5 months old) were exposed in a static system over a period of 96 hours to nominal concentrations of 18, 32, 56, 100, 180, 320, 560, and 1000 mg/L. In addition a water control was tested.
Mortality and sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour-LC_{50} was 254 mg/L (95% confidence limits 202 - 317 mg/L), the 96-hour-NOEC was determined to be 100 mg/L.

Materials and Methods:

Test item: Hoe 092944 – substance, technical; identification code: Hoe 092944 00 ZD99 0001; common name: 2-amino-4,6-dimethoxypyrimidine; analysed purity: > 99 % w/w; analytical certificate No.: AZ 04888.

Oncorhynchus mykiss (5 months old) were exposed to AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE F092944 00 ZD99 0001; purity >99.0%) in a static system over a period of 96 hours. Nominal concentrations were 18, 32, 56, 100, 180, 320, 560, and 1000 mg/L. In addition a water control was tested. Each vessel (stainless steel tanks; 300 L) served as one replicate filled with 200 L Test water was a well aerated water mixture of 60% filtered tap water and 40% deionized water passed through sand and activated charcoal filters. 10 fishes were used per replicate. Length of fishes at test start was 5.83 cm (mean of ten fishes). Body weight of fishes at test start was 3.03 g (mean of ten fishes). The static biological loading was 0.15 g/L or 0.29 cm/L. The test was conducted with one replicate per treatment level.

For analytical verification of the test item concentrations samples were taken at days 0, 2 and 4 from systems exposed to concentrations of 18, 100 and 1000 mg/L. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: September 07, 1992 – September 11, 1992

Results:

Validity Criteria:
The validity criterion of control mortality less than 10% is fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

Analytical findings:
Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table 8.2.1-2: Nominal and measured concentrations of AE F092944

Nominal test concentrations	**18 mg/L**	**100 mg/L**	**1000 mg/L**
Nominal a.i. (mg/L)	17.82	99	990
Day 0	18.012	48.796	494.1
Day 2	18.257	104.4	879.8
Day 4	17.929	102.5	---
Mean a.i.	**18.07**	**85.25**	**686.95**
% recovery day 0	101.1	49.3	49.9
% recovery day 2	102.5	105.5	88.9
% recovery day 4	100.6	103.5	---
% recovery mean	**101.4**	**86.1**	**69.4**

Biological findings:
Mortality was observed as listed below.

Table 8.2.1-3: Effect of AE F092944 on mortality of *Oncorhynchus mykiss*

Exposure time	24 h	48 h	72 h	96 h	
Test level mg / L	no. of dead	no. of dead	no. of dead	**no. of dead**	**% dead**
Control	0	0	0	**0**	**0**
18	0	0	0	**0**	**0**
32	0	0	0	**0**	**0**
56	0	0	0	**0**	**0**
100	0	0	0	**0**	**0**
180	0	0	0	**1**	**10**
320	5	6	8	**8**	**80**
560	10	10	10	**10**	**100**
1000	10	10	10	**10**	**100**

Biological endpoints derived:
From the results presented above the following biological endpoints can be derived:

96-hour-figures:
highest concentration with no effect (NOEC): 100 mg/L
LC50: 254 mg/L (95% confidence limits 202 - 317 mg/L)

Conclusions:

The acute effect of AE F092944 (2-amino-4,6-dimethoxypyrimidine; AE 092944 00 ZD99 0001) on rainbow trout (*Oncorhynchus mykiss*) can be quantified as a 96-hour-LC_{50} of 254 mg/L (95% confidence limits 202 - 317 mg/L). The highest concentration with no observed mortality and no sublethal behavioural effects can be set to 100 mg/L.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

Two chronic studies on different fish species were performed. The maximum tested dose levels were 100 mg a.s./L in the chronic study with rainbow trout, and 10.5 mg a.s./L in the study on early life stage exposure with fathead minnow. In both studies no relevant treatment related effects were observed at the maximum dose level, resulting in a NOEC of 100 or 10.5 mg a.s./L.
Details of the studies are provided in the following table.

Table 8.2.2-1: Chronic toxicity data of foramsulfuron to fish presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Oncorhynchus mykiss (rainbow trout)	chronic	28 d	NOEC 100	[redacted] 1999 C004117 M-187354-01-1 KCA 8.2.2.1 /01
Pimephales promelas (fathead minnow)	Early Life Stage flow-through	35 d	NOEC **10.5**	[redacted] 2004 B004606 M-241508-01-1 KCA 8.2.2.1 /02

Bold letters: Values considered relevant for risk assessment in the MCP document

Report:	K[redacted];[redacted];1999;M-187354-01
Title:	Prolonged toxicity to rainbow trout, *Oncorhynchus mykiss*, in a flow through system AE F130360 technical 95.8 % w/w Code: AE F130360 011C96-0002
Report No:	C004117
Document No:	M-187354-01-1
Guidelines:	**OECD: 204;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
NOEC = 100 mg/L

Report:	K[redacted];[redacted];2004;M-241508-01
Title:	Early Life Stage Toxicity of Foramsulfuron (AE F130360) Technical to the Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions
Report No:	B004606
Document No(s):	Report includes Trial Nos.: EBFSX001 (A3841201) M-241508-01-1
Guidelines:	**OECD: 210; USEPA (=EPA): 72-4, OPPTS 850.1400;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The effects of foramsulfuron on fathead minnow (*Pimephales promelas)* embryos and larvae were evaluated in a 35 days (30 days post-hatch) toxicity test under flow-through conditions. The nominal test item concentrations were 0.63, 1.25, 2.50, 5.00 and 10.0 mg a.s./L (corresponding to 0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L mean measured concentrations over the course of the study). In addition, a dilution water control was tested. Four replicates were used for each test item concentration and the control. Thirty five embryos were impartially selected and distributed to each of 24 embryo incubation cups, one of which was then suspended in each test aquarium per exposure concentration and the control.

On the morning of day 33, during the diluter routine check, it was observed that one syringe pump was not operating. The diluter could have only been operating under these conditions for a maximum of 16 hours, and corrective action was quickly taken to insure that the diluter was operating correctly as soon as possible. This relatively brief deviation from nominal concentrations occurred at a late stage of the study, and most likely had no impacts on the study results.

Observations were made on the survival of organisms at hatch and on the survival and growth (dry weight, total length) of larvae after 30 days of post-hatch exposure. Observations of abnormal

behaviour, abnormal physical changes and mortality were recorded daily by visually inspecting the organisms in each growth chamber. Effects were determined based on the mean measured concentrations of the test substance.
With regard to survival of fathead minnows, no statistically significant differences between treatment rates and control were detected. This applies both to the hatching period (5 days) and to the post-hatch exposure period (30 days). At test termination (30 days post-hatch), no statistically significant differences of mean total length and mean dry weight between treatment rates and control were found.
In conclusion, no treatment related effects occurred in the early life stage exposure of the fathead minnow to foramsulfuron technical at the tested concentrations. The NOEC was 10.5 mg a.s./L and the LOEC was >10.5 mg a.s./L for all endpoints.

Materials and Methods:

Test material: Foramsulfuron Technical; purity: 97.3%, Batch number: AAIR04430, CAS No.: 173159-57-4;
The exposure of fathead minnow (*Pimephales promelas*) embryos and larvae to foramsulfuron was initiated with fertilised embryos. Thirty five embryos were impartially selected and distributed to each of 24 embryo incubation cups, one of which was then suspended in each quadruplicate test aquarium per exposure concentration and the control. The nominal test item concentrations were 0.63, 1.25, 2.50, 5.00 and 10.0 mg a.s./L (corresponding to 0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L mean measured concentrations over the course of the study). In addition, a dilution water control was tested. Four replicates were used for each test item concentration and the control. Dead embryos were counted daily until hatching was complete. Hatching was complete on exposure day 5 at which all viable eggs had hatched.
Calculations of percentage survival of organisms at hatch were based on the number of live larvae and embryos per incubation cup after hatching was complete, compared to the number of embryos per cup on test day 0. To initiate the post-hatch larval exposure, 20 live larvae were impartially selected from the surviving larvae in each incubation cup on test day 5 and placed into their respective exposure aquaria.
Behaviour and appearance of larvae were observed and recorded daily and larval survival was analysed on study day 5 and study day 35. Effects were determined based on the mean measured concentrations of the test substance.
The control and the high, middle and low test concentrations were each sampled once and analysed for foramsulfuron concentrations prior to the start of the definitive exposure.
During the in-life phase of the definitive study, water samples were removed from the test solutions on days 0, 7, 14, 21, 27, 33 and 35 for analysis of foramsulfuron.

Dates of experimental work: October 30, 2003 – December 04, 2003

Results:

Analytical findings:
Results of the analyses (HPLC) of the test solutions during the in-life phase of the study (days 0, 7, 14, 21, 27, 33 and 35) demonstrated that mean measured concentrations of foramsulfuron were generally consistent between replicate solutions and sampling intervals. The concentration range established was generally consistent with the expected concentration gradient (i.e. 50% dilutions between treatment levels). Based on the results of the weekly solution analyses, the exposure solutions were defined as

0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L (i.e. mean measured concentrations over the course of the study).

Biological findings:
During hatching period survival of fathead minnows in the five treatment levels (0.69 - 10.5 mg a.s./L) ranged from 81 to 92%, hence it was similar to the survival of the control organisms. No statistically significant differences between treatment groups and control were found. At the end of the post-hatch exposure period (30 days) the survival rates of larvae exposed to the five concentrations of foramsulfuron (0.69 - 10.5 mg a.s./L) ranged from 81% to 94% and, thus, they were in the same range as survival rates of the pooled control larvae. Again, no statistically significant differences between treatment groups and control could be revealed.

Growth data (total length and dry weight) were determined at test termination (30 days post-hatch). The mean total length and dry weight of larvae exposed to the five treatment levels (0.69 - 10.5 mg a.s./L) ranged from 21.0 to 22.2 mm and 38.6 to 46.0 mg, respectively. Growth in these treatment levels was statistically comparable to the control data, no significant differences were found. Based on these data, it was suggested that exposure to foramsulfuron concentrations up to 10.5 mg a.s./L did not adversely affect larval growth.

Table 8.2.2-2: Survival of fish at hatch (test day 5) and survival, total length and dry weight of fathead minnow (*Pimephales promelas*) larvae after 30 days post-hatch exposure to foramsulfuron

Mean measured concentration [mg a.s./L]	Survival of organism at hatch* [%]	30 days post-hatch		
		Larval survival [%]	Total length [mm]	Dry weight [g]
Control	88	84	21.1	42.6
0.69	85	93	21.0	38.6
1.23	92	81	21.9	45.6
2.72	83	93	21.9	44.3
5.01	85	89	21.7	42.0
10.5	81	94	22.2	46.0

* Mean values of four replicates

Conclusions:

In conclusion, no treatment related effects occurred in the early life stage exposure of the fathead minnow to foramsulfuron technical at the tested concentrations. The NOEC was 10.5 mg a.s./L and the LOEC was >10.5 mg a.s./L for all endpoints.

CA 8.2.2.2 Fish full life cycle test

A fish full life cycle test with foramsulfuron is not triggered as the compound has no potential for bioconcentration and is not persistent in water-sediment systems.

CA 8.2.2.3 Bioconcentration in fish

Due to the low P_{OW} foramsulfuron has no potential for bioconcentration.

CA 8.2.3 Endocrine disrupting properties

Based on the definition of the WHO/IPCS on endocrine disruption presented in Point CA 8.1.5 following results concerning relevant adverse effects of foramsulfuron on fish are presented below.

Fish

Population relevant effects of foramsulfuron on fish were studied in an early life-stage test (ELS). No effects were seen at the highest tested concentration of 10 mg/L.
No further testing is indicated to evaluate the endocrine disrupter potential of foramsulfuron to fish.

Conclusion:

There were no indications for adverse endocrine activity observed in fish. Therefore further special testing for endocrine disrupting behaviour is not warranted.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

For foramsulfuron one acute study on *Daphnia magna* was performed. No mortality occurred at the tested dose level of 100 mg a.s./L, resulting in a NOEC of 100 mg a.s./L and an EC_{50} >100 mg a.s./L.
For the metabolite AE F092944 one acute study on *Daphnia magna* was conducted. The tested dose level ranged from 10 to 560 mg/L, the determined EC_{50} was 233 mg/L.
Details of all studies are provided in the following table.

Table 8.2.4.1-1: Acute toxicity data of foramsulfuron and metabolite to *Daphnia magna* presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Foramsulfuron-sodium				
Daphnia magna (water flea)	static acute	48 h	EC_{50} **> 100**	[redacted], (1997) A57724 & A57750 (Amendment) M-141404-02-1 KCA 8.2.4.1 /01
AE F092944				
Daphnia magna (water flea)	static acute	48 h	EC_{50} **233**	[redacted], 1993 A50353 M-131382-01-1 KCA 8.2.4.1 /02

Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on foramsulfuron

Report:	[redacted];[redacted];[redacted];1997; M-141404-02; Amended: 1997-06-05
Title:	AE F130360; technical 98.4 percent w/w; Code: AE F130360 00 1C98 0001 - The 48 hour acute toxicity to *Daphnia magna*, in a static renewal system
Report No:	A57724
Document No:	M-141404-02-1
Guidelines:	**OECD: 202; USEPA (=EPA): E 72-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
EC_{50} = 100 mg/L*

* In the Study it is noted in the conclusion: "The 48 hour EC50 of AE F130360 technical to Daphnia magna could not be determined under the conditions of this study. The no observed effect concentration (NOEC) was 100 mg/L."

Studies on the metabolites of foramsulfuron

AE F092944

Report:	[redacted]9; [redacted];1993;M-131382-01
Title:	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to *Daphnia magna* (waterflea) in a Static -Acute Toxicity Test (method OECD)
Report No:	A50353
Document No:	M-131382-01-1
Guidelines:	**OECD: 202 (1984); Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the acute effects of AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE F092944 00 ZD99 0001; purity > 99.0%) to *Daphnia magna*.
Daphnia magna (< 24 hour old neonates) were exposed in a static system over a period of 48 hours to nominal concentrations of 10, 18, 32, 56, 100, 180, 320, and 560 mg/L (corresponding to analytically verified concentrations of 100.4%). In addition a water control and solvent control was tested.
Immobilisation and sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 48-hour-EC_{50} was 223 mg/L (95% confidence limits 180 - 320 mg/L), the 48-hour-NOEC was determined to be 32 mg/L.

Materials and Methods:

Test item: Hoe 092944 = substance, technical; identification code: Hoe 092944 00 ZD99 0001; common name: 2-amino-4,6-dimethoxypyrimidine; analysed purity: > 99 % w/w; analytical certificate No.: AZ 04888.

Daphnia magna (< 24 hour old neonates) were exposed to AE F 092944 (2-amino-4,6-dimethoxy-pyrimidine; code: AE F092944 00 ZD99 0001; purity > 99.0%) in a static system over a period of 48 hours. Nominal concentrations were 10, 18, 32, 56, 100, 180, 320, and 560 mg/L. In addition a water control and solvent control was tested. Each vessel (glass jar; 300 mL) served as one replicate filled with 200 mL artificial mineral medium M4 (Elendt 1990), slightly modified. 10 daphnids were used per replicate. Biological loading rate was 20 mL/animal. The test was conducted with 2 replicates per treatment level. Immobilisation of daphnids, intoxication symptoms and physical-chemical water parameters were assessed.

For analytical verification of the test item concentrations samples were taken at 0 and 72 hours from 10 mg/L concentrations. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: November 10, 1992 – November 12, 1992

Results:

Validity Criteria:
The validity criterion of control mortality less than 10% is fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

Analytical findings:
Analytical verification of test solutions revealed measured concentrations of 100.4% of nominal calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table 8.2.4.1-2: Nominal and measured concentrations of ÅE F092944

Nominal Concentration	Concentration (mg /L)	Day 0 (New)		Day 2 (Old)		mean	
		Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal
10 mg/L	9.9	9.849	98.5	10.237	102.4	10.043	100.4

Biological findings:
Observations on immobilisation and sublethal intoxication symptoms are listed as follows:

Table 8.2.4.1-3: Immobilization symptoms of *Daphnia magna*

Nominal Test Concentration mg/L	Number of Immobilised Daphnids	
	24 h.	48 h.
Control	0	0
Solvent control	0	0
10	0	0
18	0	0
32	0	0
56	0	4
100	0	3
180	2	4
320	17	19
560	20	20

No sublethal behavioural changes were observed.

Biological endpoints derived:
From the results presented above the following biological endpoints can be derived:

24-hour-figures:

EC_{50}: 247 mg/L (95% confidence limits 215 - 283 mg/L)

48-hour-figures:

NOEC: 32 mg/L

EC_{50}: 223 mg/L (95% confidence limits 180 - 320 mg/L)

Conclusions:

The acute effect of AE F092944 (2-amino-4,6-dimethoxypyrimidine; AE F092944 00 ZD99 0001) on *Daphnia magna* can be quantified as a 48-hour-EC_{50} of 223 mg/L (95% confidence limits 180 - 320 mg/L). The highest concentration with no observed immobilisation and no sublethal behavioural effects can be set to 32 mg/L.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Foramsulfuron has no insecticidal activity and no effects on *Daphnia magna* have been observed. No additional testing with aquatic invertebrate species is needed.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

One reproductive study on *Daphnia magna* was performed. The active substance showed no chronic effects on the survival, growth or reproduction of the water flea at a concentration of 100 mg/L. Details of the study are provided in the following table.

Table 8.2.5.1-1: Reproductive toxicity data of foramsulfuron to *Daphnia magna* presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Daphnia magna (water flea)	chronic	21 d	NOEC **100**	[redacted], 1999 B002180 M-237962-01-2 KCA 8.2.5.1 /01

Bold letters: Values considered relevant for risk assessment in the MCP document

Report:	[redacted]7; [redacted]; [redacted]; 1999; M-237962-01
Title:	Effects on life-cycle of the water flea (*Daphnia magna*) in a static renewal system AE F130360 technical 95.8% w/w
Report No:	B002180
Document No(s):	Report includes Trial No's: CF99W237 M-237962-01-2
Guidelines:	**OECD: 202; USEPA (=EPA): 72-4(b);Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
NOEC > 100 mg/L

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

Foramsulfuron has no insecticidal activity and no chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is needed.

CA 8.2.5.3 Development and emergence in *Chironomus* species

Foramsulfuron has no insecticidal activity, is not a growth regulator, and no chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is needed.

CA 8.2.5.4 Sediment dwelling organisms

Foramsulfuron is highly water soluble and does not accumulate in the sediment. No testing with sediment dwelling organisms is triggered.

CA 8.2.6 Effects on algal growth

Potential effects of foramsulfuron on algal growth were investigated with four different algae species, a green alga, a blue-green alga and a freshwater and a marine diatom. The blue-green alga *Anabaena flos-aquae* was found to be, by a factor of 10, more sensitive than other algae species. The EC_{50} of foramsulfuron for this species is 8.1 mg a.s./L.
For metabolites AE F092944 and AE F0899095 studies were performed with green algae where in both cases the EC_{50} was above the highest tested dose level (EC_{50} > 560 and 100 mg/L, respectively) – and also clearly above the respective EC_{50} for green algae of the parent compound.

Table 8.2.6-1: Growth effect data of foramsulfuron and its metabolites to algae presented in this chapter

Test species	Test system	Test duration	Endpoint	[mg as/L]	Reference
Foramsulfuron-sodium					
Pseudokirchneriella subcapitata (syn. *Selenastrum capricornutum*) (green algae)	growth inhibition	72 h	E_rC_{50} [1)]	71.9	[redacted], 1998 A59926 M-143574-01-1 KCA 8.2.6.1 /01
		96 h	E_rC_{50} [1)]	86.2	
Navicula pelliculosa (diatom)	growth inhibition	72 h /96 h	E_rC_{50}	> 112	[redacted], 1999 C002422 M-184469-01-1 KCA 8.2.6.2 /01
Anabaena flos-aquae (blue-green algae)	growth inhibition	72 h	E_rC_{50} [1)]	8.1	[redacted], 1999 C003699 M-186627-01-1 KCA 8.2.6.2 /02
		96 h	E_rC_{50} [1)]	**8.1**	
Skeletonema costatum (marine diatom)	growth inhibition test	72 h /96 h	E_rC_{50}	> 105	[redacted], 1999 C002436 M-184494-01-1 KCA 8.2.6.2 /03
AE F092944					
Desmodesmus subspicatus (syn. *Scenedesmus subspicatus*) (green alga)	growth inhibition	72 h	E_rC_{50}	**> 560**	[redacted], 1993 A50395 M-131421-01-1 KCA 8.2.6.1 /02

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
AE F099095				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50} **> 100**	[redacted], 2005, M-254084-01-1 KCA 8.2.6.1 /03

Bold letters: Values considered relevant for risk assessment in the MCP document

[1)] Since the new aquatic GD[3] focusses on endpoints based on growth rates the old E_bC_{50} figures were omitted from the table above.

CA 8.2.6.1 Effects on growth of green algae

For foramsulfuron and its metabolites AE F092944 and AE F099095 aquatic toxicity studies on green algae, *Pseudokirchneriella subcapitata* or *Desmodesmus subspicatus*, were performed. Details of all studies are provided in table 8.2.6-1.

Studies on foramsulfuron

Report:	[redacted]h; [redacted]; [redacted];1998;M-143574-01
Title:	Effect to *Pseudokirchneriella subcapitata* (green alga) in a growth inhibition test AE F130360 (technical) 94.2% w/w
Report No:	A59924
Document No:	M-143574-01-1
Guidelines:	**OECD: 201, USEPA (=FIFRA): 40 CFR Part 160; Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Studies on the metabolites of foramsulfuron

AE F092944

Report:	[redacted]8; [redacted];1993;M-131421-01
Title:	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to *Scenedesmus subspicatus* (Green alga) in a Growth Inhibition Test (method OECD)
Report No:	A50395
Document No:	M-131421-01-1
Guidelines:	**OECD: 201 (1984); Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE 092944 00 ZD99 0001; purity ≥ 99.0%) to *Scenedesmus subspicatus*.
Cultures of *Scenedesmus subspicatus* with an initial cell density of 10 000 cells/mL were exposed in a static system over a period of 72 hours to nominal concentrations of 10, 18, 32, 56, 100, 180, 320, and 560 mg/L. In addition a water control and a solvent control were tested.

[3] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290

24, 48 and 72 hour growth rate based on cell density and visual assessment of potential cell deformations were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour-E_rC_{50} was > 560 mg/L, the 96-hour-NOEC was determined to be 56 mg/L.

Materials and Methods:

Test material: Hoe 092944 technical; purity: > 99.0%, Code: Hoe 092944 00 ZD99 0001, Analytical certificate No.: AZ 04888;
Green alga (*Scenedesmus subspicatus*) was exposed to 2-amino-4,6-dimethoxypyrimidine (code: AE 092944 00 ZD99 0001) in a static system over a period of 72 hours. Nominal concentrations were 10, 18, 32, 56, 100, 180, 320, and 560 mg/L. In addition, a water control and a solvent control were tested. Each vessel (Erlenmeyer flasks; 300 mL) served as one replicate filled with 100 mL test solution. At test initiation the cell density was 10 000 cells/mL. The test was conducted with 3 replicates per treatment level. In the controls 6 replicates were tested.
For analytical verification samples were taken at 0 and 72 hours from all concentrations from test solutions with 18 mg/L. High-performance liquid chromatography (HPLC) was used as analytical method.
Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section.

Dates of experimental work: August 11, 1992 – August 14, 1992

Results:

Validity criteria:
The validity criterion of cell density increase >16x in the controls is fulfilled.

Analytical findings:
Analytical verification of test solutions revealed measured concentrations of AE F092944, calculated as an arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table 8.2.6.1-2: Nominal and measured concentrations of AE F092944

Nominal concentration	Concentration (mg/L)	Day 0 (New)		Day 3 (Old)		Mean	
		Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal
18 mg/L	17.82	17.5	98.2	17.11	96.0	17.31	97.1

Biological findings:
Observations on growth rates are listed as follows:

Table 8.2.6.1-3: Effect of AE F092944 on growth-inhibition of *Scenedesmus subspicatus*

Nominal treatment level (mg/L)	% inhibition according to mean area under the growth curve after 72 h	% inhibition according to mean growth rate after 72 h
Control	-	-
Solvent control	-0.02	2.5
32	-1.9	0.8
56	-3.6	0.6
100	-7.4	0.2
180	22.4	7.9
320	37.4	11.2
560	67.6	26.7

No cell abnormalities were observed.

Biological endpoints derived:
From the results presented above the following biological endpoints can be derived:

72-hour-figures (growth rate):
EC_{50} - area under the growth curve: 403 mg/L (95% confidence limits 320 - 560 mg/L)
EC_{50} - growth rate: > 560 mg/L
NOEC: 56 mg/L

Conclusions:

The effect of AE F092944 (2-amino-4,6-dimethoxypyrimidine; AE 092944 00 ZD99 0001) on *Scenedesmus subspicatus* can be quantified as a 72-hour-E_bC_{50} of > 560 mg/L. The highest concentration with no observed growth inhibition and no cell deformations can be set to 56 mg/L. E_bC_{50} = 403 mg/L.

AE F099095

Report:	[redacted]0; [redacted];2005;M-254084-01
Title:	*Pseudokirchneriella subcapitata* - growth inhibition test with AE F099095 00 1B99 0001
Report No:	EBMMX092
Document No:	M-254084-01-1
Guidelines:	**Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (Feb. 18, 2004);none**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the influence of AE F099095 on exponentially growing *Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) expressed as NOEC; LOEC and EC_x for growth rate of algal biomass (cells per volume). The study was designed to meet OECD criteria. *Pseudokirchneriella subcapitata* were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg p.m.(pure metabolite)/L in comparison to an untreated control. Three replicate vessels per test level and six replicate vessels for the control were used.
Cell numbers per volume (as a surrogate for biomass per volume) were estimated photometrically at day 1, 2 and 3 of the exposure period. To detect possible cell deformations, samples were examined under a microscope. Based on analytical findings, the biological endpoints are reported as nominal

figures. The (0 – 72 h)-E_rC_{50} was > 100 mg p.m./L, the (0 – 72 h)-NOE_rC was determined to be 25 mg p.m./L.

Material and methods:

Test item. AE F099095 00 1B99 0001; Batch No.: KR363/364; purity: 99.6 % w/w; certificate of analysis-No.: AZ 10810; Analytical reference-No.: 0305473.
Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg p.m.(pure metabolite)/L in comparison to control(s). The pH values ranged from 7.7 to 8.5 in the controls and the incubation temperature ranged from 22.4°C to 23.4°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6619 lux.
Quantitative amounts of AE F099095 were measured in all treatment groups and in the control(s) on day 0 and day 3 of the exposure period.

Dates of experimental work: January 20, 2005 - May 23, 2005

Results:

Validity Criteria:
The test conditions met all validity criteria given by the mentioned guideline.

Analytical findings:
The analytical findings of AE F099095 in the treatment levels found on day 0 were 96 to 102 % of nominal (average 98.0 %). On day 3 analytical findings of 96 to 103 % of nominal (average 99.6 %) were found. All results are based on nominal test concentrations.

Table 8.2.6.1-4: Concentrations of AE F099095 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg AE F099095/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.0110	<0.0110	<0.0110	--
6.25	6.06	5.92	5.99	96
12.5	12.0	12.3	12.2	97
25	25.5	25.4	25.4	102
50	49.4	48.7	49.1	98
100	97.0	96.1	96.5	97
			Mean	98.0

Table 8.2.6.1-5: Concentrations of AE F099095 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg AE F099095/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.0110	<0.0110	<0.0110	--
6.25	6.14	6.32	6.23	100
12.5	12.0	12.1	12.0	96
25	25.0	24.9	25.0	100
50	51.8	50.7	51.3	103
100	98.6	99.2	98.9	99
			Mean	99.6

Biological findings:
Observations on growth rates are listed as follows:

Table 8.2.6.1-6: Inhibitory effects

Nominal initial 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 (%)	Doubling time of algae cells (days)
Control	812 000	1.466	--	0.473
6.25	751 000	1.439	1.8	0.482
12.5	806 000	1.463	0.2	0.474
25	788 000	1.455	0.7	0.476
50	727, 000	1.428	2.5	0.485
100	660 000	1.396	4.7	0.497

test initiation with 10 000 cells/mL

Conclusions:

The (0 - 72h)-E_rC_{50} for AE F099095as >100 mg pure metabolite /L and the (0 - 72h)-NOE_rC is 25 mg pure metabolite /L (based on nominal initial concentrations).

CA 8.2.6.2 Effects on growth of an additional algal species

For foramsulfuron, aquatic toxicity studies on three additional algal species, *Anabaena flos-aquae*, *Navicula pelliculosa* and *Skeletonema costatum*, were performed.

Report:	[redacted]2; [redacted]; [redacted];1999;M-184469-01
Title:	Effect to *Navicula pelliculosa* (freshwater diatom) in a growth inhibition test AE F130360 technical 94.8% w/w Code: AE F130360 00 1C94 0001
Report No:	C000122
Document No:	M-184469-01-1
Guidelines:	**OECD 201; USEPA (=EPA): 122-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted]g; [redacted]; [redacted];1999;M-186627-01
Title:	Effect to *Anabaena flos-aquae* (blue-green alga) in a growth inhibition test technical 94.6% w/w Code: AE F130360 00 1C94 0001
Report No:	C003699
Document No:	M-186627-01-1
Guidelines:	**OECD: 201; USEPA (=EPA): 123-1;Deviation not specified**
GLP/GEP:	**yes**

The new aquatic guidance document (EFSA 2013[4]) only regards endpoints based on growth rates as relevant. Therefore the biomass based endpoint of EC_{50} = 3.3 mg/L according to the Review Report for foramsulfuron (SANCO/10324/2002-Final) has to be revised and to be replaced by 8.1 mg/L.

Report:	[redacted]b; [redacted]; [redacted];1999;M-184494-01
Title:	Effect to *Skeletonema costatum* (Marine Diatom) in a growth inhibition test AE F130360 technical 94.6% w/w Code: AE F130360 00 1C94 0001
Report No:	C002436
Document No:	M-184494-01-1
Guidelines:	**OECD: 201; USEPA (=EPA): 122-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

CA 8.2.7 Effects on aquatic macrophytes

For foramsulfuron, toxicity studies on different aquatic macrophytes were performed. Besides *Lemna gibba*, also *Myriophyllum spicatum* was tested as a second macrophyte species. In addition, an outdoor growth inhibition study was performed with a total of ten species representing different taxonomic groups. Since *Lemna gibba* turned out to be the most sensitive species to foramsulfuron, higher-tier studies (recovery, peak exposure, long-term exposure) were performed with this species.
Studies investigating the toxicity to *Lemna gibba* were also performed for all metabolites of the residue definition for risk assessment in surface water. It was found that one metabolite, AE F130619, has a similar activity to *Lemna* as the parent compound, while all other metabolites turned out to be non-toxic to these organisms.
Details of all studies are provided in the following table.

[4] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290

Table 8.2.7-1: Effect data of foramsulfuron and metabolites to aquatic macrophytes presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Foramsulfuron-sodium				
Lemna gibba (duck weed)	growth inhibition, static	7 d	E_rC_{50} [1)] **1.31 µg/L**	[redacted], 1998 A67514 [redacted], 1998 C002148 (amendment) M-147891-02-1 KCA 8.2.7 /01
Lemna gibba (duck weed)	growth inhibition + recovery	7 d + 14 d	NOEC 5 µg/L	[redacted], 2005 MO-05-007405 M-250268-01-1 KCA 8.2.7 /05
Lemna gibba (duck weed)	growth inhibition, peak exposure	1 d + 6 d	E_rC_{50} [1)] **> 56.7 µg/L**	[redacted], 2013 EBFSN003 M-462369-01-1 KCA 8.2.7 /06
Lemna gibba (duck weed)	growth inhibition, mimicking exposure of outdoor study	42 d	E_rC_{50} [1)] 0.00118	[redacted], 2013 EBFSL014 M-464150-01-1 KCA 8.2.7 /08
Aquatic macrophytes (10 species)	growth inhibition + recovery	2 d – 5.5 weeks	NOEC (6 weeks) 0.1 µg/L NOEC (48h peak) 4.1 µg/L	[redacted], 2012 EBFSL012 M-429538-01-1 KCA 8.2.7 /07
Myriophyllum spicatum (aquatic plant)	growth inhibition	14 d	EC_{50} > 84	[redacted] et al., 2012 EBFSL004 M-431270-01-1 KCA 8.2.7 /09
AE F153745				
Lemna gibba (duck weed)	growth inhibition	7 d	EC_{50} **> 100**	[redacted], 2000 B002765 M-240924-01-2 KCA 8.2.7 /02
AE 0338795				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] **27.2**	[redacted] 2000 B002774 M-238498-01-2 KCA 8.2.7 /03
AE F092944				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] **> 100**	[redacted], 2002 C003865 M-186916-01-1 KCA 8.2.7 /10

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
AE F099095				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] **> 100**	[redacted], 2005 EBMMX091 M-254496-01-1 KCA 8.2.7 /11
AE F130619				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] **0.889 µg/L**	[redacted], 2013 EBFSL011 M-452669-01-1 KCA 8.2.7 /12
4-Amino-N-methylbenzamide				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] **> 10**	[redacted], 2013 EBFSN010 M-464163-01-1 KCA 8.2.7 /13
4-Formylamido-N-methylbenzamide				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] **> 10**	[redacted] 2013 EBFSN011 M-464321-01-1 KCA 8.2.7 /14
Foramsulfuron-sulfamic acid				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} **> 10**	[redacted], 2013 EBFSN012 M-464386-01-1 KCA 8.2.7 /15

Bold letters: Values considered relevant for risk assessment in the MCP document

[1] Since the new aquatic GD [5] focusses on endpoints based on growth rates the old E_bC_{50} figures were omitted from the table above.

Studies on foramsulfuron

Report:	[redacted]v; [redacted]; [redacted];1998;M-147891-02; Amended: 1999-04-20
Title:	Effect to *Lemna gibba* (duckweed) in a growth inhibition test AE F130360 technical 96.1% w/w Code: AE F130360 00 1C960002
Report No:	96/7514
Document No(s):	Report includes Trial Nos.: CF98W507 M-147891-02-1
Guidelines:	**US EPA (=EPA): 122-2; Deviation not specified**
GLP/GEP:	**yes**

Since the new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant, the biomass based endpoint of EC_{50} = 0.00065 mg/L according to the Review Report for foramsulfuron (SANCO/10324/2002-Final) has to be revised and replaced by 0.00101 mg/L.

[5] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290

Report:	[redacted]x; [redacted]; [redacted];2000;M-193919-01
Title:	Effects on growth of rooted aquatic macrophytes (Valisneria spec.) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002
Report No:	C006439
Document No:	M-193919-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/10324/2002-Final).

Report:	[redacted]n; [redacted];2005;M-250268-01
Title:	*Lemna gibba* G3 Exposure and recovery test with Foramsulfuron (tech.) (code: AE F130360 00 1D97 0001)
Report No:	EBFSX010
Document No:	M-250268-01-1
Guidelines:	**OECD 221 "Leman sp. Growth Inhibition Test" Revised Proposal for a New Guideline (April 2004); none**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to determine the effects of the test item foramsulfuron on exponentially growing *Lemna gibba* G3 after static exposure of 7 days. *Lemna* cultures were cultivated for 7 days at 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg a.i./L under static conditions. In addition an untreated control was tested. 3 replicates were used per treatment level. Plant frond numbers and total frond area of plants were recorded after 0, 2 or 3, 4 or 5, and 7 days. Growth and growth inhibition in percent were calculated. In the second part of the study aliquots were transferred into freshly prepared test medium without the test item and the growth rates during the recovery phase were measured. Furthermore, recovery of visual effects of treated plants was evaluated. The growth rates for frond numbers and total front area fully recovered for all test levels (up to 20 µg a.s./L) within the first phase of the recovery period (day 7-14). The previously treated plants fully recovered up to the treatment level of 5 µg a.s./L within the second phase of the recovery period (day 14-21).

Material and methods:

Test item. Foramsulfuron (AE F130360) a.s.; Batch No.: AAIR04430; CAS No.: 173159-59-4; analysed content of a.s.: 97.3 % w/w; certificate No.: AZ 11043.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed under static conditions (7-day-exposure phase of the study) to the nominal concentrations of 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg a.s./L in comparison to control to reach a graduated inhibition of growth around the expected ErC_{50}. In the second part of the study (14-day recovery phase (post exposure phase)), aliquots (12 fronds/replicate) were transferred (by rinsing with deionised water) into freshly prepared test medium without foramsulfuron. As the recovery phase lasted 14 days, the growth medium was renewed on day 7 and the culture was re-started with 12 fronds of the recovery phase to prevent starvation. The pH values ranged from 7.4 to 8.8 in the controls and the incubation temperature ranged from 23.3°C to 24.4°C (measured in an additional incubated glass vessel) over the whole period of testing, at a continuous illumination of 7.29 klx.
Foramsulfuron was quantitatively measured in all freshly prepared test levels on day 0 and, additionally, in all aged test levels on day 7 of the exposure period. Additional measurements for

foramsulfuron were done for all test levels at day 2 of the recovery phase, to show that no unintended transfer of the test item into the recovery phase occurred.

Dates of experimental work: December 01, 2004 – March 10, 2005

Results:

Validity Criteria:
The test conditions met all validity criteria, given by the mentioned guideline.

Analytical findings:
Analytical measurements for AE F130360 found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 89% and 113% (average 104 %). In aged test levels on day 7 there were analytical findings between 94% and 112% (average 103 %) of nominal. As expected in samples taken on day 2 of the post exposure period, the test item was not detectable in any test level including the control. All reported results are based on nominal initial values of the active substance during the exposure period.

Table 8.2.7-2: Concentrations of AE F130360 in the test solutions

Day	Nominal concentration [µg a.s./L]	Detection 1 [µg a.s./L]	Detection 2 [µg a.s./L]	Mean [µg a.s./L]	% of nominal
0	Control	< 0.070	< 0.070	< 0.070	--
7		< 0.070	< 0.070	< 0.070	--
9*		< 0.070	< 0.070	< 0.070	--
0	0.625	0.694	0.714	0.704	113
7		0.705	0.630	0.667	107
9*		< 0.070	< 0.070	< 0.070	--
0	1.25	1.24	1.32	1.28	102
7		1.39	1.42	1.40	112
9*		< 0.070	< 0.070	< 0.070	--
0	2.50	2.46	2.65	2.55	102
7		2.29	2.39	2.34	94
9*		< 0.070	< 0.070	< 0.070	--
0	5.00	5.09	5.25	5.17	103
7		5.00	5.13	5.06	101
9*		< 0.070	< 0.070	< 0.070	--
0	10.00	8.84	8.94	8.89	89
7		9.58	10.4	10.0	100
9*		< 0.070	< 0.070	< 0.070	--
0	20.00	22.9	22.2	22.6	113
7		21.4	20.6	21.0	105
9*		< 0.070	< 0.070	< 0.070	--

lowest standard solution (concentration multiplied with the dilution factor of 1.25) of foramsulfuron used for determination: 0,070 µg/L
* day 2 post exposure

Biological findings:
Inhibitory effects and intoxication symptoms were observed as follows:

Table 8.2.7-3: Inhibitory effects and intoxication symptoms

nominal µg a.i./L	7 day - % inhibition growth rate frond #	7 days - % inhibition growth rate frond area	first day when full recovery acc. growth was observed	first day when full recovery acc. symptoms was observed
0.625	49.0	52.0	12	16
1.25	70.3	69.1	9	16
2.5	74.9	75.7	9	16
5	78.1	80.3	12	49
10	83.6	84.0	12	
20	78.7	85.1	12	

The following observations were made: small fronds, deformed fronds and fronds clustered.

Conclusions:

The growth rates for frond number and total frond area fully recovered for all test levels (up to 20 µg a.s./L) within the first phase of the recovery period (study day 7-14).
Fronds fully recovered from all visual effects (reduction of size, deformation, decolouration and necrosis) up to 5 µg/L (formerly used test level) after 14 days.

Report:	[redacted];2013-M-462569-01
Title:	*Lemna gibba* G3 - Growth inhibition test with foramsulfuron (tech) (AE F 130360) under peak exposure conditions
Report No:	EBFSN003
Document No:	M-462569-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; Plants were firstly washed and then dipped into clean media. All plants were transferred.**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of foramsulfuron (code: AE F130360; purity 97.3 %) on the growth of duckweed (*Lemna gibba*) after a 24-hour peak exposure.
Cultures of *Lemna gibba* with an initial frond density of 12 fronds per vessel were exposed in a static system over a period of 7 days (1 day exposure; 6 days recovery) to nominal concentrations of 0.50, 1.10, 2.42, 5.32, 11.7, 25.8 and 56.7 µg a.s./L (corresponding to analytically verified concentrations of 115 % (mean at day 0) and 114 % (mean in aged solutions at day 1)). In addition a water control was tested.
Frond numbers and total frond area at each occasion were used to determine the endpoints. Based on analytical findings, the biological endpoints are reported as nominal figures. The EC_{50} regarding growth inhibition was >56.7 µg a.s./L for both, frond number and dry weight. The NOEC for growth during the period between day 2 and day 7 was determined to be 2.42 µg a.s./L.

Material and methods:

Test item: foramsulfuron tech. (AE F130360), analysed content of active substance: foramsulfuron tech. (AE F130360): 97.3 % w/w, specified by origin batch no: ELIR004294, specification number 102000011654, Tox No.: 09600-00.

Duck weed (*Lemna gibba*) was exposed to foramsulfuron (code: AE F130360; purity 97.3 %) in a static system over a period of 7 days (1 day exposure; 6 days recovery). Nominal concentrations were 0.50, 1.10, 2.42, 5.32, 11.7, 25.8 and 56.7 µg a.s./L . In addition a water control was tested. Each vessel (glass dishes; 470 mL) served as one replicate filled with 200 mL 20xAAP with an initial pH of 7.5 ± 0.1. At test initiation the number of fronds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level. Temperature was regulated at 24 ± 2°C.

Dates of experimental work: 20 FEB 2013 to 22 APR 2013

Results:

Environmental conditions:

Measurements of physical and chemical parameters of the test solutions are summarised as follows:

Test temperature:	mean 25.1°C (range: 25.0°C to 25.3°C)
pH:	7.5 to 8.5 during peak exposure (day 1) and 7.5 to 8.9 during recovery period.
Light intensity:	mean 6633 lux (range: 6501 to 6739 lux)

Analytical results:

Analytical verification of test solutions revealed measured concentrations of 115 % (mean at day 0) and 114 % (mean in aged solutions at day 1) calculated as arithmetic mean. Based on these analytical findings, the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table:

Table 8.2.7.4: Concentrations of AE F130360 in test solutions

Nominal test levels [µg form/L]	measured day 0		measured day 1 (aged media)		measured day 7 (old)	
	µg a.s./L	% nominal	µg a.s./L	% nominal	µg a.s./L	% nominal
control	<0.051		<0.051		<0.051	
0.5	0.587	117%	0.566	113%	<0.051	n.a.
1.1	1.28	116%	1.26	114%	<0.051	n.a.
2.42	2.94	122%	2.97	123%	<0.051	n.a.
5.32	6.38	120%	6.22	117%	<0.051	n.a.
11.7	12.9	110%	13.1	112%	<0.051	n.a.
25.8	28.5	111%	28.3	110%	<0.051	n.a.
56.7	62.6	110%	61.1	108%	<0.051	n.a.

Biological results:
Growth inhibition was observed as listed below.

Table 8.2.7-5: Survey of biological findings

Nominal test levels [µg a.s./L]	Final frond no. (replicate means, day 7)	Final total frond area of plants (replicate means) [mm^2]	% inhibition (growth rate for frond no.)	% inhibition (growth rate for total frond area of plants)
control	209.7	1643.7	-	-
0.5	214	1568.7	-0.8	1.2
1.1	180.3	1351.7	5.2	9
2.42	131.3	989	16.2	16.3
5.32	106.7	792	23.6	23.2
11.7	107.3	783.3	23.3	23.5
25.8	94	662	28.1	34.1
56.7	84.7	599	31.7	35.6

The validity criterion of a doubling time less than 60 hours (2.5 days) in the control is fulfilled.

Conclusions:

From the results presented above the following biological endpoints can be derived:

7-day-figures (growth rate frond number):
highest concentration with no effect (NOEC) (day 0-7): 0.5 µg a.s./L
highest concentration with no effect (NOEC) (day 2-7): 2.42 µg a.s./L
EC_{50}: > 56.7 µg a.s./L

7-day-figures (growth rate frond area):
highest concentration with no effect (NOEC) (day 0-7): 0.5 µg a.s./L
highest concentration with no effect (NOEC) (day 2-7): 2.42 µg a.s./L
EC_{50}: > 56.7 µg a.s./L

The EC_{50} regarding growth inhibition was > 56.7 µg a.s./L for both, frond number and dry weight. The NOEC for growth between day 2 and 7 was determined to be 2.42 µg a.s./L. After a 24-hour peak exposure up to 2.42 µg a.s./L the growth rate of duck weed does not differ significantly from an untreated control. Therefore, this NOEC can be regarded as relevant for the risk assessment.

Report:	[redacted]v; [redacted]2012;M-429538-01
Title:	Outdoor growth inhibition and recovery of aquatic plants exposed to foramsulfuron WG 50 percent
Report No:	EBFSI012
Document No:	M-429538-01-1
Guidelines:	**not applicable; not specified**
GLP/GEP:	**yes**

Executive Summary:

The objective of the study was to evaluate the toxicity of foramsulfuron WG 50% to ten aquatic plants in small, outdoor, replicated ponds under natural atmospheric conditions. Plants were placed in the ponds for a 1 to 4 week acclimation period prior to continuous exposure to nominal (initial measured) concentrations of 0.10, 0.25, 0.63, 1.6, 3.9, 9.8, 24 and 61 µg a.s./L (0.10, 0.25, 0.65, 1.6, 3.9, 9.7, 24

and 65 µg a.s./L). A 2-day exposure followed by a 5.5-week recovery phase was also conducted concurrently at nominal (initial measured) concentrations of 1.6 (1.6) and 3.9 (4.1) µg a.s./L for all ten species. In addition a deionized water control was tested. During the test duration of six weeks for *Nymphaea odorata*, the emergence of tubers was low in all ponds and the biomass collected at test termination was highly variable. Due to the inconsistency in emergence and growth, statistical analysis was not performed for *N. odorata*.
For all species exposed in the outdoor ponds and all biological endpoints measured, there were no significant differences when the 2-day peak exposures (e.g., 1.6 and 4.1 µg a.s./L initial measured concentrations) were compared to the untreated controls. However, there were statistical differences in the endpoints for some species when the 2-day peak exposures were compared to the respective treatment levels with 6-week exposure. The overall NOEC for the 2-day peak exposure followed by a 5.5 week growth in untreated water is 3.9 µg a.i./L (nominal) 4.1 µg a.s./L (initially measured).

Material and methods:

Test item. Foramsulfuron WG 50%; Batch No.: 2011-001810; CAS No.: 173159-57-4; Analysed purity: 52.2 % w/w; Expiry date: 15 April 2012.

Test species: Monocotyledon: Water weed (*Elodea canadensis*), Sago pondweed (*Stuckenia pectinata*, formerly *Potamogeton pectinatus*), Reed sweetgrass (*Glyceria maxima*), Arrowhead weed (*Sagittaria latifolia*); Dicotyledon: Water lily (*Nymphaea odorata*), Coontail weed (*Ceratophyllum demersum*), Variable milfoil (*Myriophyllum heterophyllum*), Water mint (*Mentha aquatic*), Fanwort (*Cabomba caroliniana*); Fern: Water fern (*Salvinia minima*). The selected plant species were chosen because they represent a wide range of freshwater aquatic habitats and they represent both monocotyledon and dicotyledon plants and one fern.
Thirty-two, square, 3000-L, outdoor, freshwater ponds (inside dimensions 230 cm x 230 cm x 60 cm deep) were constructed by stacking 15 cm x 15 cm x 240 cm pressure-treated timbers. The frames were lined with liners designed for use in aquatic horticulture. Each pond contained a 5 cm layer of sandy loam soil to serve as sediment. The percent sand:silt:clay of the soil was determined to be 75:19:6%, respectively, the percent organic matter was 5.2% and the pH was 6.9. Each pond was filled with approximately 1850 liters (25 cm depth) of unchlorinated well water and fortified in hardness to approximately 160 mg/L as $CaCO_3$. The ponds received full sunlight throughout the day. The covers were temporarily installed over the ponds when heavy rain was forecast, in order to prevent major dilution of the test solutions.
Each rooted species was planted in an appropriately sized plastic pot. The sandy loam, mixed in a 1:1 ratio by volume with commercial potting soil (e.g., Sun-Gro Coir® Metro Mix 560), was added as the substrate to the pots. Slow-release pelleted fertilizer (Scotts Osmocote PlusTM, 15-9-12) was added to mid-depth of the soil in each pot and the soil surface was covered with masonry sand. *Ceratophyllum*, which does not typically root in sediment, was placed in plastic mesh bags to contain the shoots, and the bag was anchored to the sediment with a small stone. The floating water fern, *Salvinia minima*, was placed in two, 30 cm diameter floating corrals to contain the plants.
Plants were placed in the ponds for a 1 to 4 week acclimation period prior to exposure to the test substance, as follows:

Table 8.2.7-6: Survey of species-specific characteristics of methods

Plant Species	Pot Diameter (cm)	Number Plants per Pot	Number Pots Per Pond	Total Number Plants per Pond
Elodea canadensis	20	5	3	15
Stuckenia pectinata	20	5	3	15
Glyceria maxima	30	3	3	9
Sagittaria latifolia	30	3	3	9
Nymphaea odorata	30	1	5	5
Ceratophyllum demersum	mesh bag	5	3	15
Myriophyllum heterophyllum	20	5	3	15
Mentha aquatica	30	5	3	15
Cabomba caroliniana	20	5	3	15
Salvinia minima	30 cm corral	20 (leaves)	2	40

Additional pots were planted and placed in two additional ponds containing the same water, and sediment, to serve as replacement plants for any plants that did not grow normally during the acclimation phase. Specific details concerning the culture of each species are provided below.

For analytical verification, a minimum of four water column samples were removed at test initiation and during weeks 2, 4 and 6 (test termination) from each pond. The sampling device collected a vertical water column sample from near the sediment to the water surface. The water samples for an individual pond were combined into the 20 L bucket assigned to that pond. A subsample of the composite sample was removed for analysis of foramsulfuron concentration. Additionally, a second set of water samples was collected from each composite sample and held frozen for future analysis, if necessary. The peak dose pond solutions were also collected and analysed on day 3, one day after the water exchange, to characterize remaining residues of foramsulfuron. Water samples were also collected from each pond during weeks 1, 3 and 5, as described above, and stored frozen for future analysis, if necessary.

Exposure, control and QC samples were analysed for foramsulfuron using a liquid chromatography/mass spectrometry (LC/MS/MS) procedure based on methodology validated at Smithers Viscient. The method validation study was conducted prior to the initiation of the test and established an average recovery of 97.7% ± 1.84% for foramsulfuron from pond water. The QC acceptance range was set at 80 to 120%. Conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation study.

On the day of test substance application, mean shoot length and mean shoot dry weight data was collected for each species. These values represented the initial shoot length and shoot dry weight values later used to calculate growth rates from test initiation to test termination.

The outdoor health observations were performed on submerged, emergent and floating plants during weeks 2, 4 and 6. An additional observation period at week one was conducted for *Salvinia* due to its rapid growth rate. In addition, the plants in the peak dose ponds were also observed on exposure days 2 and 7. Visual observations such as chlorosis, leaf curl and necrosis were recorded. Effects observed were rated as percentage effect against the control plants. The number of *Nymphaea odorata* leaves emerged from the water surface was counted weekly. Flowering was noted when observed for all plant species tested. Additionally, plants were inspected daily for caterpillars or other insects that may graze

or damage the plants, and the insects were removed if observed. General observations of the ponds were made weekly (e.g., water clarity, algal blooms). Additionally, plant cover was mapped on day 0 and at test termination (week 6). Water lost from the ponds due to evaporation was replaced when necessary in order to maintain the pond depth within 10% of the required depth, 35 cm. When filamentous algae were observed, it was noted and the algae carefully removed from the ponds.

Dates of exposure: May 31, 2011 – July 15, 2011

Results:

Environmental conditions:
The environmental conditions maintained throughout the test period were within acceptable limits for the growth and survival of the test species. Total rainfall during the exposure period was 22.5 cm. Due to the use of temporary covers, approximately 9.25 cm of rainfall was prevented from entering the ponds on several occasions between 9 June and 9 July 2011. The remaining rainfall entering the ponds (e.g., 13.25 cm) generally replenished water evaporated during the study. Water in the ponds did not evaporate more than 10% of the initial depth (e.g., 35 cm).

Analytical results:
Initial measured concentrations ranged from 99 to 110% of nominal concentrations and defined the treatment levels as 0.10, 0.25, 0.63, 1.6, 3.9, 9.7, 24 and 65 µg a.i./L. Initial measured concentrations of the Peak 1.6 and Peak 3.9 µg a.i./L treatments were both 100% of nominal concentrations and defined the treatment levels as 1.6 and 4.1 µg a.i./L.

Table 8.2.7-7: Measured concentrations of foramsulfuron (µg a.i./L) in pond water with static exposure over 6-weeks

Nominal Conc. (µg a.i./L)	Day 0	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
0.1	0.10	**100**	0.061	**61.25**	0.047	**47.25**	0.0383	**38.25**
0.25	0.25	**100**	0.145	**58.25**	0.1178	**45.75**	0.0833	**33.5**
0.63	0.63	**100**	0.3733	**59.67**	0.3133	**49.0**	0.2133	**34.0**
1.6	1.6	**100**	0.9467	**59.33**	0.7367	**46.0**	0.5133	**32.3**
3.9	3.9	**100**	2.35	**60.50**	1.95	**50.5**	1.3	**33.5**
9.8	9.7	**99**	6.0	**62.0**	5.0	**51.0**	3.25	**30.5**
24	24	**100**	14.5	**61.0**	12.0	**50.0**	7.4	**31.0**
61	65	**110**	38.5	**62.5**	31.5	**52.0**	19.0	**31.0**

Table 8.2.7-8: Measured concentrations of foramsulfuron (µg a.i./L) in pond water with peak exposure over 2-days

Nominal Conc. (µg a.i./L)	Day 0	% Nom.	Day 3	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
Peak 1.6	1.6	**100**	0.0707	4.43	0.07	4.37	0.057	3.57	0.0423	2.67
Peak 3.9	4.1	**100**	0.2267	5.8	0.2033	5.2	0.170	4.37	0.1167	2.97

Biological results:

The exposure concentrations in the following text are expressed as initial measured concentrations. The EC_{50} values and No-Observed-Effect Concentration (NOEC) values were calculated using nominal and initial measured concentrations for each species, with the exception of *Nymphaea odorata*.

Growth inhibition was observed as listed below.

Table 8.2.7-9: 6-week NOEC and EC_{50}-figures (µg a.i./L) for nine aquatic macrophytes tested in the outdoor ponds based on nominal concentrations

	Week 6 Mean Shoot Length		Week 6 Growth Rate Based on Mean Shoot Length		Week 6 Mean Shoot Dry Weight		Week 6 Growth Rate Based on Dry Weight	
	NOEC	EC_{50} (95% CL[a])	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Elodea canadensis	NC[b]	NC	NC	NC	0.10	1.5 (0.94-2.1)	0.10	1.5 (0.97-2.1)
Stuckenia pectinata	NC	NC	NC	NC	3.9	8.1 (6.9-9.5)	3.9	7.7 (6.5-9.0)
Glyceria maxima	24	>61 (NA[c])	24	38 (25-53)	61	>61 (NA[c])	61	60 (46-NA[d])
Sagittaria latifolia	1.6	>61 (NA[c])	1.6	2.4 (1.9-2.9)	3.9	5.7 (2.1-8.3)	3.9	4.6 (2.5-7.5)
Ceratophyllum demersum	NC	NC	NC	NC	61	>61 (NA[c])	61	21 (9.0-NA[d])
Myriophyllum heterophyllum	24	>61 (NA[c])	24	>61 (NA[c])	61	44 (34-54)	61	41 (31-50)
Mentha aquatica	61	>61 (NA[c])	61	>61 (NA[c])	61	>61 (NA[c])	61	>61 (NA[c])
Cabomba caroliniana	61	>61 (NA[c])	61	>61 (NA[c])	61	>61 (NA[c])	61	>61 (NA[c])
	Week 6 Mean Leaf Density		**Week 6 Growth Rate Based on Leaf Density**		**Week 6 Mean Leaf Dry Weight**		**Week 6 Growth Rate Based on Leaf Dry Weight**	
	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Salvinia minima	1.6[e]	2.8 (0.16-3.4)	1.6	5.5 (5.0-5.8)	1.6[e]	2.8 (1.4-3.3)	1.6[e]	2.8 (1.8-3.3)

[a] CL = Confidence level.

[b] NC = Not calculated and not a required endpoint for this species. Due to the constant branching or the fact that stems could not be associated with an individual plant, plant lengths were not measured.

[c] NA = Not applicable. EC_{50} value was empirically estimated, therefore 95% confidence limits could not be calculated.

[d] Corresponding 95% confidence interval could not be calculated.

[e] Due to substantial % inhibition at the higher treatment levels, the 1.6 µg a.i./L treatment was used as a conservative NOEC value.

Note: Due to the inconsistency in emergence and growth, statistical analysis was not performed for *Nymphaea odorata* (See Protocol Amendment #2 of the report).

Table 8.2.7-10: 6-week NOEC and EC_{50}-figures (µg a.i./L) for nine aquatic macrophytes tested in the outdoor ponds based on initial measured concentrations

	Week 6 Mean Shoot Length		Week 6 Growth Rate Based on Mean Shoot Length		Week 6 Mean Shoot Dry Weight		Week 6 Growth Rate Based on Dry Weight	
	NOEC	EC_{50} (95% CL[a])	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Elodea canadensis	NC[b]	NC	NC	NC	0.10	1.5 (0.96-2.2)	0.10	1.5 (0.94-2.1)
Stuckenia pectinata	NC[b]	NC	NC	NC	3.9	8.0 (6.8-9.5)	3.9	7.7 (6.7-9.0)
Glyceria maxima	24	>65 (NA[c])	24	39 (26-57)	65	>65 (NA[c])	65	64 (48-NA[d])
Sagittaria latifolia	1.6[c]	>65 (NA[c])	1.6	2.3 (1.8-2.9)	3.9	5.7 (3.1-8.1)	3.9	4.6 (2.5-7.8)
Ceratophyllum demersum	NC[b]	NC	NC	NC	65	>65 (NA[c])	65	21 (7.7-NA[d])
Myriophyllum heterophyllum	24	>65 (NA[c])	24	>65 (NA[c])	65	46 (35-57)	65	43 (31-52)
Mentha aquatica	65	>65 (NA[c])	65	>65 (NA[c])	65	>65 (NA[c])	65	>65 (NA[c])
Cabomba caroliniana	65	>65 (NA[c])	65	>65 (NA[c])	65	>65 (NA[c])	65	>65 (NA[c])
	Week 6 Mean Leaf Density		**Week 6 Growth Rate Based on Leaf Density**		**Week 6 Mean Leaf Dry Weight**		**Week 6 Growth Rate Based on Leaf Dry Weight**	
	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Salvinia minima	1.6[e]	2.8 (0.33-3.4)	1.6	5.5 (4.9-5.8)	1.6[e]	2.8 (1.8-3.3)	1.6[e]	2.8 (1.6-3.2)

[a] CL = Confidence level.

[b] NC = Not calculated and not a required endpoint for this species. Due to the constant branching or the fact that stems could not be associated with an individual plant, plant lengths were not measured.

[c] NA = Not applicable, EC_{50} value was empirically estimated, therefore 95% confidence limits could not be calculated.

[d] Corresponding 95% confidence interval could not be calculated.

[e] Due to substantial % inhibition at the higher treatment levels, the 1.6 µg a.i./L treatment was used as a conservative NOEC value.

Note: Due to the inconsistency in emergence and growth, statistical analysis was not performed for *Nymphaea odorata* (See Protocol Amendment #2 of the report).

During the exposure phase for *Nymphaea odorata*, the emergence of tubers was low in all ponds and the biomass collected at test termination was highly variable. Due to the inconsistency in emergence and growth, statistical analysis was not performed for *N. odorata*.

For all species exposed in the outdoor ponds and all biological endpoints measured, there were no significant differences when the 2-day peak exposures (e.g., 1.6 and 4.1 µg a.s./L initial measured concentrations) were compared to the untreated controls. However, there were statistical differences in

the endpoints for some species when the 2-day peak exposures were compared to the respective treatment levels with 6-week exposure. The overall NOEC for the 2-day peak exposure followed by a 5.5 week growth in untreated water is 3.9 µg a.s./L (nominal) 4.1 µg a.s./L (initially measured).

Conclusions:

The initial measured concentrations of foramsulfuron in the treated ponds closely approximated the desired nominal concentrations indicating each pond was dosed correctly. After six weeks of exposure, the concentrations of foramsulfuron declined to approximately 30 to 40% of the nominal concentrations. The 6-week geometric mean measured concentrations ranged from 54 to 58% of nominal concentration, indicating continuous, measurable concentrations of foramsulfuron were present in all treatments throughout the six week exposure. The peak dose exposure ponds which were renewed with untreated water on day 2, resulted in a > 90% reduction in test concentrations on day 3 and slowly declined for the remaining five weeks of testing.

Seven of the ten aquatic plants exposed to foramsulfuron WG 50% in outdoor ponds indicated sensitivity in reduced plant biomass or morphological abnormalities over the range of concentrations tested. Based on initial measured concentrations and the lowest NOEC and EC_{50} values, 0.10 µg a.s./L and 1.5 µg a.s./L, respectively, water weed (*Elodea canadensis*) was the most sensitive plant tested. Based on a comparison of the EC_{50} values for the nine species tested in outdoor plants, the most sensitive to least sensitive species rank as follows: *Elodea canadensis* < *Sagittaria latifolia* < *Salvinia minima* < *Stuckenia pectinata* < *Ceratophyllum demersum* < *Glyceria maxima* < *Myriophyllum heterophyllum* < *Mentha aquatica* < *Caloomba caroliniana*. The EC_{50} values ranged from 1.5 µg a.s./L to > 65 µg a.i./L.

Recovery was observed in the peak dose ponds which underwent a 2-day exposure followed by a 5.5-week recovery period for the following species: *Elodea canadensis*, *Salvinia minima*, and *Sagittaria latifolia*, based on statistical comparisons of the continuous dose vs. peak dose data. Recovery could not be assessed for the remaining six species, since they were generally unaffected at the continuous dose and equivalent peak dose concentrations. The overall NOEC for the 2-day peak exposure followed by a 5.5 week growth in untreated water is 3.9 µg a.s./L (nominal) 4.1 µg a.s./L (initially measured).

In general, the health and survival of the control plants for each species indicated the exposure systems were appropriate for use. Additionally, the results demonstrated that the plant species selected were appropriate to detect responses to the test substance.

Report:	[redacted]b; [redacted]2013;M-464150-01
Title:	*Lemna gibba* G3 - Prolonged growth inhibition test with foramsulfuron (AE F130360) with stepwise decreasing concentrations over an 6 week test duration
Report No:	EBFSL014
Document No:	M-464150-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; none**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the long-term influence over a total period of six weeks of the test item foramsulfuron 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. The objective of this study was to obtain 6-week endpoints for *Lemna gibba* by mimicking the outdoor-

concentrations under laboratory conditions. These endpoints are directly comparable to endpoints obtained from the outdoor-pond study (KCA 8.2.7 /07; [redacted].; 2012; M-429538-01-1).

Material and methods:

Test item: foramsulfuron tech. (AE F130360), analysed content of active substance: foramsulfuron tech. (AE F130360): 97.3 % w/w, specified by origin batch no: ELIR004294, specification number 102000011654, Tox No.: 09600-00.
3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for six times 7 days under static exposure conditions to the nominal concentrations of the following listed concentrations. The concentrations were derived from the analytical results of an outdoor pond-study (*Lemna gibba* could not be tested under outdoor-conditions).
The objective of this study was to obtain 6-week endpoints for *Lemna gibba* by mimicking the outdoor-concentrations under laboratory conditions.
After each week, preferably 12 fronds were transferred into the respective following concentration (e.g. fronds from the samples of 3.20 µg/L, the highest concentration of week 1 were transferred into the replicates of 2.50 µg/L, the highest concentration of week 2, fronds from the test concentration of 0.20 µg/L, the lowest concentration of week 1, were transferred into the replicates of 0.080 µg/L, the lowest concentration of week 2, etc. In cases where the number of fronds after a 7-day period was below 12 due to damages caused by the tested substance only the remaining fronds were transferred.

Table 8.2.7-11: Intended concentrations per week and treatment level

nominal **initial** test levels foramsulfuron [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1*	100	78.1	60.3	54.0	48.4	40.3
0.20	0.20	0.156	0.121	0.108	0.097	0.081
0.40	0.40	0.312	0.241	0.216	0.193	0.161
0.80	0.80	0.624	0.483	0.432	0.387	0.322
1.60	1.60	1.25	0.965	0.864	0.774	0.644
3.20	3.20	2.50	1.93	1.73	1.55	1.29

* Percentage figures in this row were obtained from analytical measurements in the outdoor pond study. The intended concentrations were derived as the respective percentages of each nominal initial concentration.

Dates of experimental work: November 19, 2012 – June 27, 2013

Results:

Environmental conditions:
Temperature varied between 23.8 and 24.4°C. pH varied between 7.5 and 7.7 at the start of each 7-day period and between 8.5 and 9.2 at the end of each 7-day period. Mean light intensity was 8038 Lux.

Analytical results:

Table 8.2.7-12: Analytical findings of foramsulfuron

	day 0	day 7
week1	103 and 114 % (average 109 %)	108 and 116 % (average 111 %)
week 2	105 and 114 % (average 110 %)	110 and 135 % (average 123 %)
week 3	160 and 165 % (average 162 %)	159 and 182 % (average 171 %)
week 4	105 and 108 % (average 106 %)	107 and 121 % (average 115 %)
week 5	108 and 160 % (average 126 %)	108 and 161 % (average 130 %)
week 6	104 and 106 % (average 106 %)	106 and 116 % (average 109 %)

Biological results:

According to the objective of this study the endpoints were referred to nominal, initial test concentrations and not to weekly treatment levels. As in weeks three and five the analytical recovery was > 120% concentrations were expressed as mean measured, while in the other weeks nominal figures were used.

Table 8.2.7-13: Weekly inhibition with regard to the mean growth rates of frond numbers

nominal **initial** test levels	% inhibition of mean growth rate of **frond numbers**					
Foramsulfuron [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	78.1	60.3	54.0	48.4	40.3
control	--	--	--	--	--	--
0.20	9.3	-2.0	3.9	5.0	3.5	10.2
0.40	25.0	6.2	12.7	10.2	8.0	10.3
0.80	49.3	47.9	53.8	35.5	30.8	16.5
1.60	57.6	69.2	80.2	75.4	84.3	76.9
3.20	64.6	85.6	95.2	89.2	98.2	99.3
NOEC	<0.20	0.40	0.199	<0.20	0.257	<0.20
EC50	1.22	1.08	0.813	1.03	0.579	**1.18**

Table 8.2.7-14: Weekly inhibition with regard to the mean growth rates of frond areas

nominal **initial** test levels	% inhibition of mean growth rate of **frond area**					
foramsulfuron [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	78.1	60.3	54.0	48.4	40.3
control	--	--	--	--	--	--
0.20	6.4	1.0	1.2	2.0	3.3	9.2
0.40	24.1	15.0	8.5	3.7	0.6	6.1
0.80	54.2	61.7	61.8	24.4	20.5	12.0
1.60	64.7	86.4	91.9	96.3	79.8	75.9
3.20	74.4	92.2	100.4	118.2	95.9	98.0
NOEC	<0.20	0.40	0.199	1.60	0.257	n.d.
EC50	0.960	0.712	0.728	0.975	0.644	**1.23**

Conclusions:

The six week exposure of *Lemna gibba* to foramsulfuron led to decreasing effects when the dissipation of foramsulfuron in a static water-sediment system is mimicked.

Based on initial nominal concentrations the following **6-week endpoints** can be derived:

Table 8.2.7-15: Endpoints obtained after the 6-week test period

6-week end point	mean growth rate	
	effect on frond no [µg a.s./L]	effect on total frond area of plants [µg a.s./L]
EC_{50} (CI 95%)	**1.18** (0.746 – 1.77)	**1.23** (0.903 – 1.56)
EC_{20} (CI 95%)	0.830 (0.200 – 1.10)	0.901 (0.429 – 1.13)
EC_{10} (CI 95%)	0.691 (0.0901 – 0.956)	0.691 (0.277 – 0.998)
LOEC	<0.20	<0.20
NOEC	<0.20	<0.20

n.d.: not determined due to mathematical reasons or inappropriate data

Report:	[redacted];[redacted];[redacted];2012;M-431270-01
Title:	Toxicity of foramsulfuron technical to the aquatic macrophyte, *Myriophyllum spicatum*
Report No:	EBFSL004
Document No:	M-431270-01-1
Guidelines:	**OCSPP Guideline Number 850.SUPP; not specified**
GLP/GEP:	**yes**

Executive Summary

The objective of this study was to determine the dose-response effect of foramsulfuron to the rooted aquatic macrophyte, *Myriophyllum spicatum*, over an exposure period of 14 days under static conditions.
5 plants (thinned to 3 shoots on day 0) per replicate (3 replicate test vessels per treatment group) were exposed to nominal (geometric mean measured) concentrations of control (< LOQ), 1.0 (1.1), 3.0 (3.4), 9.0 (10), 27 (30) and 81 (84) µg a.s./L. 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. Toxicity values were calculated based on mean measured concentrations. The statistical NOEC, LOEC and E_yC_{50} for all endpoints were 84, > 84 and > 84 µg a.s./L, respectively.

Material and Methods:

Test item: Foramsulfuron (technical); Batch code: AE F130360-01-01; Origin Batch No.: ELIR004130; CAS No.: 173159-57-4; Customer Order No: Tox-No: 09032-00; LIMS No.: 1014240; analysed purity: 97.6%; certificate No.: AZ 16624.

Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed to a control (< LOQ) and to nominal (mean measured) concentrations of 1.0 (1.1), 3.0 (3.4), 9.0 (10), 27 (30), and 81 (84) µg a.s./L for 14 days under static conditions. Mean measured concentrations are determined based on results of the recoveries from days 0, 7, and 14 sampling and ranged from 104 to 113% of the nominal concentration. The toxicity values were calculated based on these mean measured concentrations.
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. Dissolved oxygen content and pH value were measured on days -7, 0, 7 and 14. Visual observations were conducted on a daily basis.
Following 14 days of exposure, all plants were removed from the test system. Length of the main shoot and all side shoots was measured, wet weights were measured, and following drying of plants for at least 72 hours, dry weight measurements were collected. Temperature during the test ranged between 19.37 and 20.51 °C, pH was 7.9 to 9.9, the photoperiod was 16 hours light : 8 hours dark and the light intensity was 9270 to 12,330 lux (mean = 10,443 lux). All test vessels were contained in an environmentally controlled study area.
Yield (NOEC, LOEC and EC_x) of total shoot lengths, total plant wet weight and total plant dry weight were the parameters measured in the study.

Dates of experimental work: September 29, 2011 – October 13, 2011

Results:

Validity Criteria:
Not applicable, higher tier study.

Analytical findings:
The concentration of the test item was stable within the test vessels during the 14 day exposure period (within 20% of initial measured concentrations).

Biological findings:
Active growth of the control plants during the 14 day exposure period was demonstrated by an average total shoot length yield of approximately 33.5 cm. Plants in the control vessels and all treatment groups appeared normal throughout the study. At study termination roots and shoots appeared normal in the controls. In the 1.1, 3.4, 10, and 30 µg a.s./L treatment groups, the plant shoots appeared normal, but brown tips on the roots were observed on 15 of 36 plants within various test replicates throughout these treatment groups. In the 84 µg a.s./L treatment group; six plants, throughout all test replicates, were observed as having brown tips on the roots and six plants, throughout all test replicates, were observed to have brown terminal buds on the side shoots. However, growth data for all plants was included in the data analysis.

Total shoot length growth rate:
Shoot length yield was analysed at test termination on study day 14. Data analysis showed no statistically significant difference, in comparison to the control data, in any of the treatment levels. Percent inhibitions as compared to the control group were 15.1, 13.1, -1.7, 19.6 and 13.6% for the 1.1, 3.4, 10, 30, and 84 µg a.s./L test groups, respectively.

Total plant wet weight growth rate:
Total plant wet weight yield was analysed at test termination on study day 14. Data analysis showed no statistically significant difference, in comparison to the control data, in any of the treatment levels. Percent inhibitions, as compared to the control group, were 2.1, -10.5, -18.5, 2.1, and 10.1% for the 1.1, 3.4, 10, 30, and 84 µg a.s./L test groups, respectively.

Total plant dry weight growth rate:
Plant dry weight yield was analysed at test termination on study day 14. Data analysis was performed utilizing a one tailed test, one sided distribution which will not capture the differences; showing no statistically significant difference, in comparison to the control data, in any of the treatment levels. Percent inhibitions, as compared to the control group, were -7.5, -18.4, -18.0, -32.7, and -20.3% for the 1.1, 3.4, 10, 30, and 84 µg a.s./L test groups, respectively.

Table 8.2.7-16: Toxicity to *Myriophyllum spicatum*

Test Substance	Foramsulfuron technical		
Test Object	*Myriophyllum spicatum*		
Exposure	14 Day – Static Exposure		
Endpoint Units	(µg a.i./L)		
Endpoint results	Day 14 Shoot Length Yield	Day 14 Wet Weight Yield	Day 14 Dry Weight Yield
Highest Concentration Without an Effect (NOEC)	84	84	84
Lowest Concentration With an Effect (LOEC)	> 84	> 84	> 84
E_yC_{50}	> 84	> 84	> 84

Conclusions:

Statistical analysis of the growth data of shoot length, wet weight and dry weight yield indicated no statistical differences from the controls. The statistical NOEC, LOEC and E_yC_{50} for all endpoints were 84, > 84 and > 84 µg a.i./L, respectively.

Studies on the metabolites of foramsulfuron

AE F153745

Report:	[redacted];2000;M-240924-01
Title:	Effect to Lemna gibba (duckweed) in a growth inhibition test: AE F153745 technical 97.8% w/w
Report No:	R002765
Document No(s):	Report includes Trial Nos.: CF99W565 M-240924-01-2
Guidelines:	**USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

AE 0338795

Report:	[redacted];2000;M-238498-01
Title:	Effect to Lemna gibba (duckweed) in a growth inhibition test: AE 0338795 technical 90.2 percent w/w; AE 0338795 00 1C90 0001
Report No:	R002764
Document No(s):	Report includes Trial Nos.: CF99W502 M-238498-01-2
Guidelines:	**USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

AE F092944

Report:	[redacted];[redacted];[redacted];2000;M-186916-01
Title:	Duckweed (*Lemna gibba* G3) growth inhibition test AE F092944 (metabolite of ethoxysulfuron and amidosulfuron) substance technical Code: AE F092944 00 IC99 0001
Report No:	C003865
Document No:	M-186916-01-1
Guidelines:	**ASTM: E 1415-91; OECD: Draft June 1998; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The objective of this test was conducted to determine the effect of the metabolite AE F092944 on a higher freshwater plant under semi-static conditions according to draft OECD guideline, US-EPA Pesticide Assessment Guidelines J 123-2 and according to ASTM E 1415-91 guideline under GLP.

Triplicate *Lemna* cultures with an initial frond number of 12 fronds per replicate were exposed to the test substance in 20X-AAP medium at five nominal treatment levels (i.e. 10, 18, 32, 56 and 100 mg/L). Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7.

Analyses of freshly prepared water for AE F092944 resulted in concentrations ranging from 94.0% to 103.2% of nominal values. Analyses of aged water for AE F092944 at experimental termination resulted in concentrations ranging from 93.9% to 102.6% of nominal values. Therefore, nominal treatment levels of AE F092944 are reported in this study.

The concentration of the test substance leading to a 50% inhibition of the growth regarding frond numbers (μ) in comparison to the untreated control (E_rC_{50}) after 7 days test duration was nominal >100 mg/L. The concentration of the test substance leading to a 50% inhibition of the growth regarding biomass (dry weight) increase (Δb) in comparison to the untreated control (E_bC_{50}) after 7 days test duration was nominal >100 mg/L. Intoxication symptoms were not observed.
A significant inhibition of growth both related on frond number or total biomass increase was not observed at a significance level of alpha = 0.05 at any treatment level.
The no observed effect concentration (NOEC), defined as no significant growth inhibition and no changes in plant appearance and development, was set to nominal 100 mg/L.

Material and methods:

Test item. AE F092944; Code: AE F092944 00 IC99 0001; analysed content: 99.8 % w/w; certificate No.: AZ 06326.

Three replicates of *Lemna gibba* G3 per test concentration with 12 fronds were exposed for 7 days under semi-static conditions to the nominal concentrations of 10, 18, 32, 56 and 100 mg/L in comparison to untreated control. Separate vessels were prepared for chemical analysis of the test substance. The test media were analysed for chemical and physical parameters (pH, temperature, oxygen content and conductivity) on day 0, 3, 5 and 7.
Although the freshly prepared test water was adjusted to pH 7.5 there was a deviation to pH 8.6 to 9.0 in the aged test water. The temperature ranged from 24.5°C to 25.0°C at a constant light intensity of 59.7 µE*m-2*s-1.
Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7.

Dates of experimental work: February 19, 1999 – February 26, 1999

Results:

Validity Criteria:
The validation results and chromatograms demonstrate sufficient reliability of the method for the desired application: The lowest concentration level is above the LOQ and all concentrations of the analyte solution prepared for HPLC are within the linearity range. The repeatability precision is sufficient expressed by a mean CV of duplicate determinations < 20 % for all concentration levels. The accuracy is within 80 - 120 % recovery with a CV < 20 %. The specificity of the method is sufficient: The chromatograms display no matrix interference > LOQ of the determined compound and their identity is established by co-chromatography with the corresponding certified reference substance.

Analytical findings:
Analyses of freshly prepared water for AE F092944 resulted in concentrations ranging from 94.0% to 103.2% of nominal values. Analyses of aged water for AE F092944 at experimental termination resulted in concentrations ranging from 93.9% to 102.6% of nominal values. Therefore, nominal treatment levels of AE F092944 are reported in this study.

Table 8.2.7-17: Analytical findings in test solutions

Nominal concentration [µg a.s./L]	Day	Fresh water		Aged water	
		[mg test item/L]	% of nominal	[mg test item/L]	% of nominal
Control	0	0.00	96.7*	0.00	97.5*
	3	0.00	97.5*	0.00	98.0*
	5	0.00	98.6*	0.00	99.3*
	Mean	0.00	97.6*	0.00	98.3*
	Variability	--	--	--	--
10.00	0	9.98	100.0	9.67	96.9
	3	9.38	94.0	9.37	93.9
	5	10.23	102.5	9.39	94.1
	Mean	9.86	98.8	9.48	95.0
	Variability	1.09	--	1.03	--
18.00	0	17.41	96.9	17.79	99.0
	3	17.63	98.1	17.76	98.9
	5	18.35	102.2	17.80	99.1
	Mean	17.80	99.1	17.79	99.0
	Variability	1.05	--	1.00	--
32.00	0	30.73	96.2	30.75	96.3
	3	32.30	101.1	31.16	97.6
	5	32.12	100.6	31.01	97.1
	Mean	31.72	99.3	30.97	97.0
	Variability	1.05	--	1.01	--
56.00	0	54.66	97.8	55.56	99.4
	3	55.84	99.9	52.83	94.1
	5	55.69	99.7	55.06	98.5
	Mean	55.40	99.1	54.40	97.3
	Variability	1.02	--	1.06	--
100.00	0	98.44	98.6	102.41	102.6
	3	103.02	103.0	99.34	99.5
	5	98.71	98.9	98.45	98.6
	Mean	100.06	100.3	100.07	100.3
	Variability	1.05	--	1.04	--

* Concurrent recovery rate of laboratory fortifications prepared

The test results are within 80 - 120 % of the nominal concentration and the variability is < 1.5.

Biological findings:

The concentration of the test substance leading to a 50% inhibition of the growth regarding frond numbers (µ) in comparison to the untreated control (E_rC_{50}) after 7 days test duration was nominal >100 mg/L.

The concentration of the test substance leading to a 50% inhibition of the growth regarding biomass (dry weight) increase (Δb) in comparison to the untreated control (E_bC_{50}) after 7 days test duration was nominal >100 mg/L.

Table 8.2.7-18: Mean values of absolute and percentual growth inhibition compared to the solvent control

treatment level (mg/L)	mean growth rate (d-1)	percentual inhibition of growth rate	mean increase in biomass (mg)	percentual inhibition of biomass increase
untreated control	0.374	0.00	19.4	0.00
10	0.373	0.31	20.3	-4.46
18	0.369	1.26	19.8	-2.06
32	0.370	1.03	19.3	0.69
56	0.387	-3.48	21.7	-11.84
100	0.377	-0.81	21.2	-8.92

No intoxication symptoms were observed.
A significant inhibition of growth both related on frond number or total biomass increase was not observed at a significance level of alpha = 0.05 at any treatment level.
The no observed effect concentration (NOEC) defined as no significant growth inhibition and no changes in plant appearance and development was set to nominal 100 mg/L.

Table 8.2.7-19: Comparison of specific growth rates (μ), doubling times (Td) and biomass increase (Δb) after 7 days test duration with DUNCAN's Multiple Range Test at a significance level of alpha = 0.05.

Concentration in mg/L	growth rate μ (d^{-1})		doubling time (d)		change of biomass Δb (mg)	
untreated control	0.374	A	1.854	A	19.4	A
10	0.373	A	1.861	A	20.3	A
18	0.369	A	1.879	A	19.8	A
32	0.370	A	1.879	A	19.3	A
56	0.387	A	1.795	A	21.7	A
100	0.377	A	1.840	A	21.2	A

Concentrations with the same letter within each column are not significantly different.

Conclusions:

The concentration of AE F092944 leading to a 50% inhibition of the growth regarding frond numbers (μ) in comparison to the untreated control (E_rC_{50}) after 7 days test duration was nominal >100 mg/L.
The concentration of test substance leading to a 50% inhibition of the growth regarding biomass (dry weight) increase (Δb) in comparison to the untreated control (E_bC_{50}) after 7 days test duration was nominal >100 mg/L.
A significant inhibition at a significance level of alpha = 0.05 of growth both related on both frond number and biomass increase was not observed up to a nominal concentration of 100 mg/L. which was the highest tested treatment level.
The no observed effect concentration (NOEC), defined as no significant growth inhibition and no changes in plant appearance and development, was nominal 100 mg/L.

AE F099095

Report:	[redacted]ü; [redacted];2005;M-254496-01
Title:	*Lemna gibba* G3 - growth inhibition test with AE F099095 under static conditions (Code: AE F099095 00 1B99 0001)
Report No:	EBMMX091
Document No:	M-254496-01-1
Guidelines:	**OECD 221 "Lemna sp. Growth Inhibition Test" Revised Proposal for a New Guideline (April 2004);only minor (see temperature measurements) not influencing the outcome of this study negatively**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the influence of AE F099095 on exponentially growing *Lemna gibba* G3 expressed as NOEC, LOEC and EC_x for growth rate of both response variables, frond number and total frond area of plants. The pH values ranged from 7.4 to 8.5 in the control and the incubation temperature ranged from 23.4°C to 26.2°C (measured in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels) over the whole period of testing at a continuous illumination of 7.03 klx. The measured values for the temperature ranged within typical tolerances of calibrated measuring devices and showed only slight deviations from defined guideline recommendations. This did not influence the outcome of the study negatively.
Plant frond numbers and total frond area of plants are recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 percent (EC_{50}) was determined where possible. The overall E_rC_{50} for AE F099095 was > 100 mg/L and the NOEC was <100 mg/L.

Material and methods:

AE F099095, (code: AE F099095 00 1B99 0001) purity: 99.6 % was tested, specified by batch-no.: KR363/3b4, certificate of analysis: AZ 10810.
3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7days under static exposure conditions to the nominal concentration of 100 mg pure metabolite/L in comparison to control. The pH values ranged from 7.4 to 8.5 in the control and the incubation temperature ranged from 23.4°C to 26.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7.03 klx.
Quantitative amounts of AE F099095 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.

Dates of experimental work: January 26, 2005 – May 24, 2005

Results:

Validity criteria:
Test conditions met all validity criteria, given by the mentioned guideline.

Analytical findings:
The analysed quantity of AE F099095 in the treatment level found on day 0 was 102 % of nominal. On day 7 an amount of 107 % of nominal was found.
All reported results are based on nominal initial values of the pure metabolite.

Table 8.2.7-20: Measured concentrations of AE F099095 in test solutions

Day	Nominal concentration [mg/L]	Actual concentration of AE F099095			
		Detection 1 [mg/L]	Detection 2 [mg/L]	Mean [mg/L]	% of nominal
0	Control	< 0.01102	< 0.01102	< 0.01102	--
7		< 0.01102	< 0.01102	< 0.01102	--
0	100.000	100.950	103.126	102.038	102
7		106.563	106.656	106.609	107

Lowest standard solution of AE F099095 used for determination: < 0.01102 mg/L

Growth rate:
Results for the effects of the static 7 day growth inhibition test are listed in the table below.

Table 8.2.7-21: Survey of biological findings and the derived inhibitions of growth rate

Nominal test levels [mg/L]	Final frond no. (replicate means, day 7)	Total frond area of plants (replicate means) [mm2]	% inhibition	
			Average growth rate for frond no.	Average growth rate for total frond area of plants
control	87	705	--	--
100	72	672	9.7	7.9

Observed visual effects:
Observed visual effects are listed in the table below.

Table 8.2.7-22: Survey of visual effects

Test level [mg/L]	Observations
Control	no visual effects observed
100	

The results based on nominal concentrations of the test item are shown in the table below.

Table 8.2.7-23: Survey of 7-day endpoints for AE F099095

End point (0-7 day)	Effect on frond no. [mg/L]	Effect on total frond area of plants [mg/L]
E_rC_{50}	**> 100**	**> 100**
LOE_rC	100	100
NOE_rC	< 100	< 100

Conclusions:

The overall E_rC_{50} for AE F099095 was > 100 mg/L in this study.
The $NOEC_r$ (< 100 mg/L) was based on statistical analysis.

AE F130619

Report:	[redacted] ·; [redacted] ;2013;M-452669-01
Title:	*Lemna gibba* G3 - Growth inhibition test with AE F130619 (metabolite of foramsulfuron) under static conditions
Report No:	EBFSL011
Document No:	M-452669-01-1
Guidelines:	**EU Council Directive 91/414/EEC; OECD Guideline 221 *Lemna* sp. Growth Inhibition Test (March 23, 2006); none**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the effects of AE F130619 on exponentially growing *Lemna gibba* G3 exposed under defined conditions for 7 days. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The lowest NOEC was 0.179 µg p.m./L.

Material and methods:

Test item: AE F130619 (metabolite of foramsulfuron) analysed purity: 94 % was tested, specified by batch code: AE F 130619-01-02, origin batch no.: SES 10641-3-3, CAS-No: 190520-75-3, certificate no.: AZ16327 and LIMS no.: 0936695.
3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditrons to the nominal concentrations of 0.179, 0.572, 1.84, 5.86 and 18.7 µg p.m./L in comparison to controls. The pH values ranged from 7.5 to 9.0 in the controls and the incubation temperature ranged from 23.9°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8914 lux.
AE F130619 was quantitatively determined in all freshly prepared test levels on day 0 and, additionally, in all aged test levels on day 7 of the exposure period.

Dates of experimental work: October 17, 2012 – November 22, 2012

Results:

Validity Criteria:
The study met all validity criteria requested by the mentioned guideline.

Analytical findings:
On day 0, between 84 and 110% (average 100%) of nominal were found. On day 7 there were analytical findings between 83 and 112% (average 101%) of nominal. Therefore all results are based on nominal concentrations of the metabolite.

Table 8.2.7-24: Measured concentrations of AE F130619 in test solutions

Nominal (µg/L)	measured day 0		measured day 7 (old)	
	µg a.i./L	% nominal	µg a.i./L	% nominal
control	<0.0106	--	<0.0106	--
solvent control	<0.0106	--	<0.0106	--
0.179	0.178	99	0.172	96
0.572	0.602	105	0.641	112
1.84	2.02	110	2.06	112
5.86	6.01	103	5.9	101
18.7	15.7	84	15.6	83

Growth rate:
Results for the effects of the static 7 day growth inhibition test are listed in the table below.

Table 8.2.7-25: Survey of biological results and derived inhibition percentages according to growth rates

nominal test concentration [µg p.m./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm2]	% inhibition (compared to pooled control)	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	257.7	1956.3	--	--
solvent control	254.7	1898.0	--	--
pooled control	256.2	1927.2	--	--
0.179	355	2125.7	-10.8	-4.6
0.572	78.7 *	439.7 *	38.6	50.2
1.84	39.3 *	248.7 *	61.3	70.6
5.86	24.7 *	166.7 *	76.5	82.9
18.7	25.0 *	155.3 *	76.1	85.8

* Results which were significantly different (based on Williams Multiple sequential t-test Procedure) from pooled control

Observed visual effects:
On day 7 the fronds observed at 0.572 µg p.m./L were smaller than the control plants.

The results based on nominal concentrations of AE F130619 are shown in the table below.

Table 8.2.7-26: Survey of 7-day endpoints for AE F130619

end point (0-7 day)	effect on mean growth rate of frond no. [µg p.m./L]	effect on mean growth rate of total frond area of plants [mg form./L]
E_rC_50 (CI 95%)	**1.50** (0.026 – 42.4)	**0.889** (n.d. – n.d.)
E_rC_{20} (CI 95%)	**0.272** (n.d. – 0.982)	**0.244** (n.d. – n.d.)
E_rC_{10} (CI 95%)	**0.111** (n.d. – 0.537)	**0.124** (n.d. – n.d.)
LOE_rC	0.572	0.572
NOE_rC	0.179	0.179

Conclusions:

The most sensitive response variable in this study was total frond area of plants resulting in a (0-7 day) - E_rC_{50} of 0.889 µg AE F130619/L.
The lowest NOE_rC was 0.179 µg AE F130619/L and was based on statistical data analysis of frond number and the total frond area of plants.

4-Amino-N-methylbenzamide

Report:	[redacted]t; [redacted];2013;M-464163-01
Title:	*Lemna gibba* G3 - Growth inhibition test with BCS-CV29520 (metabolite of foramsulfuron) under static conditions
Report No:	EBFSN010
Document No:	M-464163-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; none**
GLP/GEP:	**yes**

Executive Summary:

The aim of this growth inhibition test was, to verify the assumption that the metabolite 4-amino-N-methylbenzamide (BCS-CV29520) will cause no adverse effects on the growth of *Lemna gibba* G3 at the limit test item concentration of 10 mg pure metabolite/L. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 percent (EC_{50}) was determined where possible. Based on the low difference compared to the controls, which are far below 10 % difference, this statistically significance can be judged as biologically non-relevant. The overall threshold level has been set as ≥ 10 mg/L.

Material and methods:

Test item: BCS-CV29520 (technical metabolite of foramsulfuron); Origin Batch No.: GSE 61195-2-2; Batch ID: BCS-CV29520-PU-01; Customer Order No.: TOX09972-00; analysed content: 97.6 % w; Certificate No.: AZ-18627; LIMS No.: 1308405

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a water control. The pH-values ranged from 7.5 to 9.0 in the control and the incubation temperature ranged from 24.7°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.92 klux (average of nine measurements).
4-amino-N-methylbenzamide (BCS-CV29520) was quantitatively determined in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: April 15, 2013 – August 19, 2013

Results:

Validity Criteria:
The study met all validity criteria requested by the mentioned guideline.

Analytical findings:
The analytical finding of 4-amino-N-methylbenzamide (BCS-CV29520) found on day 0 was 109 % of nominal and 115 % of nominal on day 7. All reported results are based on nominal values of the test item.
The static 7 day growth inhibition test provided the following tabulated effects:

Table 8.2.7-27: Survey of biological results and derived inhibition percentages based on growth rates

nominal test concentration [mg p.m./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm²]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	212.3	1639.5		
10.0	208.8	1574.8	0.6	2.5

Observed visual effects:
There were no visual effects observed in any of the test concentrations.
Observed visual effects on the test item: none

The results based on nominal concentrations of the test item 4-amino-N-methylbenzamide are shown in the table below.

Table 8.2.7-28: Survey of 7-day endpoints for 4-amino-N-methylbenzamide

end point (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}	**>10.0**	**>10.0**
LOE_rC	>10.0	10.0
NOE_rC	10.0	< 10.0*)

*) The statistical evaluation yielded a statistical significant effect for the mean growth rate of total frond area after 7 days. The actual inhibition for this endpoint was obviously below 10 % compared to the controls.

Conclusions:

4-Amino-N-methylbenzamide (BCS-CV29520) caused no adverse effects on the growth of *Lemna gibba* G3 up to a test item concentration of 10 mg pure metabolite/L.
For the endpoint mean growth rate of total frond area of plants a significant difference to the controls was statistically evaluated. The observed difference in comparison to the control was 2.5 %. Due to the low variability of the data a Minimum Detectable Difference of –2 % was evaluable by the student-t-test. After 2 and 4 days the endpoint related no statistically significant difference to the controls. Based on the low difference compared to the controls, which are far below 10 % difference, this statistically significance can be judged as biologically non-relevant. Therefore the overall threshold level has been set by the study director as ≥ 10 mg/L.

4-Formamido-N-methylbenzamide

Report:	[redacted]8; [redacted];2013;M-464321-01
Title:	*Lemna gibba* G3 - Growth inhibition test with BCS-CW90756 (metabolite of foramsulfuron) under static conditions
Report No:	EBFSN011
Document No:	M-464321-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; OECD Guideline 221 (March 23, 2006); A slight deviation concerning the inoculum of replicate D at the limit test concentration of 10 mg/L is explained and discussed within chapter 4 (Method)**
GLP/GEP:	**yes**

Executive Summary:

The objective of this growth inhibition test was to verify the assumption that the test item 4-formamido-N-methylbenzamide (BCS-CW90756) will cause no adverse effects on the growth of *Lemna gibba* G3 at the limit test item concentration of 10 mg pure metabolite/L. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 percent (EC_{50}) was determined where possible. The test item caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

Material and methods:

Test item: BCS-CW90756 (technical metabolite of foramsulfuron); Origin Batch No.: GSE 61182-2-1; Batch ID: BCS-CW90756-PU-01; Customer Order No.: TOX09974-00; analysed content: 99.0 % w; Certificate No.: AZ 18623; LIMS No.: 1308248.

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a water control. The pH values ranged from 7.5 to 9.0 in the control and the incubation temperature ranged from 24.7°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.92 klux (average of nine measurements).
4-formamido-N-methylbenzamide (BCS-CW90756) was quantitatively determined in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: April 15, 2013 – August 20, 2013

Results:

Validity Criteria:
The study met all validity criteria requested by the mentioned guideline.

Analytical findings:
The analytical finding of 4-formamido-N-methylbenzamide (BCS-CW90756) found on day 0 was 108% of nominal and 114 % of nominal on day 7. All reported results are based on nominal values of the test item.

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

Table 8.2.7-29: Survey of biological results and derived inhibition percentages based on growth rates

nominal test concentration [mg p.m./L]	final frond number (replicate means, day 7)	final total frond area of plants (replicate means) [mm²]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	212.3	1639.5	-	-
10.0	219.2	1660.5	-0.6	-1.1

Observed visual effects:
There were no visual effects observed in any of the test concentrations.
Observed visual effects on the test item: none

The results based on nominal concentrations of the test item 4-formamido-N-methylbenzamide (BCS-CW90756) are shown in the table below.

Table 8.2.7-30: Survey of 7-day endpoints for 4-formamido-N-methylbenzamide

end point (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}	**>10.0**	**>10.0**
LOE_rC	>10.0	>10.0
NOE_rC	≥10.0	≥10.0

Conclusions:

4-formamido-N-methylbenzamide (BCS-CW90756) caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

Foramsulfuron-sulfamic acid

Report:	[redacted]u[redacted]2013;M-464386-01
Title:	*Lemna gibba* G3 - Growth inhibition test with BCS-AW41401 under static conditions
Report No:	EBFSN012
Document No:	M-464386-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; OECD Guideline 221 (March 23, 2006); none**
GLP/GEP:	**yes**

Executive Summary:

The objective of this growth inhibition test was, to verify the assumption that the metabolite foramsulfuron-sulfamic acid (BCS-AW41401) will cause no adverse effects on the growth of *Lemna gibba* G3 at the only test item concentration of 10 mg pure metabolite/L. Fronds of *Lemna gibba* G3 were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10 mg pure metabolite in comparison to a water control. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 percent (EC_{50}) was determined where possible.

Foramsulfuron-sulfamic acid (BCS-AW41401) caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

Material and methods:

Test item: BCS-AW41401 (technical metabolite of foramsulfuron); Origin Batch No.: GSE 61222-2-3; Batch ID: BCS-AW41401-01-01; Customer Order No.: TOX09976-00; analysed content: 89.7 %; Certificate No.: AZ 18815; LIMS No.: 1320720.

6 x 12 fronds of *Lemna* gibba G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg pure metabolite/L in comparison to a water control. The pH values ranged from 7.6 to 8.8 in the control and the incubation temperature ranged from 24.4°C to 24.9°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.70 klux (average of nine measurements). Foramsulfuron-sulfamic acid (BCS-AW41401) was quantitatively determined in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: July 10, 2013 – August 20, 2013

Results:

Validity Criteria:
The study met all validity criteria, requested by the mentioned guideline.

Analytical findings:
The analytical finding of BCS-AW41401 found on day 0 was 113 % of nominal and 115 % of nominal on day 7. All reported results are based on nominal values of the test item foramsulfuron-sulfamic acid (BCS-AW41401).

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

Table 8.2.7-31: Survey of biological results and derived inhibition percentages based on growth rates

nominal test concentration [mg p.m./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm²]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	194.0	1400.8	--	--
10.0	207.8	1469.7	-2.5	-2.1

Observed visual effects:
There were no visual effects observed in any of the test concentrations.
Observed visual effects on the test item: none

The results based on nominal concentrations of the test item foramsulfuron-sulfamic acid (BCS-AW41401) are shown in the table below.

Table 8.2.7-32: Survey of 7-day endpoints for foramsulfuron-sulfamic acid

end point (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}	**>10.0**	**>10.0**
LOE_rC	>10.0	> 10.0
NOE_rC	≥ 10.0	≥ 10.0

Conclusions:

Foramsulfuron-sulfamic acid (BCS-AW41401) caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

CA 8.2.8 Further testing on aquatic organisms

One acute study in a static system on Grass shrimp (*Palaemonetes pugio*) and one acute study under flow-through conditions on Eastern oyster (*Crassostrea virginica*) were performed. Details of all studies are provided in the following table.

Table 8.2.8-1: Effect data of foramsulfuron to aquatic organisms presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Palaemonetes pugio (grass shrimp)	static acute	96 h	EC_{50} >100	[redacted], (1998) A59902 M-143552-01-1 KCA 8.2.8 /02
Crassostrea virginica (eastern oyster)	flow-through	96 h	EC_{50} 118	[redacted], 1998 C000906 M-181443-01-1 KCA 8.2.8 /01

Studies on foramsulfuron

Report:	[redacted]5; [redacted]; [redacted]; [redacted];1998;M-181443-01
Title:	Flow-through mollusk shell deposition test AE F130360
Report No:	C000906
Document No:	M-181443-01-1
Guidelines:	**USEPA (=EPA); FIFRA 72-3, SEP 540/9-85-011;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted]3; [redacted]; [redacted];1998;M-143552-01
Title:	96 hour acute toxicity to the Grass Shrimp, Palaemonetes pugio, in a static system. AE F130360 technical 94.2% w/w Code: AE F130360 00 1C94 0001
Report No:	A59902
Document No:	M-143552-01-1
Guidelines:	**OECD: 203; USEPA (=EPA): E 72-3;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

Foramsulfuron has a low acute toxicity to honey bees, with LD_{50} (oral and contact) above the highest tested dose level (oral: LD_{50} > 110.1 µg a.s./bee, contact: LD_{50} > 100 µg a.s./bee). The low risk is confirmed by calculated Hazard Quotients for foramsulfuron all well below the validated trigger value which would indicate the need for a refined risk assessment, no adverse effects on honey bee mortality are to be expected. This conclusion is confirmed by the results of the bee brood feeding study as well as by the results of the semi-field study which, which covered the maximum application rates of 60 g foramsulfuron a.s./ha.

Regarding potential side effects of foramsulfuron on immature honey bee life stages, the conducted bee brood feeding study ([redacted] *et al.*, 1992) found a slightly, but statistically significantly increased termination rate of young and old larvae; as the observed slightly elevated termination rate of larvae was in absolute terms low; this observation - if at all test item related - was as such biologically not relevant. The bee brood feeding study further did not reveal adverse effects on the survival of adult bees and pupae, behaviour, colony strength, colony development as well as the condition of the colonies. Nonetheless, to clarify whether the observations in the honey bee brood feeding study are due to natural variability or test-item related, foramsulfuron was subjected to *in-vitro* larval testing. The potential effects on larval development were investigated at a level of 100 µg a.s./larva, i.e. the (highest) dose recommended for a limit test and revealed no adverse effects on larval mortality: the performance of the test item groups was identical as in the control group whereas the toxic reference performance was fully in line with the guideline specification. Based on the findings of the *in-vitro* larvae study, the observations in the honey bee brood feeding study are rather to be attributed to natural variability than being test-item related (intrinsic).

In parallel, foramsulfuron was subjected to confined semi-field testing (according to the provisions of OECD Guidance Document No. 75) by applying the maximum rate of Foramsulfuron + Isoxadifen-ethyl OD 45 (i.e. 2.68 L) to full-flowering *Phacelia* during honey bees actively foraging on the crop.

The results of this higher tier study confirmed all conclusions made above on the basis of the outcome of the lower-tiered studies, as no adverse direct or delayed effects on mortality of worker bees or pupae, foraging activity, behaviour, nectar- and pollen storage, queen survival, colony strength, colony development as well as the development of bee brood were observed, even under aggravated, forced exposure conditions and by digitally following-up in a very detailed manner the fate of individually marked brood cells (digital photographic assessment) from egg stage until emergence.

Overall, it can be concluded that foramsulfuron, when applied at the maximum application rate of 60 g a.s./ha even during the flowering period of potentially bee-attractive weeds inside the cropping area, does not pose an unacceptable risk to honey bees and honey bee colonies.

For information on studies already evaluated during the first EU review of this compound, please refer to corresponding section in the Monograph and in the baseline dossier (KCA: 8.3.1.1.1/01 and KCA 8.3.1.1.2/01) provided by Bayer CropScience.

Table 8.3.1- 1: Honey bee toxicity of foramsulfuron (tech.) and formulated foramsulfuron to bees

Test substance, Test species	Test system	Endpoints	Reference
Foramsulfuron, tech.			
Honey bee (*Apis mellifera*)	oral 48/72 h	LD_{50} > 163 µg a.s./bee	[redacted], 1998 M-143626-01-1 KCA 8.3.1.1.1 /01
Honey bee (*Apis mellifera*)	contact 48/72 h	LD_{50} > 1 µg a.s./bee	[redacted], 1997 M-143215-01-1 KCA 8.3.1.1.2 /01
Honey bee (*Apis mellifera*)	oral 48 h contact 48 h	LD_{50} > 110.1 µg a.s./bee LD_{50} > 100 µg a.s./bee	[redacted], 2012 M-144765-01-1 KCA 8.3.1.1.1/02 & KCA 8.3.1.1.2 /02
Foramsulfuron WG 50			
Honey bee (*Apis mellifera*)	10 d chronic adult feeding study	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted], (2013) M-470639-01-1 KCA 8.3.1.2/01
Honey bee (*Apis mellifera*)	*In vitro* honey bee larvae laboratory study, single exposure test design	LD_{50} > 100 µg a.s./larva NOED ≥ 100 µg a.s./larva	[redacted], (2013) M-470485-01-1 KCA 8.3.1.3/01
Honey bee (*Apis mellifera*)	Honey bee brood feeding ([redacted], 1992)	Slightly, but statistically significantly increased termination rate of young and old larvae, which is not biologically relevant; no adverse effects on the survival of adult bees and pupae, behaviour, colony strength, condition of the colonies, brood index and brood compensation index by feeding honey bee colonies sugar syrup at a foramsulfuron concentration typically present in the spray tank (100 ppm)	[redacted], (2013) M-465326-01-1 KCA 8.3.1.3/02
Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5 + 22.5)			
Honey bee (*Apis mellifera*)	Semi-field honey bee brood study (acc. to OECD 75; forced exposure conditions) in *Phacelia*; application during full-bloom and bees actively foraging	No adverse effects on mortality, flight intensity, behaviour, brood development (brood termination rate, brood index, compensation index) as well as on colony vitality at maximum application rate (2.67 L product/ha)	[redacted] (2013) M-468794-01-1 KCA 8.3.1.3/03

CA 8.3.1.1 Acute toxicity to bees

In addition to the already available acute laboratory studies with technical foramsulfuron ([redacted]; 1998 and 1997, Doc.-No.: M-143626-01-1 and M-143215-01-1; KCA 8.3.1.1.1/01 and KCA 8.3.1.1.2/01), a further laboratory study on acute oral and contact toxicity to honey bees has been performed with technical foramsulfuron according to current guidelines and requirements.

In addition, a chronic 10 day adult feeding limit test was conducted with Foramsulfuron WG 50. The respective study summaries are presented below.

CA 8.3.1.1.1 Acute oral toxicity

Report:	[redacted]4;[redacted];1998;M-143626-01
Title:	Code: AE F130360 00 1C48 0001 - Substance, technical - Oral toxicity (LD 50) to honey bees (*Apis mellifera* L.)
Report No:	A59983
Document No:	M-143626-01-1
Guidelines:	**EPPO: 170;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted]1;[redacted];[redacted];2012;M-444765-01
Title:	Effects of foramsulfuron tech. (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No:	75201035
Document No:	M-444765-01-1
Guidelines:	**OECD 213 and 214 (1998); none**
GLP/GEP:	**yes**

The study is summarised in detail in KCA 8.3.1.1.2/02. The endpoint from this study is:

48 h-LD_{50}-contact > 100.0 µg a.s./bee

48 h-LD_{50}-oral > 110.1 µg a.s./bee

CA 8.3.1.1.2 Acute contact toxicity

Report:	[redacted];1997;M-143215-01
Title:	Code: AE F130360 00 1C48 0001 - Contact toxicity (LD50) to honey bees (*Apis mellifera* L.)
Report No:	A59584
Document No:	M-143215-01-1
Guidelines:	**EPPO: 170; US-EPA (=EPA): L 141-1;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted];2012;M-444765-01
Title:	Effects of foramsulfuron tech. (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No:	75201035
Document No:	M-444765-01-1
Guidelines:	**OECD 213 and 214 (1998);none**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the acute contact and oral toxicity of foramsulfuron tech to the honey bee (*A. mellifera* L.) under laboratory conditions. For this purpose female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 110.1 µg a.s./bee for feeding (oral limit test, value based on the actual intake of the test item). In addition to the oral limit toxicity test, in another oral dose response test 30 female worker bees per dose were exposed for 48 hours to 81.4, 54.2 and 27.9 µg a.s./bee for feeding (values based on the actual intake of the test item). Mortality of the bees was used as the toxic endpoint. Sub-lethal effects, such as changes in behaviour, were also assessed.
The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 110.1 µg a.s./bee. The contact NOED was ≥ 100 µg a.s./bee. The oral NOED was estimated in an additional dose response toxicity test. The oral NOED was 81.4 µg a.s./bee.

Material and methods:

Test item. Foramsulfuron technical; Batch code: AE F130360-01-02; Origin Batch No.: ELIR004294; LIMS No.: 1138112; Customer Order No.: TOX-No.: 09600-00; Article No.: 06360890; CAS No.: 173159-57-4; Specification No.: 102000011654, analysed content of a.s.: 97.3 % w/w.

Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x height x width). 10 bees were used per test unit. 5 test units for the limit tests and 3 test units for the oral dose response test were used per test item dose level, control and reference item dose level, respectively. 50 female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and 50 female worker bees (*Apis mellifera*) were exposed for 48 hours for feeding to a single dose of 110.1 µg a.s./bee (oral limit test, value based on the actual intake of the test item). In addition to the oral limit toxicity test, in another oral dose response test 30 female worker bees per dose were exposed for 48 hours to 81.4, 54.2 and 27.9 µg a.s./bee for feeding (values based on the actual intake of the test item).
For the contact test a single 5 µL droplet of foramsulfuron tech., dissolved in tap water with 0.5 % Adhäsit, was placed on the dorsal bee thorax. Likewise for the toxic reference (dimethoate) and the control (tap water). For both oral tests aqueous stock solutions of the test item and reference item were prepared and mixed with ready-to-use sugar syrup (30 % sucrose, 31 % glucose, 39 % fructose) at a concentration of 50 % (w/w). For the control, tap water and sugar syrup was used at the same ratio 50% (w/w) tap water, 50% (w/w) ready-to-use sugar syrup. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 1 hour 50 minutes (limit test) or 2 hours 15 minutes (dose response test) the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.
The number of dead bees was determined after 4 (± 0.5 h) hours (first day); 24 and 48 (± 2 h) hours. Behavioural abnormalities (e.g. vomiting, apathy, intensive cleaning) were assessed after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours. Temperature during the test was 25 °C; relative humidity

was 53 - 89% for the contact and oral limit test and 51 - 75% for the oral dose response test. Bees were kept in darkness (except during observation).

Dates of experimental work: August 21, 2012 – August 29, 2012 (contact and oral limit test)
October 09, 2012 – October 11, 2012 (oral dose response test)

Results:

Table 8.3.1.1.2-1: Validity criteria

Validity Criteria		Recommended	Obtained
Control mortality	Contact Test		
	CO_2/water control	< 10%	4.0 %
	Oral Test		
	water/sugar syrup control	10%	0.0 % (limit and dose response tests)
LD_{50} of reference item (24 h)	Contact Test		
		0.10 - 0.30 µg a.s./bee	0.17 µg a.s./bee
	Oral Test		
		0.10 - 0.35 µg a.s./bee	0.16 µg a.s./bee (limit test) 0.11 µg a.s./bee (dose response test)

All validity criteria for the study were met.

Biological results:

Contact toxicity test:
At the end of the contact toxicity test (48 hours after application), no mortality occurred at 100.0 µg a.i./bee. There was 4.0 % mortality in the control group (water + 0.5 % Adhäsit).
At the last assessment (48 hours following treatment) two bees were found apathetic. This was the only occurrence of behavioural abnormalities during the trial.

Oral limit toxicity test:
In the oral limit toxicity test, the maximum nominal test level of foramsulfuron tech. (i.e. 100 µg a.i./bee) corresponded to an actual intake of 110.1 µg a.i./bee. This dose level resulted in 10.0 % mortality after 48 hours. In the control group (50 % aqueous sugar syrup solution), no mortality occurred.
In the oral limit test, no test item induced behavioural abnormalities occurred.

Oral dose response toxicity test:
An additional oral dose response test with 75.0, 50.0 and 25.0 µg a.i./bee (nominal values) was performed in order to determine a NOED. The actual oral doses of 81.4, 54.2 and 27.9 µg a.i./bee resulted in 3.3, 3.3 and 6.7 % mortality, respectively, at the end of the test (after 48 hours). No mortality occurred in the control group (50 % aqueous sugar syrup solution).
No test item induced behavioural abnormalities occurred.

Table 8.3.1.1.2-2: Acute toxicity to honey bees; contact and oral laboratory test

Test Item	Foramsulfuron, tech.
Test Object	*Apis mellifera*

Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sugar syrup solution)
Application rate µg a.s./bee	100.0	110.1
LD_{50} µg a.s./bee	> 100.0	> 110.1
LD_{20} µg a.s./bee	> 100.0	> 110.1
LD_{10} µg a.s./bee	> 100.0	> 110.1
NOED µg a.s./bee*	≥ 100.0	81.4

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

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.17 and 0.16 µg a.s./bee, respectively. In the additional oral dose response test the oral LD_{50} (24 h) value of the reference item (dimethoate) was 0.11 µg a.s./bee.

Conclusions:

The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 110.1 µg a.s./bee. The contact NOED was ≥ 100 µg a.s./bee. The oral NOED estimated in an additional dose response toxicity test was 81.4 µg a.s./bee.

CA 8.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Foramsulfuron WG 50 as technical foramsulfuron was not well soluble in water.

Report:	[redacted]p; [redacted];2013;M-470639-01
Title:	Foramsulfuron WG 50 W - Assessment of chronic effects to the honeybee, *Apis mellifera* L., in a 10 days continuous laboratory feeding limit test
Report No:	S13-00153
Document No:	M-470639-01-1
Guideline:	**not applicable;not applicable**
GLP/GEP:	**yes**

Objective:
To investigate the potential chronic effects of foramsulfuron on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory and to investigate whether the LC50-/NOEC- value is greater than the tested concentration.

Materials and methods:
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 Foramsulfuron WG 50 W 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 exposure period. Furthermore, the daily food uptake was determined.

Dates of experimental work: July 02, 2013 – July 30, 2013

Results:
After 10 days of continuous exposure, mortality at the test item treatment level of 120 mg a.s./kg of Foramsulfuron WG 50 W was not statistically significantly different when compared to the control

group. The cumulative control mortality was 3.0 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg Foramsulfuron WG 50 W was 2.0 % at the final assessment. At 120 mg a.s./kg Foramsulfuron WG 50 W, 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 Foramsulfuron WG 50 W at the treatment level of 120 mg a.s./kg was 52.44 µg a.s./bee, the corresponding average daily dose was therefore 5.2 µg a.s./bee.
The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different (lower) when compared to the untreated control group (43.6 mg/bee at 120 mg a.s./kg, compared to 40.2 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).

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 Foramsulfuron WG 50 W at the treatment level of 120 mg a.s./kg caused no adverse effect regarding mortality, sub-lethal effects and behaviour.
The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly 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 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 LC50 was determined to be >120 mg a.s./kg (nominal).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

An *in vitro* honey bee larvae laboratory study, following the single exposure test design according to the Draft-OECD 237 guideline was conducted with Foramsulfuron WG 50, as technical foramsulfuron was not well soluble in water. The study summary his presented below.
In addition, a honey bee brood feeding study ([redacted] *et al.*) has been conducted ([redacted], 2013, M-465326-01-1) with WG 50-formulated foramsulfuron, the corresponding study summary is presented below.
Finally, foramsulfuron was subjected to confined semi-field testing (according to the provisions of OECD Guidance Document No. 75) by applying the maximum rate of Foramsulfuron + Isoxadifen-ethyl OD 45 (i.e. 2.68 L) to full-flowering *Phacelia* during honey bees actively foraging on the crop. Although performed with the representative formulation Foramsulfuron + Isoxadifen-ethyl OD 45 the study is considered to provide valuable information on the active substance foramsulfuron. Therefore, the corresponding study summary is presented here, see KCA 8.3.1.3/03.

Report:	[redacted]j; [redacted]; [redacted];2013; M-470485-01
Title:	Foramsulfuron WG 50 W: Effects of a single exposure to spiked diet on honey bee larvae (*Apis mellifera carnica*) in an in vitro laboratory testing design
Report No:	E3174533-6
Document No:	M-470485-01-1
Guidelines:	**EU Directive 91/414/EEC** **Regulation (EC) No. 1107/2009** **US EPA OCSPP 850.supp; not specified**
GLP/GEP:	**yes**

Objective:
This oral toxicity test was performed as a limit test with a single exposure in an in vitro laboratory testing design. Synchronized first instar larvae of *Apis mellifera carnica*, from three different honey bee colonies, each representing a replicate, were tested in an in vitro laboratory testing design, according to the OECD Draft Test Guideline on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure (Version of 21 February 2013) and the current draft version of the Post–WNT25 Approved Larval Honey Bee Test, dated April 2013.

Materials and methods:
Test item: Foramsulfuron WG 50 W (TOX-No.: 09720-01; Batch ID: 2012-001517; Specification no.: 102000026995; content of a.s. (analysed): 49.4% w/w).
At day +1*, first instar bee larvae (*Apis mellifera carnica*) were transferred from their bee hive into an artificial in vitro testing system. The larvae were fed with standardised amounts of artificial diet on day +1, +3, +4, +5 and +6. On day +4, the artificial diet was treated according to the respective test group. In the test item treatment group, foramsulfuron WG 50 W was incorporated into the artificial diet at the nominal test dose of 100 µg a.s./larva, corresponding to the nominal test concentration of 3030.3 mg a.s./kg diet. In the reference item treatment group dimethoate was incorporated into the artificial diet at a nominal dose of 8 µg a.s./ larva, corresponding to 266.7 mg a.s./kg diet. In the control group water was incorporated into the artificial diet.

(*) Day 0 was the anticipated day of larval hatching.

The actual concentration of foramsulfuron in the stock solution was determined according to Analytical Method 01340 for the determination of residues of foramsulfuron and its metabolite AE F153745 in/on plant matrix (sugar beet body and leaf) by HPLC-MS/MS.

During their development the honey bee larvae were incubated at about +35°C. The relative humidity inside the incubator was on average 95 ± 5% from day +1 to +8. As Assessment endpoint mortality of the honey bee larvae was recorded on day +5, day +6, day +7 (according to the study plan), and additionally on day +8 (according to amendment no. 6). Dead test animals were discarded for sanitary reasons. A first run of the study, conducted with the test item foramsulfuron tech. (TOX 09600-00), was stopped on 03 June 2013 (day +4 of the study) due to solubility problems of the technical material. It was therefore decided to conduct the study with the above-mentioned straight formulation.

Dates of experimental work:
Experimental Starting Date (1st run of biological part): 27 May 2013
Experimental Starting Date (2nd run of biological part): 05 June 2013
Experimental Completion Date (Biological part): 14 June 2013
Experimental Starting Date (Analytical part): 10 June 2013
Experimental Completion Date (Analytical part): 24 September 2013

Results:

The validity criteria of the study (Table 1) were met (i.e. larval mortality in the control group from day +4 to day +7 was ≤ 15% and the larval mortality in the reference group was ≥ 50% from day +4 until day +7). In the control group, as well as in the test item treatment group, no larvae died until day +7. Until day +8, one single larva died in the control and in the test item treatment group, respectively.

Table 8.3.1.3-1: Validity criteria

Validity criteria	Validity threshold	Obtained results
Larval mortality in the control group from day +4 until day +7	≤ 15%	0.0%
Larval mortality in the reference item group from day +4 until day +7 (Abbott)	≥ 50%	89.6%

Table 8.3.1.3-2: Control, test item and reference item performance and associated statistical evaluation

Test object	Honey bee larvae *(Apis mellifera carnica)*		
	Control (untreated diet)	**Test Item** (Foramsulfuron WG 50 spiked diet)	**Reference Item** (dimethoate, tech. spiked diet)
Test concentration (nominal) [mg a.s./kg diet]	---	**3030.3**	**266.7**
Feeding dose (nominal) [µg a.s./larva]	---	**100**	**8.8**
Total larval mortality until day +7 [%]	0.0	0.0	89.6
Abbott-corrected total mortality until day +7 [%]	0.0	0.0	89.6
[1] Statistical comparison to the control at day +7	---	n.s.	---
NOED at day +7 [µg a.s./larva]	---	≥ 100	---
LOED at day +7 [µg a.s./larva]	---	> 100	---
LD_{50} at day +7 [µg a.s./larva]	---	> 100	---
Total larval mortality until day +8 [%]	2.1	2.1	100
Abbott-corrected total mortality until day +8 [%]	---	0.0	100
[1] Statistical comparison to the control at day +8	---	n.s.	---
NOED at day +8 [µg a.s./larva]	---	≥ 100	---
LOED at day +8 [µg a.s./larva]	---	> 100	---
LD_{50} at day +8 [µg a.s./larva]	---	> 100	---

[1] Fisher´s Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$
n.s.: mean value is not statistically significantly different compared to the control
a.s.: active substance

The chemical analysis of the Foramsulfuron WG 50 stock solution, which was equivalent to the test item spiking solution used to treat the larval diet in the test item treatment group, revealed that the actual foramsulfuron concentration was well in line with the nominal foramsulfuron concentration (118% of nominal).

Conclusions:

This *in vitro* honey bee larvae study, conducted with three replicates, complies with the validity criteria according to the OECD Draft Test Guideline on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure (Version of 21 February 2013) and the current draft version of the Post-WNT25 Approved Larval Honey Bee Test, dated April 2013. The chemical analysis of the test item treated stock solution, which was equivalent to the test item spiking solution used to treat the larval diet in the test item treatment group, revealed that the actual foramsulfuron concentration was well in line with the nominal concentration. The statistical processing of the data as obtained in the study, revealed that mortality of exposed honey bee larvae until day +8 (end of the test) did not differ significantly between the control and the test item treatment group of nominal 100 µg foramsulfuron a.s./larva, corresponding to nominal 3030.3 mg foramsulfuron a.s./kg diet (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

Overall, it can be concluded that the No Observed Effect Dose (NOED) determined in this *in vitro* honey bee larvae study is ≥ 100 µg foramsulfuron a.s./larva (based on nominal) and the Lowest Observed Effect Dose (LOED) as well as the LD_{50} is > 100 µg foramsulfuron a.s./larva (based on nominal).

Report:	[redacted]1; [redacted];2013;M-465326-01
Title:	Foramsulfuron WG 50 - A honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae)
Report No:	20110170
Document No:	M-465326-01-1
Guidelines:	[redacted] **(1992). Method for honeybee brood feeding tests with insect growth-regulating insecticides. EPPO Bulletin, 22, 613-616 [1].; not specified**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to evaluate potential effects of Foramsulfuron WG 50 administered together with the herbicide safener Cyprosulfamide SC 500 G on brood development and mortality of adult worker honey bees, *Apis mellifera* L..

To assess the potential effects of Foramsulfuron WG 50 on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.198 g formulated test item/L (=0.1 g foramsulfuron/L) + 0.101 mL formulated herbicide safener/L (0.05 g cyprosulfamide/L) per colony in summer 2012. Mortality of worker bees, larvae and pupae and behavior around the hive were observed for a period of 21 days after application. Condition of the colonies and brood development were also assessed. The method of investigating the development of the honey bee brood is based on the method of [redacted]. (1992).

The administration of foramsulfuron WG 50 + the herbicide safener cyprosulfamide SC 500 G to honey colonies caused no adverse effects on the survival of adult bees and pupae, behaviour, colony strength, condition of the colonies, brood index and brood compensation index. In contrast, brood

termination rate of young and old larvae was statistically significantly increased when compared to the control treatment.
Despite of the slightly elevated termination rates in the test item treatment group, overall colony performance was normal and not impaired. Overall, due to the colony development progress during the course of the study, the observed effects in the test item group can be considered as biologically not relevant.

Materials and Methods:

Test item: Foramsulfuron WG 50; Sample code: 12004220; Batch No.: 2012-001517; Sample description: A.12000301; Specification No.: 102000026995; Nominal content of a.s.: 500 g/kg; analysed content of a.s.: 506 g/kg.

Herbicide safener: Cyprosulfamide SC 500 G; Batch No.: 2012-002411; Sample description: TOX09783-00; Specification No.: 102000014017-01; Nominal content of a.s.: 500 g/L; analysed content of a.s.: 493.4 g/L.

Three healthy, queen-right bee colonies were used per treatment group (control, test item treatment administered with the herbicide safener, and reference item). In total, nine colonies were treated. All treatments were administered in 1 L 50% (w/v) aqueous sucrose solution per colony.

Treatments:

Control: 50 % (w/v) aqueous sucrose solution, 1 L per colony.
Test item treatment: the test item foramsulfuron WG 50 and the herbicide safener Cyprosulfamide SC 500 G were both mixed together in 50 % (w/v) aqueous sucrose solution, at a final concentration of 0.1 g foramsulfuron/L and 0.05 g cyprosulfamide/L, 1 L per colony.
Reference: 0.75 g fenoxycarb a.s./L, corresponding to 3.0 g (nominal) Insegar® 25 WG in 1 L 50% (w/v) aqueous sucrose solution, 1 L per colony.

The treatment administration was conducted simultaneously to all hives in the afternoon at the time of low flight activity via commercial bee feeder as a single treatment. The feeder was placed beneath the hive roof over the hole on top of the crown board. The bee feeders were left at the colonies until total consumption of the feeding solution.

Endpoints:
Mortality of worker bees, larvae and pupae: between 3 days before to 21 days after application (= end of the trial) in the bee traps;
Behaviour around the hive: between 3 days before to 21 days after application (= end of the trial);
Condition of the colonies was assessed two times during the study: 2 days before and 20 days after application (study termination);
Detailed brood assessments (brood termination rate, brood index and brood compensation index of 197 to 210 marked eggs, 150 to 200 young larvae and 199 to200 old larvae): one day before (= BFD0) and 5 (= BFD 6), 10 (= BFD 11), 14 (= BFD 15), 20 (= BFD 21) days after the application.

Dates of work: June 05, 2012 – June 08, 2012 (pre-treatment phase, DAT -3 to 0)
June 09, 2012 – June 29, 2012 (exposure phase (DAT 1 to 21)

Results:

Validity:
The overall daily mean adult and pupae mortality of the reference item was significantly greater when compared to the control, indicating that sufficient exposure of the honeybees had taken place and thus the suitability of the test system to detect potential effects on the bee brood. The mortality of adult honeybees and brood stages in the control treatment during the course of the study remained low. In addition, the mean brood termination rate in the toxic reference treatment of all monitored brood stages on BFD 21 (eggs: 85.4%, young larvae: 43.9%, old larvae: 51.8%) was considerable increased and statistically significantly greater when compared to the control (eggs: 41.1%, young larvae: 7.7%, old larvae: 5%). Regarding the overall performance of the reference item and control treatment, the study validity criteria were fulfilled.

Biological results:

Table 8.3.1.3-3: Effects of Foramsulfuron WG 50 (+ Cyprosulfamide SC 500 G) on honeybee mortality and honeybee brood development

Test item	Foramsulfuron WG 50 (+ Cyprosulfamide SC 500 G)		
Test object	Honeybee *Apis mellifera* L. (complete colonies)		
Exposure	Via treated 50 % (w/v) aqueous sucrose solution		
Assessment	**Control** **n = 3**	**Test item** **n = 3**	**Reference Item** **n = 3**
	Mean mortality of worker bees + freshly emerged worker bees/colony		
Pre-application(DAT -3 to 0)	22.8 ± 6.5	33.4 ± 7.7	31.0 ± 16.9
Post-application(DAT 1 to 21)	11.2 ± 0.9	18.9 ± 6.3[a]	23.6 ± 7.4[a]
	Mean mortality of pupae/colony		
Pre-application(DAT -3 to 0)	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.3
Post-application(DAT 1 to 21)	0.5 ± 0.2	0.3 ± 0.2	34.8 ± 17.9[a]
	Mean values of brood development (eggs)		
Brood termination rate (%) at BFD 21 (DAT 20)	41.1 ± 32.2	43.6 ± 33.3	85.4 ± 10.9[b]
Brood index at BFD 21 (DAT 20)	2.9 ± 1.7	2.8 ± 1.7	0.7 ± 0.5
Compensation index at BFD 21 (DAT 20)	3.7 ± 0.7	3.5 ± 0.9	1.0 ± 0.8
	Mean values of brood development (young larvae)		
Brood termination rate (%) at BFD 21 (DAT 20)	7.7 ± 4.5	27.0 ± 24.9[b]	43.9 ± 35.6[b]
Brood index at BFD 21 (DAT 20)	4.6 ± 0.2	3.6 ± 1.2	2.8 ± 1.7
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.1	3.8 ± 1.2	2.9 ± 1.8
	Mean values of brood development (old larvae)		
Brood termination rate (%) at BFD 21 (DAT 20)	5.0 ± 4.3	11.0 ± 14.1[b]	51.8 ± 13.4[b]
Brood index at BFD 21 (DAT 20)	4.7 ± 0.2	4.4 ± 0.7	2.4 ± 0.7[c]
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.2	4.5 ± 0.8	2.8 ± 0.3[c]

Values are mean ± SD

[a] Statistically significantly greater when compared to the control (Mann-Whitney, α=0.05, alternative one-sided smaller)

[b] Statistically significantly greater when compared to the control (Fisher's exact test, α=0.05, alternative one-sided smaller)

[c] Statistically significantly smaller when compared to the control (t-test, α=0.05, alternative one-sided greater)

DAT Days After Treatment
BFD Brood area Fixing Day
SD Standard Deviation

Mortality (adult and young worker bees)
The overall daily mean bee mortality observed on the days before application was similar in all treatments (22.8 to 33.4 bees per colony per day) indicating well adapted colonies.
The overall daily mean bee mortality after application of all treatments was 11.2, 18.9 and 23.6 in the control, test item and reference item treatment, respectively. Both, test item and the reference item treatment was statistically significantly greater when compared to the control.
Furthermore, the mortality was statistically significantly increased on DAT 2 (test item) and on DAT 5, 7 and 19 (reference item) when compared to the control treatment.

Mortality (pupae)
The overall daily mean pupae mortality observed on the days before application was low and similar in all treatments (0.1 to 0.2 pupae per colony per day).
The overall daily mean pupae mortality after application of all treatments was 0.5, 0.3 and 34.8 in the control, test item and reference item treatment respectively. The reference item treatment was statistically significantly greater when compared to the control. Furthermore, statistically significant increased pupae mortality was observed in the reference item treatment at DAT 10 to 21 (6.7 to 105 mean pupae per colony). This indicated that honey bee brood was well exposed during the test and that the test system was sensitive to detect potential brood effects of plant protection products.

Behaviour
In all treatments, no abnormal behavioural symptoms were observed during the whole study period.

Colony strength
The mean colony strength before treatment administration was 13600, 13617 and 13267 bees/colony in the control, test item and reference item treatment, respectively, and was thus similar in all treatments.
During the course of the study, the mean colony strength in the control, test item and reference item treatment displayed a relative increase of 22%, 15% and -27%, respectively, and was at study termination 16617, 15683 and 9700 bees per colony, respectively. No distinct differences between the control and test item treatment were observed.

Brood nest (eggs/larvae/pupae)
At the 1st assessment a healthy queen was present and the brood nest was similar in all colonies indicating healthy colonies.
During the course of the study the proportion of the brood nest in the control, test item and reference item displayed a relative decrease of 13%, 16% and 41%, respectively. The brood nest decrease in the test item treatment was similar to the control treatment, whereas the reference item showed a distinct decrease when compared to the control.

Stores (pollen/nectar/honey)
At the 1st assessment (DAT -2) a sufficient amount of nectar, honey and pollen was available in all colonies.
During the course of the study the proportion of stores in the control, test item and reference item displayed a relative decrease of 1%, 2% and 1%, respectively. Thus, stores remained similar in all treatments during the course of the study.

Brood termination rate
Selected eggs at BFD 0:
The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 41.1%, 43.6% and 85.4%, respectively.

Selected young larvae at BFD 0:
The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 7.7%, 27% and 43.9%, respectively.

Selected old larvae at BFD 0:
The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 5%, 11% and 51.8%, respectively.

Overall, the mean brood termination of the test item was statistically significantly greater for young and old larvae, whereas the selected eggs at BFD 0 were not statistically significantly different when compared to the control. In the reference item treatment, brood termination rate was statistically significantly higher in all selected brood stages (eggs, young and old larvae) when compared to the control. This indicated that the test system was sensitive to detect potential brood effects of plant protection products.

Brood index
Brood indices generally correlate with the termination rates, the higher the termination rates the lower the brood indices and vice versa.

Selected eggs at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 2.9, 2.8 and 0.7, respectively.

Selected young larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.6, 3.6 and 2.8, respectively.

Selected old larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.7, 4.4 and 2.4, respectively.

Overall, the brood indices of the control and test item displayed a continuous and comparable increase, indicating a successful development of the brood. In contrast, the mean brood indices of the reference item were distinctly lower when compared to the control.

Brood compensation index
Generally the brood compensation indices of all treatment groups were slightly higher than the corresponding brood-indices at all days indicating that cells with terminated brood were at least partially refilled with new eggs, which developed successfully.

Selected eggs at BFD 0:
The mean brood compensation index of the control, test item and reference item treatment at the last assessment (BFD 21) was 3.7, 3.5 and 1.0, respectively.

Selected young larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.8, 3.8 and 2.9, respectively.

Selected old larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.8, 4.5 and 2.8, respectively.

Overall, the brood indices of the control and test item displayed a continuous and comparable increase, indicating a successful development of the brood. In contrast, the mean brood indices of the reference item were distinctly lower when compared to the control.

Conclusions:

To assess the potential effects of Foramsulfuron WG 50 on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.198 g formulated test item/L (= 0.1 g foramsulfron/L) + 0.101 mL formulated herbicide safener/L (0.05 g cyprosulfamide/L) per colony in summer 2012.
The administration of Foramsulfuron WG 50 + the herbicide safener Cyprosulfamide SC 500 G to honey colonies caused no adverse effects on the survival of adult bees and pupae, behaviour, colony strength, condition of the colonies, brood index and brood compensation index. In contrast, brood termination rate of young and old larvae was statistically significantly increased when compared to the control treatment.
Despite of the slightly elevated termination rates in the test item treatment group, overall colony performance was normal and not impaired. Overall, due to the colony development progress during the course of the study, the observed effects in the test item group can be considered as biologically not relevant.

Report:	[redacted]8; [redacted];2013;M-468794-01
Title:	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L): Effects on honey bee brood (*Apis mellifera* L.) under semi-field conditions - Tunnel test -
Report No:	79091033
Document No:	M-468794-01-1
Guidelines:	**GLP compliant study based on OEPP/EPPO guideline No. 170 (4) (OEPP/EPPO, 2010), OECD Number 75 (2007) and current recommendations of the AG Bienenschutz (2011); The post-application exposure phase in the tunnel was reduced to 4 days due to the herbicide mode of action of the test item against the *Phacelia*-crop; at the end of the 4th day after application, the *Phacelia*-crop was no longer attractive to bees (faded) and did not longer support the confined colonies**
GLP/GEP:	**yes**

Material and Methods

Test Item:

Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L): foramsulfuron (AE F130360): 2.33 % w/w (22.41 g/L) (analysed), isoxadifen-ethyl (AE F122006): 2.29 % w/w (21.96 g/L) (analysed); Batch ID.: EFKM002442; Sample Description: TOX10129-00; Material No.: 06321801; Specification No.: 102000011304 - 06; density: 0.961 g/cm³ (20 °C).

Test Species:

Honey bees (*Apis mellifera carnica* L.); small bee colonies, maintained according to normal beekeeping practice, containing 11 combs with honey, pollen and brood. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply with nectar and pollen. The mean strength of the colonies per treatment group, one day before the application, was very similar and ranged between 4736 and 5018 adult bees per colony.

Test Design:

The test was conducted under forced/confined exposure conditions (tunnel), in order to assess potential effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) to honey bee colonies including brood development under semi-field conditions. Tunnels (20 m length x 5.5 m width x 2.5 m height) were set up on a *ca.* 75 m² plot of *Phacelia tanacetifolia* (2 x 36 m²). Small bee colonies were introduced to the tunnels 3 days before the application. One honey bee colony was used per tunnel.

The test item, water and a reference item were applied on the whole plot of plants in two operations, with foraging bees present. The trial was carried out using four tunnels (*i.e.* replicates) for the test item treatment, the control and the reference item treatment (Insegar, 250 g/kg fenoxycarb), respectively. The confined exposure phase of the honey bees inside the treated crop was 4 days following the test item application. At the end of the 4th day after application, due to the herbicide mode of action of the test item, the *Phacelia*-crop was no longer attractive to bees (faded) and did not longer support the confined colonies. Thus, all bee colonies (i.e. the colonies from the test item, the water and the reference item group, respectively) were relocated after 4 complete days of confined exposure from their respective tunnels and placed in an area with no main flowering, bee attractive crops.

After foliar (spray) application of the water (control), test item and the reference item, ontogenesis of a defined number of honey bee eggs was observed for each group and colony. Mortality of adult bees and pupae/larvae as well as foraging activity of the adult bees was also assessed. The condition of the colonies was assessed in regular intervals until the end of the trial.

Ontogenesis of the bees from egg to adult workers was observed for a period of 22 days (i.e. one complete honey bee brood cycle). This was done one day before the application by taking out a brood comb and taking a digital picture of the brood comb. After saving the file on a computer, 220 - 270 eggs per colony were marked at this first brood area fixing day BFD0 (BFD = Brood Area Fixing Day). For each subsequent brood assessment (BFDn), again, the respective comb was taken out of the hive and another digital photo was taken in order to investigate the progress of the brood development until day 21 following the application (BFD22 following BFD0).

Test Parameters:

- Mortality of adult bees and pupae: 2 days before to 27 days after application (= end of the trial);
- Behavioural abnormalities: 2 days before to 27 days after application (= end of the trial);
- Foraging activity of the bees: 2 days before to 4 days after application;

- Condition of the colonies (food stores, brood status and colony strength): 1 day before and 5, 9, 15, 21 and 27 days after application;
- Bee brood development (eggs): 1 day before (= BFD0) and 5 (= BFD 6), 9 (= BFD 10), 15 (= BFD 16), 21 (= BFD 22) days after the application

Application Rates (during full flowering when honey bees were actively foraging on the crop):

Control: 400 L tap water/ha;
Test Item: 60 g foramsulfuron a.s./ha; 2.68 L (2575.5 g) product in 400 L tap water/ha (corresponding to 6.439 g product/L);
Reference Item: 300 g fenoxycarb a.s. (1200 g product)/ha in 400 L spray solution/ha (corresponding to nominally 3.00 g product/L),

Test Conditions:

Natural field conditions. On the application day, due to the warm and sunny weather, there was a very high honeybee foraging activity on the crop within the tunnels. Mean temperature during the whole experiment was between 12.9 and 29.1°C. First precipitation (28 mm) occurred in the night on day 2 (ca. 35 hours following the application). Thereafter rain occurred on days 6 (13 mm), 8 (2 mm), 9 (7 mm), 10 (6 mm) and 14 (6 mm).

Statistics:

Statistical evaluation was done for mortality, foraging activity, colony strength and the brood termination rate using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student or Welch t- test (pairwise comparison) (software: TOX Rat Professional, Version 2.10.05 ® ToxRat Solutions GmbH).

Dates of experimental work: June 17, 2013 – July 16, 2013

Results:

Mortality of the adult bees (worker bees)

Pre-application phase (day- 2 to day 0 before application):

Mortality of the pre-application phase in the control and the test item group was 24.8 and 17.6 dead bees/colony/day, respectively. The mortality in the reference item was 74.3 dead bees/colony/day. This was not statistically significantly different compared to the water control (Student t-test, pairwise comparison to the control, two-sided, $\alpha = 0.05$).

Exposure phase in the tunnels (day 0 after application to day 4):

There was no sign of an acute effect on the mortality of the bees following the test item treatment. Average control mortality of adult bees during the exposition phase (day 0 to day 4 following the application) was 19.9 dead bees/colony/day. The average mortality in the test item group was slightly higher with 26.0 dead bees/colony/day, but not statistically significant to the control values (Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$). Reference Item mortality was 36.2 dead

bees/colony/day (no statistical significant difference, Student t-test, pairwise comparison one-sided greater, α = 0.05; *Nota bene*: The absence of acute effects of the Reference Item is in line to its mode of action).

Phase outside the tunnels (day 5 after application to day 27):

An overall comparison of the mean number of dead bees found in the traps and on the gauze after the application from day 5 to day 27 did also not show a statistical significant difference between the control and the Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) - treatment (Student t-test, pairwise comparison, one-sided greater, α = 0.05). A mean of 3.8 dead bees per day and tunnel was found for the period from day 5 to day 27 after treatment in the test item group, whereas a mean of 5.4 dead bees were found in the control group.
There was no impact of the reference item to the adult bee mortality, which is not to be expected due to mode of action of the reference item.

Mortality of pupae

Pre-application phase (day -2 to day 0 before application):

Mortality of pupae in the control, test item and reference item groups was 2.3, 0.1 and 3.4 dead pupae/colony/day, respectively. There was no statistically significant different between the groups control (Student t-test, pairwise comparison to the control, two-sided, α = 0.05).

Exposure phase in the tunnels (day 0 after application to day 4):

Mean pupae mortality during the exposure phase in the test item treated group was 0.6 dead pupae/day/colony and therefore lower compared to the mean value of the control group (0.8 dead pupae/day/colony). Accordingly, this was not statistically significantly different to the control group (Student t-test, pairwise comparison one-sided greater, α = 0.05). The application of the reference item resulted in a higher number of dead pupae following the application: 5.3 dead pupae/day/colony, which was statistically significantly different to the control group.

Phase outside the tunnels (day 5 after application to day 27):

The same as observed for the confinement period holds true for the phase outside the tunnels: the test item treated group showed a lower pupae mortality rate compared to the control group, whereas pupae mortality in the reference item group was increased and statistically significant different to the control group. Mean pupae mortality from day 5 to day 27 was 0.1 dead pupae/colony/day in the test item group and 0.4 dead pupae/colony/day in the control group. Reference item induced pupae mortality was 22.3 dead pupae/colony/day.

Foraging Activity

Pre-application phase (day -2 to day 0 before application):

The mean foraging activity in the intended test item group and reference item groups was comparable to the control group, resulting in overall daily mean values of 15.4, 17.3 and 19.6 bees/m²/day in the

control, test item group and reference item groups, respectively. No statistically significant differences were found between the control, the test and reference item treatment groups at the overall daily mean comparison of this period.

Exposure phase in the tunnels (day 0 after application to day 4):

There was a slight decrease in foraging activity after application in the test item group. Mean foraging activity on each occasion was lower compared to the control values on these days. Nevertheless, these lower flight activities were not statistically significant different (Student t-test, pair-wise comparison to the control, one-sided smaller, $\alpha = 0.05$). The overall daily mean foraging activity from day 0 to day 4 in the test item group was 11.4 bees/m²/day compared to 15.7 bees/m²/day the control group.

The reference item (Insegar) resulted in no reduction of the foraging activity on the day of application and on all following days.

Behavioural abnormalities

After application of Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L) no behavioural abnormalities occurred at any time during the whole assessment period (up to day 27). No behavioural abnormalities were observed in the control group and in the reference item group.

Condition of the Colonies

At the beginning of the trial, all brood stages (eggs, larvae and closed brood), as well as a sufficient amount of nectar and pollen storage, was found in all colonies as an indication of healthy colonies.

All queens and/or a sufficient presence of eggs were found in the test item treated colonies during all brood checks indicating that the queens were alive and healthy.

After application, no indication of a test item related effect on the condition of the colonies was observed. All test item treated colonies remained vital with increasing bee numbers and healthy brood. There was no indication of any hazard of the test item on the condition of the bee colonies.

Colony Strength

The mean number of honey bees per colony in all treatment groups was very similar one day before application and did not differ statistically (mean of 4736 to 5018 per colony). The subsequent development of the colony strength among the colonies in the control and test item treatment groups followed the same pattern. There was a continuous increase of colony strength observable, which was stronger in the test item group compared to the control group. No statistical significant difference in the colony strength between the test item treated colonies and the control colonies occurred at any assessment date. Overall, no adverse effects of the test item on colony strength and population development have been observed throughout the study.

Considering the initial mean number of bees per treatment group before the application as 100 %, the following relative mean numbers of bees were determined:

Table 8.3.1.3-4: Colony strength

Treatment Group	Day -1	Day +5	Day +9	Day +15	Day +21	Day 27
Control	100%	123%	144%	159%	161%	148%
Test Item	100%	151%	161%	176%	175%	169%
Reference Item	100%	141%	152%	140%	137%	108%

Development of Bee Brood

Brood Termination Rate:

Following the assessment of single cells from the egg stage to the successfully hatched worker bee, the mean termination rate at BFD (Brood Fixing Day) 22 in the test item group was 40.3 %. Although the termination rate in the test item group was slightly higher compared to the control group (30.2 %), this difference was not statistically significantly different compared to the control group.

Treatment with the reference item Insegar (a.s.: fenoxycarb) caused a clear decrease of brood development of the marked eggs, resulting in a termination rate of 82.3 %. This decrease was statistically significantly different compared to the control group.

Table 8.3.1.3-5: Effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on honey bee brood under semi-field conditions (Tunnel Test)

Parameter	Treatment group[1)]		
	Control	Test Item [2.68 L/ha]	Reference Item Insegar [300 g a.s./ha]
Mean mortality of worker bees/colony/day [%] during			
pre-application phase [2)]	24.8 ± 11.6	17.6 ± 8.7 (n.s.)	74.3 ± 30.1 (n.s.)
exposure phase in the tunnels [2)]	19.9 ± 17.6	26.0 ± 13.9 (n.s.)	36.2 ± 16.9 (n.s.)
phase outside the tunnels [3)]	5.4 ± 4.9	3.8 ± 4.9 (n.s.)	6.8 ± 8.7 (n.s.)
overall after application	8.0 ± 9.9	7.8 ± 11.1 (n.s.)	12.0 ± 15.3 (n.s.)
Mean mortality of larvae and pupae [n] during			
pre-application phase [4)]	2.2 ± 2.7	0.1 ± 0.1 (n.s.)	3.4 ± 2.1 (n.s.)
exposure phase in the tunnels [4)]	0.8 ± 0.8	0.6 ± 0.5 (n.s.)	5.3 ± 3.7 (*)
phase outside the tunnels [5)]	0.4 ± 0.7	0.1 ± 0.2 (n.s.)	22.3 ± 28.6 (*)
overall after application	0.5 ± 0.7	0.2 ± 0.3 (n.s.)	19.3 ± 26.7 (*)
Mean foraging activity/m² /colony/day[n] during			
pre-application phase	15.4 ± 6.7	17.3 ± 6.3 (n.s.)	19.6 ± 7.2 (n.s.)
exposure phase in the tunnels	15.7 ± 5.3	21.4 ± 6.2 (n.s.)	16.3 ± 6.6 (n.s.)
Mean brood termination rate [%] [6)]	30.2	40.3 (n.s.)	82.3 (*)

1) each with four tunnels (replicate)
2) mean number of dead honey bees per day and colony found in dead bee traps and on gauze strips in the tunnels
3) mean number of dead honey bees per day and colony found in dead bee traps, only
4) mean number of dead pupae/larvae per day and colony found in dead bee traps and on gauze strips in the tunnels
5) mean number of dead pupae/larvae per day and colony found in dead bee traps, only
6) at BFD 22; n.s. = not statistically significant compared to the control; * = statistically significant compared to the control
Statistic: Student or Welch t-test, α=0.05, pairwise; before application: two-sided; after application one-sided greater (mortality and termination rate), one-sided smaller (foraging activity, colony strength)

Conclusions:

To assess the potential effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on honey bee colonies including brood development, 2.68 L product in 400 L tap water/ha (corresponding to 60 g foramsulfuron a.s./ha), a water treated control and a reference item were applied to a full-flowering and highly bee-attractive crop (i.e. *Phacelia tanacetifolia*) under semi-field (tunnel) condition during bee-flight. No adverse effects on mortality of worker or pupae, foraging activity, behaviour, nectar- and pollen storage as well as on queen survival were observed. No effects on colony development, colony strength or bee brood were observed. Based on the results of this study, it can be concluded that Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate of 2.68 L product in 400 L tap water/ha (corresponding to 60 g foramsulfuron a.s./ha) during honey bees actively foraging on a bee-attractive, flowering crop. The observed characteristic brood effects of the reference item Insegar (a.s. fenoxycarb) in terms of typicality, time of occurence and extent, showed that the prevailing test conditions allowed for a profound detection of effects on immature honey bee life stages.

CA 8.3.1.4 Sub-lethal effects

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

CA 8.3.2 Effects on non-target arthropods other than bees

For the representative formulation FSN + IDF OD 45 toxicity studies on different non-target arthropods other than bees were performed. While *Aphidius rhopalosiphi* showed some mortality in a tier 1 glass plate study (see section CA 8.3.2.1), no effects on mortality or reproduction were observed in a higher tier extended lab/aged residue study when exposed to freshly dried residues and residues aged for 3 and 7 days. An extended laboratory study with *Typhlodromus pyri* resulted in only 20% corrected mortality at the highest test rate and no effect on reproduction, confirming the results of the tier 1 glass plate study which also indicated low toxicity of FSN + IDF OD 45 to *Typhlodromus*. In addition to the tests with *Aphidius rhopalosiphi* (CA 8.3.2.1) and *Typhlodromus pyri* (CA 8.3.2.2) additional species *Chrysoperla carnea, Aleochara bilineata, Poecilus cupreus* and *Pardosa sp.* were tested. These studies showed that FSN + IDF OD 45 had no or only low effects on mortality, reproduction and feeding rate of these additional species.
Details of the studies with these additional species are provided in the table below.

Table 8.3.2-1: Toxicity data of foramsulfuron to non-target arthropods other than bees

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint			Reference
Chrysoperla carnea	FSN + IDF OD 45 Laboratory, glass plate	**LR_{50} > 4000 mL prod./ha** Corr. Mortality [%]	Eggs/Female/Day	Hatching [%]	[redacted], 2000 M-194627-01-1 KCA 8.3.2 /04 [991048098]
	control	-	15.8	81.3	
	1600 mL prod./ha	13	15.7	81.7	
	2000 mL prod./ha	2	15.0	80.8	
	4000 mL prod./ha	35	15.4	81.7	
Aleochara bilineata	FSN + IDF OD 45 Laboratory, spray deposits on quartz sand	**ER_{50} > 4000 mL prod./ha** Effect on Reproduction [%]			[redacted], 1999 M-193482-01-1 KCA 8.3.2 /03 [991048095]
	1600 mL prod./ha	0			
	2000 mL prod./ha	10			
	4000 mL prod./ha	15			
Poecilus cupreus	FSN + IDF OD 45 Laboratory, spray deposits on quartz sand	**LR_{50} > 5333 mL prod./ha** Corr. Mortality [%]	Effect on Feeding Rate [%]		[redacted], 1999 M-186968-01-1 KCA 8.3.2 /02 [CW98/112]
	2667 mL prod./ha	0	-22.5 [A]		
	5333 mL prod./ha	0	-12.4 [A]		
Pardosa sp.	FSN + IDF OD 45 Laboratory, spray deposits on quartz sand	**LR_{50} > 4000 mL prod./ha** Corr. Mortality [%]	Effect on Feeding Rate [%]		[redacted], 1999 M-188675-01-1 KCA 8.3.2 /01 [991048030]
	1600 mL prod./ha	0	8		
	2000 mL prod./ha	5	3		
	4000 mL prod./ha	0	2		

[A]: A negative value indicates a higher feeding rate in the treatment than in the control.
prod.: product
Bold letters: Values considered relevant for risk assessment in the MCP document

Report:	[redacted]ö; [redacted];1999;M-188675-01
Title:	Toxicity to the ground dwelling predator *Pardosa* spp. (laboratory) according to IOBC Guideline ([redacted] et al. 1998) Code: AE F130360 01 1K05 A304
Report No:	C004831
Document No:	M-188675-01-1
Guidelines:	**IOBC: [redacted] et al. 1998;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
5 % mortality, 8 % food consumption (120 g a.s./ha)

Report:	[redacted]6; [redacted];1999;M-186968-01
Title:	Toxicity to the ground dwelling predator *Poecilus cupreus* L. (Coleoptera: Carabidae) in the laboratory AE F130360 + AE F122006 oil dispersable 22.5 + 22.5 g/l; Code: AE F130360 01 1K05 A301
Report No:	C003899
Document No:	M-186968-01-1
Guidelines:	**BBA: VI 23-2.1.8;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted]7; [redacted];1999;M-193482-01
Title:	Toxicity to the ground dwelling predator *Neochata bilineata* Grd. (laboratory) according to IOBC Guideline ([redacted] 1992) Code: AE F130360 01 1K05 A304
Report No:	C006202
Document No:	M-193482-01-1
Guidelines:	**IOBC: [redacted], 1992;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
46 % mortality, 14 % fertility (45 g a.s./ha)

Report:	[redacted]8; [redacted];2000;M-194627-01
Title:	Toxicity to the foliage dwelling predator *Chrysoperla carnea* STEPH. (laboratory) following the IOBC Guideline ([redacted] 1988), ringtest method ([redacted] et al. 1997) and OECD Guideline proposal ([redacted] et al. 1999) Code: AE F130360 01 1K05 A304
Report No:	C006791
Document No:	M-194627-01-1
Guidelines:	**IOBC: 1988; 1999;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
35 % mortality, 13 % fertility (90 g a.s./ha)

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

For the formulation FSN + IDF OD 45 toxicity studies on the parasitic wasp *Aphidius rhopalosiphi* were performed. Details of all studies are provided in the following table.

Table 8.3.2.1-1: Toxicity data of foramsulfuron to *Aphidius rhopalosiphi* presented in this chapter

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint				Reference
Aphidius rhopalosiphi	FSN + IDF OD 45 Laboratory, glass plate	**LR_{50} 241 mL prod./ha** Corr. Mortality [%]				[redacted], 2013 M-461455-01-1 KCA 8.3.2.1 /05 [13 10 48 030 A]
	2nd test run:					
	35 mL prod./ha	0				
	62 mL prod./ha	0				
	111 mL prod./ha	12.5				
	197 mL prod./ha	50.0				
	350 mL prod./ha	57.5				
	1st test run:					
	267 mL prod./ha	61.5				
	475 mL prod./ha	89.7				
	844 mL prod./ha	100				
	1501 mL prod./ha	100				
	2670 mL prod./ha	100				
Aphidius rhopalosiphi	FSN + IDF OD 45 Laboratory, glass plate	Corr. Mortality [%]		Effect on Reproduction [%]		[redacted], 1999 M-191908-01-1 KCA 8.3.2.1 /01 [991048029]
	160 mL prod./ha	25		25		
	2000 mL prod./ha	100		n.a.		
	4000 mL prod./ha	100		n.a.		
Aphidius rhopalosiphi	FSN + IDF OD 45 Aged residues, spray deposits on potted maize plants	**LR_{50} > 2670 mL prod./ha**				[redacted], 2000 M-198973-01-1 KCA 8.3.2.1 /02 [001048067]
	107 mL prod./ha	Corr. Mortality [%]	Effect on Repr. [%]	Repellency (30 min) [%]	Repellency (2h) [%]	
	Residues aged for 0 days:	0	-3 [A]	32	39	
	Residues aged for 3 days:	0	3 [A]	-7 [B]	6	
	Residues aged for 7 days:	0	3 [A]	11	-24 [B]	
	2000 mL prod./ha					
	Residues aged for 0 days:	5	6	70		
	Residues aged for 3 days:	0	0	13	6	
	Residues aged for 7 days:	0	-6 [A]	5	-13 [B]	
	2670 mL prod./ha					
	Residues aged for 0 days:	0	3	5	21	
	Residues aged for 3 days:	5	6	-12 [B]	7	
	Residues aged for 7 days:	0	-2 [A]	5	-4 [B]	

[A]: A negative value indicates a higher reproduction rate in the treatment than in the control.
[B]: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.
prod.: product
n.a.: not assessed
Bold letters: Values considered relevant for risk assessment in the MCP document

Report:	[redacted]°; [redacted];1999;M-191908-01
Title:	Toxicity to the parasitoid *Aphidius rhopalosiphi* (Destefani-Perez) / adults under laboratory conditions according to IOBC Guidelines ([redacted]1992/1997) / Code: AE F130360 01 1K05 A304
Report No:	C005357
Document No:	M-191908-01-1
Guidelines:	**IOBC:;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
100 % mortality, - fertility (45 g a.s./ha)

Report:	[redacted]8; [redacted];2000;M-198973-01
Title:	Toxicity of AE F130360 01 1K05 A304 to the cereal aphid parasitoid *Aphidius rhopalosiphi* (Destefani-Perez) (extended laboratory test "aged residue test") / Code: AE F130360 01 1K05 A304
Report No:	C010411
Document No:	M-198973-01-1
Guidelines:	**ESCORT: [redacted]et al. 1994; IOBC [redacted]1997;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted]1; [redacted];2013;M-461455-01
Title:	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in a laboratory test
Report No:	13 10 48 030 A
Document No:	M-461455-01-1
Guidelines:	**IOBC ([redacted]et al. 2000);none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine a rate-response relationship for mortality of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in a laboratory test. Adult wasps (used within 48 hours after hatching, 4 x 7 females and 4 x 3 males for the control groups and the treatment groups) were exposed to control (deionised water) and dried spray residues of the test item with rates of 267, 475, 844, 1501 and 2670 mL product/ha (1st test run) and 35, 62, 111, 197 and 350 mL product/ha (2nd test run) in 200 L deionised water/ha applied on glass plates. Dimethoate EC 400 (0.3 mL product/ha in 200 L deionised water/ha) was used as a toxic reference item. Survival of the parasitic wasps was used as test endpoint with the aim to calculate the LR_{50}, if possible. The LR_{50} for *Aphidius rhopalosiphi* was calculated to be 241 mL product/ha in 200 L water/ha based on the results of the 1st and 2nd test run.
The test was performed following the IOBC Guideline ([redacted]*et al.* 2000) taking account of the recommendations given by [redacted]*et al.* (2001), but without performance of a reproduction assessment.

Material and Methods:

Test item: Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L); analysed active ingredients: 2.33 % w/w (22.41 g/L) Foramsulfuron (AE F130360) and 2.29 % w/w (21.96 g/L) Isoxadifen-ethyl (AE F122006); Specification No.: 102000011304 - 06, Batch ID: EFKM002442, Sample description: TOX10129-00, Material No.: 06321801, density: 0.961 g/mL (according to Certificate of Analysis)

The test item was tested under laboratory conditions after contact exposure of adults of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) to dried spray residues of the test item with rates of 267, 475, 844, 1501 and 2670 mL product/ha (1st test run) and 35, 62, 111, 197 and 350 mL product/ha (2nd test run) in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (0.3 mL product/ha in 200 L deionised water/ha) was used as a toxic reference item.
Adults of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) were exposed in 4 replicates per treatment group and 7 females and 3 males per replicate to the residues of the test item, reference item and control treatments, respectively. During the exposure phase the adult wasps were fed with 25 % w/w aqueous fructose solution. The number of surviving, affected, moribund and dead wasps was recorded over a period of 48 hours. From these data the endpoint mortality was calculated.

Climatic conditions:	Temperature:	1st test run:	19-21°C
		2nd test run:	19-21°C
	Relative humidity:	1st test run:	67-73%
		2nd test run:	68-71%
	Light – dark – cycle:	16 hours light, 8 hours dark	

Dates of work: 1st test run: June 03, 2013 – June 05, 2013
2nd test run: June 24, 2013 – June 26, 2013

Results:

Table 8.3.2.1-2: Validity criteria according to [redacted] et al. (2000)

Validity criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mortality in the control group	≤ 15 % (48 hours)	2.5 %	0 %
Corrected mortality in the reference item group	≥ 50% (48 hours)	100 %	100 %

All validity criteria according to MEAD-BRIGGS et al. (2000) were met.

The results of the control group indicated that the test organisms were in a good condition (mortality: 2.5 % in the 1st test run and 0 % in the 2nd test run). The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 100 %, both test runs). Concerning mortality in the control group and as well the susceptibility of the test organisms to the reference item the study is proved to be valid.

Mortality:
1st test run
After 48 hours, the mortality in the test item treatments ranged between 62.5 % and 100 % in the test item groups in comparison to 2.5 % in the control. Based on these results the corrected mortality for the different rates was between 61.5 % and 100 %.

2nd test run

After 48 hours, the mortality in the test item treatments ranged between 0 % and 57.5 % in the test item groups in comparison to 0 % in the control. Based on these results the corrected mortality for the different rates was between 0 % and 57.5 %.

Table 8.3.2.1-3: Effects on mortality of *Aphidius rhopalosiphi* (DESTEFANI-PEREZ)

Test item	**Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L)**	
Test object	*Aphidius rhopalosiphi* (DESTEFANI-PEREZ)	
Exposure	dried spray deposits on glass plates	
Treatment	**Mortality [2] [%]**	**Corrected mortality [3] [%]**
Application rate [1] [mL product/ha]		
1st test run		
Control	2.5	-
267	62.5*	65.5
475	90.0*	89.7
844	100*	100
1501	100*	100
2670	100*	100
2nd test run		
Control	0	
35	0 (n.s.)	0
62	0 (n.s.)	0
111	7.5 (n.s.)	7.5
197	50.0*	50.0
350	57.5*	57.5
LR_{50} [4] [95 % CL][5]	**241 mL product/ha** [216 - 269 mL product/ha]	
Reference item Dimethoate EC 400 0.3 mL product/ha (1st and 2nd test run)	100*	100

[1] Application rate in 200 L water/ha
[2] Mortality after exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using FISHER's Exact Binomial test ($\alpha = 0.05$).
[3] Corrected mortality according to [redacted] (1925)
[4] LR_{50} = lethal rate over 1st and 2nd test run
[5] 95 % CL means lower and upper 95 % confidence limits
(n.s.) not statistically significantly different compared to the control
* statistically significantly different compared to the control

No unusual observations were noted in the control and all test item treatment groups at any observation point during the test.

Conclusions

In a worst-case laboratory study with Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L) the LR_{50} for *Aphidius rhopalosiphi* was calculated to be 241 mL product/ha in 200 L water/ha based on the results of the 1st and 2nd test run.

All validity criteria according to [redacted] et al. (2000) were met.

CA 8.3.2.2 Effects on *Typhlodromus pyri*

For the formulation FSN + IDF OD 45 toxicity studies on the predatory mite *Typhlodromus pyri* were performed. Details of all studies are provided in the following table.

Table 8.3.2.2-1: Toxicity data of foramsulfuron to *Typhlodromus pyri* presented in this chapter

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	Reference
Typhlodromus pyri	FSN + IDF OD 45 Laboratory, glass plate 267 mL prod./ha 475 mL prod./ha 844 mL prod./ha 1501 mL prod./ha 2670 mL prod./ha	**LR_{50} > 2670 mL prod./ha** Corr. Mortality [%] 1.0 5.1 38.8 96.7 48.0	[redacted] 2013 M-457360-01-1 KCA 8.3.2.2 /03 [13 10 48 031 A]
Typhlodromus pyri	FSN + IDF OD 45 Laboratory, glass plate 267 mL prod./ha 2670 mL prod./ha	Corr. Mortality [%] 25 [A] 53	Effect on Reproduction [%] -0.8 [B] 13.9	[redacted] 1999 M-191384-01-1 KCA 8.3.2.2 /01 [CW99/003]
Typhlodromus pyri	FSN + IDF OD 45 Extended lab. exposure on detached *Polygonum convolvulus* leaves 2000 mL prod./ha 4000 mL prod./ha	**LR_{50} > 4000 mL prod./ha** Corr. Mortality [%] -1.3 [A] 20.0	Effect on Reproduction [%] 9.4 -10.4 [B]	[redacted] 1999 M-192822-01-1 KCA 8.3.2.2 /02 [CW99/092]

[A]: A negative value indicates a lower mortality in the treatment than in the control
[B]: A negative value indicates a higher reproduction rate in the treatment than in the control.
prod.: product
Bold letters: Values considered relevant for risk assessment in the MCP document

Report:	[redacted]b; [redacted];1999;M-191384-01
Title:	Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) in the laboratory AE F130060 + AE F122006 oil flowable 22.5 + 22.5 g/L Code: AE F130360 01 1K03 A30
Report No:	C005311
Document No:	M-191384-01-1
Guideline:	**Deviation not specified**
GLP/GCP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
55 % mortality, 34 % fertility (60 g a.s./ha)

Report:	[redacted]1; [redacted];1999;M-192822-01
Title:	Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L Code: AE F130360 01 1K05 A301
Report No:	C005863
Document No:	M-192822-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
20 % mortality, 0 % fecundity (90 g a.s./ha)

Report:	[redacted]§; [redacted];2013;M-457360-01
Title:	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test
Report No:	13 10 48 031 A
Document No:	M-457360-01-1
Guidelines:	**IOBC ([redacted] et al. 2000); none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine a rate-response relationship for mortality of the predatory mite *Typhlodromus pyri* SCHEUTEN in a worst-case laboratory test. Mites were exposed on glass plates to application rates of 267, 475, 844, 1501 and 2670 ml product/ha in 200 L deionised water/ha and effects on mortality were compared to those of deionised water treated controls (200 L/ha). Dimethoate (applied at 15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as reference item. Survival of the predatory mites was used as test endpoint with the aim to calculate the LR_{50} if possible. The test was performed according to the IOBC Guideline ([redacted] et al. 2000) taking account of the recommendations given by [redacted] et al. (2001), but without performance of a reproduction assessment. The LR_{50} for *Typhlodromus pyri* was estimated to be > 2670 mL product/ha in 200 L water/ha, the highest rate tested. All validity criteria according to the guideline were met.

Materials and Methods:

Test item. Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L); analysed active ingredients: 2.33 % w/w (22.41 g/L) foramsulfuron (AE F130360), 2.29 % w/w (21.96 g/L) isoxadifen-ethyl (AE F122006); Specification No.: 102000011304-06; Batch ID: EFKM002442, Sample description: TOX10129-00, Material No.: 06321801, Density: 0.961 g/mL (according to Certificate of Analysis).

The test item was tested under laboratory conditions after contact exposure of protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN to dried spray residues of the test item with rates of 267 – 475 – 844 – 1501 – 2670 mL product/ha in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item.
Protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN were exposed in 5 replicates per treatment group and 20 mites per replicate to the residues of the test item, reference item and control treatments, respectively. During the assessments the mites were fed with a mix of pine (*Pinus nigra*) and birch (*Betula pendula*) pollen, 1:1. The number of surviving, dead, trapped and escaped predatory mites was recorded over a period of 7 days. From these data the endpoint mortality was calculated.

Toxic standard: (Dimethoate EC 400): 15 mL product/ha (nominally equivalent to 6 g a.s./ha) in 200 L/ha of deionised water; control: deionised water only (200 L/ha).

Dates of work: May 21, 2013 – May 28, 2013

Results:

Table 8.3.2.2-2: Validity criteria

Validity criteria	
Mortality in the control group	≤ 20 % (dead, trapped and escaped mites) on day 7
Corrected mortality in the reference group	50 – 100 % on day 7

All validity criteria were met.

The results of the control group indicated that the test organisms were in a good condition (mortality: 2.0 %). The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 85.7 %). Concerning mortality in the control group and as well the susceptibility of the test organisms to the reference item the study is proved to be valid.
After 7 days, the mortality in the test item treatments ranged between 3.0 % and 49.0 % in comparison to 2.0 % in the control. Based on these results the corrected mortality for the different rates ranged between 1.0 % and 48.0 %. The LR_{50} for Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L) was estimated to be > 2670 mL product/ha in 200 L water/ha.

Table 8.3.2.2-3: Effects on mortality of *Typhlodromus pyri* SCHEUTEN

Test item	**Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5 + 22.5 g/L)**	
Test organism	*Typhlodromus pyri* SCHEUTEN	
Exposure on	Dried spray deposits on glass plates	
Treatment	**Mortality[2] [%]**	**Corrected mortality[3] [%]**
Control	2.0	-
Application rate[1] [mL product/ha]		
267	3.0 (n.s.)	1.0
475	7.0 (n.s.)	5.1
844	40.0*	38.8
1501	38.0*	36.7
2670	49.0*	48.0
LR_{50}	**> 2670 mL product/ha**	
Reference item Dimethoate EC 400 150 mL product/ha	86.0*	85.7

[1] Application rate in 200 L water/ha
[2] Mortality after exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Fisher´s Exact Binomial test ($\alpha = 0.05$).
[3] Corrected mortality according to Abbott (1925)
(n.s.) not statistically significantly different compared to the control: Fisher`s Exact Binomial test with Bonferroni correction ($\alpha = 0.05$)
* statistically significantly different compared to the control: Fisher`s Exact Binomial test with Bonferroni correction ($\alpha = 0.05$) for test item and Fisher`s Exact Binomial test ($\alpha = 0.05$) for reference item

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusions:

In a worst-case laboratory study with Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L) the LR_{50} for *Typhlodromus pyri* was estimated to be > 2670 mL product/ha in 200 L water/ha, the highest rate tested.

CA 8.4 Effects on non-target soil meso and macrofauna

In the new European dossier format/data requirements there is no data point that corresponds to acute toxicity to earthworms. Two acute studies are added here, the study on the active substance was submitted and reviewed for the first inclusion but the second study on the metabolite, AE F153745, as not previously evaluated at EU level and has been added here for completeness.

Active substance

Report:	[redacted] <; [redacted] ;1998;M-142934-01
Title:	Acute toxicity to earthworms (*Eisenia fetida*) AE F130360 substance, technical Code: AE F130360 00 1G98 0002
Report No:	A59245
Document No:	M-142934-01-1
Guidelines:	**EU (=EEC): 92/69; OECD: 207; Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

AE F153745

Report:	[redacted] B; [redacted] ; [redacted] ;1999;M-192813-01
Title:	Acute toxicity to earthworms (*Eisenia fetida*) AE F153745 (impurity of AE F130360) substance, technical Code: AE F153745 00 1G98 0001
Report No:	C005899
Document No:	M-192813-01-1
Guidelines:	**EU (=EEC): 92/69/EWG Part C; OECD: 207; Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The acute toxicity of AE F153745 to earthworms of the species *Eisenia fetida* was examined in an artificial soil test according to OECD guideline 207. The following concentrations were tested: 100, 180, 320, 560 and 1000 mg test substance/kg artificial soil (dry weight) and an untreated control with 10 worms in each of the 4 replicates. Mortality and intoxication symptoms were determined 7 and 14 days after application. Weight of worms was determined at start and end of testing. Weight changes were compared with the untreated control. No mortality occurred and no intoxication symptoms were observed in any of the tested concentrations and in the untreated control. The LC_{50} value after 7 and 14 days test duration was >1000 mg test substance/kg dry soil. The no observed effect concentration (NOEC) regarding to mortality, weight loss and intoxication symptoms was ≥ 1000 mg test substance/kg dry soil after 14 days test duration.

Material and methods:

Test item. AE F153745; substance, technical (metabolite of AE F130360); Code: AE F153745 00 1C98 0001; Analysed purity: 97.8 % w/w; Analytical certificate No.: AZ 07716

Principles of the testing procedure: Adult *Eisenia fetida* (older than 2 months and showing a clitellum), 4 x 10 animals for the control group and for each test concentration of the treatment group were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 100, 180, 320, 560 and 1000 mg test substance/kg test substrate (dry weight). The test substance was mixed in industrial sand. Mortality and intoxication symptoms were determined 7 and 14 days after application. Weight of worms was determined at start and end of testing. Weight changes were compared with the untreated control.

Dates of experimental work: September 24, 1999 – October 08, 1999

Results:

Physical and chemical parameters:
At the start of testing the moisture content of the control substrate was 25.13% and 25.06% at experimental termination. At the beginning of the test the pH value of the test substrate ranged from 6.1 to 6.4. At the end of the test the pH value of the test substrate was between 5.8 and 6.4. The pH value of the basic substrate was 6.3.

Biological results:
No mortality occurred and no intoxication symptoms were observed in any of the tested concentrations and in the untreated control (see Table below).

Table 8.4.1-6: % mortality and symptoms

Nominal concentration in mg/kg (dry weight)	Jar No.	7 d test duration (mean values)		14 d test duration (mean values)	
		% mortality	symptoms	% mortality	symptoms
Control	1-4	0	-	0	-
100	5-8	0	-	0	-
180	9-12	0	-	0	-
320	13-16	0	-	0	-
560	17-20	0	-	0	-
1000	21-24	0	-	0	-

Based on the absence of mortality no LC_{50} calculation could be determined and no concentration-effect relationship could be plotted.
The LC_{50} value in comparison with the untreated control group after 7 and 14 days test duration was >1000 mg/kg (dry weight).
Average worm weight of each replicate at the start and the end of testing with their weight differences are shown in the table below.

Table 8.4.1-6: Weight of *Eisenia fetida* after treatment with AE F153745

	Mean start weight in g	Mean weight difference start - end in g	Mean percent weight loss[1]
Control	5.3650 A	0.071250 A B	13.340 A B
100 mg/kg	5.4075 A	0.071750 A B	13.277 A B
180 mg/kg	5.3975 A	0.070750 A B	13.187 A B
320 mg/kg	5.4375 A	0.061000 B	11.201 B
560 mg/kg	5.4925 A	0.076500 A B	13.922 A B
1000 mg/kg	5.4325 A	0.086250 A	15.824 A

[1] mean percent weight loss = mean weight difference (start - end) in percent of mean start weight
Concentrations with the same letter within each column are not significantly different.

At the start of the test there was no significant difference of worm weights between the treatments and the control.
At the end of the test the mean weight of surviving worms was reduced by 11.2 - 15.8% of initial weight in the treated groups and the control group. This weight loss increased with the dose of the test substance. There were no significant differences between the treatments and the control regarding the absolute or the percent weight loss compared to controls.
The no observed effect concentration (NOEC) regarding to mortality, weight loss and intoxication symptoms was 1000 mg test substance/kg dry soil after 14 days test duration.

Conclusions:

In a 14-day Artificial Soil Test (CE99/141-1, method OECD / EU) to determine the effects of AE F153745 to *Eisenia fetida* (earthworm) the LC_{50} value after 7 and 14 days test duration was >1000 mg/kg (dry weight) in comparison with the untreated control group.
The highest concentration tested without mortality, without intoxication symptoms and without a significantly higher weight loss compared to the control (NOEC, no observed effect concentration) after 14 days test duration was ≥ 1000 mg test substance / kg dry test substrate.

CA 8.4.1 Earthworm, sub-lethal effects

For foramsulfuron and its metabolites AE F092944, AE F153745 and AE F130619 reproductive toxicity studies on *Eisenia fetida* were performed. In all studies no mortality occurred. No-Observable-Effect levels ranged from ≥ 2.75 mg/kg dws for the parent compound to ≥ 100 mg/kg dws for metabolite AE F153745. Details of all studies are provided in the following table.

Table 8.4.1-1: Reproductive toxicity data of foramsulfuron and metabolites to *Eisenia fetida* presented in this chapter

Test substance	Test species, test design	Endpoint	Reference
Foramsulfuron	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item sprayed on soil surface	NOEC = ≥ 600 g a.s./ha **≥ 2.75 mg a.s./kg dws** [1)]	[redacted] 2000 M-193508-01-1 KCA 8.4.1 /01
AE F092944	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **10 mg/kg dws**	[redacted], 2013 M-461051-01-1 KCA 8.4.1 /02
AE F130619	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **56 mg/kg dws**	[redacted], 2013 M-461453-01-1 KCA 8.4.1 /03
AE F153745	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-459518-01-1 KCA 8.4.1 /04

[1)] Considering a jar surface area of 283.4 cm^2 and an amount of 618 g dry soil per jar – BCS calculation results in 2.75 mg a.s./kg dws.
dws = dry weight soil
Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on foramsulfuron

Report:	[redacted];[redacted];[redacted];2000;M-193508-01-1
Title:	Effects on growth and reproduction of earthworms (*Eisenia fetida*) AE F130360 substance, technical Code: AE F130360 00 1C9 0002
Report No:	C006218
Document No:	M-193508-01-1
Guidelines:	**BBA: VL 2.2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):
NOEC = 0.6 kg a.s./ha (corresponds to 3.24 mg a.s./kg)*

* Considering a jar surface area of 283.4 cm^2 and an amount of 618 g dry soil per jar – BCS calculation results in 2.75 mg a.s./kg dws.

Studies on the metabolites of foramsulfuron

AE F092944

Report:	[redacted];[redacted];2013;M-461051-01
Title:	AE F092944 (BCS-AA25052): Effects on survival, growth and reproduction of the earthworm *Eisenia fetida* tested in artificial soil
Report No:	kra/Rg-R-147/13
Document No:	M-461051-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable; none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F092944 on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the first run and five different test concentrations in the second run.
In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 100 mg test item/ kg dry weight artificial soil.
In the second run adult *Eisenia fetida* (approx. 5 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentrations of 5.6, 10, 18, 32 and 56 mg test item/ kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined. The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).
Based on the biological and statistical significance observed on growth and reproduction, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 10 mg test item/ kg dry weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 18 mg test item/kg dry weight artificial soil. The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:

Test item. AE F092944 (BCS-AA25052); Batch code: AE F092944 00 1B99 0002; Origin Batch No.: 23503LR; CAS No.: 363915-01-2; LIMS No.: 1034970; purity: 99.8 % w/w; certificate No.: AZ 17077.

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 100 mg test item/ kg dry weight artificial soil.
In the second run adult *Eisenia fetida* (approx. 5 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentrations of 5.6, 10, 18, 32 and 56 mg test item/ kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Toxic standard (Carbendazim EC 360 G): 1.25 – 2.5 – 5.0 mg a.s./kg soil d.w. (corresponds to 3.94 – 7.89 – 15.78 mg test item/ kg soil d.w.); control: quartz sand, solvent control: none.

Dates of experimental work: July 10, 2012 – September 17, 2012 (first run)
April 12, 2013 – June 14, 2013 (second run)

Results:

Table 8.4.1-2: Validity criteria

Validity criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mortality of adults in the control	≤ 10 %	0 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	391, 335, 260, 313, 330, 399, 371, 387	246, 350, 278, 228, 285, 232, 254, 287
Coefficient of variance of reproduction in the control	≤ 30 %	13.7 %	14.7 %

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

In a separate toxic standard reference test (Study No. Rg-R-Ref 19/12, Report No.: kra-Rg-R-Ref 19/12; Non-GLP; performed from September 21 to November 28, 2012) the EC_{10}, EC_{20} and EC_{50} (reproduction) of the reference item Carbendazim EC 360 G were calculated to be 3.06, 3.22 or 3.54 mg a.s./kg artificial soil dry weight. The results of the reference test indicated that the test system was sensitive to the reference item.

Table 8.4.1-3: Effects on mortality and changes in body weight of the adults of *Eisenia fetida* after an exposure period of 28 days and the number of offspring per test vessel after 56 days (values in this table are rounded values)

Test object			***Eisenia fetida***					
	1st run		**2nd run**					
Test item	**Control**	**AE F092944**	**Control**	**AE F092944**				
mg test item/kg dry weight artificial soil	---	100	---	5.6	10	18	32	56
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	31.75	39.10*	13.17	6.14*	9.75	17.29	14.30	12.72
Standard Deviation	4.05	7.13	3.31	6.27	2.82	3.52	8.29	3.81
Mean number of offspring per test vessel after 56 days **	348.3	312.5	270.0	271.8	267.8	201.8**	232.3**	223.5**
Standard Deviation	47.8	42.2	39.7	55.2	23.3	19.9	20.6	10.7
Coefficient of variance (%)	13.7	13.5	14.7	20.3	8.7	9.9	8.9	4.8
% of control	---	89.7	---	100.6	99.2	74.7	86.0	82.8

	Reproduction
EC_{10} (mg test item/kg dry weight soil [1]) (95% confidence limits)	**15.35 (n.d.)**
EC_{20} (mg test item/kg dry weight soil [1]) (95% confidence limits)	**54.06 (n.d.)**
EC_{50} (mg test item/kg dry weight soil [1]) (95% confidence limits)	**n. d.**

* statistical significance compared to the control
(1st run: Student t-test, 2nd run: Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$)

** statistical significance compared to the control
(1st run: Student t-test, 2nd run: Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$)

1) Probit analysis

n.d. not determined due to mathematical reasons or inappropriate data

Mortality:

After 28 days of exposure no worms died in the control groups of both test runs and no mortality was observed at any test item concentration.

Effects on growth:

Statistically significant different values for the growth relative to the control were observed in the 1st run and the lowest concentration of the 2nd run. Since in all higher concentrations of the test item no significant differences to the control were observed this is considered not to be treatment related.

Therefore, based on biological and statistical significance (for both test runs):
NOEC related to growth: 56 mg test item/kg dry weight artificial soil
LOEC related to growth: 100 mg test item/kg dry weight artificial soil

Effects on reproduction:
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 5.6 and 10 mg test item/kg dry weight artificial soil (2nd run). Statistically significant different values for the number of juveniles per test vessel relative to the control were observed in the three highest test concentrations of the 2nd run.

Therefore, based on biological and statistical significance (for both test runs):
NOEC related to reproduction: 10 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 18 mg test item/kg dry weight artificial soil

Conclusions:

Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 10 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 18 mg test item/kg dry weight artificial soil.

AE F130619

Report:	[redacted]S; [redacted];2013;M-461453-01
Title:	AE F130619 (BCS-AU59648): Effects on survival, growth and reproduction of the earthworm *Eisenia fetida* tested in artificial soil
Report No:	kra/Rg-R-138/13
Document No:	M-461453-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: Not Applicable; none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F130619 on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the first run and five different test concentrations in the second run.
In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the test concentration of 100 mg pure metabolite/ kg dry weight artificial soil (corresponding to 106 mg test item/ kg dry weight artificial soil).
In the second run adult *Eisenia fetida* (approx. 10 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10 % peat content) to the test concentrations of 5.6, 10, 18, 32 and 56 mg pure metabolite/ kg dry weight artificial soil (corresponding to 6, 10.7, 19.0, 33.7 and 59.9 mg test item/ kg dry weight artificial soil). The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined. The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).
Based on the biological and statistical significance observed on growth and reproduction, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 56 mg pure metabolite/ kg dry

weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 100 mg pure metabolite/kg dry weight artificial soil. The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:

Test item. AE F130619 (BCS-AU59648); Batch code: AE F130619-01-01; Origin Batch No.: SES 10641-3-3; purity: 94 % w/w; certificate No.: AZ 16327 (1st run), AZ 18416 (2nd run).

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in an artificial soil (with 10 % peat content) to the test concentration of 100 mg pure metabolite/ kg dry weight artificial soil (corresponding to 106 mg test item/ kg dry weight artificial soil).
In the second run adult *Eisenia fetida* (approx. 10 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10 % peat content) to the test concentrations of 5.6, 10, 18, 32 and 56 mg pure metabolite/ kg dry weight artificial soil (corresponding to 6, 10.7, 19.0, 33.7 and 59.9 mg test item/ kg dry weight artificial soil). The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Toxic standard (Carbendazim EC 360 G): 1.25 – 2.5 – 5.0 mg a.s./kg soil d.w. (corresponds to 3.94 – 7.89 – 15.78 mg test item/ kg soil d.w.); control: quartz sand, solvent control: none.

Dates of experimental work: July 10, 2012 – September 17, 2012 (first run)
March 04, 2013 – May 06, 2013 (second run)

Results:

Table 8.4.1.4: Validity criteria

Validity criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mortality of adults in the control	≤ 10 %	0 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	448, 354, 314, 269, 374, 299, 424, 422	280, 292, 286, 268, 316, 316, 245, 321
Coefficient of variance of reproduction in the control	≤ 30 %	13.7 %	9.1 %

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

In a separate toxic standard reference test (Study No. Rg-R-Ref 19/12, Report No.: kra-Rg-R-Ref 19/12; Non-GLP, performed from September 21 to November 28, 2012), the EC_{10}, EC_{20} and EC_{50} (reproduction) of the reference item Carbendazim EC 360 G were calculated to be 3.06, 3.22 or 3.54 mg a.s./kg artificial soil dry weight. The results of the reference test indicated that the test system was sensitive to the reference item.

Table 8.4.1-5: Effects on mortality and changes in body weight of the adults of *Eisenia fetida* after an exposure period of 28 days and the number of offspring per test vessel after 56 days (values in this table are rounded values)

Test object	***Eisenia fetida***							
	1st run		**2nd run**					
Test item	**Control**	**AE F130619**	**Control**	**AE F130619**				
mg pure metabolite/kg dry weight artificial soil	---	100	---	5.6	10	18	32	56
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%]	31.75	38.29*	34.35	33.7	42.06	38.10	37.90	40.15
Standard Deviation	4.05	6.83	4.53	3.42	2.19.0	1.91	6.80	6.44
Mean number of offspring per test vessel after 56 days **	348.3	363.0	290.5	317.8	279.8	291.0	322.8	280.3
Standard Deviation	47.8	65.4	26.6	57.5	39.1	22.3	32.1	65.2
Coefficient of variance (%)	13.7	18.1	9.1	18.1	14.0	7.6	10.0	23.3
% of control	---	104.2	---	109.4	96.3	100.2	111.1	96.5

	Reproduction
EC_{10} (mg pure metabolite/kg dry weight soil [1]) (95% confidence limits)	**n.d.**
EC_{20} (mg pure metabolite/kg dry weight soil [1]) (95% confidence limits)	**n.d.**
EC_{50} (mg pure metabolite/kg dry weight soil [1]) (95% confidence limits)	**n.d.**

* statistical significance compared to the control
(1st run: Student t-test, 2nd run: Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$)

** no statistical significance compared to the control
(1st run: Student t-test, 2nd run: Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$)

1) Probit analysis

n.d. not determined due to mathematical reasons or inappropriate data

Mortality:

After 28 days of exposure no worms died in the control groups of both test runs and no mortality was observed at any test item concentration.

Effects on growth:

Statistically significant different values for the growth relative to the control were observed in the 1st run.

Therefore, based on biological and statistical significance (for both test runs):

NOEC related to growth: 56 mg pure metabolite/kg dry weight artificial soil
LOEC related to growth: 100 mg pure metabolite/kg dry weight artificial soil

Effects on reproduction:
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at all tested concentrations the first and second run. Therefore, based on biological and statistical significance (for both test runs):

NOEC related to reproduction: > 100 mg pure metabolite/kg dry weight artificial soil
LOEC related to reproduction: > 100 mg pure metabolite/kg dry weight artificial soil

Conclusions:

Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 56 mg pure metabolite/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 100 mg pure metabolite/kg dry weight artificial soil.

Report:	[redacted]8; [redacted],2013,M-459518-01
Title:	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Report No:	kra/Rg-R-140/13
Document No:	M-459518-01-1
Guidelines:	**ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; US EPA OCSPP: None; none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F153745 on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration (limit test).
Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 100 mg test item/ kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined. The test was performed as a limit test according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).
The overall No-Observed-Effect-Concentration (NOEC) related to reproduction was determined to be ≥ 100 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) related to reproduction was determined to be > 100 mg test item/kg soil dry weight. The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:

Test item. Foramsulfuron-AE F153745 (BCS-AU80017); Batch code: AE F153745 00 1B98 0001; Origin Batch No.: ZER0234; CAS No.: 173159-94-9; LIMS No.: 1131929; Analysed content: 98.2 % w/w; certificate No.: AZ 17717.

Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 100 mg test item/ kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Toxic standard (Carbendazim EC 360 G): 1.25 – 2.5 – 5.0 mg a.s./kg soil d.w. (corresponds to 3.94 – 7.89 – 15.78 mg test item/ kg soil d.w.); control: quartz sand, solvent control: none.

Dates of work: July 10, 2012 – September 17, 2012

Results:

Table 8.4.1-7: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	348.3
Coefficient of variance of reproduction in the control	≤ 30 %	13.7 %

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

In a separate toxic standard reference test (Study No. Rg-R-Ref19/12, Report No.: kra-Rg-R-Ref 19/12; Non-GLP; performed from September 21 to November 28, 2012), the EC_{10}, EC_{20} and EC_{50} (reproduction) of the reference item Carbendazim EC 360 G were calculated to be 3.06, 3.22 or 3.54 mg a.s./kg artificial soil dry weight. The results of the reference test indicated that the test system was sensitive to the reference item.

Table 8.4.1-8: Effects on mortality and changes in body weight of the adults of *Eisenia fetida* after an exposure period of 28 days and the number of offspring per test vessel after 56 days

Test object	**Control**	***Eisenia fetida***
Test item		**AE F053745**
mg test item/kg dry weight artificial soil	---	100
Mortality of adult earthworms [%] after 28 days	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	31.75	35.91
Standard Deviation	4.05	6.84
Mean number of offspring per test vessel after 56 days	348.3	342.1
Standard Deviation	47.8	32.3
Coefficient of variance (%)	13.7	9.5
% of control	---	98.2

Mortality:
After 28 days of exposure no worms died in the control group and no mortality was observed at the tested concentration of 100 mg test item/kg dry weight soil.

Effects on growth:
No statistically significant different value for the growth, relative to the control, was observed at the tested concentration of 100 mg test item/kg dry weight soil.

Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 100 mg test item/kg dry weight artificial soil
LOEC related to growth: > 100 mg test item/kg dry weight artificial soil

Effects on reproduction:
No statistically significant different value for the number of juveniles per test vessel relative to the control was observed at the tested concentration of 100 mg test item/kg dry weight artificial soil.

Therefore, based on biological and statistical significance:
NOEC related to reproduction: ≥ 100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: > 100 mg test item/kg dry weight artificial soil

Conclusions:

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

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

CA 8.4.2.1 Species level testing

For foramsulfuron and its metabolites AE F092944, AE F153745 and AE F130619 reproductive toxicity studies on *Hypoaspis aculeifer* and *Folsomia candida* were performed.
In the tests with the soil mite *Hyposapis aculeifer* no effects were observed at the highest tested dose levels when either the parent compound or the metabolites were tested. Resulting NOEC values were ≥ 1000 mg a.s./kg dws for foramsulfuron and ≥ 100 mg/kg dws for the soil metabolites.
The collembolan species *Folsomia candida* was slightly more sensitive to the parent compound foramsulfuron than the soil mite, with a NOEC of 178 mg a.s./kg dws, while for the metabolites again no effects were observed at the highest tested dose level of 100 mg/kg dws.
Details of all studies are provided in the following table.

Table 8.4.2-1: Reproductive toxicity data of foramsulfuron and metabolites to other non-target macro-organisms presented in this chapter

Test substance	Test species	Endpoint	Reference
Foramsulfuron	*Hypoaspis aculeifer*	NOEC **≥ 1000 mg a.s./kg dws**	[redacted], 2012 M-443308-01-1 KCA 8.4.2.1/01
	Folsomia candida	NOEC **178 mg a.s./kg dws**	[redacted], 2012 M-443369-01-1 KCA 8.4.2.1/02
AE F092944	*Hypoaspis aculeifer*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-454043-01-1 KCA 8.4.2.1/03
	Folsomia candida	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-451142-01-1 KCA 8.4.2.1/04
AE F130619	*Hypoaspis aculeifer*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-454051-01-1 KCA 8.4.2.1/05
	Folsomia candida	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-450824-01-1 KCA 8.4.2.1/06
AE F153745	*Hypoaspis aculeifer*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-447606-01-1 KCA 8.4.2.1/07
	Folsomia candida	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-450830-01-1 KCA 8.4.2.1/08

dws = dry weight soil
Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on foramsulfuron

Report:	[redacted];[redacted];2012;M-443308-01
Title:	Foramsulfuron (AE F130360) a.s.: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	KRA-HR-78/12
Document No:	M-443308-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis* (Geolaelaps) *aculeifer*) reproduction test in soil; not applicable**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effects of foramsulfuron (AE F130360) a.s. on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. 10 adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.

The LC_{50} could not be calculated and it is considered to be > 1000 mg test item/kg dry weight artificial soil. The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 1000 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 1000 mg test item/kg dry weight artificial soil. The EC_{50}-value could not be calculated and it was considered to be > 1000 mg test item/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

Materials and Methods:

Test item: Foramsulfuron (AE F130360) a.s. (BCS-AH47626); Batch code: AE F130360-01-02; Origin Batch No.: EHR004294; Customer order No.: TOX-No.: 09600-00; CAS No.: 173159-57-4; LIMS No.: 1138112; analysed content of a.s.: 97.3 % w/w.

10 adult, fertilized, female *Hypoaspis aculeifer* were exposed to control and to concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil containing 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C and a photoperiod: light : dark = 16 h : 8 h (400 - 800 lux). In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg-laying). During the test, they were fed with *Tyrophagus putrescentiae* (cheese mites) which were bred on brewer´s yeast. 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.

Toxic standard: (Dimethoate EC 400): 1.0 – 1.8 – 3.2 – 5.6 – 10.00 mg a.s./kg dry weight artificial soil; control: artificial soil moistened with deionised water, solvent control: none.

Dates of experimental work: August 24, 2012 – September 14, 2012

Results:

Table 8.4.2-2: Validity criteria

Validity criteria (control values)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	5.0 %
Mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	356.0
Coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	8.5 %

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

In a separate study ([redacted], kra/HR-O-14/12, February 29, 2012), the LC_{50} (mortality) of the reference item, dimethoate, was calculated to be 3.894 mg a.s./kg dry weight artificial soil. The NOEC is calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the LOEC is 5.6 mg a.s./kg dry weight artificial soil. Dimethoate EC 400 E G showed a EC_{50} (reproduction) of 6.62 mg a. s./kg dry weight artificial soil. The results of the reference test demonstrate the sensitivity of the test system.

Table 8.4.2-3: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Foramsulfuron (AE F130360) a.s. *Hypoaspis aculeifer* Artificial Soil			
mg test item/Kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	5.0	356.0 ± 30.2	100	
100	5.0	363.5 ± 55.4	102.1	_
178	12.5	340.5 ± 23.9	95.6	_
316	2.5	363.8 ± 32.8	102.2	-
562	0.0	397.8 ± 19.8	111.7	-
1000	2.5	402.8 ± 38.6	113.1	-
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			**≥ 1000**	
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			**> 1000**	

(*)= Williams-t -test one sided smaller, α=0.05

Mortality

In the control group 5 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg dry weight artificial soil.

Reproduction

Concerning the number of juveniles statistical analysis (William`s t-test one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 1000 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 1000 mg test item/kg dry weight artificial soil. The EC_{50}-value could not be calculated and is considered to be > 1000 mg test item/kg dry weight artificial soil.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be ≥ 1000 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be > 1000 mg test item/ kg dry weight artificial soil.

Report:	[redacted]t; [redacted];2012;M-443369-01-1
Title:	Foramsulfuron (AE F130360) a.s.: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Report No:	FRM-Coll-147/12
Document No:	M-443369-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil** **US EPA OCSPP None; not specified**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effects of Foramsulfuron (AE F130360) a.s. on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil, by comparing control and treatment.
10 collembolans (10 - 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight. After a period of 28 days, mortality and reproduction were determined.
The No-Observed-Effect-Concentration (NOEC) for reproduction is 178 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 316 mg test item/kg dry weight artificial soil. All validity criteria for the untreated control of the study according to the OECD Guideline 232 have been fulfilled.

Materials and Methods:

Test item. Foramsulfuron (AE F130360) a.s. (BCS-AH47626); Batch code: AE F130360-01-02; Origin Batch No: ELIR00429I; Customer order No.: TOX-No.: 09600-00; CAS No.: 173159-57-4; LIMS No.: 1138112; analysed content of a.s.: 97.3 % w/w.

10 collembolans (10 - 12 days old) were exposed to untreated control and to concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil containing 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C and a photoperiod: light : dark = 16 h : 8 h (400 - 800 lux). Each test vessel of the 8 control and the 4 treatment replicas plus the one for measurement purpose was filled up with 30±1 g wet weight artificial soil. During the test, the collembolans were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard 44 - 67 - 100 - 150 - 225 mg boric acid/kg soil d.w.; control: artificial soil with deionised water, solvent control: none.

Dates of experimental work: August 24, 2012 – September 27, 2012

Results:

Table 8.4.2-4: Validity criteria

Validity criteria (untreated control)	Recommended	Obtained
Mean adult mortality	≤ 20 %	10 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1395.4
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	17.5 %

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

In a separate most recent non-GLP study (FRM-Coll-Ref-1912, [redacted], May 25, 2012), the EC_{50} (mortality) of the reference item, boric acid, was calculated to be 116 mg test item/kg dry weight artificial soil. The $NOEC_{reproduction}$ is calculated to be 67 mg test item/kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 100 mg test item/kg dry weight artificial soil. These results demonstrate that the test organisms are sufficiently sensitive.

Table 8.4.2-5: Effects on mortality and reproduction of *Folsomia candida*

Test item	Foramsulfuron (AE F130360) a.s.					
Test object	*Folsomia candida*					
Exposure	Artificial soil					
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD			Reproduction (% of control)	
Control	10.0	1395.4	±	244.5	-	
100	7.5	1343.0	±	150.3	96.2 n.s.	
178	10.0	1172.5	±	368.7	84.0 n.s.	
316	17.5	1044.3	±	147.5	74.8*	
562	15.0	1175.0	±	187.4	84.2*	
1000	7.5	1101.3	±	199.9	79.6*	
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)					178	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)					316	

The calculations were performed with un-rounded values
SD = Standard deviation
* = statistically significant (William's-t test one-sided-smaller, $\alpha = 0.05$)
n.s. = statistically not significant (William's-t test one-sided-smaller, $\alpha = 0.05$)

Mortality

In the control group 10.0 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. The LC_{10}, LC_{20} and the LC_{50} values could not be determined and are considered to be > 1000 mg test item/kg artificial soil dry weight.

Reproduction

Concerning the number of juveniles, statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) has revealed a significant difference between control and the treatment groups with 316, 562 and 1000 mg test item/kg artificial soil dry weight.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 178 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 316 mg test item/kg artificial soil dry weight. The EC_{10}, EC_{20} and LC_{50} values could not be determined since no clear dose response relation was observed.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) for reproduction is 178 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 316 mg test item/ kg dry weight artificial soil.

Studies on the metabolites of foramsulfuron

AE F092944

Report:	[redacted] h; [redacted] ;2013;M-454043-01
Title:	AE F092944 (BCS-AA25052): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Report No:	13 10 48 044 S
Document No:	M-454043-01-1
Guidelines:	**OECD 226 (2008); none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of AE F092944 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.
10 adult soil mites (females) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to 100 mg test item/kg soil dry weight. Two weeks after start of exposure, the number of juveniles and surviving parental mites was determined. The test was performed as a limit test in accordance with the OECD Guideline 226 (2008).
The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

Materials and Methods:

Test item. AE F092944 (BCS-AA25052); Batch code: AE F092944 00 1B99 0002; Origin Batch No.: 23503LR; CAS No.: 36315-01-2; LIMS No.: 1034970; analysed purity: 99.8 % w/w; certificate No.: AZ 17077.

Per test vessel 10 adult soil mites (females) were exposed to untreated control and to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.5 – 21.5 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 400): 4.10 – 5.12 – 6.40 – 8.00 – 10.00 mg a.s./kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: January 15, 2013 – February 04, 2013

Results:

Table 8.4.2-6: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	7.5 %
Mean number of juveniles per replicate	≥ 50	263.9
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	16.4 %

All validity criteria for the study were met.

In a separate study (BioChem project No. R 13 10 48 001 S, dated February 04, 2013), the EC_{50} (reproduction) of the reference item, Dimethoate EC 400, was calculated to be 6.64 mg a.s./kg soil dry weight. The results of the reference test demonstrate sensitivity of the test system.

Table 8.4.2-7: Effects of AE F092944 on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE F092944 *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC_{10}	-	-
EC_{20}	-	-
LC_{50}/EC_{50}	> 100	> 100
95 % confidence limit	-	-

Endpoint	AE F92944 (mg metabolite/kg soil d.w.)	
	control	100
Mortality of soil mites after 14 days (%)	7.5	8.8
Mean number of juveniles after 14 days	263.9	244.3
CV %	16.4	17.4
Reproduction (% to control)	100	93

No statistically significant differences compared to the control were calculated (Chi² 2x2 Test for mortality, α = 0.05; Student t-test for reproduction, α = 0.05)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using non-rounded values
Percent reproduction: (R_t / R_c) * 100 %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group

In the control group and in the test item treatment group a parental mortality of 7.5 % and 8.8 %, respectively, could be observed at the end of the 14-day exposure period.
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 263.9 in the control and 244.3 in the test item treatment group.
The test item caused no statistically significantly adverse effects on adult mortality (Chi² 2x2 Test, α = 0.05, one-sided greater) and reproduction (Student t-test, α = 0.05, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight.

Conclusions:

The test item AE F092944 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 dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight.

Report:	[redacted]p; [redacted];2013;M-451142-01
Title:	AE F092944 (BCS-AA25052): Effects on the reproduction of the collembolan *Folsomia candida*
Report No:	13 10 48 045 S
Document No:	M-451142-01-1
Guidelines:	**OECD 232 (2009), ISO 11267 (1999); none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of the test item on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days.
10 juvenile collembolans (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to untreated control and to 100 mg test item/kg soil dry weight. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans was counted. The test was performed as a limit test in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).
The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

Materials and Methods:

Test item: AE F092944 (BCS-AA25052); Substance code: AE F092944; Batch code: AE F092944 00 1B99 0002; Origin Batch No.: 23503LR; CAS No.: 36315-01-2; LIMS No.: 1034970; analysed purity: 99.8 % w/w; certificate No.: AZ 1707.

10 juvenile collembolans (9-12 days old) per test vessel were exposed to untreated control and to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.1 – 20.7 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44 – 67 – 100 – 150 – 225 mg boric acid/kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: February 01, 2013 – March 01, 2013

Results:

Table 8.4.2-8: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	< 2.5 %
Mean number of juveniles per replicate	≥ 100	563
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	7.6 %

All validity criteria for the study were met.

In a separate study (BioChem project No. R 12 10 48 003 S, dated May 24, 2012), 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.

Table 8.4.2-9: Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE F092944 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	> 100	> 100
LC_{50}/EC_{50} 95 % confidence limit	> 100	> 100

Endpoint	AE F092944 (mg test item/kg soil d.w.)	
	control	100
Mortality of parental collembolans after 4 weeks (%)	2.5	2.5
Mean number of juveniles after 4 weeks	563	580
CV %	7.6	14.3
Reproduction (% to control)	100	103

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test, $\alpha = 0.05$, one-sided greater) and reproduction (Student-t-test, $\alpha = 0.05$, one-sided smaller)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using unrounded values
Percent reproduction: (Rt /Rc) * 100 %
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 2.5 % parental mortality at a concentration of 100 mg test item/kg soil d.w. 2.5 % parental mortality was observed in the control.
No statistically significant effect (Fisher`s Exact Binomial Test, α = 0.05, one-sided greater) on parental mortality was found for the concentration tested.
No effects on behaviour of the collembolans were observed during the test.
The mean number of juvenile springtails counted four weeks after introduction of the parental collembolans into the test vessels was on average 563 in the control and 580 at 100 mg test item/kg soil d.w. No statistically significant effects (Student-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg test item/kg soil d.w.
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dry weight.

Conclusions:

The test item AE F092944 (BCS-AA25052) showed no statistically significant adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* 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.

AE F130619

Report:	[redacted]b; [redacted];2013;M-454051-01
Title:	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Report No:	13 10 48 046 S
Document No:	M-454051-01-1
Guidelines:	**OECD 226 (2008); none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of AE F130619 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. 10 adult soil mites (females) per replicate (8 control replicates and 8 replicates for the test item concentration) were exposed to 100 mg metabolite/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight). After 2 weeks the number of juveniles and surviving parental mites was determined. The test was performed as a limit test in accordance with the OECD Guideline 226 (2008).
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil d.w.. The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg metabolite/kg soil d.w.. All validity criteria (for the control group) according to the guideline were accomplished.

Materials and Methods:

Test item: Foramsulfuron-AE F130619 (BCS-AU59648); Batch code: AE F130619-01-01; Origin Batch No.: SES 10641-3-3; CAS No.: 190520-75-3; LIMS No.: 1238224; analysed purity: 94 % w/w; certificate No.: AZ 18416.

10 adult soil mites (females) were exposed to 100 mg metabolite/kg soil dry weight containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.5 – 21.2 °C and a photoperiod: light : dark = 16 h : 8 h (540 lx) and were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 400): 4.10 – 5.12 – 6.40 – 8.00 – 10.00 mg a.s./kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: February 27, 2013 – March 15, 2013

Results:

Table 8.4.2-10: Validity criteria

Validity criteria (for control group)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	1.3 %
Mean number of juveniles per replicate	≥ 50	268.4
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	8.0 %

All validity criteria for the study were met

In a separate study (BioChem project No. R 13 10 48 001 S, dated February 04, 2013), the EC_{50} (reproduction) of the reference item Dimethoate EC 400 was calculated to be 6.64 mg/kg soil d.w.. The results of the reference test demonstrate the sensitivity of the test system.

Table 8.4.2-11: Effects of AE F130619 on mortality and reproduction of *Hypoaspis aculeifer*

Test item	AE F130619	
Test object	*Hypoaspis aculeifer*	
Exposure	Artificial soil	
	Adult mortality	Reproduction
	(mg metabolite/kg soil d.w.)	
NOEC	100	100
LOEC	> 100	> 100
EC_{10}	-	-
LC_{50}/EC_{50}	> 100	> 100
95 % confidence limit	-	-

Endpoint	AE F130619 (mg metabolite/kg soil d.w.)	
	control	100
Mortality of soil mites after 14 days (%)	1.3	0.0
Mean number of juveniles after 14 days	268.4	256.1
CV %	8.0	11.8
Reproduction (% to control)	100	95

No statistically significant differences compared to the control were calculated (Fisher's Exact Binomial Test for mortality, $\alpha = 0.05$; Student-t-test for reproduction, $\alpha = 0.05$)
Calculations were done using unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated group(s)
R_c = mean number of juveniles observed in the control group

In the control group, 1.3 % parental mortality could be observed at the end of the 14-day exposure period.
In the test item treatment group no parental mortality could be observed at the end of the test.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 268.4 in the control and 256.1 in the test item treatment group.

The test item caused no statistically significantly adverse effects on adult mortality (Fisher`s Exact Binomial Test, $\alpha = 0.05$, one-sided greater) and reproduction (Student t-test, $\alpha = 0.05$, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg metabolite/kg soil dry weight.

Conclusions:

The test item AE F130619 showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg metabolite/kg soil dry weight.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg metabolite/kg soil dry weight.

Report:	[redacted]k; [redacted];2013;M-450824-01
Title:	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the reproduction of the collembolan *Folsomia candida*
Report No:	13 10 48 047 S
Document No:	M-450824-01-1
Guidelines:	**OECD 232 (2009), ISO 11267 (1999); none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of AE F130619 on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. 10 collembolans (9-12 days old) per replicate (8 control replicates and 8 replicates for the test item concentration) were exposed to 100 mg metabolite/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight). After 4 weeks the number of offspring (juveniles) and surviving parental collembolans was counted. The test was performed as a limit test in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil d.w.. The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg metabolite/kg soil d.w.. All validity criteria (for the control group) according to the guideline were fulfilled.

Materials and Methods:

Test item: Foramsulfuron-AE F130619 (BCS-AU59648); Batch code: AE F130619-01-01; Origin Batch No.: SES 10641-3-3; CAS No.: 190520-75-3; LIMS No.: 1238224; analysed purity: 94 % w/w; certificate No.: AZ 18416.

10 Collembola (9-12 days old) were exposed to 100 mg metabolite/kg soil dry weight containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, 18.3 – 21.0 °C and a photoperiod: light : dark = 16 h : 8 h (650 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard 44 - 67 - 100 - 150 - 225 mg boric acid/kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: February 07, 2013 – March 07, 2013

Results:

Table 8.4.2-12: Validity criteria

Validity criteria (for control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	6.3 %
Mean number of juvenile per replicate	≥ 100	778
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	14.4 %

All validity criteria for the study were met

The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 2.1%.

In a separate study (BioChem project No. R 12 10 48 003 S, dated May 24, 2012), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 104 mg/kg soil d.w.. The results of the reference test demonstrate the sensitivity of the test system.

Table 8.4.2-13: Effects of AE F130619 on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE F130619 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	(mg metabolite/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥ 100	≥ 100
LC_{50}/EC_{50} 95 % confidence limit	> 100 -	> 100 -

Endpoint	**AE F130619** (mg metabolite/kg soil d.w.)	
	control	**100**
Mortality of parental collembolans after 4 weeks (%)	6.3	3.8
Mean number of juveniles after 4 weeks	778	768
CV %	14.4	14.2
Reproduction (% to control)	100	99

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test, $\alpha = 0.05$, one-sided greater) and reproduction (Student-t-test, $\alpha = 0.05$, one-sided smaller)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated groups
R_c = mean number of juveniles observed in the control group

The test item caused 3.8 % parental mortality at a concentration of 100 mg metabolite/kg soil d.w. 6.3 % parental mortality was observed in the control.
No statistically significant effect (Fisher`s Exact Binomial Test, $\alpha = 0.05$, one-sided greater) on parental mortality was found for the concentration tested.
No effects on behaviour of the collembolans were observed during the test.
The mean number of juvenile springtails counted four weeks after introduction of the parental collembolans into the test vessels was on average 778 in the control and 768 at 100 mg metabolite/kg soil d.w. No statistically significant effects (Student-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg metabolite/kg soil d.w..
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil d.w..

Conclusions:

The test item AE F130619 showed no statistically significant adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at 100 mg metabolite/kg soil d.w.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg metabolite/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg metabolite/kg soil d.w..

AE F153745

Report:	[redacted]2; [redacted];2013;M-447606-01
Title:	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Report No:	13 10 48 048 S
Document No:	M-447606-01-1
Guidelines:	**OECD 226 (2008);not specified**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of AE F153745 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.
Ten adult soil mites (females) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to 100 mg test item/kg soil dry weight. Two weeks after start of exposure, the number of juveniles and surviving parental mites was determined. The test was performed as a limit test according to the OECD Guideline 226 (2008).
The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

Materials and Methods:

Test item. Foramsulfuron-AE F153745 (BCS-AU80017); Substance code: AE F153745; Batch code: AE F153745 00 1898 0001; Origin Batch No.: ZER0234; CAS No.: 173159-94-9; LIMS No.: 1131929; analysed purity: 98.2 % w/w; certificate No.: AZ 17717.

Per test vessel 10 adult soil mites (females) were exposed to untreated control and to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.5 – 21.5 °C and a photoperiod: light : dark = 16 h : 8 h (457 lx) and were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 409): 4.10 – 5.12 – 6.40 – 8.00 – 10.00 mg a.s./kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: January 17, 2013 – February 04, 2013

Results:

Table 8.4.2-14: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	1.3 %
Mean number of juveniles per replicate	≥ 50	242.3
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	19.9 %

All validity criteria for the study were met.

In a separate study (BioChem project No. R 12 10 48 002 S, dated March 05, 2012), the EC_{50} (reproduction) of the reference item Dimethoate EC 400 was calculated to be 6.87 mg a.s./kg soil dry weight. The results of the reference test demonstrate sensitivity of the test system.

Table 8.4.2-15: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE F153745 *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC_{10}	-	-
EC_{20}	-	-
LC_{50}/EC_{50}	> 100	> 100
95 % confidence limit	-	

Endpoint	**AE F153745** (mg metabolite/kg soil d.w.)	
	control	**100**
Mortality of soil mites after 14 days (%)	1.3	3.8
Mean number of juveniles after 14 days	242.3	246.5
CV %	19.9	13.1
Reproduction (% to control)	100	102

No statistically significant differences compared to the control were calculated (Fisher`s Exact Binomial Test for mortality, $\alpha = 0.05$; Student t-test for reproduction; $\alpha = 0.05$)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group

In the control group and in the test item treatment group a parental mortality of 1.3 % and 3.8 %, respectively, could be observed at the end of the 14-day exposure period.
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 242.3 in the control and 246.5 in the test item treatment group.
The test item caused no statistically significantly adverse effects on adult mortality (Fisher`s Exact Binomial Test, $\alpha = 0.05$, one-sided greater) and reproduction (Student t-test, $\alpha = 0.05$, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight.

Conclusions:

The test item AE F153745 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 dry weight. Therefore the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight.

Report:	[redacted]1; [redacted];2013;M-450830-01
Title:	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the reproduction of the collembolan *Folsomia candida*
Report No:	13 10 48 049 S
Document No:	M-450830-01-1
Guidelines:	**OECD 232 (2009), ISO 11267 (1999);none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of AE F153475 on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days.
Ten collembolans (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to untreated control and to 100 mg test item/kg soil dry weight. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans was counted. The test was performed as a limit test in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).
The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

Materials and Methods:

Test item: Foramsulfuron-AE F153745 (BCS-AU80017); Substance code: AE F153745; Batch code: AE F153745 00 1B98 0001; Origin Batch No.: ZER0234; CAS No.: 173159-94-9; LIMS No.: 1131929; analysed purity: 98.2 % w/w; certificate No.: AZ 17717.

Ten juvenile collembolans (9-12 days old) per test vessel were exposed to untreated control and to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$ at 19.1 – 20.7 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44 – 67 – 100 – 150 – 225 mg boric acid/kg soil d.w.; control: quartz sand, solvent control: none

Dates of work: February 01, 2013 – March 01, 2013

Results:

Table 8.4.2-16: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juvenile per replicate	≥ 100	639
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	13.5 %

All validity criteria for the study were met.

In a separate study (BioChem project No. R 12 10 48 003 S, dated May 24, 2012), 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.

Table 8.4.2-17: Effects of AE F153745on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE F153745 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥ 100	≥ 100
LC_{50}/EC_{50} 95 % confidence limit	> 100 -	100 -

Endpoint	**AE F153745 (mg test item/kg soil d.w.)**	
	control	**100**
Mortality of parental collembolans after 4 weeks (%)	3.8	2.5
Mean number of juveniles after 4 weeks	639	646
CV %	13.7	12.6
Reproduction (% to control)	100	101

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test, $\alpha = 0.05$, one-sided greater) and reproduction (Student-t-test, $\alpha = 0.05$, one-sided smaller)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using unrounded values
Percent reproduction: (Rt / Rc) * 100 %
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 2.5 % parental mortality at a concentration of 100 mg test item/kg soil d.w. 3.8 % parental mortality was observed in the control.
No statistically significant effect (Fisher's Exact Binomial Test, $\alpha = 0.05$, one-sided greater) on parental mortality was found for the concentration tested.
No effects on behaviour of the collembolans were observed during the test.
The mean number of juvenile springtails counted four weeks after introduction of the parental collembolans into the test vessels was on average 639 in the control and 646 at 100 mg test item/kg soil d.w. No statistically significant effects (Student-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg test item/kg soil d.w.
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dry weight.

Conclusions:

The test item AE F153745 showed no statistically significant adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* 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.

CA 8.5 Effects on soil nitrogen transformation

For foramsulfuron and its metabolites AE F092944, AE F153745 and AE F130619 studies on the effect on soil nitrogen transformation were performed. In none of the studies unacceptable effects were found at the highest tested dose level which ranged from 0.137 mg/kg dws to 0.735 mg/kg dws. Details of all studies are provided in the following table.

Table 8.5-1: Toxicity data of foramsulfuron and metabolites to soil non-target micro-organisms presented in this chapter

Test item	Test design	Ecotoxicological endpoint		Reference
N-transformation				
Foramsulfuron	28 d	no unacceptable effects	≥0.3 mg a.s./kg dws	[redacted], 1997, M-142972-01-1, KCA 8.5/01
Foramsulfuron + bound residues	28 d	no unacceptable effects	≥0.735 mg a.s./kg dws	[redacted], 2000, M-193916-01-1, KCA 8.5/02
AE F153745	28 d	no unacceptable effects	≥0.240 mg/kg dws	[redacted], 2013, M-453508-01-1, KCA 8.5/07
AE F130619	28 d	no unacceptable effects	≥0.375 mg/kg dws	[redacted], 2013, M-453568-01-1, KCA 8.5/06
AE F092944	28 d	no unacceptable effects	≥0.137 mg/kg dws	[redacted], 2013, M-453511-01-1, KCA 8.5/05

dws = dry weight soil

Studies on foramsulfuron

Report:	[redacted];[redacted];1997;M-142972-01
Title:	AE F130360; substance, technical; Code: AE F130360 00 1C98 0002 - Effects on soil microbial activity (nitrogen turn-over)
Report No:	A59388
Document No:	M-142972-01-1
Guidelines:	**BBA: VI/1-1;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted];[redacted];[redacted];2000;M-193916-01
Title:	Effects on soil microbial activity (nitrogen turn-over) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002
Report No:	C059438
Document No:	M-193916-01-1
Guidelines:	**OECD 216 (draft);Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

The two studies below are carbon transformation studies submitted in the original European dossier. These studies are no longer required under Regulation 1107/2009 but have been included here for completeness.

Report:	[redacted]t; [redacted];1998;M-142971-01
Title:	AE F130360; substance, technical; Code: AE F130360 00 1C98 0002 - Effects on soil microbial activity (short-term respiration)
Report No:	A59287
Document No:	M-142971-01-1
Guidelines:	**BBA: VI, 1-1;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Report:	[redacted]v; [redacted]; [redacted];2000;M-193914-01
Title:	Effects on soil microbial activity (short-term respiration) - bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002
Report No:	C006437
Document No:	M-193914-01-1
Guidelines:	**OECD: Draft 217 JAN 1999;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

Studies on the metabolites of foramsulfuron

AE F092944

Report:	[redacted]; [redacted];2013;M-453511-01
Title:	AE F092944 (BCS-AA25052): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	13 10 48 018 N
Document No:	M-453511-01-1
Guidelines:	**OECD 216 adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; not applicable**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine the effects of AE F092944 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.
A loamy sand soil (DIN 4220) was exposed for 28 d to concentrations of 0.028 and 0.137 mg test item/kg soil dry weight. Application rates were equivalent to 0.021 and 0.103 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.5 %) to stimulate nitrogen transformation. No adverse effects of AE F092944 (BCS-AA25052) on nitrogen transformation in soil could be observed in both test concentrations (0.028 mg/kg dry soil and 0.137 mg/kg dry soil) after 28 days. Differences from the control of +7.9 % (test concentration 0.028 mg/kg dry soil) and +9.2 % (test concentration 0.137 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28). AE F092944 (BCS-AA25052) caused no adverse effects (difference to control < 25 %, OECD 216) on

the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Material and methods:

Test item. AE F092944 (BCS-AA25052); BCS-code: BCS-AA25052; Batch code: AE F092944 00 1B99 0002; Origin batch No.: 23503LR; CAS No.: 36315-01-2; LIMS No.: 1034970; Analysed purity: 99.8 % w/w; certificate of analysis-No.: AZ 17077

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.028 and 0.137 mg test item/kg soil dry weight. Application rates were equivalent to 0.021 and 0.103 kg test item/ha. Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of work: January 17, 2013 – February 14, 2013

Results:

Validity Criteria:
The coefficients of variation in the control (NO_3-N) were, maximum, 5.1 % and thus fulfilled the demanded range (≤15 %).

In a separate study the reference item Dinoterb (BioChem study code: R 13 10 48 001 N) caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 10.00 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.

Nitrogen transformation:
No adverse effects of AE F092944 (BCS-AA25052) on nitrogen transformation in soil could be observed at both test concentrations (0.028 mg/kg dry soil and 0.137 mg /kg dry soil) after 28 days. Differences from the control of +7.9 % (test concentration 0.028 mg/kg dry soil) and +9.2 % (test concentration 0.137 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table 8.5.2: Effects on nitrogen transformation in soil after treatment with AE F092944

Time Interval (days)	Control			0.028 mg test item/kg soil dry weight equivalent to 0.021 kg test item/ha				0.137 mg test item/kg soil dry weight equivalent to 0.103 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.16	±	0.29	3.23	±	0.05	**+2.3** [n.s.]	3.35	±	0.09	**+5.9** [n.s.]
7-14	1.30	±	0.15	1.26	±	0.24	**-3.3** [n.s.]	1.26	±	0.33	**-3.3** [n.s.]
14-28	0.93	±	0.04	1.00	±	0.14	**+7.9** [n.s.]	1.02	±	0.15	**+9.2** [n.s.]

The calculations were performed with unrounded values
[1)] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
[n.s.] = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE F092944 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.137 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.103 kg test item/ha.

AE F130619

Report:	[redacted] <; [redacted] ;2013;M-453568-01
Title:	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 019 N
Document No:	M-453568-01-1
Guidelines:	**OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine the effects of AE F130619 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.
A loamy sand soil (DIN 4220) was exposed for 28 d to concentrations of 0.075 and 0.375 mg test item/kg soil dry weight. Application rates were equivalent to 0.056 and 0.281 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.5 %) to stimulate nitrogen transformation. No adverse effects of Foramsulfuron-AE F130619 (BCS-AU59648) on nitrogen transformation in soil could be observed in both test concentrations after 28 days. Differences from the control of +6.6 % (test concentration 0.075 mg/kg dry soil) and +21.3 % (test concentration 0.375 mg /kg dry soil) were measured at the end of the 28-day incubation period. (time interval 14-28). Foramsulfuron-AE F130619 (BCS-AU59648) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Material and methods:

Test item: Foramsulfuron-AE F130619 (BCS-AU59648); BCS-code: BCS-AU59648; Batch code: AE F130619-01-01; Origin batch No.: SES 10641-3-3; CAS No.: 190520-75-3; LIMS No.: 1238224; Analysed purity: 94 % w/w; certificate of analysis-No.: AZ 18416.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.075 and 0.375 mg test item/kg soil dry weight. Application rates were equivalent to 0.056 and 0.281 kg test item/ha. Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of work: February 08, 2013 – March 14, 2013

Results:

Validity Criteria:
The coefficients of variation in the control (NO_3-N) were maximum 1.7 % and thus fulfilled the demanded range (≤15 %).

In a separate study the reference item Dinoterb (BioChem study code: R 13 10 48 001 N) caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 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.

Nitrogen transformation:
The test item Foramsulfuron-AE F130619 (BCS-AU59648) caused a temporary stimulation of the daily nitrate rate at the tested concentrations of 0.075 mg/kg dry soil and 0.375 mg/kg dry soil at time interval 7-14 days after application.
However, no adverse effects of Foramsulfuron-AE F130619 (BCS-AU59648) on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +6.6 % (test concentration 0.075 mg/kg dry soil) and +21.3 % (test concentration 0.375 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table 8.5-3: Effects on nitrogen transformation in soil after treatment with AE F130619

Time Interval (days)	Control			0.075 mg test item/kg soil dry weight equivalent to 0.056 kg test item/ha				0.375 mg test item/kg soil dry weight equivalent to 0.281 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.18	±	0.11	3.67	±	0.37	**+15.6** [n.s.]	3.81	±	0.40	**+19.9** [n.s.]
7-14	1.87	±	0.09	1.39	±	0.17	**-26.0** [*s.]	1.15	±	0.24	**-38.4** [*s.]
14-28	0.91	±	0.08	0.97	±	0.01	**+6.6** [n.w.]	1.10	±	0.15	**+21.3** [n.s.]

The calculations were performed with unrounded values
[1)] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
[n.w.] = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)
[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, $p \leq 0.05$)

Conclusions:

AE F130619 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.375 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.281 kg test item/ha.

AE F153745

Report:	[redacted]*; [redacted];2013;M-453508-01
Title:	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	1321048020N
Document No:	M-453508-01-1
Guidelines:	**OECD 216 adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; not applicable**
GLP/GEP:	**no**

Executive Summary:

The purpose of this study was to determine the effects of AE F153745 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.
A loamy sand soil (DIN 4220) was exposed for 28 d to concentrations of 0.048 and 0.240 mg test item/kg soil dry weight. Application rates were equivalent to 0.036 and 0.180 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.5 %) to stimulate nitrogen transformation. No adverse effects of AE F153745 (BCS-AU80017) on nitrogen transformation in soil could be observed in both test concentrations (0.048 mg/kg dry soil and 0.240 mg/kg dry soil) after 28 days. Differences from the control of -5.3 % (test concentration 0.048 mg/kg dry soil) and +10.9 % (test concentration 0.240 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28). Foramsulfuron-AE F153745 (BCS-AU80017) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Material and methods:

Test item. Foramsulfuron-AE F153745 (BCS-AU80017); BCS-code: BCS-AU80017; Batch code: AE F153745 00 1B98 0001; Origin batch No.: ZER0234; CAS No.: 173159-94-9; LIMS No.: 1131929; Analysed purity: 98.2 % w/w; certificate of analysis-No.: AZ 17717.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.048 and 0.240 mg test item/kg soil dry weight. Application rates were equivalent to 0.036 and 0.180 kg test item/ha. Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of work: January 17, 2013 – February 14, 2013

Results:

Validity Criteria:
The coefficients of variation in the control (NO_3-N) were maximum 7.0 % and thus fulfilled the demanded range (≤ 15 %).

In a separate study the reference item Dinoterb (BioChem study code: R 13 10 48 001 N) caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 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.

Nitrogen transformation:

The test item Foramsulfuron-AE F153745 (BCS-AU80017) caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.048 mg/kg dry soil at time interval 7-14 days after application.

However, no adverse effects of Foramsulfuron-AE F153745 (BCS-AU80017) on nitrogen transformation in soil could be observed at both tested concentrations (0.048 mg and 0.240 mg test item/kg dry soil) at the end of the test, 28 days after application (time interval 14-28). Differences from the control of -5.3 % (test concentration 0.048 mg/kg dry soil) and +10.9 % (test concentration 0.240 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table 8.5-4: Effects on nitrogen transformation in soil after treatment with AE F153745

Time Interval (days)	Control			0.048 mg test item/kg soil dry weight (equivalent to 0.036 kg test item/ha				0.240 mg test item/kg soil dry weight equivalent to 0.180 kg test item/ha			
	Nitrate-N[1]			Nitrate-N[1]			% difference to control	Nitrate-N[1]			% difference to control
0-7	3.73	±	0.39	3.28	±	0.21	**-12.0** [n.s.]	3.56	±	0.32	**-4.5** [n.s.]
7-14	1.22	±	0.42	1.64	±	0.13	**+34.8** [n.s.]	1.43	±	0.26	**+17.2** [n.s.]
14-28	0.94	±	0.12	0.89	±	0.07	**-5.3** [n.s.]	1.04	±	0.10	**+10.9** [n.s.]

The calculations were performed with unrounded values

[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

[n.s.] = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE F153745 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.240 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.180 kg test item/ha.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

For foramsulfuron, a screening study on higher plant species was performed. As expected for a sulfonyl urea herbicide the compound showed significant herbicidal activity to several plants. Details of the study are provided in the following table.

Table 8.6-1: Effect data of a straight foramsulfuron WP20 formulation to higher terrestrial plants

Test design	Test species	Ecotoxicological endpoint	Reference
Foramsulfuron, formulated as WP20			
Greenhouse, seedling emergence and growth, 28 d	Crop plants (8 species) Broadleaf plants (17 species) Grass plants (11 species)	Post-emergence application: grass plants more susceptible than broadleaf plants Pre-emergence application: some broadleaf plants are quite susceptible to dosage range of ≥ 20 g a.s./ha	[redacted] (2009) M-191762-01-1 KCA 8.6.1 /01

Report:	[redacted]1; [redacted];1999;M-191762-01
Title:	Effectivity of the herbicide AE F130360 on higher plant species as applied under greenhouse conditions
Report No:	C005291
Document No:	M-191762-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

CA 8.6.2 Testing on non-target plants

Test results of studies on non-target plants are, by nature, related to the tested formulation. Concerning the submission for the Renewal of the Approval of foramsulfuron, the tests have been performed with the representative formulation, foramsulfuron + isoxadifen-ethyl OD45. These studies are presented and discussed in Section 10 of MCP document. Nevertheless, results are repeated here for sake of completeness.

For seedlings emergence, a tier 1 and a tier 2 study have been performed with the representative formulation. For vegetative vigour, only a tier 2 study was performed, as a tier 1 study was considered unprofitable due to the proven herbicidal activity of the compound.

From both tier2- studies a most sensitive species and a respective lowest EC_{50} could be derived (see table 8.6.2-1). The risk assessment based on these endpoints is presented in Section 10 of MCP document (**C**hemical **P**roduct dossier).

Table 8.6.2-1: Survey of non-target plant tests performed with FSN + IDF OD 45

Terrestrial Non-Target Plants			
Number of species tested (species)	**Test method** **Test substance** **Application rate**	**Effects**	**Reference**
Dicotyledoneae: 6 (bean, cabbage, radish, tomato, soybean, lettuce) Monocotyledoneae: 4 (rye grass, corn, wheat, onion)	Seedling emergence FSN + IDF OD 45 0 (control) and 60 g prod./ha with observations of emergence on Days 10, 14 and 21, with observations of height and condition on Day 21 and measurement of dry weight on Day 21	Reduction > 25 % (emergence) in onion and rye grass; reductions > 25 % (height and weight of seedlings, signs of phytotoxicity) in cabbage, lettuce, onion, radish, rye grass, tomato and wheat seedlings	[redacted], 1999; B003873 M-238408-01-2 KCP 10.6.2/01
Dicotyledoneae: 6 (bean, cabbage, radish, tomato, soybean, lettuce) Monocotyledoneae: 4 (rye grass, corn, wheat, onion)	Tier 2 vegetative vigour FSN + IDF OD 45 0 (control), 0.25, 0.74, 2.2, 6.7, 20 and 60 g prod./ha with height and condition observations on Days -1 or 0 (prior to application), 7, 14 and 21, dry weight measurements on Day 21	most sensitive species: radish; **lowest EC 50 = 1.88 g sum of a.i./ha**[1]	[redacted], 1999; B002710 M-238444-01-2 KCP 10.6.2/02
Dicotyledoneae: 4 (cabbage, radish, tomato, lettuce) Monocotyledoneae: 3 (rye grass, wheat, onion)	Tier 2 seedling emergence FSN + IDF OD 45 0 (control), 0.25, 0.74, 2.2, 6.7, 20 and 60 g prod./ha with observations of emergence on Days 10 and 14, with observations of height and condition on Day 14 and measurement of dry weight on Day 14	most sensitive species: lettuce; **lowest EC 50 = 38.8 g sum of a.i./ha**[1]	[redacted], 2000; B002819 M-238550-01-1 KCP 10.6.2/03

[1] In all studies endpoints are given in g a.i./ha. Descriptions of the experimental design in the two seedling emergence studies (page 9 in each report) indicate that the endpoints are given as g (FSN + IDF) per hectare.

The literature search revealed a paper by [redacted] et al. (2005) which presented effects of 22 ALS-inhibitors, one of which was foramsulfuron, on different mutants of *Arabidopsis thaliana*.

Although the paper as a whole can be regarded as reliable, the endpoints presented in this paper are not considered in the risk assessment for foramsulfuron for the following reasons:

1. The test was conducted with strains which were susceptible to ALS-inhibitors and not to naturally occurring phenotypes of A. thaliana.
2. As far as described in the paper the test method used does not fully apply to OECD 2017. Especially the plant density (40 plants in a 1 L pot) was exceptionally high.

For sake of completeness and as supplementary information a summary of this paper is presented here:

Report:	KCA 8.6.2 /04; [redacted]; [redacted]; [redacted];2005;M-458576-01
Title:	Response of Arabidopsis thaliana to 22 ALS inhibitors: Baseline toxicity and cross-resistance of csr1-1 and csr1-2 resistant mutants.
Report No:	M-458576-01-1
Document No:	M-458576-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive Summary:

Acetolactate synthase (ALS) is the target site of the herbicide family known as ALS inhibitors. The intensive use of the ALS inhibitors, together with an apparently high weed mutation rate and/or a wide range of resistance, have resulted in an increased occurrence of weed population resistance.

The aim was to study the relationships among 22 ALS-inhibiting herbicides using two *Arabidopsis thaliana* susceptible lines and to assess the cross-resistance pattern of chlorsulfuron- and imazapyr-resistant lines to these 22 ALS-inhibiting herbicides.

Two susceptible (S) and two resistant (R) lines of A. thaliana: Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. ED_{50} values for the Col and Ler susceptible lines of *A. thaliana* were 333 mg/ha and 506 mg/ha, respectively.

Material and methods:

A. Material

1. Test material

Test item: Foramsulfuron was obtained directly from the marketing company who provided a formulation containing the ALS inhibitor as the single herbicide active ingredient.
Active substance(s): Foramsulfuron
Adjuvant / Surfactant: Not given
Source of test item: [redacted]
Lot/Batch number: Not given
Purity: 22.5% a.i. (wt/wt)
Stability of test item: Not given
Water solubility: Not given

2. Test organism(s)

Species: Two susceptible (S) and two resistant (R) lines of *A. thaliana*: Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. The *A. thaliana* chlorsulfuron-resistant (csr1-1 or GH50) and imazapyr-resistant (csr1-2 or GH90) mutants isolated by [redacted] (1986, 1990)[6] from ethylmethane- sulfonate (EMS) mutagenized populations of the wild-type susceptible Col line were used. The csr1-1 mutant is resistant due to a point mutation resulting in a Pro to Ser substitution at the 197th amino acid, while the csr1-2 mutant is resistant due to a point mutation resulting in a Ser to Asn substitution at the 653rd amino acid ([redacted] et al., 1988; [redacted] et al., 1990, 1991)[7].

6 [redacted] (1986) Sulfonylurea-resistant mutants of Arabidopsis thaliana. Molecular and General Genetics 204, 430–434.
[redacted] (1990) A mutation causing imidazolinone resistance maps to the csr1 locus of Arabidopsis thaliana. Plant Physiology 92, 1081–1085.

7 [redacted] (1988) Transformation with a mutant Arabidopsis acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. Molecular & General Genetics 211, 266-271.
[redacted] (1990) Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant Arabidopsis thaliana var. Columbia. Nucleic Acids Research 18, 2188.

Cultivar:	Not given
Source of test species:	All *A. thaliana* lines were provided by the [redacted] (Nottingham, UK).
Crop growth stage at treatment:	Post-emergence

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory assays
Guideline/method:	Not specified
Duration of study:	From seedlings to 20 days after 4 to 5 leaf stage
Conduction:	Seeds of *A. thaliana* were sown in 1-L plastic pots filled with a commercial soil ([redacted] France). They were grown in the greenhouse at 20/25°C (night/day) under natural light supplemented by artificial sodium light to provide a 16-h photoperiod. The pots were regularly rotated during the growing period. The plants were watered twice a week with a standard nutrient solution.
Application rates:	Applied post-emergence at rates: 0.034, 0.103, 0.309, 0.926, 2.778, 8.33 and 25 g a.i./ha
Number of replicates:	3 (randomized)
Plot size:	Before spraying, plants were thinned to 40 per pot.
Application / device / nozzles:	Laboratory track sprayer delivering 1 spray solution with a 110-04 nozzle operated at 400 kPa
Water volume:	300 L ha
Verification of dispersion:	Not speicified

2. Test conditions

Soil type at study site:	Commercial soil ([redacted], France)
pH:	Not specified
Organic matter (C_{org}):	Not specified
Others:	Not specified

2. Observations and measurements:

Treatment at end of test:	Two weeks after treatment plants were cut off at soil level and shoots were oven-dried at 70°C for 48 h.
Biological parameters measured:	An observation corresponded to the dry shoot biomass of 40 plants per pot.
Statistical analyses:	Data were expressed as percentages of their untreated respective controls to standardise comparisons between Col and Ler lines. For each line a non-linear regression was used to describe the response of lines to ALS inhibiting herbicides. Following [redacted] (1993)[8], we used the equation given below and fitted the dose–response curve

[redacted] (1991) Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var. Columbia. Plant Physiology 97, 1044–1050.

8 [redacted] (1993) Formulations and adjuvants. In: Herbicide Bioassays (eds [redacted]), 99-116. CRC Press, Boca Raton,, FL, USA.

using SYSTAT[9]. An F-test (P = 0.05) was used to test significant differences of the regression parameters. Bonferroni's correction was applied to adjust the observed significance level for the fact that multiple comparisons were made (1984)[10]. Comparisons of ED_{50} values among herbicides were carried out by examining the overlap between the 95% Wald's confidence limits. Wilcoxon's signed-rank test was then performed to test the effect of the Col or Ler genetic background of the S line on the ED_{50} (1984).

Results:

1. Biological findings:

Baseline toxicity: For each susceptible line the herbicide application rates were sufficient to establish the dose–response curve. ED_{50} was used to characterize the baseline toxicity of the ALS-inhibiting herbicides studied for *A. thaliana*. Results for foramsulfuron are shown in table 8.6.2-2.

Table 8.6.2-2: ED_{50} for the Col and Ler susceptible lines and resistance ratios (R:S) for the chlorsulfuron-resistant csr1-1 and imazapyr-resistant csr1-2 lines of *Arabidopsis thaliana* treated with 22 ALS-inhibiting herbicides – results for foramsulfuron

Herbicide	*Arabidopsis thaliana*				csr1-1 R:S	csr1-2 R:S
	Col		Ler			
	ED_{50} [mg/ha]	CL* [mg/ha]	ED_{50} [mg/ha]	CL* [mg/ha]		
Foramsulfuron	333	95-570	506	291-722	2	1

*CL: 95% Wald confidence limits
R = resistant, S = susceptible

Data from 14 species were considered to be suitable for the study of the relationships between ED_{50} for *A. thaliana* and other weed species. Foramsulfuron was not included in the comparison.

Cross-resistance: A cross-resistance pattern could be directly assessed by the inhibition of ALS enzyme activity. Here, the cross-resistance pattern on the 22 ALS-inhibiting herbicides, including foramsulfuron, used in the study was assessed for the homozygous chlorsulfuron- and imazapyr-resistant lines by recording plant dry matter. The resistance ratios for the csr1-1 and csr1-2 lines are indicated in Table 8.6.2-1. The csr1-2 imazapyr-resistant line conferred little or no resistance to some sulfonylurea herbicides, including foramsulfuron (R:S ratio < 5). The same was observed for the csr1-1 chlorsulfuron-resistant line.

Results summary:

ED_{50} values (dry shoot biomass) for the Col and Ler susceptible lines of *Arabidopsis thaliana* were 333 mg/ha and 506 mg/ha, respectively.

9 SYSTAT 10 (2000) SYSTAT, Release 10 for Windows. SPSS, Chicago, IL, USA.

10 (1984) Biostatistiques (ed.), 593–596. Gaetan Morin Editeur, Quebec, Canada.

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

For foramsulfuron a screening study on entomology species was performed. Details of the study are provided in the following table.

Table 8.7-1: Effect data of a straight foramsulfuron WG 50 to entomology screening species presented in this chapter

Test design	Test species	Ecotoxicological endpoint	Reference (sec IIA, Point 8)
Foramsulfuron, formulated as WG 50			
Root systemicity test, different treated stages (eggs, larvae, all stages), 6 d	*Spodoptera littoralis*, *Heliothis virescens*, *Apis fabae*, *Nilaparvata lugens*, *Diabrotica undecimpunctata*, *Meloidogyne incognita*, *Tetranychus urticae*, *Aphis fabae* (root systemic activity)	The test item is not effective on any tested species, most sensitive species: *Meloidogyne incognita* (larvae)	[redacted], 2000 M-194770-01-1 KCA 8.7 /01

Report:	KCA 8.7/01 [redacted];;2000;M-194770-01
Title:	Effectivity of the herbicide AE F150360 on entomology screening species
Report No:	C006863
Document No:	M-194770-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

CA 8.8 Effects on biological methods for sewage treatment

For foramsulfuron, one study with activated sludge has been conducted. Details of all studies are provided in the following table.

Table 8.8-1: Effect data of foramsulfuron to activated sludge presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Foramsulfuron			
Activated sludge	Respiration inhibition, 3 h, static (OECD 209)	Activated sludge, inhibition of respiratory activity : EC_{20} > 625.0 mg/L EC_{50} > 625.0 mg/L EC_{80} > 625.0 mg/L	[redacted], 1997 M-142587-01-1 KCA 8.8. /01

Report:	[redacted]g; [redacted];1997;M-142587-01
Title:	Testing the respiration inhibition of activated sludge: Bacteria toxicity. Test substance: AE F130360, substance technial
Report No:	A58873
Document No:	M-142587-01-1
Guidelines:	**EU (=EEC): 88/302 part C; ISO: 8192; OECD: 209;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

CA 8.9 Monitoring data

Monitoring data concerning adverse effects of the active substance to non-target organisms are not available.