Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Mesosulfuron-methyl

Data Requirements

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

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

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Author(s)

[redacted]

Bayer CropScience

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Version history

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

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

This document provides detailed summaries of new ecotoxicological studies which were not available at the time of the first EU review of mesosulfuron-methyl and were therefore not evaluated for the Annex I inclusion of this active substance. Existing studies already submitted for the first EU review are found evaluated in the Monograph or its Addenda, in the present document these studies are therefore only briefly referenced, marked in grey shade. Complete reports to all studies are found included in the electronic dossier provided by Bayer CropScience. The numbering and the headlines correspond to latest EU requirements.

For transparent overall data interpretion and risk assessment, key endpoints derived from both, old and new studies, are listed in the overview tables. For easy discrimination, new information is printed black, whilst existing information is marked in grey shaded font.

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

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

Compartment	Compound / Code
Soil	Mesosulfuron-methyl AE F154851 AE F160459 AE F099095 AE F092944 AE F160460 AE F140584 AE F147447
Groundwater	Mesosulfuron-methyl AE F154851 AE F160459 AE F099095 AE F092944 AE F160460 AE F140584 AE F147447
Surface water	Mesosulfuron-methyl AE F154851 AE F160459 AE F099095 AE F092944 AE F160460 AE F140584 AE F147447
Plant material	Mesosulfuron-methyl

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

In addition to the studies on metabolites listed in Table CA 8-1 as being relevant for risk assessments of products, ecotoxicological tests have also been conducted on further components (BCS-CO60720, BCS-CV14885, BCS-CO60721) for specific purposes discussed in the respective document chapters. [MCA Sec.7, Points CA 7.1.4.2, CA 7.2.2.3, and Document N4].

Metabolite designations

For historic reason, different coding or naming systems have been used for the designation of metabolites in study reports and associated documents. Equivalence information for all substance designations appearing is provided in the list of metabolites (Document N3).

For better transparency and readability, a single primary identifier for each component will be used consistently throughout the present document. To maintain comparability to documents from the first submission for Annex I inclusion, this will be (a) the AgrEvo/Aventis CropScience substance code (AE xxxxxx), or, where newly assigned, (b) the Bayer CropScience substance code (BCS-XXxxxxx).

Where applicable, substances will be addressed in the following compound sequence. This applies for tabulated information, as well as for the order of appearance of study summaries in the document text.

1) mesosulfuron-methyl (parent substance)
2) AE F154851
3) AE F160459
4) AE F099095
5) AE F092944
6) AE F160460
7) AE F140584
8) AE F147447
9) BCS-CO60720
10) BCS-CV14885
11) BCS-CO60721

Metabolite testing for aquatic organisms

Data of the parent compound show that aquatic macrophytes are the clearly most sensitive group of organisms in the aquatic environment. The sensitivity of macrophyte species is clearly driving the risk assessment for mesosulfuron-methyl. All acute studies with fish and aquatic invertebrates led to effect doses above the highest tested concentration (100 mg/L). The most sensitive algae species was *Pseudokirchneriella subcapitata* (E_rC_{50} >290 µg/L).

Based on the aquatic ecotoxicological profile of mesosulfuron-methyl, testing of metabolites was confined to *Lemna gibba* and *Pseudokirchneriella subcapitata*. Only for the terminal product AE F092944, shared with other sulfonylurea-type herbicides, additional acute tests with fish and daphnia were performed, but did not reveal notable activity.

Metabolite testing for soil organisms

The sensitivity of soil macro- and microorganisms to the active ingredient mesosulfuron-methyl is generally low. The No Observed Effect Concentrations were above the highest tested concentration for collembola, soil mites and N-transformation. The NOEC for the earthworm *Eisenia fetida* was also high with 125 mg a.s./kg dws. Consequently, the most sensitive species for the active ingredient is *Eisenia fetida*. As the NOEC for collembolan *Folsomia candida* for the representative formulation is 17 mg/kg dws, collembola is the most sensitive species regarding the formulation. Therefore, all soil metabolites were tested with earthworms, *Folsomia candida*, and N-transformation in soil. In addition, the last metabolite in the pathway, AE F092944 with an occurrence of 10.1% was tested with the predatory mite *Hypaspis aculeifer*.

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 CA 8.1.1.1- 1: Avian acute oral toxicity data of mesosulfuron-methyl presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	acute, oral	LD_{50} > **2000** [1] mg as/kg bw $LD_{50\ extrapol}$ > 3776	[redacted], 1998 M-180378-01-1 KCA 8.1.1.1 /01
Mallard duck	acute, oral	LD_{50} > **2000** mg as/kg bw $LD_{50\ extrapol}$ > 3776 [2]	[redacted], 1998 M-147788-01-1 KCA 8.1.1.1 /02

[1] 10 birds per group

[2] LD_{50} extrapolated according to EFSA GD Birds & Mammals (2009) by applying a factor of 1.888 to the top dose in case 10 animals have been tested and no mortality occurred

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

Report:	[redacted]v; [redacted];1998;M-180378-01
Title:	Bobwhite quail acute oral toxicity test AE F130060 substance, technical Code: AE F130060 00 TC95 0001
Report No:	C000232
Document No(s):	M-180378-01-1
Guidelines:	**OECD: Draft Guidel., 1992; USEPA (=EPA): Subdiv. E, 71-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

LD_{50} > 2000 mg as/kg bw

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted]., 1998a, 8.1.1/1.

- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-1 (1982) and OECD Draft Guideline for testing of chemicals "Avian acute toxicity test-oral toxicity" (1992).

- **GLP compliance**: Yes.

- **Methods**: The acute oral toxicity of AE F130060 technical (94.6% w/w) was determined in adult bobwhite quail aged, at the start of the study, of approximately 8 months. A single dose of the test substance was administered by oral gavage to a group of 10 (5 males + 5 females) birds at the rate of 2 000 mg a.s./kg body weight. During a 15 day-observation period, any sign of toxicity, mortality rate and time of death were recorded. The birds were weighted individually on days 1, 4, 8 and 15 after treatment. Food consumption was recorded for days 1-4, 4-8 and 8-15 of the observation period. All birds were finally dissected for macroscopic observations. Control consisted in birds administered the vehicle (deionised water) only.

- **Results**: No clinical signs, or effects on body weight, food consumption and no morphological changes were recorded.

  LD50 > 2 000 mg/kg b.w.
  NOEL = 2 000 mg/kg b.w.

- **Comments (RMS)**: the study is acceptable.

Further study information supplementing the original Monograph summary:

Validity Criteria:
Fulfilled

Analytical findings:
Analysis of trial mixes (5 and 25 % w/v of the test substance in double dist. water) showed that they were homogenous and stable over a period of 4 hours. According to the report of Analytical Toxikology from July 28, 1995 the achieved concentrations were entirely acceptable (95 to 99 % of nominal). Detailed analytical results are presented in the following table:

Table CA 5.1.1- 2: Homogenicity and stabilityof trial mixes

vehicle	Conc. mg/mL	Conc. %	sample	found in mg/mL		found in % of nominal	
				0 h	5 h	0 h	5 h
Double dist. water	50	5	A	48.2	48.1	96	96
			B	49.5	48.8	99	98
			C	48.8	48.8	98	98
Double dist. water	250	25	A	241	245	96	98
			B	242	240	97	96
			C	239	238	96	95

Remark: The results were calculated on the basis of one determination and have not been corrected for recoveries.

Biological findings:
There was no mortality and no clinical signs of toxicity were observed. Body weight and food consumption were not affected by the test substance. No macroscopically visible findings were seen at necropsy.

Table CA 8.1.1.1- 3: Incidence of mortality

Sex	Dose mg/kg	Total after 15 days
Males	control	0/5
	2000	0/5
Females	control	0/5
	2000	0/5

Conclusions:

The acute oral LD_{50} of AE F130060; technical substance to the adult Bobwhite Quail was above 2000 mg/kg body weight. Therefore the test substance is of very low acute toxicity to this avian species.

Table CA 8.1.1.1- 4: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / confusion about its Reliability
M-180378-01-1 KCA 8.1.1.1 /01	EPA Pesticide Assessment Guidelines § 71-1, Subdivision E, (October 1982)	(not EU relevant)	N/A	N/A
	OECD Draft Guideline for testing of chemicals - Avian acute oxicity test - oral toxicity 1992	OECD 223 (2009)	No major difference to old guidelines with regards to limit tests, the new guideline reduce the number of birds to be tested in limit tests.	The study, performed as limit test, is in compliance with actual guidelines.

Report:	[redacted] k, [redacted] 1998; M-147788-01
Title:	Mallard duck acute oral toxicity study AE F130060 substance, technical Code: AE F130060 00 TC95 008
Report No:	A62303
Document No(s):	M-147788-01-1
Guidelines:	**OECD Draft Guideline, 1992; USEPA (=EPA): E 71-1, PB83-153908;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
LD_{50} > 2000 mg as/kg bw

Study summary and RMS evaluation copied from the original Monograph:

❑ **Reference**: [redacted],1998b, 8.1.1/2.

- **Test guideline:** US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-1 (1982) and OECD Draft Guideline for testing of chemicals "avian acute toxicity test-oral toxicity" (1992).

- **GLP compliance**: Yes.

- **Methods**: The acute oral toxicity of AE F130060 technical (94.6% w/w) was determined in adult mallard ducks aged, at the start of the study, of approximately 4 months. A single dose of the test substance was administered by oral gavage to a group of 10 (5 males + 5 females) birds at the rate of 2 000 mg a.s./kg body weight. During a 15 day-observation period, any sign of toxicity, mortality rate and time of death were recorded. The birds were weighted individually on days 1, 4, 8 and 15 after treatment. Food consumption was recorded for days 1-4, 4-8 and 8-15 of the observation period. All birds were finally dissected for macroscopic observations. Control consisted in birds administered the vehicle (deionised water) only.

- **Results**: No clinical signs, or effects on body weight, food consumption and no morphological changes were recorded.
  LD50 > 2 000 mg/kg b.w.
  NOEL = 2 000 mg/kg b.w.

- **Comments (RMS)**: the study is acceptable.

Further study information supplementing the original Monograph Summary :

Validity Criteria:
fulfilled

Analytical findings:
Analysis of a trial mixes (5 and 25 g/l w/v of the test substance in double dist. water) showed that they were homogenous and stable over a period of 4 hours. According to the report of Analytical Toxikology from July 20, 1999 the achieved concentrations were entirely acceptable (95 to 99 % of nominal). Detailed analytical results are presented in the following table:

Table CA 8.6.1.1- 5: Homogeneity and stability of trial mixes

vehicle	conc. mg/mL	conc. %	samples	found in mg/mL		found in % of nominal	
				0 h	5 h	0 h	5 h
Double dist. water	200	5	A	48.2	48.1	96	96
			B	49.5	48.8	99	98
			C	48.8	48.8	98	98
Double dist. water	250	25	A	245	245	96	98
			B	242	240	97	96
			C	239	238	96	95

Remark: The results were calculated on the base of one determination and have not been corrected for recoveries.

Biological findings:
Neither mortalities nor clinical signs of toxicity were observed. Body weight and food consumption were not affected by the test substance. No macroscopically visible findings were seen at necropsy.

Table CA 8.1.1.1- 6: Incidence of mortality

Sex	Dose mg/kg	Total after 15 days
Males	control	0/5
	2000	0/5
Females	control	0/5
	2000	0/5

Conclusions:

The acute oral LD_{50} of AE F130060; substance identical to the adult Mallard Duck was above 2000 mg/kg body weight. Therefore the test substance is of very low acute toxicity to this avian species.

Table CA 8.1.1.1- 7: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / Conclusion about its Reliability
M-147788-01-1 KCA 8.1.1.1 /02	EPA Pesticide Assessment Guidelines § 71-1, Subdivision E, (October 1982)	(not EU relevant)	N/A	N/A
	OECD Draft Guideline for testing of chemicals - Avian acute oxicity test - oral toxicity 1992	OECD 223 (2009)	No major difference to old guidelines with regards to limit test, the new guideline reduces the number of birds to be tested in limit tests	The study, performed as limit test, is in compliance with actual guidelines.

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 1359 mg a.s./kg bw/d. Details of the studies are provided in the following table.

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

Test species	Test design	Ecotoxicological endpoint			Reference
Bobwhite quail	5-day dietary	LC_{50} ≡ LDD_{50} $LDD_{50\ extrapol}$ [2]	> 5000 [1] ≥ 720 1359	ppm mg as/kg bw mg as/kg bw/d	[redacted], 1999, M-184416-01-1 KCA 8.1.1.2 /01
Mallard duck	5-day dietary	LC_{50} ≡ LDD_{50} $LDD_{50\ extrapol}$ [2]	> 5000 [1] ≥ 1210 2284	ppm mg as/kg bw mg as/kg bw/d	[redacted], 1998, M-181332-01-1 KCA 8.1.1.2 /02

[1] 10 birds per group; no mortality occurred during study
[2] LD50 and LDD50 extrapolated according EFSA GD B&M 2009

Report:	[redacted]q; [redacted];1999;M-184416-01
Title:	Bobwhite quail dietary LC50 study ACF130060 substance, technical Code: ACF130060 00 1C95 0001
Report No:	C002392
Document No(s):	M-184416-01-1
Guidelines:	**OECD: 205; USEPA: EPA; §71-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
LC_{50} > 5000 mg as/kg food

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 1999, 8.1.2.1/
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-2 (1982) and OECD Draft Guideline for testing of chemicals "avian dietary toxicity test" (1984).
- **GLP compliance**: Yes
- **Methods**: The short-term cumulative toxicity of ACF130060 technical (94.6% w/w) to bobwhite quail was determined by providing chicks with food spiked with the a.s. for a 5-day period. Bobwhite quails (approximately 14 days old at the start of the study) were assigned at random to 7 groups of 10, each of which was offered one dietary concentration (0, 312.5, 625, 1 250, 2 500 or 5 000 mg/kg test substance). Control was made in duplicate. The exposure period was followed by a 3-day recovery period during which untreated food was provided. Clinical signs and mortality rate were recorded during the whole test period. Body weight was checked at days -3/1, 6 and 9 and food consumption was recorded for both exposure and recovery periods. At the end of the study, the surviving chicks were dissected for macroscopic observations.
- **Results**: No clinical signs, or effects on body weight, food consumption and no morphological changes were recorded.
  LC50 > 5 000 mg/kg
  NOEL = 5000 mg/kg.
- **Comments (RMS)**: the study is acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:
fulfilled

Analytical findings:
Directly after preparation three samples from each of the lowest and highest concentrations were analysed for achieved test substance content, homogeneity and, after 10 days storage at room temperature, for stability. Directly prior to the start of treatment three samples from each concentration were analysed for content at the start of administration. All samples which were stored deep frozen prior to analysis were analysed in duplicate to determine the content of the test substance by the Analytical Toxicology.
The mean concentrations analysed for homogeneity, stability and achieved content were in a range of 94 - 117 % of the nominal concentration and were thus within the acceptable range of 80-120 %. The results (mean values) of chemical analysis are summarised in the following table.

Table CA 8.1.1.2- 2: Results (mean values) of the chemical analysis

Dose level (ppm)	Content (% of nominal concentration)		
Date of preparation	Pre check content	Pre check stability *	Content at administration **
July 24, 1998	July 24, 1998	August 03, 1998	August 04, 1998
312.5	102	100	-
625	-	-	117
1250	-	-	108
2500	-	-	94
5000	96	94	96
*	= after 10 days storage at room temperature		
**	= after 11 days storage in a deep freezer		
-	= no sample taken		
A, B, C	= samples taken from the top, middle and bottom of the mix		

Biological findings:
No mortality occurred in any dose group. Based on these results the short-term dietary LC50 in the Bobwhite quail was greater than 5000 ppm, which was equivalent to a mean daily test substance intake of approximately 720 mg/kg body weight.
No clinical signs of toxicity occurred in any dose group at any time during the study up to and including the 5000 ppm dietary level.
Food consumption and body weight gain remained unaffected by the test substance up to and including the 5000 ppm dietary level.
Dissection of the surviving chicks killed at the end of the study revealed no macroscopically visible morphological abnormalities.

Table CA 8.1.1.2- 3: Mortality, body weight, food and test substance consumption

Dose group	Mortality rate	Bodyweight (g)		Relative food consumption	Substance intake (mg/kg/day)
	Day 1-9	Day 1	Day 6	Day 1-6	Day 1-6
Control	0/10	30.4	45.1	0.167	-
Control	0/10	29.2	42.0	0.174	-
312.5 ppm	0/10	28.3	41.6	0.146	45.6
625 ppm	0/10	31.4	45.8	0.150	93.6
1250 ppm	0/10	30.5	44.8	0.138	172.5
2500 ppm	0/10	29.5	44.0	0.127	317.5
5000 ppm	0/10	29.4	42.7	0.144	720

Conclusions:

The dietary LC_{50} of AE F130060; SUBSTANCE TECHNICAL in the Bobwhite quail was greater than 5000 ppm, equivalent to a mean daily test substance intake of approximately 720 mg/kg body weight.
The "No Observed Effect Level (NOEL)" was 2500 ppm, equivalent to a mean daily test substance intake of approximately 720 mg/kg body weight.

Table CA 8.1.1.1- 8: Summary table

Reference	Followed guidance	Guidance currently in force	Difference	Critical assessment of the study / Deviations / conclusion about its Reliability
	US-EPA Pesticide Assessment Guidelines, Subdivision E, Birds 71-2 §71-2 (1982)	(not EU-relevant)	N/A	N/A
KCA 8.1.1.2 /01	OECD Draft Guideline for testing of chemicals No. 205 "avian dietary toxicity test" (1984)	OECD Guideline for testing of chemicals No. 205 "avian dietary toxicity test" (1984)	No changes	The study is in compliance with the guideline

Report:	[redacted]5; [redacted];1998;M-181332-01
Title:	Mallard duck dietary LC_{50} study AE F130060 substance, technical Code: AE F130060 00 1C95 0001
Report No:	C000801
Document No(s):	M-181332-01-1
Guidelines:	**OECD: 205, 1984; USEPA (=EPA): §71-2,540/9-82-024;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
LC_{50} > 5000 mg as/kg food

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted],1998c, 8.1.2.2/1.

- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-2 (1982) and OECD Draft Guideline for testing of chemicals "avian dietary toxicity test " (1984).

- **GLP compliance**: Yes.

- **Methods**: The short-term cumulative toxicity of AE F130060 technical (94.6% w/w) to mallard duck was determined by providing ducklings with food spiked with the a.s. for a five-day period. Thirty-two quail (approximately 10 days old at the start of the study) were assigned at random to 7 groups of 10, each of which was offered one dietary concentration (0, 312.5, 625, 1 250, 2 500 or 5 000 mg/kg test substance). Control was made in duplicate. The exposure period was followed by a 3-day recovery period during which untreated food was provided. Clinical signs and mortality rate were recorded during the whole test period. Body weight was checked at days -3, 1, 6 and 9 and food consumption was recorded for both exposure and recovery periods. At the end of the study, the surviving chicks were dissected for macroscopic observations.

- **Results**: No clinical signs, effects on body weight and on morphological changes were recorded. Food consumption was lower in all groups exposed to mesosulfuron-methyl but no biological significance could be assigned to this observation.
  LC50 > 5 000 mg/kg.
  NOEL = 5 000 mg/kg.

- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:
fulfilled

Analytical findings:
Directly after preparation three samples from each of the lowest and highest concentrations were analysed for achieved test substance content, homogeneity and, after 12 days storage at room temperature, for stability. Directly prior to the start of treatment three samples from each concentration were analysed for content at the start of administration. All samples which were stored deep frozen prior to analysis were analysed in duplicate to determine the content of the test substance by the Analytical Toxicology.
The mean concentrations analysed in preparation were in a range of 92 -104 % of the nominal concentration and were thus within the acceptable range of 80-120 %. The stability of the test substance in the diet after 12 days storage at room temperature was also acceptable (98 and 93 % of the nominal at the lowest and highest concentrations respectively).
Sampling and analysis regimes of the test diet mixes are summarised in the following Table.

Table CA 8.1.1.2- 4: Sampling and analysis regimes of the test diet mixes

Dose level (ppm)	Content (% of nominal concentration		
	Pre check homogeneity	Pre check stability*	Content at administration**
312.5	98	98	99
625	-	-	104
1250	-	-	96
2500	-	-	85
5000	97	93	95
*	= after 12 days storage at room temperature		
**	= after 12 days storage in a deep freezer		
-	= no sample taken		
A, B, C	= samples taken from the top, middle and bottom of the mix		

Biological findings:

No mortality occurred in any dose group. Based on these results, the short-term dietary LC_{50} in the Mallard duck was greater than 5000 ppm, which was equivalent to a mean daily test substance intake of approx. 1210 mg/kg body weight.

No clinical signs of toxicity occurred in any dose group at any time during the study up to and including the 5000 ppm dietary level.

Food consumption was lower in all dosing groups as compared with the controls during the treatment period; no differences were noted during the recovery period. In the absence of any dose-dependency and any impairment of the body weight development no biological significance could be assigned to this observation.

Dissection of the ducklings killed at the end of the study revealed no macroscopically visible abnormalities.

Table CA 8.1.1.2- 5: Mortality, body weight, food and test substance consumption

Dose group	Mortality rate	Bodyweight		Relative food consumption	Substance intake (mg/kg/day)
	Day 1-9	Day 1	Day 6	Day 1-6	Day 1-6
Control 1	0/10	167.7	382.2	0.342	-
Control 2	0/10	162.6	385.7	0.355	-
312.5 ppm	0/10	158.8	289.1	0.248	77.5
625 ppm	0/10	156.9	289.0	0.232	145.1
1250 ppm	0/10	162.7	364.9	0.261	326.3
2500 ppm	0/10	154.7	279.1	0.218	545.0
5000 ppm	0/10	164.1	387.3	0.242	1210.0

Conclusions:

The dietary LC_{50} of AE F100060; substance, technical in the Mallard duck was greater than 5000 ppm, equivalent to a mean daily test substance intake of greater than approx. 1210 mg/kg body weight.

The No Observed Adverse Effect Level (NOAEL) was considered to be 5000 ppm, the regulatory limit dose.

Table CA 8.1.1.1- 9: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-181332-01-1 KCA 8.1.1.2 /02	US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-2 (1982)	(not EU-relevant)	N/A	N/A
	OECD Draft Guideline for testing of chemicals No. 205 "avian dietary toxicity test " (1984)	OECD Guideline for testing of chemicals No. 205 "avian dietary toxicity test " (1984)	No changes	The study is in compliance with the guideline.

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 93 mg a.s./kg bw/d. Details of the studies are provided in the following table.

Table CA 8.1.1.3- 1: Avian reproductive toxicity data of mesosulfuron-methyl presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	20-weeks feeding chronic, reproduction	NOEC 1000 ppm = NOEL 93 mg as/kg bw/d	[redacted], 2000 M-198082-01-1 KCA 8.1.1.3 /01
Mallard duck	22-weeks feeding chronic, reproduction	NOEC 1000 ppm NOEL 126 mg as/kg bw/d	[redacted] et al., 1999 M-191369-01-1 KCA 8.1.1.3 /02

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

Report:	[redacted];[redacted];2000;M-198082-01
Title:	Bobwhite quail dietary reproduction study AE F130060 substance technical Code: AE F130060 00 1C9 9001
Report No:	200908
Document No(s):	M-198082-01-1
Guidelines:	**OECD: 206(1984);USEPA (=EPA): §71-4;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
NOEC = 1000 mg as/kg food

Study summary and RMS's evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000, 8.1.3.1/1.

- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-4 (1982) and OECD Draft Guideline for testing of chemicals "avian reproduction test " (1984).

- **GLP compliance**: Yes.

- **Methods**: Effects of AE F130060 technical (94.6% w/w) on the reproduction of bobwhite quail were determined by exposing chicks (F0) to the a.s. via the diet for 20 weeks. Bobwhite quails were approximately 7 months old at the start of the study. A total of 4 test groups of 16 pairs were constituted, each to which was offered one dietary concentration (0, 40, 200, or 1 000 mg a.s./kg). Behaviour, general health and mortality were checked daily. Body weight was recorded at the start of the acclimatisation period and every two weeks until week 8, and then on week 20. Food consumption was checked weekly. First generation quails were finally dissected at the end of the study for macroscopical and pathological observations and organ's weight (heart, liver, spleen, testes and oviduct) was measured. Effects on reproduction were assessed through the number and quality of the eggs produced (visual observations, weight, shell thickness). Effects on the F1 were assessed through the hatching rate of eggs, the survival of chicks, body weight and food consumption over the first two weeks after hatching. General health observations were performed.

- **Results**: There were no effects of a long term dietary exposure of adult bobwhite quails on health, body weight gain, food consumption and egg production. Viability of embryos was unaffected except in groups exposed to 40 and 1 000 mg a.s./kg food, but the difference observed was significant in the last group only, and was assumed to rather reflect the exceptional fertility that occurred in controls (95.0%, compared to usual 75-90% fertility rates). No effects were observed on hatching, survival, health, bodyweight and food consumption in F1. A summary of reproduction data is proposed in table B.9.1.3.1-1.

Table B.9.1.3.1-1. Reproduction data on *Colinus virginianus*

Dietary concentration	Control	40 ppm	200 ppm	1 000 ppm
Eggs laid	880	850	813	875
Eggs laid per female	50.6	53.1	54.2[1]	54.7
Eggs damaged	6	4	4	17
Eggs damaged of eggs laid (%)	0.81	0.13	0.53	2.1
Mean egg shell thickness (mm)	0.215	0.214	0.217	0.214
Eggs set	793	842	809	854
Viable embryos	707	768	717	689
Viable embryos of eggs set (%)	88.6	88.9	88.6	80.7*
Live 3-wk embryos	698	653	714	670
Live 3-wk embryos of viable embryos (%)	98.7	97.8	99.6	97.2
Normal hatchling	649	585	675	617
Normal hatchling of viable embryos (%)	91.8	87.6	94.1	89.6
Normal hatchling of live 3-wk embryos (%)	93.0	89.6	94.5	92.1
14-d old survivors	612	502	600	559
14-d old survivors of eggs laid (%)	63.2	59.1	73.8	63.9
14-d old survivors of normal hatchling (%)	78.9	85.8	88.9	90.6
14-d old survivors per female	32.0	31.4	40.0	34.9
Chick bodyweight at hatching (g)	6.7	6.7	6.8	6.9
Chick bodyweight at 14 d (g)	28.0	27.6	28.5	30.0

* significantly different from the control at $p \leq 0.05$.
[1] 15 females.

NOEC 20 wks = 1 000 mg a.s./kg diet (nominal concentration).

- **Comments (RMS)**: The study is acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:

The definitive test criteria for control groups as set out in the respective testing guidelines and the corresponding values obtained in this study are shown in the table below.

Table CA 8.1.1.3- 2: Definitive test criteria for control groups

	Definitive test criteria	Present study
Mortality of adult birds	≤10 %	0 %
14-day survivors/pen/10 weeks	≥ 12	32
Egg shell thickness (mm)	≥ 0.19	0.215

These definitive test criteria have been clearly met in the present study.

Analytical findings:

Analysis of the diet preparations showed that they were homogeneous and the achieved mean concentrations analyzed directly after preparation ranged between 94 and 100 % of the nominal concentration and were entirely acceptable.

Since the homogeneity of the diet mixtures of week 8 was not considered to be fully satisfactory due to the occurrence of "hot spots", these mixtures were replaced by new mixtures in week 10.

The homogeneity and stability of the test substance in the diet mixtures at room temperature was confirmed for all dietary concentrations over a period of 35 days.

Table CA 8.1.1.3- 3: Mean values (in %) of the nominal content of the test substance in the individual examination times

	Content (% of nominal concentration)							
Diet concentration	Week 1	Week 4	Week 8	Week 10	Week 12	Week 16	Mean
	Day 2	Day 25						
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
40 mg/kg	86	94	97	92	96	105	97	95
200 mg/kg	93	96	91	107	96	87	97	94
1000 mg/kg	96	104	104	106	93	96	101	100
				Study overall				96

n.d. = not detectable

Biological findings:

Adult Birds (F_0 generation)

Behaviour and general health was unaffected by the test substance in all groups up to and including 1000 ppm. No clinical signs and no mortality attributable to the test substance were observed.

No changes in body weight gain and food consumption were observed in any treated group up to and including 1000 ppm.

Table CA 8.1.1.3- 4: Group body weight, food consumption and test substance intake

Parameter	0 ppm	40 ppm	200 ppm	1000 ppm
Male body weight at termination (g)	222	229	218	222
Female body weight at termination (g)	248	247	248	247
Group mean food consumption (g/bird/day)	19.4	19.6	21.5	19.9
Group mean food consumption (g/pen/day)	38.7	39.2	42.9	39.8
Group mean body weight (g/replicate/day)	421.4	426.5	423.1	428.0
Replicate food consumption (g/1 OOg bw/day)	9.2	9.1	10.1	9.3
Test substance intake (mg/kg bw/day)	-	3.7	20.5	93.9

Necropsy, including gastrointestinal tract and the major organs of the birds which had died or of those killed during or at termination of the study, indicated no pathological changes attributable to the test substance. In particular, no changes in the reproductive organs were detected.

Statistical analysis of absolute and relative weights of heart, liver, spleen, testes and oviduct (without developing eggs) revealed no significant changes. In summary, it is concluded that the test substance caused no specific effects on the organ weights.

Table CA 8.1.1.3- 5: The relative organ weights (group mean values)

Organ	Sex	0 ppm (control)	40 ppm	200 ppm	1000 ppm
Heart (%)	Male	0.55	0.55	0.54	0.52
	Female	0.42	0.43	0.42	0.41
Liver (%)	Male	2.5	2.5	2.2	2.3
	Female	3.1	3.1	2.9	2.8
Spleen (%)	Male	0.037	0.042	0.034	0.035
	Female	0.032	0.029	0.033	0.031
Testes (%)	Female	0.97	1.04	0.92	0.91
Oviduct (%)	Male	2.8	2.8	2.8	2.7
No statistically significant difference from controls ($p<0.05$) observed in any parameter.					

Adult birds (F_0 generation) – Reproduction toxicity:
Egg production was unaffected in all treatment groups. The numbers of eggs laid, broken or cracked eggs and abnormal eggs as well as the egg weight and shell thickness indicated no substance-related changes. The group egg data (pen mean) are summarised in the following table:

Table CA 8.1.1.3- 6: Group egg data (pen mean)

Parameter	0 ppm	40 ppm	200 ppm	1000 ppm
Number laid/female	53.8	53.1	54.2	54.7
Cracked eggs/number laid (%)	0.81	0.13	0.53	2.1
Number normal/female	50.2	52.9	53.9	53.4
Mean eggshell thickness (mm)	0.218	0.214	0.217	0.214
Mean egg weight (g)	9.81	9.88	9.89	9.94
No statistically significant difference from controls ($p<0.05$) observed in any parameter.				

Treatment up to and including 1000 ppm had no adverse effects on fertility, embryonic development and hatchability.
The group mean incubation data (pen mean) are summarised in the following table:

Table CA 8.1.1.3- 7: Group mean incubation data (pen mean)

Parameter	0 ppm	40 ppm	200 ppm	1000 ppm
Fertile eggs/eggs incubated (%)	95.4	85.6	96.0	87.5
Live 3-week embryos/fertile eggs (%)	98.7	97.8	99.6	97.2
Chicks hatched/live 3-week embryos (%)	93.0	89.6	94.5	92.1
Chicks hatched/eggs incubated (%)	87.6	75.0	90.4	78.4

Examination of the unhatched eggs revealed fully developed chicks in most cases, which died shortly before or during hatching after pecking the eggshell, and very few late embryonic deaths.

Chicks (F_1 generation):
No changes in behaviour, general health condition and 14-day survival rate were observed in the hatchlings of the treated groups. In addition, there was no increased number of chicks with malformations, e.g. of the pelvis.
The descriptive overall statistical parameter for reproductive performance (expressed as percentages of 14-day survivors / eggs set and comprising all core steps of embryonic and offspring development such as fertility, embryonic development, hatching and viability of the offspring) also indicated no substance-related changes in any treated group.
The chick data (pen mean) are summarized in the following table.

Table CA 8.1.1.3- 8: Chick data (pen mean)

Parameter	0 ppm	40 ppm	200 ppm	1000 ppm
14-day survivors / female [A]	32.0	31.4	40.0	34.5
14-day survivors / chicks hatched (%)	79.1	87.7	89.0	90.2
14-day survivors / fertile eggs (%)	64.8	63.7	69.1	64.2
14-day survivors / eggs incubated (%)	69.6	64.8	80.6	69.5

[A] : statistically not examined;
No statistically significant difference from controls ($p \leq 0.05$) observed in any parameter.

Chick body weights at hatching and at day 14 were unaffected by treatment. There were no statistically significant changes in any treated group at hatching or after completion of the 14-day rearing period as shown in the following table.

Table CA 8.1.1.3- 9: Chick body weight

Parameter	0 ppm	40 ppm	200 ppm	1000 ppm
Chick weight at hatching (g)	6.7	6.7	6.8	6.9
Chick weight on day 14 (g)	25.0	27.6	28.5	30.0
No statistically significant difference from controls ($p \leq 0.05$) observed in any parameter.				

Food consumption during the 14-day rearing period determined roughly as total feed consumption by group and presented as daily food consumption per 14-day chick, was not indicative of treatment-related changes.

Table CA 8.1.1.3- 10: Group food consumption as daily food consumption per-day chick

Parameter	0 ppm	40 ppm	200 ppm	1000 ppm
Group food consumption (g/chick/day)	2.6	2.5	2.6	2.8

Conclusions:

The NOEL of AE F130060, technical in this 20-week reproduction study with adult Bobwhite Quail

was 1000 ppm (diet) which is the limit dose according to the relevant OECD testing guideline.

Table CA 8.1.1.1- 10: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its reliability
M-198082-01-1 KCA 8.1.1.3 /01	US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-4 (1982)	(not EU-relevant)	NA/	N/A
	OECD Draft Guideline for testing of chemicals No. 206 “avian reproduction test ” (1984)	OECD guideline 205 for testing of chemicals "Avian Dietary Toxicity Test (1984)	No change	The study is in compliance with the guidelines

Report:	[redacted], 1999;M-191369-01
Title:	Mallard duck dietary reproduction toxicity study AE F130060 substance technical Code: AE F130060 00 1693 0000
Report No:	C005103
Document No:	M-191369-01-1
Guidelines:	**OECD: 206; USEPA (=EPA): §71-4;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
NOEC = 1000 mg as/kg food

Study summary and/or MS evaluation copied from the original Monograph:

- **Reference**: [redacted] 1999, 8.1.1.2/1.
- **Test guideline**: US-EPA Fifra Guidelines (1982), OECD Guideline 206 and the A.S.T.M. guideline “Standard practice for conducting reproductive studies with avian species” (1983).
- **GLP compliance**: Yes.
- **Methods**: Effects of AE F130060 technical (96.6% w/w) on the reproduction of the mallard duck was determined by exposing ducklings (F0) to the active substance via the diet, over a 22 weeks period. All birds were approximately 24 weeks old at test initiation. A total of 4 test groups of 16 pairs were constituted, each of which was offered the dietary concentration (0, 40, 200, or 1 000 mg a.s./kg). Behaviour, general health and mortality were checked daily. Body weight was recorded at the start of the acclimatisation period and every two weeks until week 20 and then on week 22. Food consumption was checked weekly. First generation quails were finally dissected at the end of the study for macroscopical and pathological observations. Effects on reproduction were assessed through the number and quality of the eggs produced (visual observations, shell thickness). Effects on the F1 were assessed through the hatching rate of eggs, the survival of chicks, body weight and food consumption over the first two weeks after hatching. General health observations were performed.
- **Results**: There were no effects of a long term dietary exposure of adult mallard ducks on their health, body weight gain and food consumption. A slight but significant increase in male body weight was observed in the 1

000 mg/kg exposed group ($p < 0.01$), but was not found to be of biological signigficance. Similarily, a significant decrease in food consumption was recorded in the 40 mg/kg exposed group during week 16 that could not be interpreted as treatment-related. Egg production was unaffected and neither was hatchin rate survival, health, bodyweight and food consumption in F1. Slight differences were recorded in the survival rate during the embryonic stage and in the hatchling rate, that could not be interpreted as treatment-related. A summary of reproduction data is proposed in table B.9.1.3.1-2.

Table B.9.1.3.1-2. Reproduction data on ***Anas platyrhynchos***

Dietary concentration	Control	40 ppm	200 ppm	1 000 ppm
Eggs laid	810	805	693	801
Eggs laid per female	50.6	50.3	43.3	50.1
Eggs damaged	17	12	10	16
Eggs damaged of eggs laid (%)	2.1	1.5	1.4	2.0
Mean egg shell thickness (mm)	0.386	0.384	0.374	0.379
Eggs set	715	711	623	698
Viable embryos	655	641	587	655
Viable embryos of eggs set (%)	91.6	90.5	95.9	85.8
Live 3-wk embryos	653	626	534	591
Live 3-wk embryos of viable embryos (%)	99.7	98.0*	99.7	98.7
Normal hatchling	598	502	474	513
Normal hatchling of viable embryos (%)	91.3	78.3**	80.1	82.0
Normal hatchling of live 3-wk embryos (%)	91.6	79.8**	90.6	87.1
14-d old survivors	592	497	475	513
14-d old survivors of eggs laid (%)	73.1	61.5	68.5	64.0
14-d old survivors of normal hatchling (%)	99.0	98.8	98.1	99.6
14-d old survivors per female	37.0	30.9	29.3	32.1
Ducklings bodyweight at hatching (g)	34.0	34.0	35.0	35.0
Ducklings bodyweight at 14 days (g)	268.0	271.0	272.0	274.0

* significantly different from the control $p < 0.05$
** significantly different from the control at $p \leq 0.01$
[1] 15 females

NOEC 22 wks ≥ 1 000 mg a.s./kg diet (nominal concentration).

- **Comments (RMS)**: the studies acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:
This study was of excellent technical quality as evidenced by a very high reproductive performance of the control pairs, which was at the upper limit of the typical biological range as specified in the respective testing guidelines. In particular, the definite test criteria for acceptability of the test, i.e. 14-d survivors / hen / 10-weeks ≥ 10, egg shell thickness (> 0.34 mm) and adult mortality (≤ 10%) were clearly met by the control group.

Analytical findings:
The quantification of the test substance in the diet was performed by HPLC separations (reverse phase, UV-detection) of organic solvent (acetonitrile) extracts of the test substance-diet mixtures.
The achieved concentration, stability and homogeneous distribution of the test substance in the diet for 7 days at room temperature were confirmed as acceptable, i.e. in the range of 83-107 % of nominal.

Table CA 8.1.1.3- 11: Mean values (in %) of the nominal content of the test substance at the individual examination times

	Concentration of AE F1 30060 in the diet (mean % of nominal)							
Diet concentration	Pre study		Reproduction study					
			Week 1					
	Day 0	Day 7	Day 0	Day 7	Week 4[a]	Week 8	Week 12	Week 20
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40 mg/kg	94[b]	90[b]	88[b]	83[b]	93	97	94	101
200 mg/kg	92[b]	88[b]	86[b]	86[b]	97	91	99	83
1000 mg/kg	106b	95[b]	90[b]	93[b]	99	106	94	107

[a] diets prepared from the first premix and stored for deep frozen for 4 weeks
[b] samples collected from the top, middle and bottom layers of feed from the feeders to determine homogeneity,
n.d. = not detectable (estimated detection limit = 1 mg/kg)

Biological findings:

Adult Birds

There were no mortalities in any of the treatment or control groups at any time during the test.

No clinical signs of toxicity were seen at any of the concentrations tested. Incidental clinical observations such as foot lesions, leg swelling and feather loss, that normally are associated with pen wear or interactions among penmates were observed. Other clinical signs such as lameness and lower limb weakness also were noted occasionally, and typically were associated with the incidental injuries. Except for such incidental findings, all birds appeared normal throughout the test. Thus there were no treatment-related findings.

There were no adverse effects upon adult body weight at any of the concentrations tested. Any differences in female body weight between the control group and each of the treatment groups were not statistically significant at any of the body weight intervals. A slight, but statistically significant (p<0.01) increase in male body weight in the 1000 ppm treatment group at adult termination was not considered to be of any biological significance. Since the difference observed was slight (< 10%), and represented an increase in body weight, it was considered not to be treatment-related.

Due to wastage by some birds, feed consumption was variable among pens. However, there were no treatment-related effects upon feed consumption at the 40, 200 or 1000 ppm test concentrations. At the 40 ppm test concentration, there was a slight decrease in feed consumption during Week 16 that was statistically significant at p < 0.05. Since the difference from the control group observed at the 40 ppm test concentration was not consistent over the test period and was not concentration-dependent, it was not considered to be treatment-related. Any other differences in feed consumption between the control and any of the treatment groups were not statistically significant.

The estimated test substance intakes for mallard ducks during the test were 4.6, 25.8 and 126 mg AE F130060 /Kg body weight/day for the 40, 200 and 1000 ppm treatment groups, respectively.

All surviving adults were subjected to gross necropsy following adult termination. All findings were considered incidental to treatment. In particular, no findings were observed in the reproductive organs.

Reproduction data

There were no treatment-related effects upon reproductive performance in the 40, 200 or 1000 ppm treatment groups. There was a slight decrease in live three-week embryos as a percentage of viable embryos in the 40 ppm treatment group that was statistically different from the control value at $p < 0.05$. However, the difference was due, primarily, to exceptional performance by the control group (100%). Since the percentage of live three-week embryos was consistent with the historical control value of 98%, and the reduction observed was not concentration-dependant, the difference from the control value was not considered to be treatment-related.
Additionally, at the 40 ppm test concentration there was a slight decrease in hatchlings as a percentage of live three-week embryos that was statistically different from the control group at $p < 0.05$. Again the difference observed was primarily the result of exceptional performance by the control group, which achieved 93% hatchability. Hatchability in the 40 ppm treatment group (76%) was comparable to the historical control value for this parameter of 73 +/- 13%. Since the slight reduction in hatchability was not concentration-dependant, and hatchability in the 40 ppm treatment group was comparable to the historical control value, the difference observed was not considered to be treatment-related.
Any other differences between the control group and any of the treatment groups were not statistically significant for any of the other reproductive parameters measured.

There were no treatment-related effects upon egg shell thickness at the 40, 200 or 1000 ppm test concentrations, and any differences from the control group were not statistically significant.

There were no treatment-related effects upon the body weights of hatchlings or 14 day old survivors at any of the concentrations tested. Any differences between the control group and any of the treatments groups were not statistically significant.

Conclusions:

The no observed effect concentration for mallard ducks treated with AE F130060 in the diet during this reproduction study was 1000 ppm (equivalent to an achieved daily intake of 126 mg AE F130060 /kg body weight/day).

Table CA 8.1.1.3- 12: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-191369-01-1 KCA 8.1.1.3 /02	US-EPA Pesticide Assessment Guidelines, Subdivision E, series 71, §71-4 (1982)	(not EU-relevant)	N/A	N/A
	OECD Draft Guideline for testing of chemicals No. 206 “avian reproduction test ” (1984)	OECD guideline 205 for testing of chemicals "Avian Dietary Toxicity Test (1984)	No change	The study is in compliance with the guidelines
	ASTM Standard E1062-86 Standard Practice for Conducting Reproductive Studies with Avian Species (1986)	(not EU-relevant)	N/A	N/A

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 CA 8.1.2.1-1: Mammalian acute oral toxicity data of mesosulfuron-methyl presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	acute, oral	LD_{50} > 5000 [1] mg as/kg bw	[redacted], 1996 M-140405-01-1 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 mesosulfuron-methyl (SANCO/10298/2003-Final):
LD_{50} > 5000 mg as/kg bw

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

Several subchronic and chronic studies were performed with mesosulfuron-methyl. As the 90-day dietary as well as the long-term carcinogenesis study resulted in endpoints equal or greater than the highest tested concentration of the two generation reproduction study, 12000 and 16000 ppm, respectively, there is no reason to deviate from the reproduction study as endpoint for risk assessment.

The reproduction study resulted - like the long-term carcinogenesis study - in an endpoint equal or greater than 16000 ppm, the highest tested concentration. According to the slightly higher food uptake in the reproduction study the corresponding NOEL is also slightly higher than in the long-term carcinogenesis study, underlining the low toxicity of mesosulfuron-methyl. Details of the studies are provided in the following table.

Table CA 8.1.2.2- 1: Mammalian reproductive toxicity data of mesosulfuron-methyl presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Rat	90-d chronic dietary	NOEC ≡ $NOEL_{male}$ ≡ $NOEL_{female}$ $NOEL_{geomean}$ 1)	12000 907.5 976.5 941	ppm mg as/kg bw/d mg as/kg bw/d mg as/kg bw/d	[redacted], 1999 M-187497-01-1 KCA 5.3.2 /01
Rat	Combined, chronic (long-term, carcinogenesis study)	NOEC ≡ NOEL	16000 956	ppm mg as/kg bw/d	[redacted], 2000 M-198434-01-1 KCA 5.5/01
Rat	2-generation dietary reproduction study	NOEC ≡ $NOEL_{male}$ ≡ $NOEL_{female}$ $NOEL_{geomean}$ 1)	16000 1175 1388 **1277**	ppm mg as/kg bw/d mg as/kg bw/d mg as/kg bw/d	[redacted], 2000 M-198366-01-1 KCA 5.6.1 /02

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

NOEC values (12000 ppm and 16000 ppm) of the studies of [redacted] (1999) and [redacted] (2000) on rats were listed as ecotoxicological endpoints in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). For the reasons discussed above, it is proposed for the updated list of endpoints to specify as endpoint relevant for long-term mammalian risk assessment the geomean NOEL value of 1277 mg as/kg bw/day derived from the results of the 2 generation dietary reproduction study.

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

As the log P_{ow} of the active substance mesosulfuron-methyl 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 mesosulfuron-methyl 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 mesosulfuron-methyl to wildlife presented below.

<u>Wild Mammals</u>

Potential endocrine activity and potential population relevant effects of mesosulfuron-methyl 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 observed that could be related to primary endocrine activity.
Based on the absence of any indication of relevant effects it can be concluded that mesosulfuron-methyl is not an endocrine disrupter.

<u>Birds</u>

The population relevant effects of mesosulfuron-methyl 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 mesosulfuron-methyl. No additional studies seem necessary.

<u>Amphibians and Reptiles</u>

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test method exists, 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 this group 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 substance and the metabolites included in the residue definition for aquatic risk assessment (see MCA Section CA 7.4.1).

Metabolite testing in each case included *Lemna*, which represents the standard aquatic organism by far the most sensitive to the parent active substance mesosulfuron-methyl. Moreover, green algae (*Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus*) were tested in addition for all but two

[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.

intermediate components, as a standard aquatic testing organism of general relevance for herbicides. Nevertheless, in case of mesosulfuron-methyl, algae were found significantly less sensitive to parent active substance than aquatic vascular plant.

The terminal metabolite AE F092944 is a degradate shared with several sulfonyl urea type herbicides. Tests with further aquatic species (Fish, Daphnia) have been performed on this metabolite.

In addition, Lemna and algae tests have been conducted on two components BCS-CO60720 and BCS-CO60721, supportive to evaluation of artifact degradates that were observed in a water/sediment study, but not considered relevant for inclusion in the aquatic residue definition. Details hereon are summarised in document MCA Section 7.2.2.3.

CA 8.2.1 Acute toxicity to fish

For mesosulfuron-methyl, three acute toxicity studies on three different fish species were performed. The maximum tested dose level in all studies was 100 mg a.s./L. No mortality or sublethal effects were observed in the treatments, 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 CA 8.2.1- 1: Acute toxicity data of mesosulfuron-methyl and metabolite to fish presented in this chapter

Test species	Test system	Test duration	Endpoint	[mg as/L]	Reference
Mesosulfuron-methyl					
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50}	**> 100**	[redacted] et al., 1999 M-186666-01-1 KCA 8.2.1 /01
Lepomis macrochirus (bluegill sunfish)	static acute	96 h	LC_{50}	**> 100**	[redacted] et al., 1999 M-186597-01-1 KCA 8.2.1 /02
Cyprinodon variegatus (sheephead minnow)	static acute	96 h	LC_{50}	**> 100**	[redacted], 2001 M-238810-01-1 KCA 8.2.1 /03
AE F092944					
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50}	**254**	[redacted], 1993 M-131422-01-1 KCA 8.2.1 /04

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

Studies on Mesosulfuron-methyl

Report:	[redacted]v;[redacted];[redacted];[redacted];1999;M-186666-01
Title:	Acute toxicity to rainbow trout (Oncorhynchus mykiss) AE F130060 substance, technical Code: AE F130060 00 1C95 0001
Report No:	C003718
Document No:	M-186666-01-1
Guidelines:	**EU (=EEC): C.1; OECD: 203; USEPA (=EPA): E § 72-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
LC_{50} > 100 mg/L

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 1999a, 8.2.1.1/1.
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 72, §72-1 (1982) OECD guideline no 203 (1992) and EU directive 92/69 Annex Part C: C.1.
- **GLP compliance**: Yes.
- **Methods**: The acute toxicity of AE F130060 (technical substance, purity 96% w/w) was assessed in juvenile rainbow trouts exposed for 4 days under static conditions. Fish were approximately 5 months old at the start of the study. Exposure was performed in 50 L containers containing control and test water (respectively 0 and 10, 18, 32, 56 or 100 mg/l, nominal, in 50% filtered tap water/50% deionised water). Ten fish were allocated randomly per container. Mortality and abnormal responses of fish were recorded at 24 h intervals throughout the exposure period.
  A fine sediment was observed on the bottom of test containers within the first 48 h, that had disappeared 72 h after the start of the test. Analytical measurements demonstrated satisfying exposure rates over this period (above 87.9% of nominal a.s.).
- **Results**: No mortality and no abnormal behaviour were recorded in fish exposed to mesosulfuron-methyl, as well as in control fish. Because of the absence of mortality over the range of concentrations tested, no concentration-effect relationship, and therefore no LC50, could be established.
  LC50 – 96 h > 100 mg a.s./l.
- **Comments (RMS)**: The study is acceptable.

Further study information supplementing the original Monograph summary :

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:
Chemical analysis of the freshly prepared and aged (96 hours old) test solutions indicated that the actual exposure concentration ranged from 87.9% to 100.1% at the start of the test, and ranged from 93.7% to 99.7% at the end. The mean measured concentration over the time of exposure ranged from 96.7% to 102.1% of the nominal values. As all analysed concentrations were above 80% of nominal, the nominal concentrations were used for reporting the results.

Biological findings:
No mortality and no intoxication symptoms occurred in any of the tested concentrations and in the untreated control.

The concentration of 50% mortality of the test animals (LC_{50}) is given below:

Table CA 8.5 - 2: Endpoint (LC_{50} value)

	24 - 96 hours
LC_{50} [mg test substance/L] nominal	> 100

Conclusions:
In a static-acute toxicity test (method EPA / OECD / EU) to determine the effect of AE F130060; substance, technical, Code: AE F130060 00 1C95 0001 to rainbow trout (*Oncorhynchus mykiss*) a lethal concentration for 50% of the test animals (LC_{50}) after 24-96 hours test duration was >100 mg test substance/L (corresponding to 94.6 mg a.s./L).
The concentration without mortality and without any observed effects (NOEC, no observed effects concentration) was found at 100 mg animals (LC_{50}) after 24 - 96 hours test duration was >100 mg test substance/L (corresponding to 94.6 mg a.s/L).

Table CA 8.2.1- 3: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-186666-01-1 KCA 8.2.1 /01	OECD No. 203 (1992)	OECD No. 203 (1992)	no changes	no deviations from current guideline
	US EPA, E, § 72-1	(not EU-relevant)	N/A	N/A
	92/69/EWG, C.1	(not relevant)	N/A	N/A

Report:	[redacted]8; [redacted]; [redacted]; [redacted]; 1999; M-186597-01
Title:	Acute toxicity to bluegill sunfish (Lepomis macrochirus) AE F130060 substance, technical Code: AE F130060 00 1C95 0001
Report No:	C009682
Document No:	M-186597-01-1
Guidelines:	**EU (=OECD): 1.1; OECD: 203; US EPA (=EPA): E § 72-1; Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final), but is available from the Monograph evaluation (B.9.2.1.2):
$LC_{50} > 100$ mg/L

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 1999, 8.2.1.2.1.
- **Test guideline**: US EPA Pesticide Assessment Guidelines, Subdivision E, series 72, §72-1 (1982), OECD guideline no 203 (1992) and EU directive 92/69 Annex Part C: C.1.
- **GLP compliance**: Yes.
- **Methods**: The acute toxicity of AE F130060 (technical substance, purity 94.6% w/w) was assessed in bluegill sunfish exposed for 4 days under static conditions. Fish were approximately 9 months old at the start of the study. Exposure was performed in 50 l containers containing control and a limit test concentration, respectively 0 and 100 mg/l (nominal, prepared in 50% filtered tap water/50% deionised water). Ten fish were allocated randomly per container and the limit concentration was tested in triplicate. Mortality and abnormal responses of fish were recorded at 24h intervals throughout the exposure period.
- **Results**: No mortality and no abnormal behaviour were recorded in fish exposed to mesosulfuron-methyl, as well as in control fish. Because of the absence of mortality over the range of concentrations tested, no concentration-effect relationship, and therefore no LC50, could be established.
  LC50 – 96 h > 100 mg a.s./l.
- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary :

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:
Chemical analysis of the freshly prepared and aged (96 hours old) test solutions indicated that the actual exposure concentration ranged from 97.2% to 105.1% at the start of the test, and ranged from 99.4% to 105.5% at the end. The mean measured concentration over the time of exposure ranged from 98.3% to 105.3% of the nominal value. As all analysed concentrations were above 80% of nominal, the nominal value was used for reporting the results.

Biological findings:
No mortality and no intoxication symptoms of the test animals occurred in any of the treated tanks and the untreated control.
Based on this limit test without mortality the LC_{50} could not be determined and a concentration-effect relationship could not be plotted.

The concentration of test substance lethal to 50% of the test animals (LC_{50}) after 24, 48, 72 and 96 hours test duration lay above the single concentration tested of 100 mg test substance /L (corresponding to 94.6 mg a.s./L).

Table CA 8.2.1- 4: Endpoint (LC_{50} value)

	24, 48, 72 and 96 hours
LC_{50} [mg test substance/L] nominal	> 100

Conclusions:
In a static acute toxicity test (method EPA / OECD / EU) to determine the effect of AE F130060; substance, technical, Code: AE F130060 00 1C9503001 to bluegill sunfish (*Lepomis macrochirus*) a lethal concentration for 50% of the test animals (LC_{50}) after 24, 48, 72 and 96 hours test duration was > 100 mg test substance/L (corresponding to 94.6 mg a.s./L).
The concentration without mortality and without any observed effects (NOEC, no observed effect concentration) was found at 100 mg test substance/L (corresponding to 94.6 mg a.i./L).

Table CA 8.2.1- 5: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-186597-01-1	OECD No. 203 (1992)	OECD No. 203 (1992)	no changes	no deviations from current guideline
	US EPA, FIFRA § 72-1	(not EU-relevant)	N/A	N/A
KCA 8.2.1 /02	92/69/EWG, C.1	(no longer relevant)	N/A	N/A

Report:	[redacted]3;[redacted];[redacted];[redacted];2001;M-238810-01
Title:	96 Hour acute toxicity to the sheepshead minnow, Cyprinodon variegatus, in a static system AE F130060 technical 95.7 percent w/w
Report No:	B003157
Document No(s):	M-238810-01-1
Guidelines:	**OECD: 203; USEPA (=EPA): 72-3;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary

The aim of the study was to determine the acute effects of mesosulfuron-methyl (AE F130060; code: AE F130060 00 1C95 0001; purity 95.7% w/w) to sheepshead minnow (*Cyprinodon variegatus*). Juvenile *Cyprinodon variegatus* were exposed in a static system over a period of 96 hours to the nominal concentration of 100 mg a.s./L. In addition a dilution 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} of AE F130060 technical to sheepshead minnows could not be determined under the conditions of this study, and is greater than 100 mg/L. The no observed effect concentration (NOEC) was 100 mg/L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.

Material and Methods

Test item: AE F130060, technical, Batch No.: Pfl. 35316; Code No.: AE F130060 00 1C95 0001; Sample No.: ZBA806; Analysed purity: 95.7% w/w; Certificate of Analysis: AZ 08063.

Juvenile sheepshead minnows (*Cyprinodon variegatus*) were exposed AE F130060 in a static system over a period of 96 hours to a nominal concentration of 100 mg a.s./L in synthetic sea water (mean temperature of 22.1°C). In addition a dilution water control was tested. Each vessel (glass fish tanks; 20 L) served as one replicate filled with 15 L solution. 10 fish were used per test vessel (i. e., 30 fish per treatment level). Mean weight and length of fish taken at the end of the study were 0.307 g (range 0.169 to 0.502 g) and 2.2 cm (range 1.9 to 2.5 cm). The organism loading during the study was 0.205 g/L. The test was conducted with 3 replicates per treatment level.
Observations for death, abnormal appearance and behavior were performed at 3, 6, 24, 48, 72, and 96 hours (± 1 hour).
For analytical verification of the test item concentrations samples were taken at test initiation and termination. All samples were analysed for AE F130060 by reverse-phase High Performance Liquid Chromatography with ultraviolet detection (HPLC/UV) under isocratic conditions.
Test methodology was in agreement with OECD 203 and USEPA 72-3 guidelines.

Dates of experimental work: June 1, 2000 – June 5, 2000

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:
Results of analyses revealed that the AE F130060 concentration was 105% of nominal over the course of the study. There was no AE F130060 residues found in the dilution water or control samples greater than the limit of quantitation (5.0 mg/L). All toxicity values were calculated based on the nominal

concentrations of test substance added to the test water. Detailed analytical results are presented in the following table:

Table CA 8.2.1- 6: measured concentrations of AE F130060

Sample identification (mg/L)	Measured AE F130060 Concentration (mg/L)			
	on day 0	on day 4	Mean (combined)	% Nominal
Dilution water	< LOQ*	< LOQ	< LOQ	--
Control	< LOQ	< LOQ	< LOQ	--
100	107.3780	101.7925	104.59	105 (Std. Dev. = 3.9%)

[a] Limit of Quantitation (5.0 mg/L)

Biological findings:
No mortality or sublethal effects were observed in the control or 100 mg/L treatments during the study.

Conclusion

The 96-hour LC_{50} of mesosulfuron-methyl technical to sheepshead minnows could not be determined under the conditions of this study, and is greater than 100 mg/L. The no observed effect concentration (NOEC) was 100 mg/L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.

Studies on the metabolites of mesosulfuron-methyl

AE F092944

Report:	[redacted]9;[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, metabolite of mesosulfuron-methyl; code: AE F092944 00 ZD99 0001; further code: Hoe 092944; 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 (synonym: AE F092944); 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 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 CA 8.2.1- 7: 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	18.252	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 CA 8.2.1- 8: 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	1	**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
LC_{50}: 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

One chronic study on Rainbow trout was performed. The maximum tested dose level was 32 mg a.s./L. No relevant treatment related effects were observed at the maximum dose level, resulting in a NOEC of 32 mg a.s./L. Details of the study are provided in the following table.

Table CA 8.2.2- 1: Chronic toxicity data of mesosulfuron-methyl 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 **32**	[redacted] et al., 2000 M-187567-01-1 KCA 8.2.2 /01

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

Report:	[redacted];2000;M-187567-01
Title:	Effects on juvenile growth of rainbow trout (Oncorhynchus mykiss) in a 28 days static renewal system AE F130060 substance, technical Code: AE F130060 00 1C95 (00)
Report No:	C004237
Document No:	M-187567-01-1
Guidelines:	**ISO: 10229; OECD: Draft, No. 204;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
NOEC (28 d) = 32 mg/L

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000, 8.2.2.1/1.
- **Test guideline**: OECD guideline no 204 (1984), OECD draft guideline "Fish, Juvenile Growth Test - 28 days" (Nov. 1994), ISO 10229.
- **GLP compliance**: Yes.
- **Methods**: The effects of AE F130060, technical substance (purity 94.6% w/w) on growth of juvenile rainbow trout was assessed in a static renewal system over a 28-day exposure period. Fish were approximately 2 months old at the start of testing. Exposure was performed in 50 L containers of which 1/3 of the volume of test water was renewed daily. In each of these containers twenty fish were randomly allocated to 0 (control) 0.32, 1, 1.32, 10 and 32 mg/l (nominal). No treatment was replicated. Mortality and intoxication symptoms were recorded daily throughout the study and fish growth rates were calculated at the end of the test.
- **Results**: No mortality and no intoxication symptoms were observed in fish exposed to mesosulfuron-methyl over the exposure period. The growth rate of the test fish was not statistically different in mesosulfuron-methyl exposed fish from the growth rate in the control.
NOEC 28 d 32 mg a.s./l.
- **Comments (RMS)**: the study is acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:
The overall survival in the controls was greater than 10%. The water temperature did not differ by more than ± 1°C between chambers at any time and ± 2°C within the range for the test species. Concentrations of test item were above 80% of nominal.

Analytical findings:
Analyses of test substance concentrations which were based on AE F130060 revealed that mean measured concentrations over the time of exposure ranged from 92.1% to 107.8% of nominal values for fresh water and ranged from 99.3% to 108.9% of nominal values for aged water. As all mean measured concentrations were above 80% of nominal, nominal concentrations were used for reporting the results.

Biological findings:
No mortality and no intoxication symptoms of the fish were observed during the time of study.
The concentration of test substance lethal to 50% of the test animals (LC_{50}) after 28 days test duration was far above the highest tested concentration of 32 mg/L.

At the start of the test, there was no significant difference (alpha = 0.05) in length and weight of the test fish between the test concentrations and the control. After 28 days test duration the test fish had increased body weight (+ 147 % compared to initial) and length (+ 37 % compared to initial). There was no significant difference (alpha = 0.05) in weight and length of the test fish at the end of the test compared to the control.
Pseudo-specific growth rates for length (growth rate based on individual end length and mean start length) and relative increase in length did not show any difference of treatments compared to the control. Pseudo-specific growth rate on weight did not show any difference of treatments compared to the control.
The highest concentration of no observed effects, NOEC, (without mortality, intoxication symptoms, effects on growth) was 32 mg/L.

Table CA 8.2.2- 2: Endpoint (LC_{50})

	After 1 - 28 days
LC_{50} [mg test substance/L] nominal	>32

Conclusions:
In a static renewal system (method OECD, ISO) to determine the effects of AE F130060 substance, technical, Code: AE F130060 00 1C95 0001 on juvenile growth of rainbow trout (*Oncorhynchus mykiss*) the highest concentration of no observed effects (NOEC) (without mortality, intoxication symptoms, effects on growth) was 32 mg/L.

Table CA 8.2.2- 3: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-187567-01-1	OECD No. 204 (1984)	The test guideline was deleted in April 2010	N/A	N/A
KCA 8.2.2 /01	OECD draft, Fish Juvenile Growth Test (1994)	OECD No. 215 (2000)	none	Starting from day 19, the test water was additional aerated in the treated tanks, due to the decrease of the oxygen saturation below 60%. The aeration has not effected the test substance analyses. Study reliable.
	ISO 5229 (1994)	no longer relevant	N/A	N/A

CA 8.2.2.1 Fish early life stage toxicity test

One chronic study on early life stage exposure with fathead minnow was performed. The maximum tested mean measured concentration was 95 mg a.s./L. No relevant treatment related effects were observed at this maximum dose level, resulting in a NOEC of 95 mg a.s./L. Details of the study are provided in the following table.

Table CA 8.2.2.1- 1: Chronic toxicity data of mesosulfuron-methyl to fish presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Pimephales promelas (fathead minnow)	Early Life Stage flow-through	32 d	NOEC 95	[redacted], 2003 B004569 M-241475-01-1 KCA 8.2.2.1 /01

Report:	[redacted]q; [redacted];2003;M-241475-01
Title:	Mesosulfuron - The Toxicity to Fathead Minnow (Pimephales promelas) During an Early Life-Stage Exposure
Report No:	B004569
Document No(s):	M-241475-01-1
Guidelines:	**USEPA (=EPA): FIFRA 72-4, OPPTS 850.1400;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary

The aim of the study was to determine the effects of mesosulfuron-methyl (code: AE F130060; purity 96.7%) on fathead minnow (*Pimephales promelas*) embryos and larvae during continuos aqueous exposure.
Eggs and fry of *Pimephales promelas* were exposed in a flow through system over a period of 32 days to nominal concentrations of 6.3, 13, 25, 50 and 100 mg a.s./L (corresponding to analytically verified concentrations of 6.6, 10, 25, 46 and 95 mg a.s./L (77 to 110% of nominal). In addition a water control was tested.
Hatching rates, sublethal symptoms, survival and growth (length and wet and dry weight) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as mean measured figures. The overall NOEC was determined to be 95 mg a.s./L.

Materials and Methods:

Test material: Mesosulfuron-methyl; Synonym: AE F130060; Batch No.: AAIC00961, CAS No.: 208465-21-8; purity: 96.7%.

Fathead Minnow (*Pimephales promelas*) eggs starting at ≤ 24 hours old were exposed to mesosulfuron-methyl (code: AE F130060; purity 96.7%) in a flow through system over a period of 32 days. Test vessels were dosed via a proportional diluter with a renewal rate of 6.6 aquarium volumes per 24-hour period; 90% replacement time of approximately 8 hours. Nominal concentrations were 6.3, 13, 25, 50 and 100 mg a.i./L. In addition a dilution water control was tested. Each vessel (glass aquaria; 39 x 20 x 25 cm) served as one replicate containing 1egg cup(s) and filled with approximately 15 L well water. 40 organisms were used per replicate (80 organisms per treatment level). Thinning of surplus alevin took place at day 4, the post-hatch phase started at day 4 (when no more than 10% unhatched viable embryos remained in any control or treatment level egg incubation cup). The dynamic biological loading did not exceed 0.14 g/L/day in any replicate exposure aquarium. The test was conducted with 2 replicates per treatment level.
During the post-hatch exposure period, observations of abnormal behavior, abnormal physical changes and were recorded daily. Larval survival was estimated at least twice weekly. At 28 days post-hatch exposure (test termination), the percentage of larval survival was determined.
For analytical verification of the test item concentrations and the control samples were taken at on days 0, 4, 11, 18, 25 and 32. Samples of the stock solution were also removed and analyzed on test day

0, 4, 11, 18, 25 and 32. High-performance liquid chromatography using ultraviolet detection (HPLC/UV) was used as analytical method.

Dates of in-life definitive exposure: July 14, 2003 – August 15 2003

Results:

Validity Criteria

The overall survival of fertilised eggs in the controls was greater than the species-specific limits given in OECD 210 and OPPTS 850.1400. The oxygen saturation was above 60% (except some short-term deviations). The water temperature did not differ by more than ± 1.5 °C between chambers or successive days. Concentrations of test item were within ± 20% of nominal.

Analytical findings:

Analytical verification of test solutions revealed measured concentrations of 6.6,10, 25, 46 and 95 mg a.s./L (77 to 110% of the nominal) calculated as arithmetic mean. Biological results are reported as mean measured. Detailed analytical results are presented in the following table:

Table CA 8.2.2.1- 2: nominal and measured concentrations of AE F130060

Nominal Concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)						Mean (SD)	Percent of Nominal
	Day 0	Day 4	Day 11	Day 18	Day 25	Day 32		
Control	<0.88	<0.92	<0.83	<0.90	<0.84	<0.88	NA (NA)	NA
6.3	6.2	6.6	6.2	7.2	6.3	7.1	6.6 (0.47)	110
13	10	9.5	10	10	10	10	10 (0.31)	77
25	24	24	24	26	25	26	25 (0.80)	100
50	45	46	43	49	45	49	46 (2.4)	92
100	91	95	91	99	95	97	95 (3.2)	95

Biological findings:

Larval survival, weight and size in the treatment levels were not statistically different from the respective figures of the control organisms.

Table CA 8.2.2.1- 3: Effect of AE F330060 on hatching success and mortality of *Pimephales promelas*

mean measured concentration (mg a.s./L)	mean survival at hatch (%)	mean larval survival at day 28 post-hatch (%)	mean length (mm) at day 28 post-hatch	mean dry weight (mg) at day 28 post-hatch
control	76	81	30.3	81
6.6	74	73	30.7	83
10	75	85	30.0	80
25	78	83	30.4	81
46	85	86	31.4	90
95	83	76	31.3	90

No sublethal behavioural changes were observed.

Biological endpoints derived:

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

NOEC (alevin survival day 4):	95 mg a.s./L
NOEC (fry survival day 32):	95 mg a.s./L
NOEC (percent hatch):	95 mg a.s./L
NOEC (growth in terms of length):	95 mg a.s./L
NOEC (growth in terms of weight):	95 mg a.s./L
NOEC (overall):	95 mg a.s./L

Conclusion:

The effect of mesosulfuron-methyl (AE F130060) on early life stages of fathead minnow (*Pimephales promelas*) can be quantified as a no observed effect concentration of 95 mg a.s./L. The Lowest-Observed-Effect Concentration (LOEC) was > 95 mg a.s./L. The geometric mean Maximum-Acceptable-Toxicant Concentration (MATC) was determined to be > 95 mg a.s./L.

CA 8.2.2.2 Fish full life cycle test

A fish full life cycle test with mesosulfuron-methyl 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} mesosulfuron-methyl 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 mesosulfuron-methyl on fish are presented below.

Fish

Population relevant effects of mesosulfuron-methyl on fish were studied in an early life-stage test (ELS). No effects on embryo survival at hatch or on survival and growth (wet weight, dry weight, and total length) of larvae at test termination were seen at the highest tested concentration of 95 mg/L.
No further testing is indicated to evaluate the endocrine disrupter potential of mesosulfuron-methyl 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 mesosulfuron-methyl one acute study on *Daphnia magna* was performed. No mortality and no intoxication symptoms 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 CA 8.2.4.1- 1: Acute toxicity data of mesosulfuron-methyl and metabolite to *Daphnia magna* presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Mesosulfuron-methyl				
Daphnia magna (water flea)	static acute	48 h	EC_{50} >100	[redacted] et al., 1999 M-186707-01-1 KCA 8.2.4.1.01
AE F092944				
Daphnia magna (water flea)	static acute	48 h	EC_{50} **233**	[redacted], 1993 M-131382-01-1 KCA 8.2.4.1.02

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

Studies on mesosulfuron-methyl

Report:	[redacted]ü; [redacted]; [redacted]; [redacted]; 1999; M-186707-01-1
Title:	Acute toxicity to waterflea (Daphnia magna) of F130060 substance, Technical Code: AE F130060 00 TC95 0009
Report No:	C003741
Document No:	M-186707-01-1
Guidelines:	**EU (=EEC): 92/69 C.2; OECD: No. 202; USEPA (=EPA): E § 72-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron (SANCO/10298/2003-Final):
EC_{50} > 100 mg/L

Study summary and GMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 1999b, 8.2.4.1/1
- **Test guideline**: US EPA Pesticide Assessment Guidelines, Subdivision E, series 72, §72-2 (1982), OECD guideline no 202 (1984) and EU directive 92/69 Annex Part C: C.2
- **GLP compliance**: Yes
- **Methods**: The acute toxicity of AE F130060 (technical substance, purity 94.6% w/w) was assessed in waterfleas exposed for 2 days under static conditions. Exposure was performed in 300 ml glass jars containing 200 ml of test water with 0 (control) or 100 mg/l (nominal) test substance, as a limit test concentration. Both control and test substance water were prepared in deionised water. Ten daphnids were allocated randomly per jar. The control was repeated two times and the limit test concentration was repeated 6 times. Immobilisation of daphnids was recorded at 24 h intervals throughout the exposure period.
- **Results**: No effects on mortality were recorded at any of the concentrations tested. Because of the absence of mortality over the range of concentrations tested, no concentration-effect relationship, and therefore no LC50, could be established.
  EC50 48 h >100 mg a.s./l
- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary :

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:
Chemical analysis of the freshly prepared and aged (48 hours old) test solutions for AE F130060 indicated that actual exposure concentrations ranged from 97.4% to 102.7% at the start of the test, and from 86.2% to 93.9% at the end. The mean measured concentrations over the time of exposure ranged from 93.2% to 98.34% of the nominal values. As all analysed concentrations were above 80% of nominal, nominal values based on 100 % purity of the test substance were used for reporting the results.

Biological findings:
No immobilisation and no intoxication symptoms occurred in the tested concentration and the untreated control.
Based on the absence of mortality an EC_{50} calculation could not be determined and a concentration-effect relationship could not be plotted.
The concentration estimated to immobilise 50% of the test animals (EC_{50}) after 24 and after 48 hours test duration was >100 mg/L.
The concentration without any observed effects (NOEC) after 48 hours test duration was 100 mg/L.

Table CA 8.2.4.1- 2: Endpoint ($E_{x}C_{50}$ value)

	after 24 and 48 hours
EC_{50} [mg test substance/L] nominal	> 100

Conclusions:
In a static-acute toxicity test (method EPA / OECD / EU) to determine the effects of AE F130060; substance, technical; Code: AE F130060 00-JC95-0001 on *Daphnia magna* (waterflea) the concentration estimated to immobilize 50% of the test animals (EC_{50}) after 48 hours test duration lay above 100 mg test substance/L.
The highest concentration tested without immobilisation and without intoxication symptoms (NOEC, no observed effect concentration) after 48 hours test duration was 100 mg/L.

Table CA 8.2.4.1- 3: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-186707-01-1	OECD No. 202 (1984)	OECD No. 202 (2004)	none	no deviations from current guideline
	US EPA FIFRA § 72-2	(not EU-relevant)	N/A	N/A
KCA 8.2.4.1 /01	92/69/EWG, C.2	(not relevant)	N/A	N/A

Studies on the metabolites of mesosulfuron-methyl

AE F092944

Report:	[redacted]n;[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%, metabolite of mesosulfuron-methyl) 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 (synonym: AE F092944); 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 CA 8.2.4.1- 4: Nominal and measured concentrations of AE F092944

Nominal Concen-tration	Concen-tration (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 CA 8.2.4.1- 5: 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

One acute study on *Mysidopsis bahia* was performed. No mortality or sublethal effects were observed at the concentration of 100 mg/L, resulting in a NOEC of 100 mg/L and a LC_{50} >100 mg/L.

Details of the study are provided in the following table.

Table CA 8.2.4.2- 1: Acute toxicity data of mesosulfuron-methyl to *Mysidopsis bahia* presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]		Reference
Mysidopsis bahia (mysid shrimp)	static acute	96 h	LC_{50} NOEC	>100 100	[redacted] et al., 2000 M-238811-01-1 KCA 8.2.4.2 /01

Report:	KCA 8.2.4.2 /01; [redacted], 2000; M-238811-01
Title:	96 Hour Acute Toxicity to the Mysid Shrimp, *Mysidopsis bahia*, in a Static System AE F130060 Technical 95.7% w/w
Report No:	B003158
Document No(s):	M-238811-01-1
Guidelines:	**USEPA (=EPA): 72-3;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the acute toxicity of mesosulfuron-methyl (AE F130060, technical; code: AE F130060 00 1C95 0001; purity: > 95.7%) to the mysid shrimp, *Mysidopsis bahia* Molenock.
Juvenile mysids (< 24 hour old neonates) were exposed in triplicate to nominal concentrations of 0 mg/L (control sample) and 100 mg/L of the test substance synthetic seawater for a 96-hour period. Results of analysis revealed that test item concentration was 111 % of nominal over the course of the study. All treatments had 10 mysids per test vessel (i.e., 30 mysids per treatment level). Test solutions were not renewed.
Mortality and sublethal behavioral effects were used to determine the endpoints. The 96-hour LC_{50} of AE F130060 technical to mysid shrimp could not be determined under the conditions of this study, and is greater than 100 mg/L. The no observed effect concentration (NOEC) was 100 mg/L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.

Materials and Methods:

Test item: AE F130060 – technical substance; Code No.: AE F130060 00 1C95 0001; Batch No.: Pfl. 35316; Sample No.: ZBA806; Analysed purity: > 95.7 % w/w; analytical certificate No.: AZ 08063.

Juvenile mysids (< 24 hour old neonates) were exposed in triplicate to nominal concentrations of 100 mg/L of the test substance in synthetic seawater (mean temperature of 25.5°C) for a 96-hour period. In addition control treatments of dilution water were tested in triplicate. Each vessel (Pyrex® beaker, 1 Liter) served as one replicate, filled with approx. 800 mL of solution. 10 mysids were tested for each replicate, i.e. 30 organisms were tested for each treatment level. Mysids were fed concentrated *Artemia nauplii* twice daily, and the test solutions were not aerated during the study. Observations of mortality and for abnormal appearance and behavior were performed at 3, 6, 24, 48, 72, and 96 hours (± 1 hour). Physico-chemical water parameters were also assessed.
Samples of the test solutions from each test chamber were taken at study initiation and at study termination. All samples were analyzed for AE F130060 by reverse-phase High Performance Liquid Chromatography with ultraviolet detection (HPLC/UV) under isocratic conditions.

Test methodology was in agreement with USEPA 72-3 guidelines.

Dates of experimental work: June 16, 2000 – June 20, 2000

Results:

Analytical findings:
Results of analyses revealed that the AE F130060 concentration was 111% of nominal over the course of the study. There were no AE F130060 residues found in the dilution water or control samples greater than the limit of quantitation (5.0 mg/L). All toxicity values were calculated based on the nominal concentrations of test substance added to the test water.

Detailed analytical results are presented in the following table:

Table CA 8.2.4.2- 2: measured concentrations of AE F130060

Sample Identification (mg/L)	Measured AE F130060 concentrations (mg/L)			
	16JUN00	20JUN00	Mean (combined)	% Nominal
Dilution water	<LOQ[a]	<LOQ	<LOQ	--
Control	ND[b]	<LOQ	<LOQ	--
100	110.3012	110.7519	110.53	111 (Std. Dev. = 0.35)

[a] Limit of Quantitation (5.0 mg/L).

[b] ND = Not Detected.

Biological findings:
One mysid in the control was not found during observations at 96 hours and was thought to have been a victim of cannabilism. No additional mortality or sublethal effects were observed in the control or 100 mg/L treatments during the study.

Biological endpoints derived:
The 96-hour LC_{50} of AE F130060 technical to mysid shrimp could not be determined under the conditions of this study, and is greater than 100 mg/L. The no observed effect concentration (NOEC) was 100 mg/L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.

Conclusions:

The 96-hour LC_{50} of mesosulfuron-methyl (AE F130060) technical to the mysid shrimp, *Mysidopsis bahia*, could not be determined under the conditions of this study, and is greater than 100 mg/L. The no observed effect concentration (NOEC) was 100 mg/L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.

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 of the water flea at a concentration of 100 mg/L. The NOEC for reproduction was 32 mg/L. The lowest NOEC (adult dry weight) was 1.8 mg/L. Details of the study are provided in the following table.

Table CA 8.2.5.1- 1: Reproductive toxicity data of mesosulfuron-methyl 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	NOECreproduction: 32 **NOECweight: 1.8**	[redacted] et al., 2000 M-197785-02-2 KCA 8.2.5.1 /01

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

Report:	[redacted]7; [redacted]; [redacted]; [redacted]; 2000; M-197785-02
Title:	Effects on growth and reproduction of Daphnia magna (waterflea) AE F130060 Substance technical Code: AE F130060 00 1C9 0001
Report No:	C008780
Document No:	M-197785-02-2
Guidelines:	**EU (=EEC): C.2; OECD: No. 202; US-EPA (FIFRA): E § 72-4; Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
NOEC (21 j*) = 1.8 mg/L

* This is most likely a typing error, should read "d" for days

Study summary and RMS evaluation copied from the original Monograph:

1st Test-rep: C008780, GLP97/081; treatment groups: 0, 10, 18, 32, 56 and 100 mg test item/L):

- **Reference**: [redacted] 2000, 8.2.5.1/1
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 72, §72-4 (1982), OECD guideline no. 202 (1984) and EU directive 92/69 Annex Part B: C.2.
- **GLP compliance**: Yes.
- **Methods**: Effects of AE F130060 (technical substance, purity = 94.6% w/w) on the reproduction of *Daphnia magna* was determined under semi-static conditions over an exposure period of 21 d. Effects on survival and growth were assessed with 0 = control-, 10, 18, 32, 56 or 100 mg test substance/ml in deionised water. Each treatment was repeated three times with 5 daphnids each. Effects on reproduction and growth were investigated in tests vessels containing 200 ml test water of similar mesosulfuron-methyl concentrations and in which 10 adult daphnids were individually allocated (10 test vessels containing one daphnid per concentration). For all concentrations test water was renewed three times a week. The mortality of adults and the number of young were recorded three times per week before renewal of the test media.
- **Results**: No immobilisation of adult females was observed in any replicates, neither with single females nor with females kept in groups of five individuals. The number of living juvenile after 21 days was significantly lower for exposure of 56 and 100 mg/l, but no mortality was observed in the neonates in any treatment level. Regarding to the length of females at test termination, the NOEC was estimated below the lower test concentration (10 mg/l) for both test designs. Regarding to their weight at this time, the NOEC was below 10 mg/l when females were tested by groups of five while it was 56 mg/l when females were tested individually.

NOEC 21 d = 32 mg a.s./l, based on the effects on reproduction.
Since the NOEC could, on the best, be estimated to be below the lower tested concentration, and regarding the contradictory results achieved for the weight of females depending on the test design, it was decided to conduct a new study using lower concentrations.

- **Comments (RMS)**: The RMS agrees that the NOEC is <NOEC 21 d < 10 mg a.s./l based on the effects on the size (weight and length) of females. Nevertheless, this study is not considered as valid because NOEC could not be estimated.

2nd Test run (C009791, CE97/098-2; treatment groups: 0, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg test item/l)

- **Reference**: [redacted] 2004, 8.2.5.1/2.
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision E, series 72-4 (1982), OECD guideline no 202 (1984) and EU directive 92/69 Annex part C.2.2.
- **GLP compliance**: Yes.
- **Methods**: Effects of AE F130060 (technical substance, purity: 94.6 % w/w) on the reproduction of *Daphnia magna* was determined under semi-static conditions over a 21-day exposure period. Effects on survival and growth were assessed with 0 -control-, 1.0, 1.8, 3.2, 5.6, 10 or 18 mg test substance/l in deionised water. Each treatment was repeated three times with 10 daphnids each. Effects on reproduction and growth were investigated in tests vessels containing 100 ml test water of similar mesosulfuron-methyl concentrations and in which 10 adult daphnids were individually allocated (10 test vessels containing one daphnid per concentration). For all concentrations, test water was renewed three times a week. The mortality of adults and the number of young were recorded three times per week before the renewal of the test media.
- **Results**: No immobilisation was observed in any replicates, neither in adult females nor in neonates. The number of living juvenile at each day of assessment and their cumulative number after 21 days did not differ from the control at any treatment levels. These data confirm the NOEC of 32 mg/l that was determined in the first study, based on the effects of mesosulfuron-methyl on the reproduction. Regarding to the length of females, both test designs lead to a NOEC determination of 5.6 mg/l. Regarding to their weight, the NOEC was estimated to 10 mg/l in females that were kept in groups but no clear concentration-effect relationship could be achieved regarding to the weight of the females that were kept individually, as significant effects were recorded at 3.2 and 5.6 mg/l but not at 1.8 mg/l and 10 mg/l.
NOEC 21 d = 18 mg a.s./l based on the effects on reproduction.
NOEC 21 d = 5.6 mg a.s./l, based on the effects on the size (weight and length) of females.
- **Comments (RMS)**: The NOEC of 10 mg/l determined on the basis of the effects induced on the weight of females that were kept individually is unsatisfying. Indeed, significant effects were recorded in two consecutive concentrations: 3.2 and 5.6 mg/l, but not at 1.8 mg/l and 10 mg/l, they may not be considered as "false positive". Therefore, the NOEC should be set at 1.8 mg/l.
NOEC 21 d = 18 mg a.s./l, based on the effects on reproduction.
NOEC 21 d = 1.8 mg a.s./l based on the effects on the size (weight and length) of females.

Further study information supplementing the original Monograph summary :

Validity Criteria:
No unforeseen circumstances were observed which may have affected the quality or integrity of this study. In both projects the validity criteria for this type of study are fulfilled:
1. No mortality occurred in the control.
2. The mean number of living offspring in the controls produced per female surviving at the end of the test was ≥ 60.
3. No ephippia were produced by any test individual.
4. Deviations of temperature were less than 1°C, deviations of pH were less than one unit during the test period.

Analytical findings:
The analyses of freshly prepared water for AE F130060 resulted in test substance concentrations ranging from 89.8% to 109.8% of nominal values with mean values per concentration between 92.4% and 103.8% of nominal. Analyses of aged water for AE F130060 resulted in test substance concentrations ranging from 88.2% to 109.1% of nominal values with mean values per concentration between 93.1% and 105.3% of nominal.
As all analysed concentrations and all mean measured concentrations were above 80% of nominal, nominal concentrations are used for reporting.

Biological findings:

Immobilisation
As in test run 1 (= CE97/098-1) no immobilisation was observed in the adult females or the neonates. In project test run 2 (= CE97/098-2) the assessment at day 9 was postponed to day 10 for logistic reasons. At day 10 first neonates were observed at any treatment level. Therefore, results from test run 2 (= CE97/098-2) are in line with those from the first project. The NOEC regarding immobilisation as reported in test run 1 (= CE97/098-1) are confirmed and need not to be corrected.

Reproduction
Reproduction continued until the last assessment at day 21 at all treatment levels. Nevertheless, a close inspection of the raw data indicate that even in the control replicates some individuals had terminated their reproductive phase at day 14 already.
Data on living juveniles per surviving female on each day of assessment and data on the cumulative number of living juveniles base on the assumption of homogeneity of variance according to Bartlett's test ($p>0.05$) with exception of day 12. The number of juveniles at each day of assessment and the cumulative number at day 21 did not differ from the control at any treatment level. Since all higher treatment levels within test run 2 (= CE97/098-2) did not differ significantly from the control, the difference at 1 mg/L can be regarded as a "false positive". Therefore, the NOEC of 32 mg/L regarding reproduction as reported in test run 1 (= CE97/098-1) is confirmed and needs not to be corrected.

Length & weight
While data on reproduction from both projects (i.e. test run 1 and 2) were non contradictory, data on length and weight were difficult to interpret. In test run 1 (= CE97/098-1) no NOEC regarding length of singled females and those kept in groups could be achieved. Regarding weight the NOEC was 56 mg/L in case of singled females and below the lowest treatment level of 10 mg/L in case of females kept in groups. This led to the decision to repeat the study with lower treatment levels.
In test run 2 (= CE97/098-2) data on length and weight of females from groups just failed the assumption of homogeneity, (p-level just below 0.05). Regarding the length of females in singled females as well as in those kept in groups, a NOEC of 18 mg/L was obtained. This is in contradiction to test run 1 (CE97/098-1) where the treatment levels of 10 and 18 mg/L differed significantly from the control.

Weight of single females did not differ from the control at treatment levels of and above 56 mg/L in test run 1 (CE97/098-1), while according to data from test run 2 (CE97/098-2) a NOEC of 1.1 mg/L was obtained. The treatment level of 10 mg/L was also within the same DUNCAN-group as the control, while the treatment levels of 3.2 and 5.6 mg/L differed significantly. This indicates the lack of a clear dose response and justifies to determine the NOEC regarding the final weight of females as 10 mg/L. This is in line with the data from females of test run 2 (CE97/098-2) kept in groups. Moreover, it should be mentioned that the weights of females from the control replicates in test run 1 (CE97/098-1) was 0.95 mg ± 0.12 (single) and 0.91 mg ± 0.10 (groups) while in test run 2 (CE97/098-2) females were considerably heavier: 1.23 mg ± 0.10 (single) and 1.16 mg ± 0.18 (groups).

The above mentioned differences in body weight between both projects did not refer to the final body length. Mean body length in the controls was between 4.26 mm (CE97/098-2; groups) and 4.40 mm (CE97/098-1; singles). Regarding the final length of females a NOEC of 18 mg/L as achieved from CE97/098-2 is in contradiction to the results from CE97/098-1 where the NOEC was the lowest concentration of 10 mg/L. In order to harmonize both outcomes the NOEC regarding the final length of females is considered to be 5.6 mg/L. This covers the findings from both projects.

An overview on the endpoints (over both test runs) is given in the following table:

Table CA 8.2.5.1- 2: Endpoints (NOEC and LOEC) after 21 days determined for survival, reproduction and growth

Parameter	NOEC nominal	LOEC nominal
Immobilisation of adults	100	-
Immobilisation of juveniles	100	-
Reproduction	32	56
Weight	10	32
Length	5.6	10

Conclusion:
In a 21-day reproduction test (method EPA / OECD) to determine the effects of AE F130060; substance, technical; code AE F130060 00 1013 0001 on immobilization, growth and reproduction of *Daphnia magna* were investigated.
The lowest no-observed effect concentration on immobilisation, growth and reproduction of the waterfleas after 21 days according to the table above is 56 mg/L. A lower NOEC of 1.8 mg/L, and the lowest observed effect concentration (LOEC) as 3.2 mg/L can be derived from table 6.7.2 on page 41 of the original report.

Table CA 8.2.5.1- 3: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-197785-02-2	OECD No. 211 (1998)	OECD No. 211 (2012)	neonate sex to be determined	no deviation from current guideline
KCA 8.2.5.1/01	US EPA, E. § 72-4 (1982)	(not EU-relevant)	N/A	N/A

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

Mesosulfuron-methyl has no insecticidal activity and no relevant chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is deemed necessary.

CA 8.2.5.3 Development and emergence in Chironomus species

Mesosulfuron-methyl has no insecticidal activity, is not a growth regulator, and no relevant chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is deemed necessary.

CA 8.2.5.4 Sediment dwelling organisms

Mesosulfuron-methyl 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 mesosulfuron-methyl 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 effect of mesosulfuron-methyl on algae in general was found moderate to low, the numeric endpoint relevant for risk assessment derived from the study on green alga *Pseudokirchneriella subcapitata*, E_rC_{50} for this species is > 0.29 mg a.s./L.

For the metabolites AE F160459, AE F154851, AE F099095, AE F092944, and AE F147447, studies were performed with green algae. All of these components were found devoid of notable effect on algae, EC_{50} in each case was above the highest tested dose level (EC_{50} > 10 to > 560 mg/L).

In addition, tests on green algae were conducted on two components BCS-CO60720 and BCS-CO60721, supportive to a discussion of artifact defects in a water/sediment study (cf. document MCA Section 7.2.2.3). Also these components were found devoid of notable effect on algae.

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

Test species	Test system	Test duration	Endpoint [mg as/L]		Reference
Mesosulfuron-methyl					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h /96 h	E_rC_{50} 1)	**> 0.29**	[redacted] et al., 1998 M-143500-01-1 KCA 8.2.6.1 /01
	growth inhibition	72 h	E_rC_{50}	**3.99**	[redacted], 2015 M-516540-01-1 KCA 8.2.6.1 /09
		96 h	E_rC_{50}	4.43	
Navicula pelliculosa (diatom)	growth inhibition	72 h /96 h	E_rC_{50} 1)	> 74.9	[redacted] et al., 2000 M-187975-01-1 KCA 8.2.6.2 /01

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Anabaena flos-aquae (blue-green algae)	growth inhibition	72 h	E_rC_{50} [1] 5.6	[redacted] et al., 2001 M-238869-01-1 KCA 8.2.6.2 /02
		96 h	E_rC_{50} [1] 4.1	
Skeletonema costatum (marine diatom)	growth inhibition test	72 h /96 h	E_rC_{50} > 100	[redacted] 2001 M-238809-01-1 KCA 8.2.6.2 /03
AE F154851				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50} **38.0**	[redacted], 2005 M-255087-01-1 KCA 8.2.6.1 /04
AE F160459				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h /96 h	E_rC_{50} [1] > 100	[redacted] et al., 2000 M-198314-01-1 KCA 8.2.6.1 /02
AE F099095				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50} [1] **> 100**	[redacted] 2005 M-254084-01-1 KCA 8.2.6.1 /05
AE F092944				
Scenedesmus subspicatus (green alga)	growth inhibition	72 h	E_rC_{50} [1] **> 560**	[redacted], 1993 M-131421-01-1 KCA 8.2.6.1 /06
AE F147447				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h /96 h	E_rC_{50} [1] > 100	[redacted] et al., 2000 M-199529-01-1 KCA 8.2.6.1 /03
BCS-CO60720				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50} [1] > 10.0	[redacted], 2011 M-414950-01-1 KCA 8.2.6.1 /07
BCS-CO60721				
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50} [1] > 10.0	[redacted], 2011 M-415112-01-1 KCA 8.2.6.1 /08

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 E_bC_{50} figures were omitted from the table above.

[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

CA 8.2.6.1 Effects on growth of green algae

For mesosulfuron-methyl and its degradates AE F154851, AE F160459, AE F0999095, AE F092944, AE F147447, BCS-CO60720 and BCS-CO60721 aquatic toxicity studies on green algae, *Pseudokirchneriella subcapitata* or *Scenedesmus subspicatus*, were performed. An overview on all studies is provided in Table CA 8.2.6-1.

Studies on mesosulfuron-methyl

Report:	[redacted];[redacted];[redacted];[redacted];1998;M-143500-01
Title:	Algal growth inhibition (Pseudokirchneriella subcapitata) AE F130060 substance, technical 94.6 percent Code: AE F130060 00 1C95 0021
Report No:	A59843
Document No:	M-143500-01-1
Guidelines:	**EU (=EEC): 92/69 C.3; OECD: 201; USEPA=EPA: J § 123-2;Deviation: not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

E_bC_{50} = 0.20 mg/L

The new aquatic guidance document (EFSA 2013) regards endpoints based on growth rates as relevant. ~~Accordingly, the listed endpoint should be revised to:~~

~~E_rC_{50} > 0.29 mg/L.~~

In the present study, due to the low top dose level tested no definite value but only 'greater than' information for ErC50 could be obtained:

E_rC_{50} 72/96 h > 0.29 mg/l (based on measured concentrations);

E_rC_{50} 72/96 h > 0.32 mg/l (based on nominal concentrations).

A definite information on ErC50 for green algae Pseudokirchneriella subcapitata could however be derived from a repeat test reported under point KCA 8.2.6.1/09 below, using a higher dosing regime. It is therefore proposed to base the new List of Endpoints value for green algae on this latter study:

E_rC_{50} (72 h) = 3.99 mg/L

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted]., 1998, 8.2.6.1/1.
- **Test guideline:** US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD guideline no 201 (1984) and EU directive 92/69 Annex Part C: C.3.
- **GLP compliance**: yes.
- **Method**: The toxicity of AE F130060 (technical substance, purity = 94.6%) to the green algae species *Pseudokirchneriella subcapitata* was determined under static conditions over an exposure period of 96 h. The test was conducted in 300 ml flasks filled with 100 ml test water, containing 0 (control), 0.032, 0.056, 0.1, 0.18 and 0.32 mg test substance/l. The cell density was 10^4 cells/ml at the start of the test. Each concentration was repeated three times, and control was repeated 6 times. Cell density was measured in 5 ml aliquots on every 24 h until the end of the test.
- **Results**: based on measured concentrations:
  E_rC_{50} 72/96 h > 0.29 mg/l

NOE_rC 96 h = 0.018 mg/l
E_bC_{50} 72 h = 0.18 mg/l; 95% CI =[0.16 – 0.29]mg/l
E_bC_{50} 96 h = 0.21 mg/l; 95% CI =[0.16 – 0.29]mg/l.

- **Comments (RMS):** analytical measurements showed a low recovery rate for the active substance up to 0.1 mg/l at the end of the test period (14.9% to 87.4%). This phenomenon was explained by the adsorption of mesosulfuron-methyl on algal cells. This hypothesis is acceptable as the recovery rate increases with the growth inhibition rate (i.e. as algal density increases). However, the fraction of the active substance that is responsible for effects is difficult to estimate (cell adsorbed a.s. may also induce toxicological effects), especially since the a.s. is systemic. Endpoints should then rather be expressed as nominal concentrations. The study is acceptable.

  E_rC_{50} 72/96 h > 0.32 mg/l
  NOE_rC 96 h = 0.032 mg/l
  E_bC_{50} 72 h = 0.20 mg/l
  E_bC_{50} 96 h = 0.23 mg/l.

Further study information supplementing the original Monograph summary :

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

The additional validity criteria defined in the recent version of OECD guideline 201 [mean coefficient of variation for section-by-section specific growth rates in control ≤35% (criterion 2) and coefficient of variation of average specific growth rates in replicate controls ≤7% (criterion 3)] are fulfilled.

Analytical findings:
Analyses of freshly prepared water for AE F130060 resulted in test substance concentrations ranging from 90.7 to 96.6% of nominal values. Analyses of aged water (96 h) for AE F130060 at experimental termination resulted in test substance concentrations ranging from 14.9 to 87.4% of nominal values. The mean measured values over the time of exposure ranged from 54.8 to 91.6% and were 0.018, 0.041, 0.084, 0.165, and 0.292 mg/L. Mean measured values were used for reporting the results.
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 %. Due to a dilution failure preparing the recovery sample on day 4, the results of day 4 were corrected by the recovery determined on day 0.
The specificity of the method is sufficient: Interferences of the determined compound with matrix above the LOQ were regarded by subtraction of the blank value of the blank control water from the sample results and its identity is established by cochromatography with the corresponding certified reference substance.
On day 0 the test results are within 80 - 120 % of the nominal concentration, whereas on day 4 the results of the low nominal concentration levels of 32, 56 and 100 µg/L are < 80 % of the nominal concentration. Therefore, the variability of the two lowest concentration levels is >1.5.
The reason for the deficiencies was strong growth of algae adsorbing considerable amounts of the test substance, which particularly influenced the results of the lower concentration level.

Biological findings:
Significant inhibition of growth (significance level of alpha = 0.05) was observed in mean measured concentrations of 0.041 mg/L and above. Therefore, the no observed effect concentration (NOEC)

defined as no significant growth inhibition and no cell deformation was 0.018 mg/L.

Table CA 8.2.6.1- 1: Endpoints (EC_{50} values)

	After 72 hours		After 96 hours	
	E_bC_{50}	E_rC_{50}	E_bC_{50}	E_rC_{50}
EC_{50} [mg/L] (mean measured)	0.18	>0.29	0.21	>0.29
95% confidence limits in µg/L, low	0.16	-	0.16	
95% confidence limits in µg/L, high	0.29	-	0.29	

Calculation method selected: binomial probability

Conclusions:
In a Growth Inhibition Test (method EPA / OECD / EU) to determine the effect of AE F136060; substance, technical, 94.6%, Code: AE F136060 00 1C9 0001 to *Pseudokirchneriella subcapitata* (Green alga) after 72 hours test duration, the E_bC_{50} was 0.18 mg/L (95% confidence limits 0.16 – 0.29 mg/L) and after 96 hours test duration 0.21 mg/L (95% confidence limits 0.16 – 0.29 mg/L) in comparison with the untreated control.
The concentration for a 50% reduction of growth based on a comparison of slopes of the growth curves (E_rC_{50}) in comparison with the untreated control after 72 and 96 hours test duration was > 0.29 mg/L.
The no observed effect concentration (NOEC) after 96 hours (defined as no significant growth inhibition and no cell deformation) was 0.018 mg/L.

Table CA 8.2.6.1- 2: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-143500-01-1 KCA 8.2.6.1 /01	OECD No. 201 (1984)	OECD No. 201 (2006, corr. 2011)	additional validity criteria	no deviation from current guideline.; the study fulfills the new OECD validity criteria
	US EPA, § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	92/69/EWG C.3	(not relevant)	N/A	N/A

*****

A repeat study on growth inhibition of green algae *Pseudokirchneriella subcapitata* [KCA 8.2.6.1 /09] was conducted upon request of a country authority, to overcome concern over the analytical detect of a small amount of test item in control samples of the previous study KCA 8.2.6.1 /01 [cf. Table 6.2 of original report].

Testing in the repeat study included higher top dose levels to enable the determination of a definite value for the risk assessment relevant parameter ErC50, where the dosing regime of the previous experiment allowed only for the derivation of a 'greater than' figure.

Report:	[redacted]k; [redacted];2015;M-516540-01
Title:	Pseudokirchneriella subcapitata growth inhibition test with mesosulfuron-methyl (tech.)
Report No:	EBMMN130
Document No:	M-516540-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; OCSPP Guideline 850.4500: Algal Toxicity (January 2012);not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the influence of the test item on exponentially growing populations of *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algae biomass (cells per volume). Algae were exposed in a chronic multigeneration test system for 72 hours with a prolongation to 96 hours to in order to cover OECD and OCSPP guideline under static exposure conditions to nominal concentrations of 0.143, 0.458, 1.46, 4.69 and 15.0 mg a.s./L in comparison to control. Four 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, 3 and 4 of the exposure period. To detect possible alterations in algae cells that might influence extinction measurements, such as unusual cell size, pooled samples were examined under a microscope. Based on analytical findings, the biological endpoints are reported as nominal figures. After 72 hours the E_rC_{50} for mesosulfuron-methyl (tech.) was determined as 3.99 mg a.s./L (95 % CI: 3.60 – 4.44 mg a.s./L) and the NOE_rC as 0.143 mg p.m./L.

Material and methods:

Test item: mesosulfuron-methyl (tech.); Batch ID: EBME000144Sample description: TOX09287-01; Specification No.: 102000013204; Purity: 97.4% w/w.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 72 hours with a prolongation to 96 hours to in order to cover OECD and OCSPP guideline under static exposure conditions to nominal concentrations of 0.143, 0.458, 1.46, 4.69 and 15.0 mg a.s./L in comparison to control. The test volume was 150 mL test medium per replicate. 4 replicate vessels per test level and 6 replicate vessels per control were used during the test. The pH values ranged from 7.9 to 9.2 in the control replicates and the incubation temperature ranged from 22.3 °C to 23.1 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 4.76 Klux (mean value).
Quantitative amounts of mesosulfuron-methyl were measured in all treatment groups and in the control at test start, after 72 hours and test end (96 hours). HPLC was used as analytical method.

Dates of experimental work: January 09, 2015 to February 18, 2015

Results:

Validity criteria:
The study conditions met all validity criteria, requested by the OECD guideline 201: Biomass increased in the control by a factor of at least 16 within the evaluation period (factor of 80.9). The mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35% (27.4%). The percent coefficient of variation of the average growth rate in each control replicate did not exceed 7% (0.7%).

For the test to be valid according to OCSPP Guideline No. 850.4500, Biomass increased in the control exponentially by a factor of at least 100 within the 96 hours test (factor of 248.8). The coefficient of variation for mean control yield at day 4 did not exceed 15% (4.5%). The percent coefficient of variation for average specific growth rate of controls at test termination did not exceed 15% (0.8%).

Analytical findings:

The analytical findings of mesosulfuron-methyl in the treatment levels found on day 0 were 97.3 % to 109 % of nominal (average 104 %). After 72 hours analytical findings of 96.7 % to 108 % of nominal (average 105 %) were found and after 96 hours analytical findings of 101 % to 107 % of nominal (average 105 %) were found. All results are based on nominal test concentrations of the test substance.

Table: Summary of analytical results

Nominal concentration [mg p.m./L]	Actual concentration (mg p.m./L)											
	0 hours				72 hours				96 hours			
	Determination		Average	%	Determination		Average	%	Determination		Average	%
	1.	2.			1.	2.			1.	2.		
control	<0.0010	<0.0010	<0.0010	--	<0.0010	<0.0010	<0.0010	--	<0.0010	<0.0010	<0.0010	--
0.143	0.152	0.153	0.153	107	0.153	0.154	0.154	108	0.154	0.152	0.153	107
0.458	0.493	0.501	0.497	109	0.492	0.492	0.492	107	0.492	0.492	0.492	107
1.46	1.52	1.52	1.52	104	1.55	1.56	1.56	107	1.54	1.54	1.54	105
4.69	4.82	4.87	4.85	103	4.86	4.87	4.87	104	4.83	4.81	4.82	103
15.0	14.5	14.7	14.6	97.3	14.6	14.4	14.5	96.7	15.2	15.1	15.2	101
			Mean	104			Mean	105			Mean	105

Biological findings:

Observations are listed as follows:

Table: Effects after 72 hour on algae growth inhibition test

Nominal concentration [mg a.s./L]	Cell number after 72 h (means) per ml	72 h average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
control	808 000	1.464	0.0
0.143	788 000	1.455	0.7
0.458	499 000	1.304	11.0*
1.46	254 000	1.078	26.4*
4.69	63 000	0.611	58.3*
15.0	32 000	0.382	73.9*

Test initiation with 10 000 cells/mL

* Significantly (α=0.05, one-sided smaller) reduced, based on Welch-t test for inhomogeneous variances with Bonferroni-Holm adjustment

Table: Effects after 96 hour on algae growth inhibition test

Nominal concentration [mg a.s./L]	Cell number after 72 h (means) per mL	72 h average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
control	2 488 000	1.379	0.0
0.143	2 464 000	1.376	0.2
0.458	1 899 000	1.312	4.9*
1.46	872 000	1.117	19.0*
4.69	127 000	0.631	54.2*
15.0	31 000	0.282	79.6*

Test initiation with 10 000 cells/mL
* Significantly (α=0.05, one-sided smaller) reduced, based on Welch-t test for inhomogeneous variances with Bonferroni-Holm adjustment

No morphological change in algae was observed in any test concentration.

Conclusions:

After 72 hours the E_rC_{50} for mesosulfuron-methyl (tech.) was determined as 3.99 mg a.s./L (95 % CI: 3.60 – 4.44 mg a.s./L) and the NOE_rC as 0.143 mg p.m./L.
After 96 hours the E_rC_{50} for mesosulfuron-methyl (tech.) was determined as 4.43 mg a.s./L (95 % CI: 4.16 – 4.72 mg a.s./L) and the NOE_rC as 0.143 mg p.m./L.

Studies on the metabolites of mesosulfuron-methyl

AE F154851

Report:	[redacted]k;[redacted];2005;M-255087-01
Title:	Pseudokirchneriella subcapitata - growth inhibition test with AE F154851 00 1B96 0001
Report No:	EBMMX093
Document No:	M-255087-01-1
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 the study was to determine the influence of AE F154851 (metabolite of mesosulfuron-methyl) on exponentially growing of the green algae *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume). Cultures of *Pseudokirchneriella subcapitata* (freshwater microalgae) with an initial cell density of 10 000 cells/mL were exposed in a chronic multi-generation 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 per 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, samples were examined under a microscope. The (0-72h)-E_rC_{50} for the metabolite AE F154851 is 38.0 mg p.m./L based on the geometric mean of measured concentrations.

Materials and Methods:

Test item: AE F154851 00 1B96 0001; Analytical ref.: 0107127; Batch No.: LOR 21029; Chemical code name: AE F154851; purity: 96.1 % w/w; certificate of analysis no.: AZ 09181.

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 7.8 to 8.5 in the controls and the incubation temperature ranged from 22.2°C to 23.4°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6341 lux.
Quantitative amounts of AE F154851 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 27, 2005 – May 30, 2005

Results:

Validity Criteria:
Test conditions met all validity criteria given by the mentioned guideline(s).

Analytical findings:
The analytical findings of AE F154851 in the treatment levels found on day 0 were 64 to 81 % of nominal (average 72.2 %). On day 3 analytical findings of 60 to 89 % of nominal (average 73.0 %) were found. All results are based on the geometric mean of measured test concentrations. Detailed analytical results are presented in the following table.

Table CA 8.2.6.1- 1: Concentrations of AE F154851 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg AE F154851/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.00562	<0.00562	<0.00562	--
6.25	3.98	4.01	4.00	64
12.5	10.2	10.2	10.2	81
25	16.0	15.9	16.0	64
50	37.0	37.0	37.0	74
100	79.4	79.1	79.3	79
			Mean	72.4

Table CA 8.2.6.1- 2: Concentrations of AE F154851 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg AE F154851/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.00562	<0.00562	<0.00562	-
6.25	4.74	4.69	4.71	75
12.5	7.48	7.46	7.47	60
25	18.6	18.0	18.3	73
50	33.8	34.5	34.1	68
100	90.7	88.2	89.5	89
			Mean	73.0

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

Table CA 8.2.6.1- 3: Effect of AE F154851 on growth inhibition of *Pseudokirchneriella subcapitata*

Geometric mean concentration (mg p.m./L)	Cell number after 72 h (mean) per mL	(0-72h) Average specific growth rates [day-1]	Inhibition of average specific growth rate (%)	Doubling time of algae cells (days)
Control	669 000	1.399	-	0.495
4.34	526 000	1.321	5.6	0.525
8.72	390 000	1.221	12.7	0.568
17.1	192 000	0.985	29.6	0.704
35.5	77 000	0.679	51.5	1.021
84.2	38 000	0.443	68.3	1.565

* test initiation with 10 000 cells/mL

A morphological change in algae was observed in test concentrations of 35.5 and 84.2 mg p.m./L.

Conclusions:

The influence of AE F154851 (mesosulfuron-methyl metabolite) on the growth of the green algae *Pseudokirchneriella subcapitata* can be quantified as a (0-72h)-E_rC_{50} of 38 mg p.m./L (95% confidence limits 31.2 - 48.0 mg/L) based on the geometric mean of measured concentrations.

AE F160459

Report:	[redacted];2000;M-198314-01
Title:	Algal growth inhibition - Pseudokirchneriella subcapitata AE F160459 (metabolite of AE F160060, substance, purity code: AE F160459 00 1B97 0001
Report No:	C010060
Document No:	M-198314-01-1
Guidelines:	**EU (=EEC): 92/69 C.3; OECD: 201; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

E_bC_{50} (72h) = 92 mg/L.

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, the listed endpoint should be revised to:

E_rC_{50} > 100 mg/L.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000d, 8.2.6.3.1/1.
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §122-2 (1982), OECD guideline no. 201 (1984) and EU directive 92/69 Annex Part C: C.3.
- **GLP compliance**: Yes.
- **Methods**: The toxicity of AE F160459 (purity = 97.8%) to the green algae species *Pseudokirchneriella subcapitata* was determined under static conditions over an exposure period of 96 h. The test was conducted in 300 ml flasks filled with 100 ml test water, containing 0 (control), 10, 18, 32, 56 and 100 mg test substance/l. The cell density was 10^4 cells/ml at the start of the test. Each concentration was treated three times, and control was repeated 6 times. Cell density was measured in 0.2 to 1 ml aliquots on every 24 h until the end of the test.
- **Results**: Growth was retarded at the highest treatment level of 100 mg/l, based on significant differences in the area under the growth curve between this treatment level and the control. The comparison of curve slopes also showed significant differences between the two groups. Based on nominal concentrations:
  ErC50 72/96 h > 100 mg/l
  NOAErC 96 h = 56 mg/l
  EbC50 72 h = 92 mg/l
  EbC50 96 h = 98 mg/l
- **Comments (RMS)**: the study is acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:
The validity criterion of cell density increased 16x in the controls fulfilled. The additional validity criteria defined in the recent OECD Guideline 201 (mean coefficient of variation for section-by-section specific growth rates (criterion 2) and coefficient of variation of average specific growth rates (criterion 3) are fulfilled.

Analytical findings:
Analyses of freshly prepared waters for AE F160459 resulted in test substance concentrations ranging from 96.7% to 103.2% of nominal values. Analyses of aged water (96 h) for AE F160459 at experimental termination resulted in test substance concentrations ranging from 98.2% to 103.4% of nominal values. The mean measured values over the time of exposure ranged from 98.8% to 102.8%. Therefore the mean measured concentrations of AE F160459 were within ± 20% of nominal at the start and the end of the study, all effect concentrations were based on nominal initial test concentrations.

Biological findings:
Significant inhibition of growth based on a comparison of areas under the growth curves (significance level of alpha = 0.05) was observed at the highest tested concentration of 100 mg/L after 72 and 96 hours test duration. Significant inhibition of specific growth rate based on a comparison of slopes of the growth curves (significance level of alpha = 0.05) was also observed in the highest tested concentration of 100 mg/L after 72 and 96 hours test duration.

72 hour- and 96 hour growth rates at 10 mg/L did not differ significantly from the control. At treatment levels between 18 and 56 mg/L a slight growth promotion was observed. A significant

growth inhibition occurred at 100 mg/L.

Table CA 8.2.6.1- 3: Endpoints (EC_{50} values)

	After 72 hours		After 96 hours	
	E_bC_{50}	E_rC_{50}	E_bC_{50}	E_rC_{50}
EC_{50} [mg/L]	92	>100	98	>100

Conclusions:

In a Growth Inhibition Test (method EPA / OECD / EU) to determine the effect of AE F160459; substance, pure, Code: AE F160459 00 1B97 0001 to *Pseudokirchneriella subcapitata* (Green alga) the nominal concentration of the test substance inhibiting the growth and the resulting E_bC_{50} in comparison with the untreated control after 72 and 96 hours was 92 and 98 mg test substance/L, respectively.

The nominal concentration of test substance inhibiting the growth and the resulting E_rC_{50} in comparison with the untreated control after 72 and 96 hours was > 100 mg test substance/L.

Therefore, the no observed adverse effect concentration (NOAEC) defined as no significant growth inhibition and no cell deformation after 96 hours was 56 mg/L.

Table CA 8.2.6.1- 4: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-198314-01-1 KCA 8.2.6.1 /02	OECD No. 201 (1984)	OECD No. 201 (2006, corr. 2011)	additional validity criteria	no deviation from current guideline.; the study fulfils the new OECD validity criteria
	US EPA J, § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	92/69/EWG, C.3	(not relevant)	N/A	N/A

AE F099095

Report:	[redacted]*;[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 (metabolite of mesosulfuron-methyl) 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 CA 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 CA 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 CA 8.2.6.1- 6: Inhibitory effects of AE F099095

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 F099095 is >100 mg pure metabolite /L and the (0 - 72h)-NOE_rC is 25 mg pure metabolite /L (based on nominal initial concentrations).

AE F092944

Report:	[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
Guideline:	**OECD: 201 (1984); Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of AE F092944 (metabolite of mesosulfuron-methyl; code: AE 092944 00 ZD99 0001; further code: Hoe 092944; 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.
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 72-hour-E_rC_{50} was > 560 mg/L, the 72-hour-NOEC was determined to be 56 mg/L.

Materials and Methods:

Test material: Hoe 092944 technical (synonym: AE F092944); purity: > 99.0%, Code: Hoe 092944 00 ZD99 0001; Analytical certificate No.: AZ 04888;

Green alga (*Scenedesmus subspicatus*) was exposed to AE 092944 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 control 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 CA 8.2.6.1- 7: 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 CA 8.2.6.1- 8: 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	17.2
560	67.6	26.7

After 48 hours test duration enlarged cells could be observed in concentrations equal to and higher than 100 mg/L.

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 on *Scenedesmus subspicatus* can be quantified as a 72-hour-E_rC_{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 F147447

Report:	[redacted];2000;M-199529-01
Title:	Algal growth inhibition - Pseudokirchneriella subcapitata AE F147447 (metabolite of AE F136060) substance technical Code: AE F147447 00 1C93 0001
Report No:	CA09927
Document No:	M-199529-01-1
Guidelines:	**EU (=EEC): 92/69; C3; OECD: 201; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
E_bC_{50} (72h) = 92 mg/L.

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, the listed endpoint should be revised to:
E_rC_{50} > 100 mg/L.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000e, 8.2.6.3.1/2.
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD guideline no 201 (1984) and EU directive 92/69 Annex Part C: C.3.

- **GLP compliance**: Yes.

- **Methods**: The toxicity of AE F147447 (technical substance, purity = 93.1%) to the green algae species *Pseudokirchneriella subcapitata* was determined under static conditions over an exposure period of 96 h. The test was conducted in 300 ml flasks filled with 100 ml test water, containing 0 (control), 10, 18, 32, 56 and 100 mg test substance/l. The cell density was 10^4 cells/ml at the start of the test. Each concentration was treated three times, and control was repeated 6 times. Cell density was measured in 0.1 to 1 ml aliquot on every 24 h until the end of the test.

- **Results**: After 72 h test duration, a significant growth inhibition was observed at the concentrations of 56 and 100 mg/l, based on the results of both the area under the growth curve and the slopes of the growth curves. These differences were not recorded after 96 h test duration. NOEC 72 h was therefore set to 32 mg/l. Based on nominal concentrations:

  ErC50 72/96 h > 100 mg/l
  NOErC 72 h = 32 mg/l
  NOErC 96 h = 100 mg/l
  EbC50 72/96 h > 100 mg/l

Further study information supplementing the original Monograph summary :

Validity Criteria:
The validity criterion of cell density increase >16x in the controls is fulfilled.
The additional validity criteria defined in the recent version of OECD guideline 201 [mean coefficient of variation for section-by-section specific growth rates in control ≤35% (criterion 2) and coefficient of variation of average specific growth rates in replicate controls ≤7% (criterion 3)] are fulfilled.

Analytical findings:
Analyses of freshly prepared water for AE F147447 resulted in test substance concentrations ranging from 97.1% to 98.8% of nominal values. Analyses of aged water (96 h) for AE F147447 at experimental termination resulted in test substance concentrations ranging from 97.3% to 99.7% of nominal values. The mean measured values over the time of exposure ranged from 97.9% to 98.8%.

Therefore the mean measured concentrations of AE F147447 were within ± 20% of nominal at the start and the end of the study. All effect concentrations were based on nominal initial test concentrations.

Biological findings:
After 72 h test duration a significant inhibition of growth based on a comparison of both the growth rates and the areas under the growth curves (significance level of alpha = 0.05) was observed at the concentrations of 56 and 100 mg/L.
After 96 h test duration no significant inhibition of growth based on a comparison of both the growth rates and the areas under the growth curves (significance level of alpha = 0.05) was observed at any treatment level up to 100 mg/L, the highest concentration tested.

Therefore, the no observed effect concentration (NOEC), defined as the concentration which had no significant effect on growth inhibition or cell morphology after 72 and 96 hours was 32 and 100 mg/L, respectively.

Table CA 8.2.6.1- 5: Endpoints (EC_{50} values)

	After 72 hours		After 96 hours	
	E_bC_{50}	E_rC_{50}	E_bC_{50}	E_rC_{50}
EC_{50} [mg/L]	>100	>100	>100	>100

Conclusions:
In a Growth Inhibition Test (method EPA / OECD / EU) to determine the effect of AE F147447; substance, technical, Code: AE F147447 00 1C93 0001 to *Pseudokirchneriella subcapitata* (green alga) the nominal concentration of test substance inhibiting the growth and the resulting E_bC_{50} in comparison with the untreated control after 72 and 96 hours test duration was > 100 mg test substance/L.
The nominal concentration of test substance inhibiting the growth and the resulting E_rC_{50} in comparison with the untreated control after 72 and 96 hours test duration was > 100 mg test substance/L.
The no observed effect concentration (NOEC) defined as no significant growth inhibition and no cell deformation after 72 and 96 hours was 32 and 100 mg/L, respectively.

Table CA 8.2.6.1- 6: Summary table

Reference	Followed guidance	Guidance currently in force	Deviations	Critical assessment of the study / deviations / conclusion about its Reliability
M-199529-01-1 KCA 8.2.6.1 /03	OECD No. 201 (1984)	OECD No. 201 (2006, corr. 2011)	additional validity criteria	no deviations from current guideline.; the study fulfils the new OECD validity criteria
	US EPA; § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	92/69/EWG/C.3	(not relevant)	N/A	N/A

BCS-CO60720

Report:	[redacted]ü; [redacted];2011;M-414950-01
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CO60720 - limit test
Report No:	EBMML012
Document No:	M-414950-01-1
Guidelines:	**OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006);none**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the influence of BCS-CO60720 (degradate of mesosulfuron-methyl) on exponentially growing of the green algae *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume). The study was designed to meet OECD criteria. Cultures of *Pseudokirchneriella subcapitata* (freshwater microalgae) with an initial cell density of 10 000 cells/mL were exposed in a chronic multi-generation limit test for 3 days under static exposure conditions to the nominal concentration of 10.0 mg pure metabolite (p.m.)/L in comparison to an untreated control and a solvent control. Six replicate vessels per test level and 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 alterations in algae cells that might influence

extinction measurements, such as unusual cell size, samples were examined under a microscope. The (0-72h)-E_rC_{50} for the metabolite BCS-CO60720 was determined to be > 10.0 mg p.m./L and the (0-72h)-NOE_rC was ≥ 10.0 mg p.m./L.

Materials and Methods:

Test item: BCS-CO60720; Origin batch No.: SES 10689-26-2; Batch code: BCS-CO60720-01-01; TOX-No.: 08551-00; Analysed purity: 98.3 % w/w; LIMS No.: 1008755; analytical certificate no.: AZ 16515.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentration of 10.0 mg pure metabolite (p.m.)/L in comparison to controls. The pH values ranged from 7.8 to 8.2 in the controls and the incubation temperature ranged from 21.3°C to 22.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7481 lux.
Quantitative amounts of BCS-CO60720 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: October 15, 2010 – May 11, 2011

Results:

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

Analytical findings:
The analytical findings of BCS-CO60720 in the treatment levels found on day 0 were 102 % of nominal. On day 3 analytical findings of 103 % of nominal were found. All results are based on nominal test concentrations of the metabolite. Detailed analytical results are presented in the following table:

Table CA 8.2.6.1-9: Concentrations of BCS-CO60720 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	Actual Concentration (mg BCS-CO60720/L)			
	1. Determination	2. Determination	Average	%
Control	<0.632	<0.632	<0.632	--
Solvent control	<0.632	<0.632	<0.632	--
10.0	10.21	10.19	10.20	102

Table CA 8.2.6.1- 10: Concentrations of BCS-CO60720 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	Actual Concentration (mg BCS-CO60720/L)			
	1. Determination	2. Determination	Average	%
Control	<0.632	<0.632	<0.632	--
Solvent control	<0.632	<0.632	<0.632	--
10.0	10.25	10.31	10.28	103

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

Table CA 8.2.6.1- 11: Effect of BCS-CO60720 on growth-inhibition of *Pseudokirchneriella subcapitata*

Nominal concentration [mg p.m./L]	Cell number after 72 h (mean) per mL	(0-72h) Average specific growth rates [day^{-1}]	Inhibition of average specific growth rate [%]
control	741 000	1.435	-
solvent control	733 000	1.432	
pooled controls	737 000	1.433	
10.0	728 000	1.429	0.3

* test initiation with 10 000 cells/mL

No morphological change in algae was observed in any test concentration.

Conclusions:

The influence of BCS-CO60720 on the growth of the green algae *Pseudokirchneriella subcapitata* can be quantified as a (0-72h)-E_rC_{50} of > 10.0 mg p.m./L and the (0-72h)-NOE_rC is ≥ 10.0 mg p.m./L.

BCS-CO60721

Report:	[redacted]; [redacted];2011;M-415112-01
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-CO60721 - limit test
Report No:	EBMML013
Document No:	M-415112-01-1
Guidelines:	**OECD Guideline 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006);none**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the influence of BCS-CO60721 (degradate of mesosulfuron-methyl) on exponentially growing of the green algae *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume). Cultures of *Pseudokirchneriella subcapitata* (freshwater microalgae) with an initial cell density of 10 000 cells/mL were exposed in a chronic multi-generation test for 3 days under static exposure conditions to the nominal concentration of 10.0 mg pure metabolite (p.m.)/L in comparison to an untreated control and a solvent control. Six replicate vessels per test level and 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, samples were examined under a microscope. The (0-72h)-E_rC_{50} for the metabolite BCS-CO60721 was determined to be > 10.0 mg p.m./L and the (0-72h)-NOE_rC was ≥ 10.0 mg p.m./L.

Materials and Methods:

Test item: BCS-CO60721; Origin batch No.: SES 10798-12-3; Batch code: BCS-CO60721-01-01; analysed purity: 95.1 % w/w; analytical certificate no.: AZ 16765.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure

conditions to the nominal concentration of 10.0 mg pure metabolite (p.m.)/L in comparison to controls. The pH values ranged from 7.8 to 8.3 in the controls and the incubation temperature ranged from 21.4°C to 22.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8260 lux.
Quantitative amounts of BCS-CO60721 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: April 01, 2011 – May 18, 2011

Results:

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

Analytical findings:
The analytical findings of BCS-CO60721 in the treatment levels found on day 0 were 104 % of nominal. On day 3 analytical findings of 103 % of nominal were found. All results are based on nominal test concentrations of the metabolite. Detailed analytical results are presented in the following table:

Table CA 8.2.6.1- 12: Concentrations of BCS-CO60721 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	Actual Concentration (mg BCS-CO60721/L)			
	1. Determination	2. Determination	Average	%
Control	<0.522	<0.522	<0.522	--
Solvent control	<0.522	<0.522	<0.522	--
10.0	10.4	10.4	10.4	104

Table CA 8.2.6.1- 13: Concentrations of BCS-CO60721 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	Actual Concentration (mg BCS-CO60721/L)			
	1. Determination	2. Determination	Average	%
Control	<0.522	<0.522	<0.522	--
Solvent control	<0.522	<0.522	<0.522	--
10.0	10.2	10.3	10.3	103

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

Table CA 8.2.6.1- 14: Effect of BCS-CO60721 on growth-inhibition of *Pseudokirchneriella subcapitata*

Nominal concentration [mg p.m./L]	Cell number after 72 h (mean) per mL	(0-72h) Average specific growth rates [day^{-1}]	Inhibition of average specific growth rate [%]
control	800 000	1.460	-
solvent control	802 000	1.462	-
pooled controls	801 000	1.461	-
10.0	820 000	1.469	-0.5

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

No morphological change in algae was observed in any test concentration.

Conclusions:

The influence of BCS-CO60721 on the growth of the green algae *Pseudokirchneriella subcapitata* can be quantified as a (0-72h)-E_rC_{50} of > 10.0 mg p.m./L and the (0-72h)-NOE_rC is ≥ 10.0 mg p.m./L.

CA 8.2.6.2 Effects on growth of an additional algal species

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

Studies on mesosulfuron-methyl

Report:	[redacted]8; [redacted]; [redacted]; [redacted]; 2000; M-187975-01
Title:	Algal growth inhibition - Navicula pelliculosa AE F130060 substance, technical code: AE F130060 00 1C9[redacted]0001
Report No:	C004457
Document No:	M-187975-01-1
Guidelines:	**EU (=EEC): 92/69/EWG C.3; OECD: 201; USEPA (=US EPA): § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Study endpoints according to Monograph evaluation (Point B.9.2.6.2.):

E_rC_{50} 96 h > 100 mg/l (nominal) or > 74.9 mg/l (mean measured).
$NOAE_rC$ 96 h = 100 mg/l
E_bC_{50} 96 h > 100 mg/l

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final).

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted]2000c, 8.2.6.2/1
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD guideline no 201 (1984) and EU directive 92/69 Annex Part C C.3.
- **GLP compliance**: yes.
- **Methods**: The toxicity of AE F130060 (technical substance, purity = 94.6%) to the diatom species *Navicula pelliculosa* was determined under static conditions over an exposure period of 96 h. The test was conducted in 300 ml flasks filled with 100 ml test water containing 0 (control), 10, 18, 32, 56 and 100 mg test substance/l. The cell density was 10^4 cells/ml at the start of the test. Each concentration was repeated three times, and control was repeated 6 times. Cell density was measured in 5 ml aliquots on every 24 h until the end of the test.
- **Results**: All treatments lead to higher cell densities in comparison with the untreated control. Analytical measurements showed recovery rates below 80% at the end of the test period: from 75.5% to 79.3%. Endpoints were then also expressed as corrected concentrations, using the overall recovery rate of 74.9% which corresponds to the lowest recovery rate that was recorded over the duration of the test.
  ErC50 96 h > 100mg/l (nominal) or > 74.9 mg/l (mean measured).
  NOAErC 96 h = 100 mg/l
  EbC50 96 h > 100 mg/l

- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary:

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

Analytical findings:
Analyses of freshly prepared water determined by chromatography for AE F130060 resulted in test substance concentrations ranging from 75.0% to 94.2% of nominal values. Analyses of aged water (96 h) at experimental termination resulted in test substance concentrations ranging from 74.8% to 79.3% of nominal values. The mean measured values over the time of exposure ranged from 74.9% to 84.7% of the nominal values. In only one case the measured concentration of the test substance was above the limit of 80% from the corresponding nominal concentration (84.7% for 56 mg/L). The measured concentrations of four test substance treatments concentrations were below 80% of the nominal treatment level.
Therefore the measured test substance concentrations were 7.6, 14.1, 25.1, 47.4 and 74.9 mg/L. These figures were obtained from mean recovery rates at each treatment level. Nominal and mean measured concentrations were used for reporting the results.

Biological findings:
Significant inhibition of growth (significance level of alpha = 0.05) was not observed in any of the nominal concentrations. All treatments lead to higher cell concentrations in comparison with the untreated control. The test substance seemed to promote the algal growth at nominal treatment levels of and above 32 mg/L during 96 hours.
Therefore, the no observed adverse effect concentration (NOAEC) defined as no significant growth inhibition and no cell deformation was nominal (mean measured) 100 mg/L (74.9 mg/L).

Table CA 8.2.6.2-1: Endpoints (EC_{50} values)

	After 72 hours		After 96 hours	
	E_bC_{50}	E_rC_{50}	E_bC_{50}	E_rC_{50}
EC_{50} [mg/L]	>100 (nominal) => 74.9 (mean measured)			

Conclusions:
In a Growth Inhibition Test (method EPA / OECD / EU) to determine the effect of AE F130060; substance, technical, Code: AE F130060 00 1595 0001 to *Navicula pelliculosa* (Bacillariophyceae) all treatments lead to higher cell concentrations in comparison with the untreated control.
No inhibition of growth regarding the areas under the growth curves (E_bC_{50}) and slopes of the growth curves (E_rC_{50}) were observed after 72 hours and 96 hours test duration.
Therefore, the no observed adverse effect concentration (NOAEC) defined as no significant growth inhibition and no cell deformation was nominal 100 mg/L corresponding to 74.9 mg dissolved test substance/L (mean measured).

Table CA 8.2.6.2- 2: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-187975-01-1 KCA 8.2.6.2 /01	OECD No. 201 (1984)	OECD No. 201 (2006, corr. 2011)	additional validity criteria	the study does not fulfill the new OECD validity criteria#2 and #3, but is nevertheless considered to provide reliable results suitable for risk assessment. (see explanative comment below)
	US EPA, J, § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	92/69/EWG, C.3	(not relevant)	N/A	N/A

Comment on validity criteria: Algae studies performed before 2006 were technically not designed to optimally address the additional validity criteria set up in the revision of the OECD 201 Guideline version in 2006 [mean coefficient of variation for section-by-section specific growth rates in control ≤35% (criterion 2) and coefficient of variation of average specific growth rates in replicate controls ≤7% (criterion 3)].

Nevertheless they still can be scientifically robust and valid. One important issue is the sectional growth rate. This criterion is heavily influenced by the time of algae cell number measurement. As before 2006 this criterion did not exist, the relevance of checking the cell counts daily at the same time was not given. This is one reason why studies performed before 2006 frequently fail this criterion. Nevertheless, as long as the algae in the controls are still growing and exposure is given during the experiment the data are still delivering robust endpoints. This is even more the case as the old studies in most cases have been based on biomass related endpoints which in general are lower compared to growth rate related figures. Therefore these values represent a worst case and should be acceptable for the risk assessment.

The new Aquatic guidance Document states as well that studies according to US guidelines can be used in the risk assessment. If this is the case, than old studies based on biomass values should be acceptable as well.

For the present case of mesosulfuron-methyl this is even more the case as the aquatic risk is clearly driven by aquatic macrophytes (Lemnaceae). This study clearly indicates that mesosulfuron-methyl has no effect on *Navicula pelliculosa*.

Report:	[redacted];[redacted];[redacted];[redacted];2001;M-238869-01
Title:	Effect to Anabaena flos-aquae (Blue-Green Alga) in a Growth Inhibition Test, AE F130060 Technical, 95.7% w/w
Report No:	B003222
Document No:	M-238869-01-1
Guidelines:	**OECD: 201; USEPA (=EPA): 132-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The toxicity of mesosulfuron-methyl (AE F130060) technical to the blue-green alga, *Anabaena flos-aquae*, was assessed in a static system over a 96 hour exposure period.

Triplicate algal cultures with an initial nominal cell count of approximately 1.0 x 10^4 cells/mL were exposed to the nominal concentration of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L of the test substance (corresponding to mean measured concentrations of 0.55, 1.1, 2.1, 4.25, and 8.6 mg/L of the test substance) in Algal Assay Procedure (AAP) media for a 96-hour period. Six replicate algal cultures were cultured without test substance as the control treatment. Cell density of each culture was counted

under a microscope at 48, 72, and 96 hours. Average specific growth rate and biomass were both calculated at each timepoint. Inhibition of growth was calculated relative to the control group. Water samples of each treatment at test initiation and at test termination were analysed by High Performance Liquid Chromatography (HPLC) with ultraviolet detection (UV) for quantification of AE F130060.
Based on analytical findings the biological endpoints are reported as nominal figures. The E_bC_{50} (biomass) values for 72 and 96 hours were calculated as 2.8 mg/L and 2.4 mg/L, respectively. The E_rC_{50} (growth rate) values for 72 and 96 hours were calculated as 5.6 mg/L and 4.1 mg/L. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC), baesd on biomass at 96 hours, were 1.0 mg/L and 2.0 mg/L, respectively.

Materials and Methods:

Test material: AE F130060, technical; Batch no.: PFL 35316; Code No.: AE F130060 00 1C95 0001; Sample number: ZBA806, CAS number: 208465-21-8, purity: 95.7% w/w; Certificate of analysis: AZ 08063.

Triplicate algal cultures with an initial nominal cell count of approximately 1.0 x 104 cells/mL were exposed to the nominal concentration of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L of the test substance in Algal Assay Procedure (AAP) media for a 96-hour period. Six replicate algal cultures were cultured without test substance as the control treatment. The cell density (cells/mL) of each culture was counted under a microscope using a hemacytometer at 48, 72, and 96 hours, average specific growth rate (rate of change in cell number with time) and biomass (the productivity of the culture determined as area under the growth curves) were both calculated at each timepoint. Inhibition of growth was calculated relative to the control group.
Water samples for chemical analysis of each treatment were taken at test initiation (0 hours) and at test termination (96 hours). At test initiation, samples were taken from the original parent stock solutions prior to the addition of alga. Samples at test termination were taken as composite samples and centrifuged at 1000 g for 10 minutes prior to analysis to remove alga and any undissolved particulates. All samples were analyzed by High Performance Liquid Chromatography (HPLC) with ultraviolet detection (UV) for quantification of AE F130060.
Test methodology was in agreement with OECD 201 and USEPA 123-2 guidelines.

Dates of experimental work: June 12, 2000 to June 16, 2000

Results:

Analytical findings:
The method efficiency from fortified study media with AE F130060 had a mean percent recovery of 111% (SD= 12.3%). The mean measured test concentrations for AE F130060 were 0.55, 1.1, 2.1, 4.25, and 8.6 mg/L (105 to 112% of nominal). There were no residues of AE F130060 in the dilution water and control samples greater than the limit of quantitation (0.5 mg/L).
The measured concentration of AE F130060 indicated that the nominal concentration was achieved at test initiation and remained stable throughout the study. All toxicity values were calculated based on the nominal concentrations. Detailed analytical results are presented in the following table:

Table CA 8.2.6.2- 1: Nominal and measured concentrations of AE F130060

Sample identification	nominal concentration (mg a.s./L)	measured concentrations (mg a.s./L)			
		day 0	day 4	mean	combined percent of nominal
Dilution water	0	<LOQ[a]	<LOQ[a]	<LOQ[a]	<LOQ[a]
Control	0	<LOQ[a]	<LOQ[a]	<LOQ[a]	<LOQ[a]
0.5	0.5	0.5	0.6	0.6	120
1.0	1.0	1.0	1.2	1.1	110
2.0	2.0	1.9	2.3	2.1	105
4.0	4.0	3.8	4.7	4.3	108
8.0	8.0	7.8	9.4	8.6	110

a LOQ = Limit of Quantitation (0.5 mg/L)

Biological findings:

The control algal growth increased by a factor of at least 16 from test initiation to 96 hours. At 72 and 96 hours, the control group had average cell densities of 8.57 x 10^4 and 2.34 x 10^5 cells/mL, respectively. The nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L had a 72 hour average cell densities of 8.67 x 10^4, 8.37 x 10^4, 6.4 x 10^4, 3.6 x 10^4, and 2.3 x 10^4 cells/mL, respectively. The nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L had a 96 hour average cell densities of 3.08 x 10^5, 2.37 x 10^5, 1.29 x 10^5, 5.3 x 10^4, and 2 x 10^4 cells/mL, respectively. Inhibition of biomass at 96 hours, relative to the control, ranged from 0 to 89%. Inhibition of specific growth rate at 96 hours, relative to the control, ranged from 0 to 77%.

The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) are presented in the following table:

Table CA 8.2.6.2- 2: NOEC and LOEC of AE F130060 at different times of observation

Time (Hours)	Specific Growth Rate (μ)		Biomass – Area Under Curve (A)	
	NOEC (mg/L)	LOEC (mg/L)	NOEC (mg/L)	LOEC (mg/L)
48	1.0	2.0	1.0	2.0
72	2.0	4.0	1.0	2.0
96	1.0	2.0	1.0	2.0

The E_rC_{50} (Specific Growth Rate) and E_bC_{50} (Area under the Curve) are presented in the following table:

Table CA 8.2.6.2- 3: Effect concentrations of AE F130060 at different times of observation

Time (Hours)	EC_{50} Method	E_rC_{50} (95% CL) (mg/L)	E_bC_{50} (95% CL) (mg/L)
48	Nonlinear Regression	3.1 (2.2 to 4.4)	2.4 (1.5 to 3.8)
72	Nonlinear Regression	5.6 (4.8 to 6.6)	2.8 (2.1 to 3.7)
96	Nonlinear Regression	4.1 (3.6 to 4.6)	2.4 (1.8 to 3.1)

Conclusion:

The E_bC_{50} (biomass) values for 72 and 96 hours, as determined by nonlinear regression, were calculated as 2.8 mg/L (95% CL = 2.1 to 3.7 mg/L) and 2.4 mg/L (95% CL = 1.8 to 3.1 mg/L), respectively. The E_rC_{50} (growth rate) values for 72 and 96 hours, as determined by nonlinear

regression, were calculated as 5.6 mg/L (95% CL = 4.8 to 6.6 mg/L) and 4.1 mg/L (95% CL = 3.6 to 4.6 mg/L), respectively. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC), based on biomass (area under curve) at 96 hours, were 1.0 mg/L and 2.0 mg/L, respectively.

Report:	[redacted].;[redacted];[redacted];2001;M-238809-01
Title:	Effect to Skeletonema costatum (Marine Diatom) in a Growth Inhibition Test AE F130060 Technical 95.7% w/w
Report No:	B003156
Document No(s):	M-238809-01-1
Guidelines:	**OECD: 201; USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to determine the toxicity of mesosulfuron-methyl (AE F130060) technical to the marine diatom, *Skeletonema costatum* in a static system over a 96 hour exposure period.
Triplicate diatom cultures with an initial nominal cell count of approximately 1.0 x 10^4 cells/mL were exposed to the nominal concentration of 13, 22, 36, 60, and 100 mg/L of the test substance in Marine Algal Assay (MAA) media for a 96-hour period (corresponding to mean measured concentrations of 12.25, 22.55, 37.25, 62.25, and 106.2 mg a.s./L). In addition six replicate cultures were tested as untreated control. At 24-hour intervals, the cell density of each culture was counted. Average specific growth rate and biomass were counted at each timepoint. Inhibition of growth was calculated relative to the control group. Based on analytical findings the biological endpoints are reported as nominal figures. The E_bC_{50} (values for 72 and 96 hours were calculated as 82 mg/L and 93 mg/L, respectively. The E_rC_{50} (growth rate) values for 72 and 96 hours were both calculated as > 100 mg/L. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC), based on biomass (area under curve) at 96 hours, were 36 mg/L and 60 mg/L, respectively.

Materials and Methods:

Test material: AE F130060, technical; Batch No.: Pfl. 35316; Code No.: AE F130060 00 1C95 0001; Sample No.: ZBA806; CAS No.: 208465-21-8, Purity: 95.7% w/w.

Triplicate diatom cultures (*Skeletonema costatum*) with an initial nominal cell count of approximately 1.0 x 10^4 cells/mL were exposed to the nominal concentration of 13, 22, 36, 60, and 100 mg/L of AE F130060 in Marine Algal Assay (MAA) for a 96-hour period. Six replicate diatom cultures were cultured without test substance as the control treatment. Each vessel (Erlenmeyer flasks; 250 mL) served as one replicate filled with 100 mL MAA (Marine Algal Assay) nutrient medium. Cell density determinations were made on each flask at study initiation, 24, 48, 72, and 96 hours during the study. Average specific growth rate (rate of change in cell number with time) and biomass (the productivity of the culture determined as area under the growth curves) were both calculated at each timepoint. Inhibition of growth was calculated relative to the control group.
Water samples for chemical analysis of each treatment were taken at test initiation (0 hours) and at test termination (96 hours). At test initiation, samples were taken from the original parent stock solutions prior to the addition of diatoms. Samples at test termination were taken as composite samples and centrifuged at 1000 g for 10 minutes prior to analysis to remove diatoms and any undissolved particulates. All samples were analyzed by High Performance Liquid Chromatography (HPLC) with

ultraviolet detection (UV) for quantification of AE F130060. Test methodology was in agreement with OECD 201 and USEPA 123-2 guidelines.

Dates of experimental work: June 5, 2000 to June 9, 2000

Results:

Analytical findings:

The method efficiency from fortified study media with AE F130060 had a mean percent recovery of 105% (SD = 7.8%). The mean measured test concentrations for AE F130060 were 12.25, 22.55, 37.25, 62.25, and 106.2 mg/L (94 to 106% of nominal). There were no residues of AE F130060 in the dilution water and control samples greater than the limit of quantitation (5.0 mg/L).

The measured concentration of AE F130060 indicated that the nominal concentration was achieved at test initiation and remained stable throughout the study. All toxicity values were calculated based on the nominal concentrations. Detailed analytical results are presented in the following table:

Table CA 8.2.6.2- 4: Analytically measured concentrations.

Nominal concentration (mg a.s./L)	Measured concentrations (mg a.s./L)			
	05JUN00 (Fresh)	09JUN00 (Old)	Mean	Combined percent of nominal
Control	< LOQ	< LOQ	< LOQ	--
13	12.8	11.7	12.25	94%
22	23	22.1	22.55	103%
36	38	37.5	37.75	105%
60	62.2	62.3	62.25	104%
100	105.7	106.6	106.15	106%

LOQ = Limit of Quantitation (5.0 mg/L)

Biological findings:

Diatom cell numbers in the control increased by a factor of at least 16 from test initiation to 72 hours. At 72 and 96 hours, the control group had average cell densities of 5.34 x 10^5 and 7.31 x 10^5 cells/mL, respectively. The nominal concentrations of 13, 22, 36, 60, and 100 mg/L had a 72 hour average cell densities of 5.48 x 10^5, 5.74 x 10^5, 5.65 x 10^5, 4.23 x 10^5, and 1.94 x 10^5 cells/mL, respectively. Inhibition of biomass at 72 hours, relative to the control, ranged from 0 to 63%. Inhibition of specific growth rate at 72 hours, relative to the control, ranged from 0 to 25%. The nominal concentrations of 13, 22, 36, 60, and 100 mg/L had a 96 hour average cell densities of 7.26 x 10^5, 7.33 x 10^5, 7.66 x 10^5, 7.23 x 10^5, and 4.47 x 10^5 cells/mL, respectively. Inhibition of biomass at 96 hours, relative to the control, ranged from 0 to 55%. Inhibition of specific growth rate at 96 hours, relative to the control, ranged from 0 to 12%.

The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) are presented in the following table:

Table CA 8.2.6.2- 5: NOEC and LOEC at different times of observation

Time (hours)	Specific growth rate (μ)		Biomass - Area under the curve	
	NOEC (mg a.s./L)	LOEC (mg a.s./L)	NOEC (mg a.s./L)	LOEC (mg a.s./L)
24	36	60	36	60
48	36	60	36	60
72	60	100	36	60
96	60	100	36	60

The E_rC_{50} (Specific Growth Rate) and E_bC_{50} (Area under the curve) are presented in the following table:

Table CA 8.2.6.2- 6: Effect concentrations at different times of observation

Time (hours)	EC_{50} method	E_rC_{50} (95 % CL) (mg a.s./L)	E_bC_{50} (95 % CL) (mg a.s./L)
24	Nonlinear regression	78 (68 to 90)	66 (54 to 81)
48	Nonlinear regression	> 100	78 (69 to 88)
72	Nonlinear regression	> 100	82 (75 to 91)
96	Nonlinear regression	> 100	93 (86 to 100)

Conclusion:

The E_bC_{50} (biomass) values for 72 and 96 hours, as determined by nonlinear regression, were calculated as 82 mg/L (95% CL = 75 to 91 mg/L) and 93 mg/L (95% CL = 86 to 100 mg/L), respectively. The E_rC_{50} (growth rate) values for 72 and 96 hours, as determined by nonlinear regression, were both calculated as > 100 mg/L (95% CL = unable to determine). The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC), based on biomass (area under curve) at 96 hours, were 36 mg/L and 60 mg/L, respectively.

CA 8.2.7 Effects on aquatic macrophytes

For mesosulfuron-methyl, toxicity studies on aquatic macrophytes clearly indicated vascular plant to represent the overall most sensitive group of aquatic organisms. To provide a thorough description of susceptibility in terms of species sensitivity distribution, and of the influence of temporal aspects of the exposure, a number of specifically designed higher tier studies on aquatic macrophytes were performed to complement the results of the standard static exposure laboratory test on *Lemna gibba*.

The following tests on active substance will be described in the following:

Standard test:

KCA 8.2.7 /01: Standard Lemna growth inhibition test with 7 days exposure time

Investigations on the influence of exposure duration & recovery potential:

KCA 8.2.7 /06: Lemna laboratory growth inhibition & recovery test with 4 days exposure phase followed by 7 days recovery phase

KCA 8.2.7 /07: Lemna laboratory growth inhibition & recovery test with 7 days exposure phase followed by 7 days recovery phase

Investigations on species sensitivity distribution:

KCA 8.2.7 /08:	8 week multispecies outdoor growth inhibition test in pond systems, 9 species
KCA 8.2.7 /09:	8 week Lemna multigeneration laboratory test, mimicking the exposure situation in the pond systems of study KCA 8.2.7 /08

Moreover, laboratory studies investigating the toxicity to *Lemna gibba* were performed for all metabolites of the residue definition for risk assessment in surface water. Even though Lemna represents the most sensitive aquatic macrophyte species for mesosulfuron-methyl, effect of all metabolites was found orders of magnitude lower than of parent substance, or fully absent.

In addition, tests on *Lemna* were conducted on two components BCS-CO60720 and BCS-CO60721, supportive to a discussion of artifact findings in a water/sediment study (cf. document MCA Section 7.2.2.3). Also these components were found devoid of notable effect on Lemna.

Details of all studies are provided in the following table.

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

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Mesosulfuron-methyl				
Lemna gibba (duck weed)	growth inhibition, static	7 d	E_rC_{50} 1) >0.0010 NOEC 0.00018	[redacted] 2000 M-195390-01-1 KCA 8.2.7 /01
Lemna gibba (duck weed)	growth inhibition + recovery	4 d + 7 d	exposure phase: 4 d E_rC_{50} 1) 0.00199 (nom) 4 d E_rC_{50} 1) 0.00151 (mm) 4 d NOEC 0.00025 (mm) recovery phase: 7 d E_rC_{50} 1) > 0.0032 (nom) 7 d E_rC_{50} 1) > 0.0038 (mm) 7 d NOEC 0.00044 (mm)	[redacted], 2001 M-201731-01-1 KCA 8.2.7 /06
Lemna gibba (duck weed)	growth inhibition + recovery	7 d + 7 d	exposure phase 7 d E_rC_{50} 1) 0.00208 (nom) 7 d E_rC_{50} 1) 0.00172 (mm) 7 d NOEC 0.00077 (mm) last 4 days of recovery phase 7 d E_rC_{50} 1) > 0.010 (nom) 7 d E_rC_{50} 1) > 0.00941 (mm) 7 d NOEC 0.00141 (mm)	[redacted], 2002 M-206814-01-1 KCA 8.2.7 /07
Aquatic macrophytes (9 species) [*Lemna* bioassay not considered; replaced by 8-week Lemna study; see below]	outdoor growth inhibition, static	8 weeks	ErC50 (dry weight) *Elodea canadensis* **0.0038** *Potamogeton pectinatus* **0.0071** *Pontederia cordata* **0.0021** *Nymphaea odorata* **>0.025** *Cabomba caroliniana* **>0.025** *Cerat. demersum* **0.0053** *Glyceria maxima* **>0.025** *Mentha aquatica* **0.012** *Myriophyll.heterophyllum* **0.022**	[redacted], 2009 M-329474-01-1 KCA 8.2.7 /08
Lemna gibba (duck weed)	growth inhibition, mimicking exposure of outdoor study	8 weeks	7-day endpoints $E_rC_{50frondnumber}$ 1) 0.00161 (nom) $E_rC_{50frondarea}$ 1) **0.00129** (nom) NOEC 0.00039 (nom) 8-week endpoints based on initial nominal concentrations $E_rC_{50frondnumber}$ 1) **0.00190 (nom)** $E_rC_{50frondarea}$ 1) 0.00210 (nom) NOEC 0.00039 (nom)	[redacted], 2013 M-445139-01-1 KCA 8.2.7 /09
Lemna gibba (duck weed)	statement; rationale for the replacement of the old 7-day *Lemna* growth inhibition study ([redacted] 2000; M-195390-01-1) with the 7-day endpoints from the *Lemna* study ([redacted] 2013; M-445139-01-1)		$E_rC_{50frondarea}$ **0.00129** (nom)	[redacted], 2014 M-487405-01-1 KCA 8.2.7 /10
Lemna gibba (duck weed)	growth inhibition, leachate water of Lysimeter	7 d	no inhibitory effects observed	[redacted], 2000 M-197850-01-1 KCA 8.2.7 /02

Test species	Test system	Test duration	Endpoint [mg as/L]		Reference
	study, semi-static				
AE F154851					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOE_rC	**0.11** 0.03	Dorherloh, 2005 M-255283-01-1 KCA 8.2.7 /11
AE F160459					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOEC	**2.6** 0.56	[redacted], 2000 M-198076-01-1 KCA 8.2.7 /03
AE F099095					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOE_rC	**> 100** < 100	[redacted], 2005 M-254496-01-1 KCA 8.2.7 /12
AE F092944					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOEC	**> 100** 100	[redacted], 2000 M-186916-01-1 KCA 8.2.7 /13
AE F160460					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOEC	**> 100** 100	[redacted], 2000 M-199266-01-1 KCA 8.2.7 /04
AE F140584					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOE_rC	**> 10.0** ≥ 10.0	[redacted], 2014 M-486658-01-1 KCA 8.2.7 /14
AE F147447					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOEC	**> 100** 100	[redacted], 2000 M-198273-01-1 KCA 8.2.7 /05
BCS-CO60720					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOE_rC	**> 11.8** ≥ 11.8	[redacted], 2013 M-449110-01-1 KCA 8.2.7 /15
BCS-CO60721					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1)] NOE_rC	**> 10.0** ≥ 10.0	[redacted], 2013 M-445154-01-1 KCA 8.2.7 /16

Bold letters: Values considered relevant for risk assessment in the MCP document
mm = mean measured, nom = nominal
[1)] Since the new aquatic GD focusses on endpoints based on growth rates the old E_bC_{50} figures were omitted from the table above.

Studies on mesosulfuron-methyl

Report:	[redacted] p; [redacted]; [redacted];2000;M-195390-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F130060 substance, technical, 95.3 % Code: AE F130060 00 1C95 0001
Report No:	C007190
Document No:	M-195390-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): J § 123-2; Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

$$E_bC_{50} = 0.00062 \text{ mg/L}$$

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, this study endpoint should be revised to

$$E_rC_{50} > 0.001 \text{ mg/L}$$

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000a, 8.2.8.1/1

- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD draft guideline for testing of chemicals on *Lemna gibba*, growth inhibition test (April 1997) and ASTM "Standard guide for conducting static toxicity test with *Lemna gibba*" G3 1415-91 (1991).

- **GLP compliance**: yes.

- **Methods**: The effects of AE F130060 (technical substance, purity = 95.3%) on the growth of the duckweed *Lemna gibba* was determined under renewal conditions. Plants were exposed to the active substance in 300 ml flasks filled with 150 ml of test water, that contained 0 (control), 0.1, 0.18, 0.32, 0.56 or 1.0 microg test substance/l. A total of 12 fronds (3-5 plants) were allocated per flask. Each concentration and the control were repeated three times. Test water was renewed on days 3 and 5 before data checks. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological sign was also recorded.

- **Results**: A significant inhibition of both growth rate and biomass production was observed at the end of the test for concentrations of 0.32 microg/l and above. Mesosulfuron-methyl concentrations in fresh test water could not be measured in the control (spiked) and 0.32 microg/l samples at day-5, because of strong interference of the media on the analysis that could not be explained. Therefore, mean recovery in control (recovery of spiked samples) and 0.32 microg/l was re-calculated from the mean of days 0 and 3 recovery. With regard to the high recovery that was observed during the whole test duration and at any concentration, this estimation of data may not have introduce any bias in the results.
  Based on nominal concentrations:
  E_rC_{50} 7 days > 1.0 microg/l
  NOE_rC 7 days = 0.18 microg/l
  E_bC_{50} 7 days = 0.62 microg/l (95% CI=[0.56 – 1.0]microg/l.

- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary :

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

Analytical findings:

Analyses of freshly prepared water for AE F130060 resulted in test substance concentrations ranging from 112.5 to 124.6% of mean nominal values. Analyses of aged water for AE F130060 at experimental termination resulted in test substance concentrations ranging from 93.1 to 114.1% of mean nominal values. Therefore, nominal values were used for reporting the results.

The test results for fresh water are partly above the range of 80-120% of the nominal concentration although the variability is < 1.5 - due to recalculation with a recovery slightly above or below 90% (but still in the accetable range of 80 -120%) for which no reasoning is available.

For the control sample (and thus for the spiked control sample for recovery evaluation) as well as for the concentration level of 320 µg/L on day 5 no results are available due to strong interferences which cannot be explained. Therefore, the mean recovery of days 0 and 3 were used for recalculation of the results of day 5.

Biological findings:

Mean values of absolute and percentual growth inhibition in comparison to the solvent control are presented in the following table:

Table CA 8.2.7- 2: Mean values of absolute and percentual growth inhibition compared to the solvent control

Treatment level [µg/L]	Mean growth rate [d^{-1}]	Percentual inhibiton of growth rate	Mean increase in biomass [mg]	Percentual inhibition of biomass increase
control	0.376	0.00	15.7	0.00
0.10	0.376	0.14	15.5	1.27
0.18	0.373	0.91	15.2	3.18
0.32	0.324	14.02	11.0	30.08
0.56	0.286	23.90	8.2	48.09
1.0	0.219	41.77	6.5	58.47

The level of 50% growth inhibition regarding frond number (E_bC_{50}) after 7 days was calculated as 0.62 µg test substance /L (95% confidence limits 0.56 - 1.0 µg/L). The level of 50% growth inhibition regarding biomass (E_rC_{50}) after 7 days was calculated above 1.0 µg test substance /L.

A significant inhibition of growth related on frond number was observed in nominal concentrations of 0.32 µg/L and above. A significant inhibition of biomass increase (dry weight) was observed in nominal concentrations of 0.32 µg/L and above.

Since intoxication symptoms (yellow coloured fronds) were observed at concentrations of and above nominal 0.32 ug/L, the no observed effect concentration (NOEC) defined as no significant growth inhibition and no changes in plant appearance and development was set to nominal 0.18 µg/L.

Table CA 8.2.7- 3: Endpoints (EC$_{50}$ values)

	E_bC_{50}	E_rC_{50}
EC-values after 7 days in µg/L (nominal)	0.62	> 1.0
95% confidence limits in µg/L, high	0.56	-
95% confidence limits in µg/L, low	1.0	-

EC$_{50}$ calculations are based on the binomial regression method.

Conclusions:

In a Growth Inhibition Test (method EPA / OECD / ASTM) to determine the effect of AE F130060; substance, technical, 95.3%, Code: AE F130060 00 1C95 0001 to *Lemna gibba* (Duckweed) the concentration of test substance inhibiting the growth (µ, frond numbers) and the resulting E_bC_{50} in comparison with the untreated control after 7 days test duration was nominal 0.62 µg/L

(95% confidence limits 0.56 - 1.0 µg/L). The E_rC_{50} was nominal >1.0 µg/L and the NOEC nominal 0.18 µg/L.

Table CA 8.2.7- 4: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-195390-01-1 KCA 8.2.7 /01	US EPA, J, § 123-2 (1982)	(not EU relevant)	N/A	N/A
	US EPA, OPPTS 850.4400 (1996)	(not EU relevant)	N/A	N/A
	ASTM, E 1415-91 (1991)	(not EU relevant)	N/A	N/A
	OECD Draft, *Lemna* growth inhibition (1997)	OECD 221, *Lemna* growth inhibition (2006)	none	no deviation from current guideline

Since the study dosing regime did not allow for the derivation of a definite numeric result for E_rC_{50} (only a 'greater than' figure) and comparable growth inhibition data is available from several further 7-day exposure tests on *Lemna gibba*, it is proposed to use these information as a basis for a definite number E_rC_{50} value to be included in the updated list of endpoints (E_rC_{50} = 0.00129 mg/L). A review of the data available, and a reasoned proposal is presented in KCA 8.2.7/10 below.

Report:	[redacted];[redacted];2001;M-201731-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test with recovery phase AE F130060 substance, pure Code: AE F130060 00 1B98 0002
Report No:	C016099
Document No:	M-201731-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft 1998; USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to determine the effects of the test item AE F130060 (mesosulfuron-methyl) on growth inhibition of *Lemna gibba* in a 4-day exposure test with subsequent 7-day recovery period 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. 0.32, 0.56, 1.0, 1.8 and 3.2 µg/L). During the treatment phase growth and abnormal appearance of fronds were determined on test days 3 and 4. At day 4 the test continued with untreated nutrient solutions (recovery phase). Growth and abnormal appearance of fronds were determined at days 7, 9 and 11. Three replicates were tested per treatment level. One control replicate was omitted from the evaluations of the recovery phase since symptoms were observed at these plants.

While intoxication syndromes were observed during the exposure phase and at the beginning of the recovery phase, at the end of the recovery phase fronds recovered from all intoxication symptoms.

Mean measured levels of 50% growth inhibition during the seven-day recovery phase after the four day exposure period were calculated as follows:

7 d E_rC_{50} > 3.8 µg/L
7 d E_bC_{50} > 3.8 µg/L
7 d NOEC 0.44 µg/L

Material and methods:

Test item: AE F130060; Code: AE F130060 00 1B98 0002; Analysed purity: 98.1 % w/w; Certificate No.: AZ 07726.

Lemna cultures with an initial frond number of 12 fronds per replicate were exposed to the test item in 20X-AAP medium at five nominal treatment levels (i.e. 0.32, 0.56, 1.0, 1.8 and 3.2 ug/L). During the treatment phase growth and abnormal appearance of fronds were determined on test days 3 and 4. At day 4 the test continued with untreated nutrient solutions (recovery phase). Again, growth and abnormal appearance of fronds were determined at days 7, 9 and 11. Three replicates were tested per treatment level. One control replicate was omitted from the evaluations of the recovery phase since symptoms were observed at these plants.
Chemical analyses were conducted on day 0 and 3 from fresh water and on day 3 and 4 from aged water from all tested concentration by chromatographic determination. The limit of detection (LOD) was 0.008 µg/L, the limit of quantification (LOQ) was 0.014 µg/L.

Dates of experimental work: August 17, 2001 – September 25, 2001

Results:

Analytical findings:
Analyses of freshly prepared water for AE F130060 resulted in concentrations ranging from 88.0% to 136.3% of nominal values at day 0 and between 72.8 % and 109.4% at day 3. Analyses of aged water for AE F130060 at day 3 resulted in concentrations ranging from 58.7% to 99.3% of nominal values. Analyses from aged water at day 4 were regarded as not reliable and were omitted from further evaluations. The mean measured concentrations between day 0 and day 3 ranged between 77.0 and 118.7%. A summary of the analytical findings are presented in the following table:

Table CA 8.2.7- 5: Overall survey of analytical results as % of nominal

Nominal conc.	0.32 µg/L	0.56 µg/L	1.0 µg/L	1.8 µg/L	3.2 µg/L
Day 0 fresh water	92.6	101.1	88.0	93.3	136.3
Day 3 aged water	65.4	58.7	90.3	81.8	99.3
Mean day 0 - 3	**79.0**	**79.9**	**89.2**	**87.6**	**117.8**
Day 3 fresh water	73.3	72.8	92.8	95.5	109.4

Biological findings:

Inhibitory effects during the exposure and recovery phase were observed as follows:

Table CA 8.2.7- 6: Mean values of absolute and percentual growth inhibition of AE F130060 compared to the control during the treatment phase

Treatment level (µg/L)	Mean growth rate (d^{-1})	Percentual inhibition of growth rate	Mean increase in biomass (mg)	Percentual inhibition of biomass increase
untreated control	0.36970	0.00	7.022	0.00
0.32	0.36569	1.08	7.254	-3.29
0.56	0.33032	10.65	7.143	-1.71
1.0	0.27636	25.25	7.193	-2.43
1.8	0.17669	52.21	4.632	34.04
3.2	0.13584	63.26	4.933	29.76

Table CA 8.2.7- 7: Mean values of absolute and percentual growth inhibition of AE F130060 compared to the control during the recovery phase

Treatment level (µg/L)	Mean growth rate (d^{-1})	Percentual inhibition of growth rate	Mean increase in biomass (mg)	Percentual inhibition of biomass increase
untreated control	0.35777	0.00	23.23500	0.00
0.32	0.34957	2.29	24.62000	-5.96
0.56	0.33682	5.86	22.07333	5.00
1.0	0.32743	8.48	20.62333	11.24
1.8	0.32751	8.46	18.63000	19.82
3.2	0.32447	9.31	18.42000	20.72

A significant inhibition of growth related on frond number during the four-day treatment phase at a significance level of alpha = 0.05 was observed at all treatment levels except nominal 0.32 µg/L (mean measured 0.25 µg/L).

Due to the high variability of individual data no significant inhibition of growth related on biomass during the four-day treatment phase at a significance level of alpha = 0.05 was observed at any treatment levels up to nominal 3.2 µg/L (mean measured 3.8 µg/L).

No significant inhibition of growth related on frond number during the 7 day-recovery phase at a significance level of alpha = 0.05 was observed at all treatment levels up to nominal 0.56 µg/L (mean measured 0.44 µg/L).

No significant inhibition of growth related on biomass during the 7 day-recovery phase at a significance level of alpha = 0.05 was observed at any treatment levels up to nominal 3.2 µg/L (mean measured 3.8 µg/L).

Non-separating fronds were observed in all replicates from the two highest treatment levels at day 3 and day 4 but not in the consecutive recovery phase. Vaulted fronds were observed at the highest treatment level during the treatment phase and at the first assessment during the recovery phase (day 7). Moreover, fronds had nearly no root at day 7 in all replicates of the highest treatment level. All these intoxication symptoms had disappeared at assessments of day 9 and 11, which refer to day 5 and day 7 of the recovery phase. Thus, up to 3.2 µg/L fronds recovered from all intoxication symptoms acquired during a four-day treatment phase.

Conclusions:

Nominal levels of 50% growth inhibition during the four-day treatment phase were calculated as follows:

4 d E_rC_{50}	1.989 µg/L	(95% confidence limits 1.513 - 3.188 µg/L)
4 d E_bC_{50}	> 3.2 µg/L	
4 d NOEC	0.32 µg/L	

Mean measured levels of 50% growth inhibition during the four-day treatment phase were calculated as follows:

4 d E_rC_{50}	1.510 µg/L	(95% confidence limits 0.890 - 1.580 µg/L)
4 d E_bC_{50}	> 3.8 µg/L	
4 d NOEC	0.25 µg/L	

Nominal levels of 50% growth inhibition during the seven-day recovery phase were calculated as follows:

7 d E_rC_{50}	> 3.2 µg/L
7 d E_bC_{50}	> 3.2 µg/L
7 d NOEC	0.56 µg/L

Mean measured levels of 50% growth inhibition during the seven-day recovery phase were calculated as follows:

7 d E_rC_{50}	> 3.8 µg/L
7 d E_bC_{50}	> 3.8 µg/L
7 d NOEC	0.44 µg/L

Report:	[redacted];[redacted];[redacted];2002;M-206814-01
Title:	Duckweed (Lemna gibba G3) - Growth inhibition test with recovery phase AE F130060 substance, pure Code: AE F130060 00 1B98 0002
Report No:	C018852
Document No:	M-206814-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): E § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to determine the effects of the test item AE F130060 (mesosulfuron-methyl) on growth inhibition of *Lemna gibba* in a 7-day exposure test with subsequent 7-day recovery period 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. *Lemna* cultures were exposed to the test substance in 20X-AAP medium at five nominal treatment levels (i.e. 1.0, 1.8, 3.2, 5.6 and 10 µg/L). During the treatment phase six replicates were involved in which growth and abnormal appearance of fronds were determined on test days 3, 5 and 7. At day 7 the test continued with three replicates but with untreated nutrient solutions (recovery phase). Again, growth and abnormal appearance of fronds were determined at days 10, 12 and 14.
Vaulted and yellow coloured fronds were observed as severe intoxication symptoms. Moreover, slight changes in plant appearance like spread fronds, small fronds and shortened roots were observed.
At treatment levels of and above 1.8 µg/L the growth was obviously inhibited during the first three days of the recovery phase, only. During the period between day 3 and day 7 of the recovery phase the growth curves obviously run more or less parallel to the control. This is likely to be linked to the observed occurrence of the test item during the first days of the recovery phase. Therefore, the evaluation of data obtained during the recovery phase were separately analysed for the whole seven-

day recovery phase and the last four days of the recovery phase. For the last four days of the seven-day static renewal recovery phase the following endpoints were observed:

Figures based on time-weighted average concentrations during:

7 d E_rC_{50}	> 9.41 µg/L	
7 d NOEC	9.41 µg/L	regarding growth rate (frond number)
7 d NOEC	1.41 µg/L	regarding severe intoxication symptoms

Material and methods:

Test item: AE F130060 (mesosulfuron-methyl); Code: AE F130060 00 1B98 0002; Analysed purity: 98.1 % w/w; Certificate No.: AZ 07726.

Lemna cultures with an initial frond number of 12 fronds per replicate were exposed to the test item in 20X-AAP medium at five nominal treatment levels (i.e. 1.0, 1.8, 3.2, 5.6 and 10 µg/L). Additionally an untreated control was tested. During the treatment phase six replicates were involved in which growth and abnormal appearance of fronds were determined on test days 3, 5 and 7. At day 7 the test continued with three replicates but with untreated nutrient solutions (recovery phase). Again, growth and abnormal appearance of fronds were determined at days 10, 12 and 14.
Chemical analyses were conducted on day 0, 3 and 5 from fresh water and on day 3, 5 and 7 of aged water from all tested concentration by chromatographic determination. Additional chemical analyses were conducted on day 10 and 12 of aged water of the untreated control, the concentration levels 5.6 mg/L and 10 mg/L by chromatographic determination of AE F130060. The limit of detection (LOD) was 0.05 µg/L for the treatment phase and 0.15 µg/L for the recovery phase, the limit of quantification (LOQ) was 0.08 µg/L for the treatment phase and 0.25 µg/L for the recovery phase.

Dates of experimental work: January 11, 2002 – January 27, 2002

Results:

Analytical findings:
Analyses of freshly prepared water for AE F130060 resulted in the means of the measured concentrations ranging from 82.5% to 93.3% of nominal values. The variability was < 1.5% at all treatment levels. Analyses of aged water for AE F130060 resulted in the means of the measured concentrations ranging from 69.2% to 103.3% of nominal values. Time-weighted average concentrations for 1.0, 1.8, 3.2, 5.6 and 10 µg/L were 77.36%, 78.42%, 95.71%, 90.36% and 94.06% of nominal, respectively. Since time-weighted average concentrations were below 80% of nominal at the two lowest treatment levels the biological results were based on time-weighted average concentrations.
Since plants were transferred from treated test solutions to untreated nutrient medium at start of the recovery phase, samples from the two highest treatment levels were analysed for AE F130060. Aged water from day 10 (day 3 of the recovery phase) contained 10.1% and 4.8% of the respective nominal concentrations of the foregoing treatment phase (5.6 and 10 µg/L, respectively). Aged water from day 12 (day 5 of the recovery phase) contained no test item anymore. Therefore, no further analytical measurements have been conducted during the recovery phase.

Biological findings:
Vaulted and/or yellow coloured fronds were observed in concentrations of and above 1.0 ug/L.

A significant inhibition at a significance level of alpha = 0.05 of growth both related on frond number and of biomass increase (dry weight) was observed at concentrations of and above nominal 1.0 µg/L (time-weighted average 0.77 µg/L) after 7 days test duration. Inhibitory effects during the exposure phase were observed as follows:

Table CA 8.2.7- 8: Mean values of absolute and percentual growth inhibition of AE F130060 compared to the control during the treatment phase

Treatment level (µg/L)	Mean growth rate (d^{-1})	Percentual inhibition of growth rate	Mean increase in biomass (mg)	Percentual inhibition of biomass increase
Control	0.394	0.00	26.70	0.00
1.0	0.298	24.43	18.87	29.34
1.8	0.212	46.22	14.03	47.44
3.2	0.154	61.00	12.13	54.56
5.6	0.130	66.96	10.20	61.80
10	0.108	72.65	9.43	64.67

At treatment levels of and above 1.8 µg/L the growth was obviously inhibited during the first three days of the recovery phase, only. During the period between day 3 and day 7 of the recovery phase the growth curves obviously run more or less parallel to the control. This is likely to be linked to the observed occurrence of the test item during the first days of the recovery phase. Therefore, the evaluation of data obtained during the recovery phase were separately analysed for the whole seven-day recovery phase and the last four days of the recovery phase.

During the whole recovery phase (test day 7 to 14) a significant inhibition at a significance level of alpha = 0.05 of growth both related to frond number and of biomass increase (dry weight) was observed at concentrations of and above nominal 1.8 µg/L. During day 10 to 14 (the last four days of the recovery phase) a significant inhibition at a significance level of alpha = 0.05 of growth related to frond number increase was observed at concentrations of and above nominal 10 µg/L / time-weighted average 9.41 µg/L.

Vaulted and yellow coloured fronds were observed as severe intoxication symptoms. Moreover, slight changes in plant appearance like spread fronds, small fronds and shortened roots were observed.

Inhibitory effects during the recovery phase were observed as follows:

Table CA 8.2.7- 9: Mean values of absolute and percentual growth inhibition of AE F130060 compared to the control during the recovery phase

Treatment level (µg/L)	Mean growth rate (d^{-1})	Percentual inhibition of growth rate	Mean growth rate (d^{-1})	Percentual inhibition of growth rate	Mean increase in biomass (mg)	Percentual inhibition of biomass increase in biomass Day 7 to 14
	Day 7 to 14		Day 10 to 14		Day 7 to 14	
Control	0.38007	0.00	0.33117	0.00	23.0	0.00
1.0	0.39664	-4.36	0.35325	-6.67	22.6	1.74
1.8	0.34250	9.88	0.39191	-18.34	17.0	26.09
3.2	0.34558	9.07	0.36161	-9.19	18.7	18.70
5.6	0.35528	6.52	0.36800	-11.12	20.3	11.74
10	0.31242	17.80	0.38237	-15.46	15.1	34.35

Conclusions:

The following endpoints were obtained:

Nominal figures during the seven-day static renewal treatment phase:

7 d E_rC_{50}	2.084 µg/L	(95% confidence limits 1.8 - 3.2 µg/L)
7 d E_bC_{50}	2.213 µg/L	(95% confidence limits 1.8 - 3.2 µg/L)
7 d NOEC	< 1.0 µg/L	regarding growth rate (frond number)
7 d NOEC	< 1.0 µg/L	regarding growth rate (biomass)
7 d NOEC	< 1.0 µg/L	regarding intoxication symptoms

Figures based on time-weighted average concentrations during the seven-day static renewal treatment phase:

7 d E_rC_{50}	1.717 µg/L	(95% confidence limits 1.41 - 3.06 µg/L)
7 d E_bC_{50}	1.863 µg/L	(95% confidence limits 1.41 - 3.06 µg/L)
7 d NOEC	< 0.77 µg/L	regarding growth rate (frond number)
7 d NOEC	< 0.77 µg/L	regarding growth rate (biomass)
7 d NOEC	< 0.77 µg/L	regarding intoxication symptoms

During the first three days of the recovery period slight effects were observed. Therefore, the recovery-endpoints were assessed after this initial recovery phase:

Nominal figures during the last four days of the seven-day static renewal recovery phase:

7 d E_rC_{50}	> 10.0 µg/L	
7 d E_bC_{50}	> 10.0 µg/L	
7 d NOEC	10.0 µg/L	regarding growth rate (frond number)
7 d NOEC	1.8 µg/L	regarding severe intoxication symptoms
7 d NOEC	1.0 µg/L	regarding slight changes in plant appearance

Figures based on time-weighted average concentrations during the last four days of the seven-day static renewal recovery phase:

7 d E_rC_{50}	> 9.41 µg/L	
7 d E_bC_{50}	> 9.41 µg/L	
7 d NOEC	9.41 µg/L	regarding growth rate (frond number)
7 d NOEC	1.41 µg/L	regarding severe intoxication symptoms
7 d NOEC	< 0.77 µg/L	regarding slight changes in plant appearance

Report:	[redacted] a; [redacted];2009;M-329474-01
Title:	Outdoor growth inhibition of aquatic plants exposed to Mesosulfuron-methyl
Report No:	13798.6220
Document No:	M-329474-01-1
Guidelines:	**special design;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The objective of this study was to evaluate the toxicity of mesosulfuron-methyl to nine aquatic plant species cultivated in outdoor pond systems under natural atmospheric conditions, over a study period of 8 weeks. The plants were placed in the ponds for a 2 to 4 week acclimation period prior to exposure to the test substance. Nominal test concentrations were 0.40, 0.78, 1.6, 3.1, 6.3, 13 and 25 µg a.s./L, and were confirmed by analytical measurement: 0.45, 0.82, 1.9, 3.1, 6.7, 14, 25 µg a.s./L (arithmetic mean values of 3-4 replicate ponds per test concentration). During the test period, a continuous dissipation of the test substance was observed in the ponds, as to be expected for mesosulfuron-methyl in a static water body.

In addition to the nine species cultivated in the ponds, the sensitivity of the duckweed, *Lemna gibba*, was evaluated during weeks 0, 2 and 7 in an environmental chamber, by exposing the plants for 7 days to nutrient enriched water samples taken from treated and control ponds. For biological reason, Lemna could not be grown directly in the pond systems. Results of this test reflect Lemna response to the actual pond water exposure situation in a particular test week, however are not equivalent endpoints to those of the species grown over the full 8-week period in the ponds.

Results are reported as the percent reduction in growth (mean shoot length, mean dry shoot weight, and the respective growth rates) of plants exposed to the test substance as compared with growth of solvent controls. For *Lemna gibba*, results are expressed as the percent reduction in growth (frond density, frond dry weight), and the respective growth rates. Visual observations for symptoms of toxicity, when present, were also made. Results are based on nominal concentrations; EC_{10}, EC_{25} and EC_{50} values and the No-Observed-Effect-Concentration (NOEC) were determined for each endpoint for each species tested. For tests with *Lemna gibba* conducted in the laboratory, the biological endpoints assessed for effects were 7-day frond numbers (density), frond dry weight biomass and growth rate based on frond density and dry weight.

Key endpoints of the study, expressed as lowest ErC_{50} (8-week) based on shoot length or dry weight for the nine species maintained in the ponds:

Elodea canadensis	0.0038 mg/L
Potamogeton pectinatus	0.0071 mg/L
Pontederia cordata	0.0021 mg/L
Nymphaea odorata	>0.025 mg/L
Cabomba caroliniana	>0.025 mg/L
Cerat. demersum	0.0053 mg/L
Glyceria maxima	>0.025 mg/L
Mentha aquatica	0.012 mg/L
Myriophyll.heterophyllum	0.022 mg/L

Material and methods:

Test item: Mesosulfuron-methyl; Synonym: AE F130060, technical; Batch No.: EFME000042; CAS No.: 208465-21-8; Analysed purity: 98.1% w/w.

Test species: Monocotyledon: Water weed (*Elodea canadensis*), Sago pondweed (*Stuckenia pectinata*, formerly *Potamogeton pectinatus*), Reed sweetgrass (*Glyceria maxima*), Pickerel weed (*Pontederia cordata*), duckweed (*Lemna gibba*). Dicotyledon: Water lily (*Nymphaea odorata*), Coontail weed (*Ceratophyllum demersum*), Variable milfoil (*Myriophyllum heterophyllum*), Water mint (*Mentha aquatica*), Fanwort (*Cabomba caroliniana*).
Thirty-one, square, 3000-L, outdoor, freshwater ponds (inside dimensions 230 cm x 230 cm x 60 cm deep) containing a 5 cm layer of sandy loam soil to serve as sediment were used. The percent sand:silt:clay of the soil was determined to be 65:24:11%, respectively, the percent organic matter was 9.4% and the pH was 4.3. Each pond was filled with approximately 1850 L (35 cm depth) of unchlorinated well water and fortified in hardness to approximately 180 mg/L as $CaCO_3$. The ponds received full sunlight throughout the day. Covers were temporarily installed over the ponds when heavy rain was forecast, in order to prevent major dilution of the test solutions.

Plants were placed in the ponds for a 2 to 4 week acclimation period prior to exposure to the test substance. After the acclimation phase the ponds were dosed with the test substance at nominal concentrations of 0.40, 0.78, 1.6, 3.1, 6.3, 13 and 25 µg a.s./L in addition with a solvent control (18.5 mL of acetone in 1850 L of pond water in each solvent control pond). Six solvent control replicates were established. Four replicates were established for the four lowest concentrations (0.40, 0.78, 1.6 and 3.1 µg a.s./L), while three replicates were established for the three highest concentrations (6.3, 13 and 25 µg a.s./L).
During the exposure, health observations were performed on emergent and, as far as possible, on submerged plants during weeks 2, 4, 6 and 8. The test was terminated after eight weeks of exposure. Plants were harvested and mean shoot length and mean shoot dry weight were determined for each species separately. Endpoints were calculated as reduction of mean shoot length and mean shoot dry weight compared to the control and as reduction of the respective growth rates.
Additionally, the sensitivity of the duckweed, *Lemna gibba*, was evaluated during weeks 0, 2 and 7, by exposing the plants in an environmental chamber for 7 days to nutrient enriched samples from treated and control ponds. The culture medium used was sterile 20X Algal Assay Procedure (AAP) medium adjusted to pH 7.5 ± 0.1. The biological endpoints assessed were 7-day frond numbers (density), frond dry weight biomass and growth rate based on frond density and dry weight.
Analytical samples of each pond were taken at test initiation and on day 14, day 28 and test termination (day 54). HPLC was used as analytical method.

Dates of experimental work (including dry weight determination):

June 4, 2008 – August 21, 2008 (outdoor exposure of nine aquatic plants)
June 5, 2008 – June 16, 2008 (*Lemna gibba* Test 1)
June 19, 2008 – July 2, 2008 (*Lemna gibba* Test 2)
July 24, 2008 – August 4, 2008 (*Lemna gibba* Test 3)

Results:

Environmental conditions:
The environmental conditions maintained throughout the test period were within acceptable limits for the growth and survival of the test species. Rainfall during the exposure period was 9.0 cm for June 2008 and 12.4 cm for July 2008. Approximately 6.4 cm of rainfall was prevented from entering the ponds on 24 to 25 July 2008 since the ponds were covered. The remaining rainfall entering the ponds generally replenished water evaporated during the study.

Analytical results:
The day 0 measured concentrations closely approximated the desired nominal concentrations indicating the ponds were dosed as intended. In all ponds, concentrations of mesosulfuron-methyl slowly decreased over the 8-week exposure period. Endpoints, however, are given in terms of nominal concentrations, since they will be used in risk assessments in combination with initial or maximum PEC-figures. Details of analytical measurements are presented in the following table:

Table CA 8.2.7- 10: Concentrations of mesosulfuron-methyl measured in the pond water at test start and three timepoints over the eight week test period

Nominal Concentration (µg a.s./L)		Measured Concentration (µg a.s./L)			
		Day 0	Day 14	Day 28	Day 54
Solvent control[a]		<0.10	<0.021	<0.021	<0.019
0.40	A	0.46	0.18	0.41	<0.019
	B	0.43	0.20	0.34	<0.019
	C	0.47	0.23	0.46	<0.019
	D	0.44	0.15	0.29	<0.019
	Mean	**0.45**	**0.19**	**0.38**	**0.0093[b]**
	SD	**0.020**	**0.033**	**0.072**	**NA**
0.78	A	0.79	0.48	0.52	0.47
	B	0.87	0.39	0.63	0.41
	C	0.84	0.47	0.55	0.48
	D	0.78	0.43	0.55	0.45
	Mean	**0.82**	**0.44**	**0.56**	**0.45**
	SD	**0.042**	**0.038**	**0.049**	**0.031**
1.6	A	2.1	1.2	1.1	0.78
	B	2.0	1.2	1.1	0.86
	C	1.7	1.1	1.0	0.75
	D	1.8	1.1	1.1	0.74
	Mean	**1.9**	**1.1**	**1.1**	**0.78**
	SD	**0.18**	**0.076**	**0.018**	**0.053**
3.1	A	3.2	2.5	1.9	1.3
	B	3.1	2.5	2.0	1.4
	C	3.0	2.4	1.9	1.4
	D	3.3	2.2	2.2	1.4
	Mean	**3.1**	**2.4**	**2.0**	**1.4**
	SD	**0.12**	**0.13**	**0.14**	**0.054**
6.3	A	6.4	4.4	3.5	2.5
	B	7.2	4.9	4.0	2.4
	C	6.6	4.6	3.5	2.4
	Mean	**6.7**	**4.6**	**3.6**	**2.4**
	SD	**0.41**	**0.23**	**0.28**	**0.038**
13	A	15	10	8.0	4.8
	B	14	9.4	7.9	4.9
	C	14	11	7.6	5.3
	Mean	**14**	**10**	**7.8**	**5.0**
	SD	**0.71**	**0.58**	**0.23**	**0.25**
25	A	24	18	14	8.5
	B	25	18	15	8.3
	C	25	18	15	9.2
	Mean	**25**	**18**	**15**	**8.6**
	SD	**0.50**	**0.52**	**0.93**	**0.48**

[a] Composite sample of all replicates

[b] When the measured concentration decreased to below the limit of quantitation (LOQ), one-half of the LOQ value was used in the calculation of the mean measured concentration.

NA = Not Applicable.

SD = Standard Deviation

Biological results:

The nine species grown in the outdoor ponds showed a wide range of sensitivity to mesosulfuron-methyl. While *Glyceria maxima* and *Cabomba carolinea* were not affected even at 25 µg a.s./L (based

on nominal concentrations), the lowest EC_{50} of 2.1 µg a.s./L (based on nominal concentrations) were obtained for growth rate of mean shoot length in case of *Ponteteria cordata*. The lowest NOEC of 0.78 µg a.s./L (based on nominal concentrations; analytically measured: 0.82 µg/L) were achieved for *Potamogeton pectinatus*.
EC_{50} values and the NOEC for the different response variables and each species tested are summarized in the following table.

Table CA 8.2.7- 11: Summary of NOEC and EC_{50} values based on nominal concentrations, for nine aquatic macrophytes exposed to mesosulfuron-methyl in outdoor ponds for eight weeks.

Exposure Period	Based on Nominal Concentrations (µg a.s./L)							
	Week 8 Mean Shoot Length		Week 8 Growth Rate Based on Mean Shoot Length		Week 8 Mean Shoot Dry Weight		Week 8 Growth Rate Based on Dry Weight	
	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Elodea canadensis	NC[a]	NC	NC	NC	1.6	4.6 (1.5-15)	1.6	3.8 (1.3-5.8)
Potamogeton pectinatus	1.6	>25 (NA[b])	1.6	18 (13-23)	0.78	8.3 (5.3-13)	0.78	7.1 (4.7-11)
Pontederia cordata	1.6	>3.1 (NA)	1.6	2.1 (1.5-3.0)	1.6	2.7 (2.1-3.4)	1.6	2.6 (1.9-3.0)
Nymphaea odorata	25[c]	>25 (NA)	25[c]	>25 (NA)	25	>25 (NA)	25	>25 (NA)
Cabomba caroliniana	25	>25 (NA)	25	>25 (NA)	25	>25 (NA)	25	>25 (NA)
Ceratophyllum demersum	6.3	>25 (NA)	6.3	5.3 (2.8-9.7	25	>25 (NA)	ND[d]	ND
Glyceria maxima	25	>25 (NA)	25	>25 (NA)	25	>25 (NA)	25	>25 (NA)
Mentha aquatica	13	20 (12-25)	13	12 (3.0-18)	25	16 (2.4-24)	25	15 (2.1-24)
Myriophyllum heterophyllum	25	>25 (NA)	25	>25 (NA)	25	>25 (NA)	25	22 (18-25)

[a] NC = Not calculated. Due to the constant branching observed and the fact that stems could not be associated with a unique plant, plant lengths were not measured for E. canadensis.
[b] NA = Not applicable
[c] Mean shoot length for water lily may not be a good indicator of sensitivity since mean shoot length is limited by the water column depth.
[d] ND = Not determined. Test termination plant dry weights were less than the initial weight, resulting in negative growth rates for the solvent control and each treatment; therefore, the EC and NOEC values were not calculated for shoot dry weight growth rates.

The following observations concerning plant health were made at test termination: *Elodea canadensis* plants exposed to the 6.3, 13 and 25 µg a.s./L treatments were stunted, exhibited shortened branches and shortened and necrotic leaves. *Potamogeton pectinatus* plants exposed to ≥0.78 µg a.s./L were generally chlorotic or necrotic. *Glyceria maxima* plants in all treatments and the solvent control were noted as healthy. *Pontederia cordata* plants exposed to replicates A and B of the 6.3 µg a.s./L treatment and all plants exposed to the 13 and 25 µg a.s./L treatments were dead. Surviving plants exposed to 3.1 and 6.3 µg a.s./L were generally necrotic at test termination. For *Nymphaea odorata* replicate B plants exposed to the 1.6 µg a.s./L treatment had not emerged, while in replicate D, one plant did not emerge and one plant died during the exposure. *Mentha aquatica* plants exposed to

higher concentrations of mesosulfuron-methyl were more fragile and contained less biomass then the solvent control. Branching was observed to be stunted and the leaves were smaller then solvent control leaves. The solvent control and the lower treatment levels of the *Cabomba caroliniana* replicates were observed to have less biomass than the higher treatment levels and may be attributable to greater competition for space and nutrients at the lower treatment levels.

The results from the three seven-day *Lemna*-bioassays are not reported in this summary for the following reasons:

1. The 7-day tests in nutrient-enriched pond water samples do not provide any new information of effects of mesosulfuron on *Lemna* compared to standard laboratory studies.
2. In order to obtain a *Lemna*-endpoint which is directly comparable to the endpoints from this outdoor study a 8-week *Lemna* study was performed in the laboratory under sterile conditions while mimicking the dissipation of mesosulfuron as observed in the pond study (see **KCA 8.2.7/09** below).

Conclusions:

Seven of the nine aquatic macrophytes tested indicated sensitivity in reduced mean shoot length, dry weight, growth rates, or morphological abnormalities to mesosulfuron-methyl over the range of tested concentrations, 0.4 to 25 µg a.s./L. EC_{50} and NOEC values for all species and response variables were reported in Table CA 8.2.7- 11 above. Based on the lowest EC_{50} value, 2.1 µg a.s./L for growth rate based on mean shoot length, pickerel weed (*Pontederia cordata*) was the most sensitive species tested. In general, the health and survival of the control plants for each species indicated the exposures 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]*; [redacted];2013;M-445139-01
Title:	Lemna gibba G3 - Prolonged growth inhibition test with mesosulfuron-methyl (AE F130060) with stepwise decreasing concentrations over an 8 week test duration
Report No:	EBMML017
Document No:	M-445139-01-1
Guidelines:	**OECD - 221 (March 23, 2006);none**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the long-term influence over a total period of eight weeks of mesosulfuron-methyl (AE F130060) 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 study is intended to complement the dataset of a multispecies growth inhibition study in outdoor ponds (KCA 8.2.7/08; [redacted] 2009; M-329474-01-1) by the generation of a corresponding 8-week growth inhibition endpoint for *Lemna*. This species could not be grown directly in the pond systems for biological reason.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for eight times 7 days under static exposure conditions, starting at nominal initial test item concentrations of 0.194, 0.388, 0.755, 1.55, and 3.1 µg/L (100 % levels, week 1). These concentrations were stepwise decreased week after week of the test period, targeting levels of 84.1% (week 2), 70.7% (week 3), 63.2% (week 4), 56.5% (week 5), 50.2% (week 6), 44.7% (week 7), and

39.7% (week 8), to mimick under laboratory conditions the dissipation of mesosulfuron-methyl observed in the outdoor pond systems of study KCA 8.2.7/08. The *Lemna* plants were transferred into new test solutions of the next concentration level every 7 days, using 12 fronds from the respective treatment level of the preceding week.

Table CA 8.2.7- 12: Key endpoints of the study, expressed as E_rC_{50} (8-week) for *Lemna gibba*:

	mean growth rate	
	effect on frond no. [µg a.s./L]	effect on total frond area of plants [µg a.s./L]
E_rC_{50} (8 week)	1.90	2.10

Material and methods:

Test item: Mesosulfuron-methyl (AE F130060), substance technical; Batch code: AE F130060-01-2; Batch No.: EFME000144; Specification No.: 102000013204; Tox No.: 08878-00; Analysed purity: 97.4% w/w; Certificate No.: AZ16385.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for eight times 7 days under static exposure conditions to the nominal concentrations listed in Table CA 8.2.7- 13. These concentration profiles were derived from the analytical results of an outdoor pond-study ([redacted] 2009; M-329474-01-1). *Lemna gibba* could not be tested under outdoor-conditions.
The objective of this study is to obtain 8-week endpoints for *Lemna gibba* by mimicking the outdoor-concentration curves under laboratory conditions.
After each week preferably 12 fronds were transferred into the respective following concentration (e.g. fronds from the samples of 3.10 µg/L, the highest concentration of week 1, were transferred into the replicates of 2.61 µg/L, the highest concentration of week 2, fronds from the test concentration of 0.194 µg/L, the lowest concentration of week 1, were transferred into the replicates of 0.163 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.
Samples were analysed for the actual concentration of mesosulfuron-methyl present in all freshly prepared test levels at the start day and in all aged test levels after 7 days of the exposure period. Samples were analysed with HPLC-MS/MS.

Table CA 8.2.7- 13: Summary of test concentrations and experimental conditions

Nominal **initial** test levels mesosulfuron-methyl [µg /L]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
% of week 1	100	84.1	70.7	63.2	56.5	50.2	44.7	39.7
0.194	0.194	0.163	0.137	0.122	0.109	0.0970	0.0870	0.0770
0.388	0.388	0.326	0.274	0.245	0.219	0.195	0.173	0.154
0.775	0.775	0.652	0.548	0.490	0.438	0.389	0.346	0.308
1.55	1.55	1.30	1.10	0.979	0.875	0.778	0.692	0.616
3.10	3.10	2.61	2.19	1.96	1.75	1.56	1.38	1.23
pH	7.5 – 8.7	7.5 – 8.7	7.5 – 8.7	7.5 – 8.7	7.5 – 8.7	7.5 – 8.7	7.5 – 8.7	7.5 – 8.7
Temperature range	23.3 – 23.8°C	23.3 – 24.1°C	23.3 – 23.9°C	23.8 – 24.4°C	24.1 – 24.4°C	23.8 – 24.4°C	23.9 – 24.3°C	24.0 – 24.1°C
Light intensity (lux)	8610	8000	9080	8760	8760	8430	8090	8320

Dates of experimental work: October 22, 2010 – February 03, 2011

Results:

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

Analytical results:
Analytical results are summarized in the following table.

Table CA 8.2.7- 14: Analytical findings

	Day 0	**Day 7**
Week 1	68 and 95% (average 79%)	79 and 116% (average 93%)
Week 2	92 and 98% (average 95%),	94 and 102% (average 100%)
Week 3	92 and 96% (average 94%)	96 and 108% (average 100%)
Week 4	96 and 104% (average 99%)	101 and 110% (average 104%)
Week 5	93 and 97% (average 96%)	103 and 108% (average 105%)
Week 6	94 and 101% (average 98%)	97 and 115% (average 103%)
Week 7	89 and 95% (average 92%)	93 and 107% (average 101%)
Week 8	91 and 101% (average 96%)	95 and 102% (average 100%)

According to the objective of this study the endpoints were referred to nominal initial test concentrations and not to weekly treatment levels.

Biological results:
Growth rate effects of the test item on *Lemna gibba* are presented in the following tables.

Table CA 8.2.7- 15: Weekly inhibition with regard to the mean growth rates of frond numbers

Nominal **initial** test levels	% inhibition of mean growth rate of **frond numbers**							
Mesosulfuron-methyl [µg /L]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	**Week 8**
% of week 1	100	84.1	70.7	63.2	56.5	50.2	44.7	39.7
Control	--	--	--	--	--	--	--	--
0.194	0.4	-0.6	-6.3	3.0	8.8	1.4	1.5	2.6
0.388	2.1	1.1	-1.1	5.5	5.9	-1.0	3.1	3.3
0.775	32.7	15.1	11.7	8.6	12.4	3.9	3.5	10.3
1.55	51.1	65.7	43.2	53.7	39.2	27.5	21.2	10.8
3.10	67.5	74.8	89.5	97.2	101	100	99.0	102
NOEC	0.388	0.775	0.388	0.388	<0.194	0.775	0.775	**0.388**
EC_{10}	0.376	0.538	0.811	0.829	1.05	1.38	1.39	**1.54**
EC_{50}	1.61	1.42	1.65	1.47	1.68	1.72	1.85	**1.90**

Table CA 8.2.7- 16: Weekly inhibition with regard to the mean growth rates of frond areas

Nominal **initial** test levels	% inhibition of mean growth rate of **frond area**							
Mesosulfuron-methyl [µg /L]	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	**Week 8**
% of week 1	100	84.1	70.7	63.2	56.5	50.2	44.7	39.7
Control	--	--	--	--	--	--	--	--
0.194	0.7	0.1	-3.4	-1.6	3.3	-1.6	0.6	1.5
0.388	-1.5	-0.7	-2.2	1.8	2.3	1.3	0.2	5.4
0.775	35.9	16.4	7.8	5.1	4.8	2.8	1.0	5.8
1.55	58.1	74.3	77.2	64.0	15.0	21.5	16.7	10.5
3.10	80.0	88.1	95.7	97.7	101	97.5	98.9	94.6
NOEC	0.388	0.388	0.388	0.388	0.388	0.775	0.775	**0.775**
EC_{10}	0.402	0.652	0.806	0.869	1.50	1.37	1.45	**1.54**
EC_{50}	1.29	1.19	1.22	1.368	1.78	1.89	1.90	**2.10**

Lemna exposed to initial nominal concentrations of 0.194 and 0.388 µg/L (and lower concentrations in the consecutive weeks) were inhibited to less than 10% during the whole study period of eight weeks. *Lemna* exposed to the initial nominal concentration of 0.775 µg/L were inhibited by 32.7% and 35.9% with regard to frond number and frond area, respectively. In the following weeks the inhibition percentages steadily dropped to 10.3% and 5.8% during the last week of the test. *Lemna* exposed to the initial nominal concentration of 1.55 µg/L were inhibited by 51.1% and 58.1% with regard to frond number and frond area, respectively. In the first following weeks the inhibition percentages increased to a maximum of 77.2% (frond area in week 3), to then decrease again to levels of 10.8% and 10.5% during the last week of the test. *Lemna* exposed to the initial nominal concentration of 3.1 µg/L were inhibited by 67.5% and 80.0% with regard to frond number and frond area, respectively. In the following weeks the inhibition percentages increased to figures greater than 95% from week 4 onwards. No recovery was observed at this highest treatment level.

Table CA 8.2.7- 17: Observed visual effects on *Lemna gibba*

nominal **initial** test levels mesosulfuron-methyl [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
0.194	-	-	-	-	-	-	-	-
0.388	-	-	1	-	-	-	-	-
0.775	1, 2,4	4	-	-	-	3	-	-
1.55	1, 3	4, 5	6	7, 10, 11	12	3	3	3
3.10	1	1	7, 8	7, 10	7, 13, 14, 15	15, 16(A+B), 17(C), 18	3, 15	3, 15

1. Overlapping fronds
2. Plants with 2 and 3 fronds
3. Small fronds
4. Thin ligaments between fronds
5. Fronds mostly of similar size, some individual fronds extremely small
6. Tiny narrow fronds with ligaments connected to big fronds
7. Big yellowish fronds
8. Small fronds of a deep green colour
9. Connections between fronds
10. Detached curled roots
11. Small fronds with elongated ligaments
12. Come curly roots
13. No new root growth
14. Roots brownish
15. Isolated fronds
16. Nearly no green fronds
17. Some green fronds
18. Brownish plant parts at the bottom of the test vessel

Conclusions:

The effects of mesosulfuron-methyl (AE F130060) to growth inhibition of *Lemna gibba* during a 8-week period simulating a steady dissipation in a static water body can be quantified by the following endpoints based on nominal initial concentrations:

Table CA 8.2.7- 18: EC_{50} for mean growth based on nominal initial concentrations

Endpoint	**Time period**	**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%)	week 1 0-7 d	1.61 (1.06 – 2.91)	1.29 (0.866 – 1.98)
EC_{50} (CI 95%)	week 2 7-14 d	1.42 (0.718 – 3.02)	1.19 (0.912 – 1.52)
EC_{50} (CI 95%)	week 3 14-21 d	1.65 (1.49 – 1.82)	1.22 (1.12 – 1.31)
EC_{50} (CI 95%)	week 4 21-28 d	1.47 (1.27 – 1.69)	1.37 (1.31 – 1.42)
EC_{50} (CI 95%)	week 5 28-35 d	1.68 (n.d.)	1.78 (n.d.)
EC_{50} (CI 95%)	week 6 35-42 d	1.72 (n.d.)	1.89 (1.78 – 2.11)
EC_{50} (CI 95%)	week 7 42-49 d	1.85 (1.68 – 16174)	1.90 (1.84 – 2.01)
EC_{50} (CI 95%)	**week 8 49-56 d**	**1.90 (n.d.)**	**2.10 (1.77 – 2.73)**

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

Report:	[redacted]g; [redacted];2014;M-487405-01
Title:	Mesosulfuron-methyl rationale for the replacement of the old 7-day Lemna growth inhibition study ([redacted]2000; M-195390-01-1) with the 7-day endpoints from the Lemna study ([redacted] 2013; M-445139-01-1)
Report No:	M-487405-01-1
Document No:	M-487405-01-1
Guidelines:	**not specified;not specified**
GLP/GEP:	**n.a.**

Executive Summary:

The inhibition on **7-day growth rate-figures** has been studied within three Lemna-tests with mesosulfuron-methyl a.i (see Table CA 8.2.7-16). The first one is a 7-day study conducted in 2000 by [redacted] according the OECD draft guideline (June 1998). Frond numbers and frond biomass was assessed during the study. While growth rate inhibition was calculated from frond numbers (N), the effects on biomass were simply evaluated from biomass increase (Δb) between day 0 and day 7.

The EbC50 calculated from biomass inhibition percentages was 0.62 µg a.i./L and has been listed in the Review Report for mesosulfuron-methy (SANCO/10298/2003-Final) as lowest Lemna-endpoint. Nowadays a 7-day ErC50 based on growth rate inhibition is used for risk assessments. The ErC50 for frond number was > 1.0 µg a.i./L in that study.

Table CA 8.2.7- 19: Survey of E_rC_{50}-figures obtained from static *Lemna*-growth inhibition tests conducted with mesosulfuron-methyl a.i.

Test system	Duration of exposure	Results (µg a.i./L)		Reference
growth inhibition	7 d	E_rC_{50} (frond number):	1.0	[redacted] (2000); M-195390-01-1
growth inhibition during the exposure phase of a recovery study	7 d	E_rC_{50} (frond number):	1.717	[redacted](2002); M-206814-01-1
growth inhibition, mimicking exposure of outdoor study	7 d	E_rC_{50} (frond number) **E_rC_{50} (frond area)**	1.61 **1.29**	[redacted], 2013 M-445139-01-1
	8 weeks	E_rC_{50} (frond number) E_rC_{50} (frond area)	1.90 2.10	

The new *Lemna* study ([redacted] 2013) shall replace the study by [redacted](2000) to derive a definite value for ErC50 (7 day) suitable for risk assessment purposes, for the following reasons:

1. In the new study two endpoints, frond number and frond area, were measured.
2. The new study has been conducted on the currently valid guideline OECD 221 (2006).
3. Within a risk assessment, sensitivities of different plant species are compared. As their growth, the test durations, and the test designs are different, a comparison of sensitivities only makes sense when growth rate related endpoints are used. This is reflected in the current

versions of the OECD guidelines for algae and *Lemna*, stating that the growth rate related endpoints are preferred.

Consequently the Review Report endpoint of 0.62 µg/L, based on frond biomass (data of study KCA 8.2.7/01) shall be replaced by the lowest 7-day ErC50 definitive value of the studies available which is 1.29 µg a.i./L based on frond area (data of study KCA 8.2.7/09).

Report:	[redacted]0; [redacted];2000;M-197850-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test. Leachate water from the lysimeterstudy Covance Muenster 1490-001 with AE F130060 Code: AE F130060
Report No:	C008847
Document No:	M-197850-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA: EPA J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

The results of this study were not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). A study evaluation is however available in the Monograph (B.9.2.8.6); it was concluded that leachates collected from the lysimeter exerted no significant herbicidal activity.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted]2000a, A.2.8.2.2/1.
- **Test guideline**: US EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD draft guideline for testing of chemicals on *Lemna gibba*, growth inhibition test (April 1997) and ASTM "Standard guide for conducting static toxicity test with *Lemna gibba*" G1415-91 (1991).
- **GLP compliance**: yes.
- **Method**: The effects of leachate water from the lysimeter study Covance [redacted] 1490-001 (study no 7.1.1.3/01) on the growth of the duckweed *Lemna gibba* was determined under renewal conditions. In the leaching study, the active substance (as a WP10 formulation, mixed with mefenpyr-diethyl as an EW10 formulation, ratio of ^{14}C AE F130060 and AE F107892 of 1:3 w/w) was applied twice on the lysimeters, in spring, at 12 months interval. The active substance was applied at the recommended field rate (15 g/ha). Analyses that were performed from the leachates revealed that AE F130060 was below the limit of detection (0.003 to 0.009 microg/l a.s. or equivalent). Two metabolites, M1 and M2, slightly exceeded 0.1 microg/l. The majority of the not identified radioactivity was of very polar nature. Leachates were pooled in 4 samples in order to constitute monthly or half year samples. Two samples of both type were constituted. Plants were exposed to the test substance (leachates) in 300 ml flasks filled with 150 ml of test water, that contained 0 or 87.5% (v/v) of test substance. A total of 10 fronds (3-5 plants) were allocated per flask. Each concentration and the control were repeated three times. Test water was renewed on days 3 and 5, before data checks. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological state was also recorded.
- **Results**: *Lemna* growth was promoted significantly in each treatment compared to the control, of 1.75 and 3.33% in the monthly samples and of 4.75 and 5.05% in the half year samples (growth rate). According to biomass, growth was promoted of 4.99 and 8.21% in the monthly samples and of 10.85 and 12.02% in the half year samples. This phenomenon was explained by the presence of unidentified compounds in leachates. It was concluded that leachates exerted no significant herbicidal activity.
- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary :

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

Analytical findings:
Since the leachates contained non-identified radioactive residues no chemical analysis of the test solutions was performed.
Although the freshly prepared test water was adjusted to pH 7.5 ± 0.1 there was a deviation to pH 7.4 to 9.0 in the aged test water.

Biological findings:
Mean values of absolute and percentual growth inhibition in comparison to the untreated control are presented in the following table:

Table CA 8.2.7- 20: Mean values of absolute and percentual growth inhibition compared to the control

Treatment group	Mean growth rate [d^{-1}]	Percentual inhibition of growth rate	Mean increase in Biomass [mg]	Percentual inhibition of biomass increase
Control	0.398682	0.00	20.73333	0.00
Treatment 1 (monthly)	0.39377	-1.75	23.86667	-4.99
Treatment 2 (half-year)	0.440520	-4.75	23.20000	-10.85
Treatment 3 (monthly)	0.39973	-3.34	24.60000	-8.21
Treatment 4 (half-year)	0.420637	-5.05	25.46666	-12.02

Probably due to unknown ingredients in the leachates *Lemna* growth was promoted significantly in each treatment compared to the control.
Inhibition according to growth rate was -1.75% and -3.34% in the monthly samples and -4.75% and -5.05% in the half-year samples. Inhibition according to biomass was -4.99% and -8.21% in the monthly samples and -10.85% and -12.02% in the half year samples.

Conclusions:
A Growth Inhibition Test (method EPPO/OECD / ASTM) was performed under semi-static conditions in order to determine the effect of leachate water from the Lysimeter study Covance Muenster 1490-001 with AE F130061 to *Lemna gibba* (Duckweed).
Growth was promoted at all four treatments tested compared to the control. Percentual inhibition rates were between -1.75 and -5.05% according to growth rate and -4.99% and -12.02% according to biomass increase.
The lack of any inhibitory effect leads to the conclusion that the non-identified residues from the leachates have no herbicidal activity.

Table CA 8.2.7- 21: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-197850-01-1 KCA 8.2.7 /02	US EPA, J § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	US EPA, OPPTS 850.4400 (1996)	(not EU-relevant)	N/A	N/A
	ASTM, E 1415-91 (1991)	(not EU-relevant)	N/A	N/A
	OECD Draft, *Lemna* growth inhibition (1998)	OECD 221, *Lemna* growth inhibition (2006)	none	no deviation from current guideline

Studies on the metabolites of mesosulfuron-methyl

AE F154851

Report:	[redacted]6; [redacted];2005;M-255283-01
Title:	Lemna gibba G3, growth inhibition test with AE F154851 under static conditions, (code: AE F154851 00 1B96 0001)
Report No:	EBMMX090
Document No:	M-255283-01-1
Guidelines:	**OECD 221 Lemna sp. Growth Inhibition Test Revised Proposal for a New Guideline (April 2004);only minor (initial pH in the lowest test level was slightly below (0.3 units) the guideline recommendations, which did not influence the outcome of this study negatively**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the influence of the test item AE F154851 (metabolite of mesosulfuron-methyl) 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. This test was conducted according to the OECD Guideline 221. As minor deviation the initial pH in the lowest test level was slightly below (0.3 units) the recommendations of the guideline. This did not influence the outcome of the study negatively.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 0.003, 0.01, 0.03, 0.10, 0.31, 0.98, 3.13, and 10.0 mg pure metabolite/L in comparison to control. 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 most sensitive endpoint in this study with AE F154851 was frond area of plants, resulting in a (0-7-day) - E_rC_{50} of 0.11 mg p.m./L. The NOEC was 0.03 mg p.m./L.

Material and methods:

Test item: AE F154851; Code: AE F154851 00 1B96 0001; Batch No.: LOR 21029; Analysed purity: 96.1% w/w; AZ No.: 09181.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 0.003, 0.01, 0.03, 0.10, 0.31, 0.98, 3.13, and 10.0 mg pure metabolite/L in comparison to control. The pH values ranged from 7.4 to 8.8 in the control and the incubation temperature ranged from 23.7°C to 24.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8.95 klx.
Quantitative amounts of AE F154851 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: February 23, 2005 – May 30, 2005

Results:

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

Analytical findings:
The test levels 0.003 and 0.01 mg pure metabolite/L were not analysed because they were below the (0-7-day)-NOE_rC.
The analytical findings of AE F154851 found in the freshly prepared test levels 0.03 – 3.13 mg pure metabolite/L on day 0 in reference to nominal concentrations ranged between 101 and 109% (average 105%). In the same aged test levels on days 7 there were analytical findings between 97 and 106% (average 101%) of nominal. The analysed quantity of AE F154851 in the highest treatment level 10 mg pure metabolite/L found on day 0 and day 7 was only 61% and 62% of nominal.
All results are based on nominal initial concentrations of the pure metabolite, whereas the test level 10.0 mg p.m./L was excluded from EC_{50}-calculations due to potential exceedance of water solubility.

Table CA 8.2.7- 22: Concentrations of AE F154851 in the test solutions

Day	Nominal concentration In mg AE F154851/L	Actual concentration of AE F154851			
		Detection 1 [mg /L]	Detection 2 [mg /L]	Mean [mg /L]	% of nominal
0 7	Control	< 0.001349 < 0.001349	< 0.001349 < 0.001349	< 0.001349 < 0.001349	-- --
0 7	0.003	* *	* *	* *	-- --
0 7	0.01	* *	* *	* *	-- --
0 7	0.03	0.0330 0.0321	0.0325 0.0316	0.0327 0.0318	109 106
0 7	0.10	0.102 0.0975	0.1010 0.0967	0.101 0.0971	101 97
0 7	0.31	0.337 0.318	0.332 0.316	0.335 0.317	108 102
0 7	0.98	1.067 1.015	1.031 1.005	1.049 1.010	107 103
0 7	3.13	3.179 3.100	3.173 3.078	3.176 3.089	101 99
0 7	10.00**	6.065 6.194	6.053 6.153	6.059 6.179	61 62

lowest standard solution of AE F154851 used for determination: 1.349 µg/L
* not determined
** excluded from EC50-calculations due to potential exceedence of water solubility

Biological findings:
Effects are summarized in the following table.

Table CA 8.2.7- 23: Effects of AE F154851 on *Lemna gibba* in a static 7-day test

Nominal test concentration [mg p.m./L]	Final frond no. (replicate means, day 7)	Total frond area of plants (replicate means) [mm^2]	% inhibition*	
			Mean growth rate for frond no.	Mean growth rate for total frond area of plants
control	167	1377	--	--
0.003	167	1393	-0.1	0.9
0.01	159	1265	2.0	3.1
0.03	180	1443	-2.9	1.9
0.10	40	307	54.3	60.9
0.31	22	190	77.7	79.4
0.98	20	175	81.3	85.5
3.13	18	146	85.7	89.3
10.0**	16	128	89.2	91.8

* negative value means growth stimulation
** excluded from EC_{50}-calculations due to potential exceedance of water solubility

Table CA 8.2.7- 24: Summary of the observed visual effects of AE F154851

Test level [mg p.m.//L]	Observations
Control 0.003 0.01 0.03	no visual effects observed
0.10	fronds grown together on days 2, 5 and 7
0.31 0.98	fronds small and grown together on day 2; fronds grown together on days 5 and 7
3.13	no visual effects observed on days 2 and 5; fronds grown together and slightly chlorotic on day 7
10.0	Single fronds on day 2; slight chlorosis and single fronds on days 5 and 7

Table CA 8.2.7- 25: Results based on nominal concentrations of AE F154851 (test level 10.0 mg/L excluded)

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [mg p.m./L]	Effect on mean growth rate of total frond area of plants [mg p.m./L]
E_rC_{50} (CI 95%)	**0.12** (0.04 – 0.34)	**0.11** (0.06 – 0.23)
LOE_rC	0.10	0.10
NOE_rC	0.03	0.03

Conclusions:

The most sensitive endpoint in this study with AE F154851 was frond area of plants, resulting in a (0-7-day) - E_rC_{50} of 0.11 mg p.m./L. The NOEC (0.03 mg p.m./L) was based on statistical analysis.

AE F160459

Report:	[redacted]1[redacted];[redacted];[redacted];2000;M-198076-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F160459 (metabolite of AE F130060) substance: pure, code: AE F160459 00 1B97 0001
Report No:	CA09078
Document No:	M-198076-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

E_bC_{50} = 1.7 mg/L.

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, the listed endpoint should be revised to

E_rC_{50} = 2.6 mg/L.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference:** [redacted]., 2000b, 8.2.8.2.1/1.

- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD draft guideline for testing of chemicals on *Lemna gibba*, growth inhibition test (April 1997) and ASTM "Standard guide for conducting static toxicity test with *Lemna gibba*" G3 1415-91 (1991).

- **GLP compliance**: Yes.

- **Methods**: The effects of AE F160459 (purity = 96.8%) on the growth of the duckweed *Lemna gibba* was determined under renewal conditions. Plants were exposed to the active substance in 300 ml flasks filled with 150 ml of test water, that contained 0 (control), 0.1, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2, 5.6 or 10 mg test substance/l. A total of 12 fronds (3-5 plants) were allocated per flask. Each concentration and the control were replicated three times. Test water was renewed on days 3 and 5, before data check. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological sign was also recorded.

- **Results**: A significant inhibition of both growth rate and biomass production was observed at the end of the test for concentrations of 1.0 mg/l and above. Based on nominal concentrations:
  ErC50 7 days > 2.6 mg/l, 95% CI =[1.8 - 3.2]mg/l
  NOErC 7 days = 0.56 mg/l
  EbC50 7 days = 1.7 mg/l, 95% CI =[1.0 - 1.8]mg/l

- **Comments (RMS)**: the study is acceptable.

Further study information supplementing the original Monograph summary :

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

Analytical findings:
Analyses of freshly prepared water for AE F160459 resulted in concentrations ranging from 91.9% to 115.1% of nominal values. Analyses of aged water for AE F160459 at experimental termination resulted in concentrations ranging from 87.7% to 109.9% of nominal values. Therefore, nominal treatment levels of AE F160459 are reported in this study.

Biological findings:
Mean values of absolute and percentual growth inhibition in comparison to the solvent control are presented in the following table.

Table CA 8.2.5.2.6: 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	0.396	0.00	22.0	0.00
0.10	0.395	0.12	21.9	0.61
0.18	0.395	0.20	21.7	1.52
0.32	0.393	0.59	21.7	1.52
0.56	0.384	2.89	21.3	3.33
1.0	0.377	4.75	20.5	6.82
1.8	0.247	37.58	9.4	57.12
3.2	0.171	56.71	4.3	80.45
5.6	0.119	69086	4.4	80.15
10.0	0.093	76.61	2.5	88.64

The concentration of 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 2.6 mg/L (95% confidence limits 1.8 - 3.2 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 1.7 mg/L (95% confidence limits 1.0-1.8 mg/L).
Intoxication symptoms (yellow coloured fronds / fronds do not fully separate, plant vaulted) were observed at concentrations of and above nominal 1.0 mg/L.
A significant inhibition at a significance level of alpha = 0.05 of growth both related on frond number was observed at nominal concentrations of 1.0 mg/L and above.
A significant inhibition at a significance level of alpha = 0.05 of biomass increase (dry weight) was observed at nominal concentrations of 1.0 mg/L and above.
The no observed effect concentration (NOEC) defined as no significant growth inhibition and no changes in plant appearance and development was set to nominal 0.56 mg/L.

The following table shows the EC_{50} values after 7 days. The calculation method selected was binomial probability.

Table CA 8.2.7- 27: EC_{50} values

	nominal	
	E_rC_{50}	E_bC_{50}
EC-values after 7 days in mg/L	2.6	1.7
95% confidence limits in mg/L, low	1.8	1.0
95% confidence limits in mg/L, high	3.2	1.8

Conclusions:
In a Growth Inhibition Test (method EPA / OECD / ASTM) to determine the effect of AE F160459, substance, pure, Code: AE F160459 00 1B97 0001 (metabolite of AE F130060) to *Lemna gibba* (Duckweed), the E_rC_{50} and E_bC_{50} after 7 days test duration were nominal 2.6 mg/L (95% confidence limits 1.8 - 3.2 mg/L) and nominal 1.7 mg/L (95% confidence limits 1.0-1.8 mg/L), respectively.
The no observed effect concentration (NOEC) was nominal 0.56 mg/L.

Table CA 8.2.7- 28: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-198076-01-1 KCA 8.2.7/03	US EPA, § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	US EPA, OPPTS 850.4400 (1996)	(not EU-relevant)	N/A	N/A
	ASTM, E 1415-91 (1991)	(not EU-relevant)	N/A	N/A
	OECD Draft, *Lemna* growth inhibition (1998)	OECD 221, *Lemna* growth inhibition (2006)	none	no deviations from current guideline

AE F099095

Report:	[redacted] ; [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 (metabolite of mesosulfuron-methyl) 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.: KR363/364, certificate of analysis: AZ10810.

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 CA 8.2.7- 29: 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

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

Table CA 8.2.7- 30: 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	572	9.7	7.9

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

Table CA 8.2.7- 31: 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 CA 8.2.7- 32: 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 (=100 mg/L) was based on statistical analysis.

AE F092944

Report:	[redacted]5; [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 1C99 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 AE F092944 (metabolite of mesosulfuron-methyl) 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 1C99 0001; 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 CA 8.2.7- 33: Analytical findings of AE F092944 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	52.57	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	103.2	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 CA 8.2.7- 34: 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 CA 8.2.7- 35: 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 F160460

Report:	[redacted]3; [redacted]; [redacted];2000;M-199266-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F160460 (metabolite of AE F130060) substance, pure Code: AE F160460 00 1B96 0001
Report No:	C009792
Document No:	M-199266-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

$E_bC_{50} > 100$ mg/L.

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, the listed endpoint should be revised to:

$E_rC_{50} > 100$ mg/L.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted]2000c, 8.2.1.1
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD draft guideline for testing of chemicals on *Lemna gibba* growth inhibition test (April 1997) and ASTM "Standard guide for conducting static toxicity test with *Lemna gibba*" G341/15-91 (1991).
- **GLP compliance**: yes.
- **Methods**: The effects of AE F160460 (substance, purity 96.1%) on the growth of the duckweed *Lemna gibba* was determined under renewal conditions. Plants were exposed to the active substance in 300 ml flasks filled with 100 ml of test water, that contained 0 (control), 3.2, 10, 18, 32, 56 and 100 mg test substance/l. A total of 12 fronds (3-5 plants) were allocated per flask. Each concentration and the control were repeated three times. Test water was renewed on days 3 and 5 before data checks. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological sign was also recorded.
- **Results**: No intoxication symptoms were observed. Based on nominal concentrations:
  E_rC_{50} 7 days > 100 mg/l
  NOE_rC 7 days = 100 mg/l
  E_bC_{50} 7 days > 100 mg/l
- **Comments (RMS)**: The study is acceptable.

Further study information supplementing the original Monograph summary :

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

Analytical findings:
Analyses of freshly prepared water for AE F160460 resulted in concentrations ranging from 89.8% to 104.4% of nominal values. Analyses of aged water for AE F160460 at experimental termination resulted in concentrations ranging from 89.0% to 102.7% of nominal values. Therefore, nominal treatment levels of AE F160460 are reported in this study.

Biological findings:
Mean values of absolute and percentual growth inhibition in comparison to the solvent control are presented in the following table:

Table CA 8.2.7- 36: Mean values of absolute and percentual growth inhibition compared to the solvent control

Treatment level [mg/L]	Mean growth rate [d^{-1}]	Percentual inhibiton of growth rate	Mean increase in biomass [mg]	Percentual inhibition of biomass increase
Untreated control	0.38888	0.00	20.63	0.00
10	0.39025	-0.35	21.06	-2.10
18	0.39073	-0.47	20.73	-0.48
32	0.39302	-1.06	20.83	-0.97
56	0.38597	0.75	20.[illegible]	-1.1[illegible]
100	0.39506	-1.59	21.10	-2.[illegible]6

The concentration of test substance leading to a 50% inhibition of the growth regarding frond numbers (u) 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.
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.

The following table shows the EC_{50} values after 7 days. The calculation method selected was binomial probability.

Table CA 8.2.7- 37: EC_{50} values

	nominal	
	E_rC_{50}	E_bC_{50}
EC-values after 7 days in mg/L	> 100	> 100

Conclusions:
In a Growth Inhibition Test (method EPA, OECD, ASTM) to determine the effect of AE F160460, substance, pure, Code: AE F160460 00 1B96 0001 (metabolite of AE F130060) to *Lemna gibba* (Duckweed), the E_rC_{50} and E_bC_{50} after 7 days test duration were both nominal >100 mg/L.
The no observed effect concentration (NOEC) was nominal 100 mg/L.

Table CA 8.2.7- 38: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-199266-01-1 KCA 8.2.7 /04	US EPA, J, § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	US EPA, OPPTS 850.4400 (1996)	(not EU-relevant)	N/A	N/A
	ASTM, E 1415-91 (1991)	(not EU-relevant)	N/A	N/A
	OECD Draft, *Lemna* growth inhibition (1997)	OECD 221, *Lemna* growth inhibition (2006)	none	no deviations from current guideline

AE F140584

Report:	[redacted];[redacted];2013;M-486658-01
Title:	Lemna gibba G3 - Growth inhibition test with BCS-AU66443 (AE F 140584) under semi static conditions
Report No:	EBMMN119
Document No:	M-486658-01-1
Guidelines:	**OECD Guideline 221 (March 23, 2006)** **US EPA OCSPP 850.4400;a slight deviation (0.1) of pH is explained and discussed with the preparation of the nutrient medium**
GLP/GEP:	**yes**

Executive Summary:

The objective of this growth inhibition test was to verify the assumption that the test item AE F140584 (metabolite of mesosulfuron-methyl) will cause no adverse effects on the growth of *Lemna gibba* G3 at the only test item concentration of 10 mg pure metabolite (p.m.)/L. This test was conducted according to the OECD Guideline 221 (2006) and is also based on US EPA Ecological Effects Test Guideline OCSPP850.4400 (2012).

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under semi static exposure conditions to the nominal concentration of 10.0 mg p.m./L in comparison to a 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 E_rC_{50} was > 10.0 mg p.m./L and the NOE_rC was >10.0 mg p.m./L.

Material and methods:

Test item: AE F140584; Batch Code: AE F140584-01-01; Origin Batch No.: SES 10678-15-2; TOX No.: 10305-00; Analysed a.s. content: 98.4% w/w AE F140584; certificate No.: AZ 19035.

6 x 12 fronds of *Lemna gibba* G3 were exposed in a chronic multigeneration test for 7 days under semi static exposure conditions to the nominal concentration of 10.0 mg p.m./L in comparison to a control.

The pH values ranged from 7.5 to 8.7 in the control and the incubation temperature ranged from 24.0°C to 24.1°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6603 lux (average of nine measurements).

Quantitative amounts of AE F140584 were measured in all freshly prepared test levels on day 0, 1, 2, 3, 4, 5 and 6 and additionally in all aged test levels on day 1, 2, 3, 4, 5, 6 and 7 of the exposure period.

Dates of experimental work: April 22, 2014 – May 20, 2014

Results:

Validity Criteria:
The doubling time of frond number in the control was 1.8, corresponding to a 15.4 fold increase. The control coefficient of variation (CV) for yield and growth rate is < 20% at test termination. Therefore the study met all validity criteria, requested by the mentioned guidelines.

Analytical findings:
The analytical findings of AE F140584 in all freshly prepared test levels ranged between 85 and 101 % of nominal. In all aged test levels analytical findings ranged between 0 and 1 % of nominal due to rapid degradation of the test substance. However, all reported results are based on nominal concentrations. During the test all efforts were made to establish a constant exposure concentration by performing a daily water exchange.

Table CA 8.2.7- 39: Measured concentrations of AE F140584 in test solutions

Day	Nominal concentration [mg p.m./L]	Actual concentration of AE F140584			
		Detection 1 [mg/L]	Detection 2 [mg/L]	Mean [mg/L]	% of nominal
0	Control	< 0.672	< 0.672	< 0.672	--
1 aged		< 0.672	< 0.672	< 0.672	--
1 new		< 0.672	< 0.672	< 0.672	--
2 aged		< 0.672	< 0.672	< 0.672	--
2 new		< 0.672	< 0.672	< 0.672	--
3 aged		< 0.672	< 0.672	< 0.672	--
3 new		< 0.672	< 0.672	< 0.672	--
4 aged		< 0.672	< 0.672	< 0.672	--
4 new		< 0.672	< 0.672	< 0.672	--
5 aged		< 0.672	< 0.672	< 0.672	--
5 new		< 0.672	< 0.672	< 0.672	--
6 aged		< 0.672	< 0.672	< 0.672	--
6 new		< 0.672	< 0.672	< 0.672	--
7		< 0.672	< 0.672	< 0.672	--
0	10.0	10.1	10.2	10.2	102
1 aged		< 0.672	< 0.672	--	--
1 new		9.50	9.71	9.61	96
2 aged		< 0.672	< 0.672	--	--
2 new		9.60	9.61	9.61	96
3 aged		< 0.672	< 0.672	< 0.672	--
3 new		9.80	9.61	9.71	97
4 aged		< 0.672	< 0.672	--	--
4 new		8.58	8.50	8.54	85
5 aged		< 0.672	< 0.672	--	--
5 new		9.95	9.43	9.69	97
6 aged		< 0.672	< 0.672	--	--
6 new		9.57	9.57	9.57	96
7		< 0.672	< 0.672	--	--

Biological findings:
Effects are summarized in the following table.

Table CA 8.2.7- 40: Effects of AE F140584 on *Lemna gibba* in a static 7-day test

Nominal test concentration [mg p.m./L]	Frond no. (day 7) mean values from 6 replicates	Total frond area of plants (day 7) Mean values from 6 replicates [mm²]	% inhibition	
			Mean growth rate for frond no.	Mean growth rate for total frond area of plants
Control	185	1641	--	--
10.0	193	1678	-1.5	-0.5

-% inhibition: increase in growth relative to the control

No sublethal effects on Lemna gibba were observed
No remarkable observations of the test item in the test medium were recorded. Over the whole test period, the media were clear and colourless.

Table CA 8.2.7- 41: Results based on nominal concentrations of AE F140584

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [mg p.m./L]	Effect on mean growth rate of total frond area of plants [mg p.m./L]
E_rC_{50}	**>10.0**	**>10.0**
LOE_rC	>10.0	>10.0
NOE_rC	10.0	10.0

The E_rC_{50}, LOE_rC and NOE_rC determination is based on statistical data analysis.

Conclusions:

AE F140584 caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

AE F147447

Report:	[redacted]n, [redacted]; [redacted];2000;M-198273-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F147447, substance, technical (Metabolite of AE F130060) Code: AE F147447 00 1C93 0001
Report No:	C009805
Document No:	M-198273-01-1
Guideline:	**ASTM: E1415-91; OECD: draft june 1998; USEPA (=EPA): J §123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

E_bC_{50} > 100 mg/L.

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, the listed endpoint should be revised to

E_rC_{50} > 100 mg/L.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000d, 8.2.8.2.1/3.
- **Test guideline**: US-EPA Pesticide Assessment Guidelines, Subdivision J, §123-2 (1982), OECD draft guideline for testing of chemicals on *Lemna gibba*, growth inhibition test (April 1997) and ASTM "Standard guide for conducting static toxicity test with *Lemna gibba*" G3 1415-91 (1991).
- **GLP compliance**: Yes.
- **Methods**: The effects of AE F147447 (substance, purity = 93.1%) on the growth of the duckweed *Lemna gibba* was determined under renewal conditions. Plants were exposed to the active substance in 300 ml flasks filled with 150 ml of test water, that contained 0 (control), 10, 18, 32, 56 and 100 mg test substance/l. A total of 12 fronds (3-5 plants) were allocated per flask. Each concentration and the control were repeated three times. Test water was renewed on days 3 and 5, before data checks. Effects on growth rate were assessed through the number of fronds measured after 3, 5 and 7 days test duration. Any abnormal morphological sign was also recorded.
- **Results**: based on nominal concentrations:
  E_rC_{50} 7 days > 100 mg/l
  NOE_rC 7 days = 100 mg/l
  E_bC_{50} 7 days > 100 mg/l
- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary:

Validity Criteria:
The validity criterium of doubling time less than 60 hours (2.5 days) in the control is fulfilled.

Analytical findings:
Analyses of freshly prepared water for AE F147447 resulted in concentrations ranging from 91.6% to 103.9% of nominal values. Analyses of aged water for AE F147447 at experimental termination resulted in concentrations ranging from 91.2% to 101.7% of nominal values. Therefore, nominal treatment levels of AE F147447 are reported in this study.

Biological findings:
Mean values of absolute and percentual growth inhibition in comparison to the control are presented in the following table:

Table CA 8.2.7- 42: Mean values of absolute and percentual growth inhibition compared to the control

Treatment level [mg/L]	Mean growth rate [d]	Percentual inhibiton of growth rate	Mean increase in biomass [mg]	Percentual inhibition of biomass increase
Untreated control	0.35913	0.00	24.10	0.00
10	0.37241	-0.91	25.87	-7.34
18	0.36897	0.04	25.14	-4.31
32	0.37110	-0.53	25.10	-4.15
56	0.36729	0.50	25.59	-6.17
100	0.36844	0.19	23.73	1.55

The concentration of 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 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.

The following table shows the EC_{50} values after 7 days. The calculation method selected was binomial probability.

Table CA 8.2.7- 43: EC_{50} values

	nominal	
	E_rC_{50}	E_bC_{50}
EC-values after 7 days in mg/L	> 100	> 100

Conclusions:
In a Growth Inhibition Test (method EPA / OECD / ASTM) to determine the effect of AE F147447, substance, pure, Code: AE F147447 00 1C95 0001 (metabolite of AE F130060) to *Lemna gibba* (Duckweed), the E_rC_{50} and E_bC_{50} after 7 days test duration were both nominal >100 mg/L.
The no observed effect concentration (NOEC) was nominal 100 mg/L.

Table CA 8.2.7- 44: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-198273-01-1 KCA 8.2.7 /08	US EPA, J, § 123-2 (1982)	(not EU-relevant)	N/A	N/A
	US EPA, OPPTS 850.4400 (1996)	(not EU-relevant)	N/A	N/A
	ASTM, E 1415-91 (1991)	(not EU-relevant)	N/A	N/A
	OECD Draft, *Lemna* growth inhibition (1997)	OECD 221, *Lemna* growth inhibition (2006)	none	no deviatons from current guideline

BCS-CO60720

Report:	[redacted];8;[redacted];2013;M-449110-01
Title:	Lemna gibba G3 - Growth inhibition test with BCS-CO60720 under static conditions
Report No:	EBMML010
Document No:	M-449110-01-1
Guidelines:	**OECD - 221 (March 23, 2006);none**
GLP/GEP:	**yes**

Executive Summary:

The objective of this growth inhibition test was to verify the assumption that the test item BCS-CO60720 (metabolite of mesosulfuron-methyl) will cause no adverse effects on the growth of *Lemna gibba* G3 up to a test item concentration of 11.8 mg pure metabolite (p.m.)/L. This test was conducted according to the OECD Guideline 221.

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 11.8 mg p.m./L 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 E_rC_{50} was > 11.8 mg pure metabolite (p.m.)/L and the NOE_rC was ≥ 11.8 mg p.m./L.

Material and methods:

Test item: BCS-CO60720; Batch Code: BCS-CO60720-01-01; Origin batch no.: SES 10689-26-2; TOX no.: 08551-00; Analysed purity: 98.3% w/w; certificate No.: AZ 16515.

6 x 12 fronds of *Lemna gibba* G3 were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 11.8 mg p.m./L in comparison to a water control. The pH value at day 0 was 7.5 in the controls and the incubation temperature ranged from 23.5°C to 23.6°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7824 lux.
Quantitative amounts of BCS-CO60720 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: November 08, 2010 – May 12, 2011

Results:

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

Analytical findings:
The analytical finding of BCS-CO60720 found on day 0 was 108% of nominal and 112% of nominal on day 7. All reported results are based on nominal values of the test item.

Table CA 8.2.7- 45: Concentrations of BCS-CO60720 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg BCS-CO60720/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.632	<0.632	<0.632	--
10.0	12.7	12.8	12.8	108

Table CA 8.2.7- 46: Concentrations of BCS-CO60720 in the test solutions at day 7

Day 7				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg BCS-CO60720/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.632	<0.632	<0.632	--
10.0	13.2	13.2	13.2	112

Biological findings:
Effects are summarized in the following table.

Table CA 8.2.7- 47: Effects of BCS-CO60720 on *Lemna gibba* in a static 7-day test

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	182.8	1318.7	--	--
11.8	188.3	1323.3	-2.7	1.0

There were no visual effects observed in any of the test concentrations.

Table CA 8.2.7- 48: Results based on nominal concentrations of BCS-CO60720

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [mg p.m./L]	Effect on mean growth rate of total frond area of plants [mg p.m.L]
E_rC_{50}	**>11.8**	**>11.8**
LOE_rC	>11.8	>11.8
NOE_rC	≥11.8	≥11.8

The LOE_rC determination is based on statistical data analysis

Conclusions:

BCS-CO60720 caused no adverse effects on the growth of *Lemna gibba* G3 up to a test item concentration of 11.8 mg pure metabolite/L.

BCS-CO60721

Report:	[redacted]1; [redacted];2013;M-445154-01
Title:	Lemna gibba G3 - Growth inhibition test with BCS-CO60721 under static conditions
Report No:	EBMML011
Document No:	M-445154-01-1
Guidelines:	**OECD - 221 (March 23, 2006);Deviations: The documentation of a 7-10 day old pre-culture is missing for this study. On the basis of the photo-documentation of Lemna plants within the study and the fulfilled validity criteria it can be stated that this deviation from the guideline had no impact on the study results and therefore on the validity of the study. The deviations from the guideline are documented within the raw data. The pH values were not determined on day 7. Based on the normal control growth it can be assumed that the pH values on day 7 were in line with the guideline requirements.**
GLP/GEP:	**yes**

Executive Summary:

The objective of this growth inhibition test was, to verify the assumption that the test item BCS-CO60721 (metabolite of mesosulfuron-methyl) will cause no adverse effects on the growth of *Lemna gibba* G3 up to a test item concentration of 10 mg pure metabolite (p.m.)/L. This test was conducted according to the OECD Guideline 221. As deviation from the defined guideline recommendations the documentation of a 7-10 day old pre-culture is missing for this study. On the basis of the photo-documentation of *Lemna* plants within the study and the fulfilled validity criteria it can be stated that this deviation from the guideline had no impact on the study results and therefore on the validity of the study. The deviations from the guideline are documented within the raw data. The pH values were not determined on day 7. Based on the normal control growth it can be assumed that the pH values on day 7 were in line with the guideline requirements.

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.
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 E_rC_{50} was > 10 mg p.m./L and the NOE_rC was ≥ 10 mg p.m./L.

Material and methods:

Test item: BCS-CO60721; Batch Code: BCS-CO60721-01-01; Origin batch No.: SES-10798-12-3; Customer order no.: TOX08552-00; Analysed purity: 95.1% w/w; Certificate no.: AZ-16765.

6 x 12 fronds of *Lemna gibba* G3 were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a water control. The pH value at day 0 was 7.5 in the control and the incubation temperature ranged from 23.5°C to 23.6°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7824 lux. Quantitative amounts of BCS-CO60721 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: November 08, 2010 – December 27, 2012

Results:

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

Analytical findings:
The analytical finding of BCS-CO60721 found on day 0 was 97% of nominal and 101% of nominal on day 7. All reported results are based on nominal values of the test item.

Table CA 8.2.7- 49: Concentrations of BCS-CO60721 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	Actual Concentration (mg BCS-CO60721/L)			
	1. Determination	2. Determination	Average	%
Control	<0.652	<0.652	<0.652	--
10.0	9.64	9.68	9.66	97

Table CA 8.2.7- 50: Concentrations of BCS-CO60721 in the test solutions at day 7

Day 7				
Nominal Concentration in mg p.m./L	Actual Concentration (mg BCS-CO60721/L)			
	1. Determination	2. Determination	Average	%
Control	<0.652	<0.652	<0.652	--
10.0	10.1	10.2	10.1	101

Biological findings:
Effects are summarized in the following table.

Table CA 8.2.7- 51: Survey of biological results and derived inhibition percentages of BCS-CO60721 according to growth rates

Nominal test concentration [mg p.m./L]	Final frond no. (replicate means, day 7)	Final total frond area of plants (replicate means) [mm^2]	% inhibition	
			Mean growth rate for frond no.	Mean growth rate for total frond area of plants
control	182.8	1318.7	--	--
10.0	187.2	1264.5	-0.9	1.5

There were no visual effects observed in any of the test concentrations.

Table CA 8.2.7- 52: Results based on nominal concentrations of BCS-CO60721

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [mg p.m./L]	Effect on mean growth rate of total frond area of plants [mg p.m./L]
E_rC_{50}	**>10.0**	**>10.0**
LOE_rC	>10.0	>10.0
NOE_rC	≥10.0	≥10.0

The LOE_rC determination is based on statistical data analysis

Conclusions:

BCS-CO60721 caused no adverse effects on the growth of *Lemna gibba* G3 up to a test item concentration of 10 mg pure metabolite/L.

CA 8.2.8 Further testing on aquatic organisms

One acute study under flow-through conditions on Eastern oyster (*Crassostrea virginica*) was performed. Details of the study are provided in the following table.

Table CA 8.2.8- 1: Effect data of mesosulfuron-methyl to aquatic organisms presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]		Reference
Mesosulfuron-methyl					
Crassostrea virginica (eastern oyster)	flow-through	96 h	EC_{50} NOEC	> 100 100	[redacted], 2000 B003104 M-238739-02-1 KCA 8.2.8 /01

Report:	[redacted]; [redacted] 2000;M-238739-02; Amended: 2000-12-07
Title:	AE F130060 00 1C96 0004 - Acute Toxicity to Eastern Oysters (Crassostrea virginica) Under Flow-Through Conditions
Report No:	B003104
Document No(s):	M-238739-02-1
Guidelines:	**USEPA (=EPA): FIFRA 72-3, OPPTS 850.1025;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to estimate the acute toxicity of mesosulfuron-methyl (AE F130060) technical to Eastern oysters (*Crassostrea virginica*) under flow-through conditions.

Crassostrea virginica (mean valve height 37 ± 4.0 mm) were exposed in a flow-through system over a period of 96 hours to nominal concentrations of 13, 22, 36, 60 and 100 mg a.s./L (corresponding to mean measured concentrations of 13, 20, 37, 57 and 100 mg a.s./L). In addition a dilution water control (natural unfiltered seawater) was tested. Shell deposition, mortality and sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as mean measured figures. The 96-hour-EC_{50} was > 100 mg a.s./L, the 96-hour-NOEC was determined to be 100 mg a.s./L.

Material and methods:

Test item: AE F130060, technical (mesosulfuron-methyl); Code: AE F130060 00 1C96 0004; CAS No.: 208465-21-8; Batch No.: OP 3/99, Purity: 95.6% w/w; Certificate of Analysis: AZ 08196.

Crassostrea virginica (mean valve height 37 ± 4.0 mm) were exposed to the test item in a flow-through system over a period of 96 hours. Nominal concentrations were 13, 22, 36, 60 and 100 mg a.s./L (corresponding to mean measured concentrations of 13, 20, 37, 57 and 100 mg a.s./L). In addition a dilution water control (natural unfiltered seawater) was tested. Each vessel (glass aquaria measuring 49.5 x 25.5 x 29 cm) served as one replicate filled with 18 L of test solution. Natural unfiltered seawater was used as dilution water. The seawater used for these solutions had a salinity of 32‰ and a pH of 8.0. 20 oysters were placed in each test aquarium. The temperature of the test solution was regulated at 20 ± 2°C. The test was conducted with 2 replicates per treatment level and the control. Prior to the test initiation, each oyster was ground with a fine-grit grinding wheel to remove approximately 3 to 5 mm of shell and form a blunt edge. Observations of stress, abnormal behavioural activity and mortality were made at the start of the test and after 24, 48, 72, and 96 hours. Oyster shell deposition was measured microscopically at the end of the study.
For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. High-performance liquid chromatography with ultraviolet detection (HPLC/UV) was used as analytical method.

Dates of experimental work: September 14, 2000 to September 18, 2000

Results:

Validity Criteria:
The validity criterion of control mortality less than 10% was fulfilled. The validity criterion of control shell growth > 2 mm was fulfilled. The validity criterion of oxygen saturation above 60% was fulfilled.

Analytical Findings:
Results of the AE F130060 technical analyses established that the measured concentrations were consistent between sampling intervals and defined the exposure concentrations as 13, 20, 37, 57 and 100 mg a.i./L. Detailed analytical results are presented in the following table:

Table CA 8.2.8- 2: Concentrations of AE F1 30060 technical measured in the exposure solutions during the 96-hour exposure of Eastern oysters (*Crassostrea virginica*).

Nominal Concentration (mg a.s./L)	Measured concentration (mg a.s./L)[1]			Percent of nominal (%)
	0-Hour	96-Hour	Mean	
Control	< 2.3	< 2.6	NA	NA
13	12	13	13	96
22	20	21	20	93
36	38	36	37	100
60	56	58	57	95
100	98	105	100	100

[1] Analytical values are reported to two significant figures
NA = not applicable

Biological results:

The percent reduction in shell growth did not exceed 12% for oysters exposed to all AE F130060 technical concentrations tested. No sublethal effects were observed among any of the exposed oysters throughout the tested concentration range.

Table CA 8.2.8- 3: Effects of AE F130060 technical on the shell deposition of Eastern oysters (*Crassostrea virginica*) after 96 hours of exposure.

Mean measured concentration (mg a.i./L)	Mortality %	Mean shell deposition[1] (mm)	SD	Percent of control[2]
Control	0	3.4	1.0	NA
13	0	3.5	1.2	0 (+6)
22	0	3.3	1.1	3
36	0	3.0	0.9	10
60	0	2.9	1.2	12
100	0	3.3	1.2	< 1

[1] Mean shell deposition represents the measurements of 40 oysters per treatment
[2] Unrounded replicate mean shell growth was compared to the control to determine treatment effects
SD = Standard deviation
NA = not applicable

Conclusions:

The 96-hour EC_{50} value was empirically estimated to be > 100 mg a.s./L, the highest concentration tested. The No-Observed-Effect Concentration (NOEC) after 96 hours was 100 mg a.s./L.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

Table CA 8.3.1- 1: Toxicity endpoints of/for mesosulfuron-methyl (tech.)

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity for honey bees			
Mesosulfuron-methyl, tech.	LD_{50}-oral, 72 h	LD_{50} 5.6 µg a.s./bee	[redacted], 1996 M-141738-01-1 KCA 8.3.1.1.1 /01
Mesosulfuron-methyl, tech.	LD_{50}-contact, 48/72 h	LD_{50} > 13 µg a.s./bee	[redacted], 1996 M-143107-01-1 KCA 8.3.1.1.2 /01
Mesosulfuron-methyl, tech.	LD_{50}-oral, 96 h LD_{50}-contact, 96 h NOED (oral/contact), 96 h	**LD_{50} > 105.6 µg a.s./bee** **LD_{50} > 100 µg a.s./bee** **NOED ≥ 105.6/100 µg a.s./bee**	[redacted], 2012 M-453998-01-1 KCA 8.3.1.1 /01
Acute contact toxicity for bumble bees			
Mesosulfuron-methyl WG 75	LD_{50}-contact, 48 h NOED (contact), 48 h	LD_{50} > 100 µg a.s./bee NOED ≥ 100 µg a.s./bee	[redacted], 2014 M-485279-01-1 KCA 8.3.1.1 /02
Chronic toxicity for adult honey bees			
Mesosulfuron-methyl, tech.	10 d chronic adult feeding study	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted], 2014 M-485655-01-1 KCA 8.3.1.2 /01
Honey bee brood feeding test			
Mesosulfuron-methyl WG 75 (+Mefenpyr-diethyl WG 15)	Honey bee brood feeding ([redacted] *et al*., 1992)	No adverse effects on adult bee mortality, bee brood development (eggs, young larvae, old larvae, pupae), behaviour, colony strength and colony development, by feeding honey bee colonies sugar syrup at a mesosulfuron-methyl concentration typically present in the spray tank (37.5 ppm)	[redacted], 2013 M-465325-01-1 KCA 8.3.1.3 /01
Mesosulfuron-methyl WG 75 (+Mefenpyr-diethyl WG 15)	Semi-field honey bee brood study (OECD No. 75; forced exposure conditions) in *Phacelia*; application during full-bloom and bees actively foraging	No adverse effects on mortality of adult bees and brood, flight intensity, behaviour, brood development (brood termination rate, brood index, compensation index) and colony vitality at 15 g mesosulfuron-methyl/ha	[redacted], 2015 M-510267-01-1 KCA 8.3.1.3 /03

In addition to the already available acute laboratory studies with technical mesosulfuron-methyl ([redacted]; 1996, Doc.-No.: M-141738-01-1 and [redacted]; 1997, M-143107-01-1; KCA 8.3.1.1.1/01 and KCA 8.3.1.1.2/01), a further laboratory study on the acute oral and contact toxicity to honey bees has been performed with technical mesosulfuron-methyl according to current guidelines and requirements. Moreover, an acute contact toxicity study with Mesosulfuron-methyl WG 75 in bumble bees has been conducted (KCA 8.3.1.1 /02) in order to benchmark potential mesosulfuron-methyl - inherent sensitivity differences to honey bees.

In addition, a chronic 10 day adult feeding limit test was conducted with technical mesosulfuron-methyl (KCA 8.3.1.2 /01). The respective study summaries are presented below.

When considering the acute toxity findings from [redacted] (1996, 1997) and when comparing these endpoints with the endpoints as obtained in the recent study by [redacted] (2012) according to current guideline requirements, the findings of an LD_{50}-contact of >13 µg a.s./bee ([redacted], 1997) and of >100 µg a.s./bee ([redacted], 2012) with 0% corrected mortality in both studies at any point in time are fully consistent and the numerical difference is just to be attributed to the different doses tested. As such, the **intrinsic acute contact toxicity** of technical mesosulfuron-methyl can be concluded **to be actually >100 µg a.s./bee**, which should also be appropriately reflected in the new List of Endpoints.

When comparing the findings regarding the intrinsic oral toxicity, the old study ([redacted], 1996) revealed a LD_{50} of 184.8 µg a.s./bee after 24 and 48 h, however, after 72 h, a marked decrease of the LD_{50} to apparently 5.6 µg a.s./bee was observed.

In order to investigate whether there is any potential mesosulfuron-methyl inherent delayed toxicity effect, the new study ([redacted], 2012) was deliberately prolonged to the maximum guideline-complient study duration of 96 h, although this was not triggered by the actual study results. The new study ([redacted], 2012), according to the latest OECD guideline requirements, revealed a LD_{50}-oral of >105.6 µg a.s./bee after 24, 48, 72 and 96 h, always associated with 0% mortality, respectively.

Moreover, in the the 10 day chronic feeding study ([redacted], 2014), the bees were continuously fed *ad libitum* over a period of 10 days with technical mesosulfuron-methyl at a concentration of 120 ppm - which was verified by daily chemical analysis - which resulted in 0% mortality after 10 days of consecutive feeding. In the old acute study by [redacted] (1996), treatment levels of 10 and 100 ppm after a one-time feeding event resulted in a mortality of 22/50 (=44%) at 10 ppm (=0.001%) and in a mortality of 23/50 (= 46%) at 100 ppm (=0.01%) after 72 h.

In addition, in the bee brood feeding study ([redacted], 2013), where entire honey bee colonies were exposed to Mesosulfuron-methyl WG 75 at a mesosulfuron-methyl concentration corresponding to 37.5 ppm, also no effects on honey bee mortality were observed.

Overall, when accounting for the findings of (i) the new acute oral toxity study with technical mesosulfuron-methyl ([redacted], 2012), which was deliberately prolonged to 96 h without any mortality, the findings of (ii) the new chronic oral toxicity study ([redacted], 2014), with a daily analytical confirmation of the exposure concentration and (iii) the findings of the bee brood feeding study ([redacted], 2013) in comparision to the old acute oral toxity study by [redacted] (1996), it appears that the observed significant increase in mortality in the study by [redacted] (1996) during 48 to 72 h must be attributed to study inherent technical shortcomings, rather than to mesosulfuron-methyl inherent substance properties. As such, it can be concluded that the the **intrinsic acute oral toxicity** of technical mesosulfuron-methyl is actually **>105.6 µg a.s./bee**, which should also be appropriately reflected in the new List of Endpoints.

CA 8.3.1.1 Acute toxicity to bees

Study with mesosulfuron-methyl

Report:	[redacted] v; [redacted] ;2012;M-433998-01
Title:	Effects of mesosulfuron-methyl tech. (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	72941035
Document No:	M-433998-01-1
Guidelines:	**OECD 213 and 214 (1998);The test was prolonged up to 96 hours**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the acute contact and oral toxicity of mesosulfuron-methyl tech. to the honey bee (*A. mellifera* L.) under laboratory conditions. For this purpose 50 female worker bees were exposed for 96 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and to a single dose of 105.6 µg a.s. per bee by feeding (oral limit test, value based on the actual intake of the test item). As deviation to the OECD Guideline 213 and 214 the test was prolonged up to 96 hours. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.
The contact LD_{50} (96 h) was > 100.0 µg a.s./bee. The oral LD_{50} (96 h) was > 105.6 µg a.s./bee. The contact NOED was ≥ 100 µg a.s./bee. The oral NOED was ≥ 105.6 µg a.s./bee.

Material and methods:

Test item: Mesosulfuron-methyl, technical; Batch code: AE F130060-01-02; Origin batch no.: EFME000144; LIMS no.: 1101337; TOX no: 09287-00; Specification no.: 102000013204; Article no.: 05748967; Analysed content: 97.4% w/w; Certificate of Analysis No.: AZ 17171.
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 were used per test item dose level, control and reference item dose level, respectively. 50 worker bees were exposed for 96 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees were exposed for 96 hours to a single dose of 105.6 µg a.s. per bee by feeding (oral limit test, value based on the actual intake of the test item).
For the contact test a single a single 5 µL droplet of mesosulfuron-methyl tech. in an appropriate carrier (acetone 90% + DMSO 10%) was placed on the dorsal bee thorax, likewise for the toxic reference (dimethoate made up in acetone), the control (tap water containing 0.5 % Adhäsit) and solvent control (acetone 90% + DMSO 10%). For the oral test the final test item feeding solution was appropriate amounts of mesosulfuron-methyl dilutions mixed with 95% (w/w) ready-to-use syrup (30 % sucrose, 31 % glucose, 39 % fructose) with solvent (2.5% acetone, 2.5% DMSO, 95% ready-to-use syrup); the feeding solution of the solvent control had the identical composition (i.e. 2.5% acetone, 2.5% DMSO, 95% ready-to-use syrup). The water control consisted of 50% (w/w) aqueous syrup solution (50% tap water, 50% ready-to-use syrup). The reference item was solved in pure acetone (5%) and thereafter mixed with water (45%) and syrup (50%). The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 4 hours 20 minutes, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food.
The number of dead bees was recorded after 4 (± 0.5 h) hours (first day); 24, 48, 72 and 96 (± 2 h) hours. Behavioural abnormalities (e.g. vomiting, apathy, intensive cleaning) were assessed after 4 (±

0.5 h) hours (first day); 24, 48, 72 and 96 (± 2 h) hours. Temperature during the test was 25 °C; relative humidity was 50 - 75% for both tests. Bees were kept in darkness (except during observation).

Dates of experimental work: May 14, 2012 – May 19, 2012

Results:

Table CA 8.3.1.1- 1: Validity criteria

Validity Criteria		Recommended	Obtained
Control mortality	Contact Test		
	CO_2/water control	< 10%	2.0%
	CO_2/acetone/DMSO control:	< 10%	0.0%
	Oral Test		
	water/sugar syrup control	< 10%	0.0%
	Acetone/DMSO/ syrup control	< 10%	0.0%
LD_{50} of reference item (24 h)	Contact Test		
		0.10 - 0.30 µg a.s./bee	0.24 µg a.s./bee
	Oral Test		
		0.10 - 0.35 µg a.s./bee	0.10 µg a.s./bee

All validity criteria for the study were met.

Biological results:

Contact Test:

At the end of the contact toxicity test (96 hours after application), there was 2.0% mortality at 100.0 µg a.s./bee. Also 2.0% mortality occured in the water control group (water + 0.5% Adhäsit) and there was no mortality in the solvent control group (acetone + DMSO).

Oral Test:

In the oral toxicity test, the maximum nominal test level of mesosulfuron-methyl tech. (i.e. 100 µg a.s./bee) corresponded to an actual intake of 105.6 µg a.s./bee. This dose level led to no mortality after 96 hours.

Also no mortality occurred in the solvent control group (acetone + DMSO) and in the water control group (50% sugar solution), respectively.

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

Table CA 8.3.1.1- 2: Acute toxicity of mesosulfuron-methyl to honey bees; contact and oral laboratory test

Test Item	Mesosulfuron-methyl tech.	
Test Object	*Apis mellifera*	
Exposure	**contact (Acetone/DMSO solution)**	**oral (sugar/acetone/DMSO/water solution)**
Application rate µg a.s./bee	100.0	105.6
LD_{50} µg a.s./bee	> 100.0	> 105.6
LD_{20} µg a.s./bee	> 100.0	> 105.6
LD_{10} µg a.s./bee	> 100.0	> 105.6
NOED µg a.s./bee*	≥ 100.0	≥ 105.6

* 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 0.24 and 0.10 µg a.i./bee, respectively.

Conclusions:

The toxicity of mesosulfuron-methyl tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (96 h) was > 100.0 µg a.s./bee. The oral LD_{50} (96 h) was > 105.6 µg a.s./bee.

Report:	[redacted]t; [redacted];2014;M-485279-01
Title:	Mesosulfuron-methyl WG 75 W: Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No:	S13-01778
Document No:	M-485279-01-1
Guidelines:	**No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of VAN DER STEEN (2001) not specified**
GLP/GEP:	**yes**

Executive summary:

The contact toxicity of Mesosulfuron-methyl WG 75 W to the bumble bee (*Bombus terrestris* L.) was determined in a limit test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of [redacted] (2001). In the test item treatment group, no mortality and no sub-lethal effects were observed until the final assessment 48 hours after start of the experimental phase. The 48 hour contact LD_{50} value for Mesosulfuron-methyl WG 75 W was determined to be > 100 µg mesosulfuron-methyl a.s./bumble bee.

Material and methods:

Test item: Name: Mesosulfuron-methyl WG 75 W
TOX-No.: 09721-01
Batch ID: 2012-001670
Specification No.: 102000027087
Content of a.s.: 74.8 % w/w (analysed)

The contact toxicity of Mesosulfuron-methyl WG 75 W to the bumble bee (*Bombus terrestris* L.) was determined in a limit test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of [redacted] (2001).
In the laboratory, bumble bees were exposed to 100 µg mesosulfuron-methyl a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after application. The control group was exposed for the same period of time under identical conditions to tap water.

Dates of work: 06 November 2013 – 08 November 2013

Findings:

In the control group, treated with tap water, no mortality was observed during the 48 h test period.

In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

Table CA 8.3.1.1- 3: LD_{50} values in the bumble bee contact toxicity test with Mesosulfuron-methyl WG 75 W

Mesosulfuron-methyl WG 75 W	Contact toxicity test [µg a.s./bumble bee]
LD_{50} (24 h)	> 100
LD_{50} (48 h)	> 100

In the test item treatment group, no mortality and no sub-lethal effects were observed until the final assessment 48 hours after start of the experimental phase. Thus, it can be concluded that the topical application of Mesosulfuron-methyl WG 75 W on bumble bees at the treatment level of 100 µg mesosulfuron-methyl a.s./bumble bee, caused no adverse effects regarding mortality, sub-lethal effects and behaviour.

Conclusion:

The 48 hour contact LD_{50} value for Mesosulfuron-methyl WG 75 W was determined to be >100 µg mesosulfuron-methyl a.s./bumble bee.

CA 8.3.1.1.1 Acute oral toxicity

Study with mesosulfuron-methyl

Report:	[redacted]x; [redacted];1996;M-141738-01
Title:	Code: Hoe F130060 00 ZC96 0003 - Oral toxicity (LD 50) to honey bees (Apis mellifera L.)
Report No:	A58014
Document No:	M-141738-01-1
Guidelines:	**EPPO 170;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

72 h-LD_{50}-oral = 5.6 µg a.s./bee

It is proposed to replace this endpoint by the following endpoint derived from new study KCA 8.3.1.1/01:

96 h-LD_{50}-oral > 105.6 µg a.s./bee

For an indepth evaluation of the old and new findings according to the provisions of current guidelines and requirements, please refer to the explanative text at the introduction of Section CA 8.3.1.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted], 1996, 8.3.1.1.1/1.
- **Test guideline**: EPPO 170 (1992).
- **GLP compliance**: Yes.
- **Methods**: Lethal effect of AE F130060 (technical substance, purity = 96.0%) to bees were assessed through oral exposure. The test substance was added to diet paste at the rate of 0 (control), 0.0001, 0.001, 0.01, 0.1 and

1% (w/w), corresponding to 0, 0.0204, 0.2038, 1.4994, 15.808 and 184.81 microg a.s./bee. Each dose and the control was repeated 5 times on groups of 10 bees each. Mortality and behavioural abnormalities, such as lethargy and uncoordinated movements, were checked daily for a 72 hours test period.

- **Results**:
  Oral LD50 - 72 h = 5.6 microg a.s./bee

- **Comments (RMS):** no mortality among exposed bees occured during the first 48 hours. Therefore the LD50s at 24 and 48 h (> 184.81 microg/bee) are greater than the LD50 72h. An additional assessment after 96 hours would perhaps have lead to a lower LD50 value. The proposed endpoint will therefore be used. The study is acceptable.

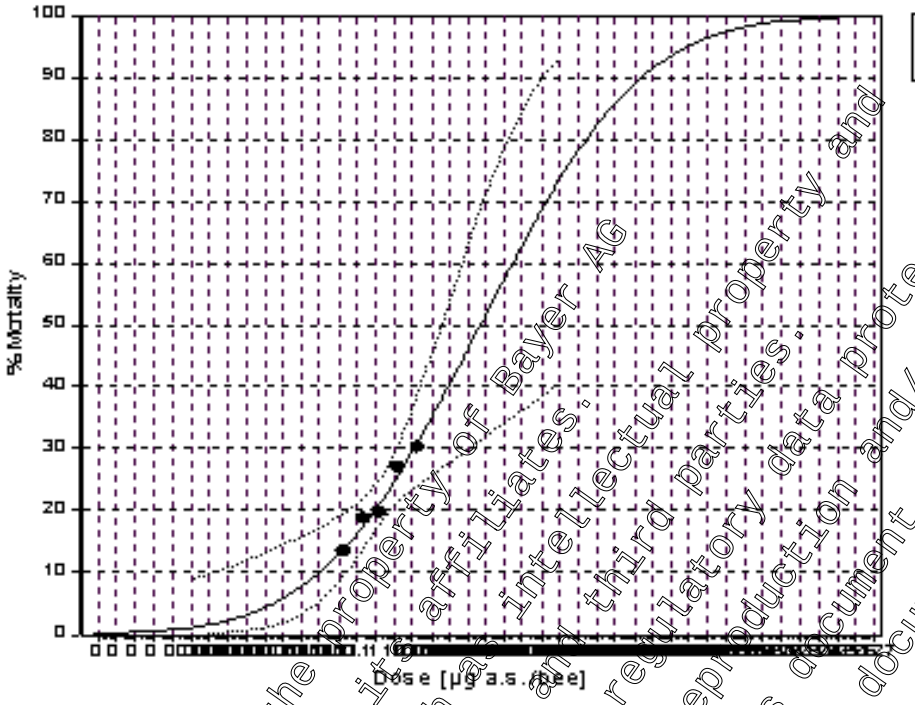
Further study information supplementing the original Monograph summary

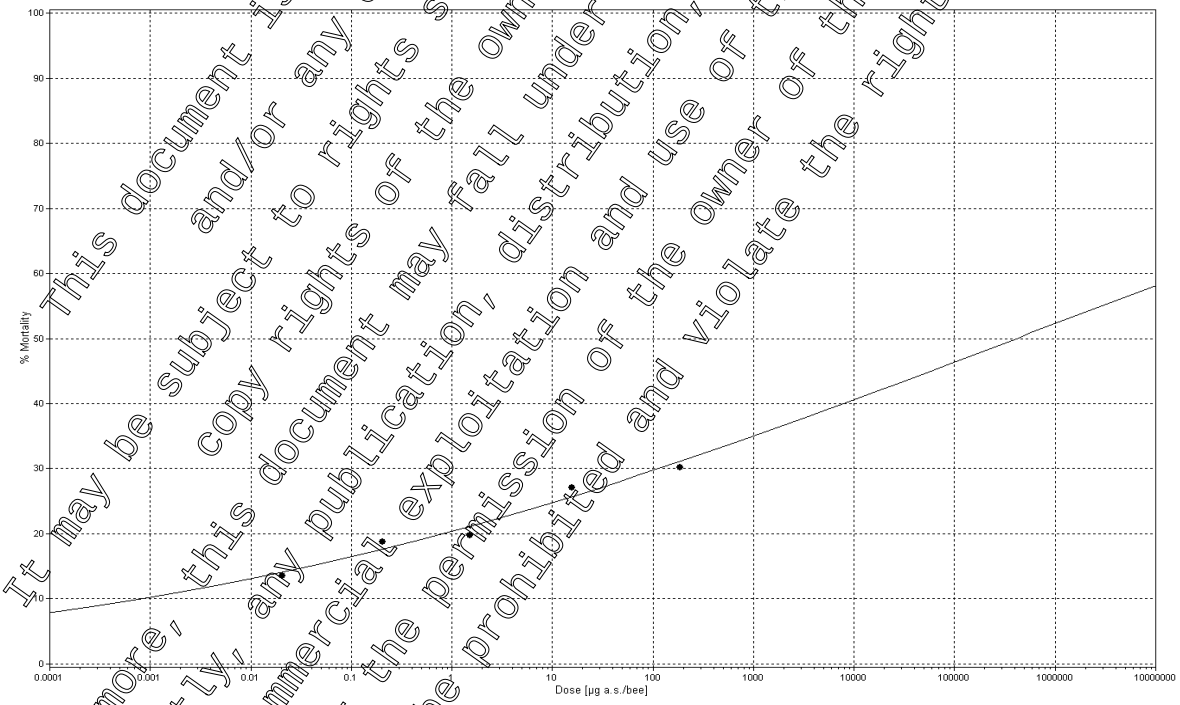
- **Results**:

1. Hoe 130060 00 ZC96 0002

% a.i. in diet	mean net food consumption per bee (g)	ingested dose per bee (µg a.i.)	% mortality after 24 h	48 h	72 h
Control	0.0244	--	0		4
0.0001	0.0204	0.0204	0	6	17
0.001	0.0204	0.2038	0	13	22
0.01	0.0150	1.4994	1	17	23
0.1	0.0158	15.8080	3	14	30
1.0	0.0185	184.8100	0	16	33

The 72-hour LD_{50} value of 5.6 µg a.s./bee as given in the study report and the DAR does not appear plausible when considering that even at the highest test dose of 184.81 µg a.s./bee a total mortality of 33% was observed, which is clearly below 50% mortality. The statistical analysis as presented in the report appears compromised and unsuitable. When entering the data into a currently available statistical program (i.e. Probit analysis with TSKRatPro Version 2.10) then an 72-hour LD_{50} value of greater than the 184.8 µg a.s./bee is derived. Therefore, the oral 72-hour LD_{50} value should be corrected and presented as >184.81 µg a.s./bee.





Validity Criteria:

The validity criterion of an average mortality for the total number of controls must not exceed 10 per cent at the end of the test, as described in OECD 213, was met. However, the validity criterion that the LD_{50} of the toxic standard meets the specified range as described in OECD 213 cannot be confirmed. In the current study Triazophos was used and an LD_{50} of 0.013µg a.s./bee was determined. OECD 213 defines dimethoate as preferred toxic standard but also allows the use of others.

A large number of deviations from the currently valid OECD guideline 213 were determined for this study and are described in detail in Table CA 8.3.1.1.1- 1 below. It is concluded that the validity of the study is no longer given.

Analytical findings:
The diet mix (containing the test item) was offered to the bees for 5 hours. After the end of this period the tubes were reweighed and the ingested substance quantity calculated (µg a.s. / bee).
The experiment was performed in a room at an average temperature of 26.5 – 28°C, a humidity of 50 - 76% and a lighting period of 16 hours light : 8 hours darkness.

Biological findings:

Table CA 8.3.1.1.1- 1: Acute oral LD50 values after up to 72 hours of exposure to the test and reference item

Time (hours)	Hoe 130060 00ZC96 0002 (treatment = mesosulfuron-methyl)	Hoe 002960 00 EC40 C661 (reference item = Trichlorphos 40EC)
	LD_{50} (95% fiducial limits) [µg/bee]	
24	> 184.81 µg a.s./bee	0.013 (0.0-0.028) µg prod./bee
48	> 184.81 µg a.s./bee	0.014 (0.001-0.028) µg prod./bee
72	> 184.81 µg a.s./bee	0.013 (---) µg prod./bee

Conclusions:

In a laboratory study to determine the effect of mesosulfuron-methyl (Hoe 130060 00 ZC96 0002) technical substance, on *Apis mellifera*, the 72 hour LD_{50} was determined to be > 184.81 µg a.s./bee.

Table CA 8.3.1.1.1- 2: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-141738-01-1 KCA 8.3.1.1.1 /01	EPPO No. 170 (1992)	OECD No. 213 (1998)	a) spacing factor b) toxic standard c) climatic conditions d) feeding time	a) The spacing factor should be at maximum 2 but was 10. b) Dimethoate should be used but was triazophos. c) Temperature should be 25±2°C but was 26.8-28°C. Relative humidity should be 50-70% but was 70-76%. Bees should be held in the dark, whereas a lightning time of 16 hours with fluorescent tubes (no further details available) is described in the report. d) Food should be offered for a period of 6 hours whereas it was only 5 hours

CA 8.3.1.1.2 Acute contact toxicity

Study with mesosulfuron-methyl

Report:	[redacted]u; [redacted];1997;M-143107-01
Title:	Code: Hoe 130060 00 ZC96 0002; identical to new AgrEvo code: AE F130060 00 ZC96 0002 - Contact toxicity (LD50) to honey bees (Apis mellifera L.)
Report No:	A59434
Document No:	M-143107-01-1
Guidelines:	**EPPO: 170; USEPA (=EPA): L 141-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

72 h-LD_{50}-contact ≥ 13 µg as/bee

It is proposed to replace this endpoint by the following endpoint derived from new study KCA 8.3.1.1/01 :

96 h-LD_{50}-contact > 100 µg as/bee

For an indepth evaluation of the old and new findings according to the provisions of current guidelines and requirements, please refer to the explanative text at the introduction of Section CA 8.3.1.

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted]1997, 8.3.1.1.2/1
- **Test guideline**: EPPO 170 (1992).
- **GLP compliance**: Yes.
- **Methods**: Lethal effect of AE F130060 00 (technical substance, purity = 95.0%) to bees were assessed through contact exposure. Bees were submitted to a topical application of the test substance, at an individual rate of 0 (control), 0.04, 0.08, 0.12, 0.16 and 0.2 microg/bee, prepared in acetone. Each dose and the control was repeated 5 times on groups of 10 bees each. Mortality and behavioral abnormalities, such as lethargy and discoordinated movements, were checked daily for a 72 hours test period.
- **Results**:
  Contact LD50 - 72 h ≥ 13 microg a.s./bee
- **Comments (RMS)**: The study is acceptable.

Further study information supplementing the original Monograph summary :

Validity Criteria:
No unforeseen circumstances were observed, which may have affected the quality or integrity of this study.

Analytical findings:
The experiment was performed in a room at a temperature of 26.2 - 27.5°C, and a humidity of 64 - 73%. During the experimental phase the test animals were kept under constant darkness. Observations were made under light.

Biological findings:

Table CA 8.3.1.1.2- 1: Acute contact LD50 values after exposure to test and reference item

Time (hours)	Hoe 130060 00ZC96 0002 (treatment = mesosulfuron-methyl)	Hoe 002960 00 EC40 0963 (reference item = Triazophos 40EC)
	LD_{50} (95% fiducial limits) [µg/bee]	
24	> 13.0 µg a.s./bee	0.250 (0.115-0.337) µg prod./bee
48	> 13.0 µg a.s./bee	0.249 (0.094-0.335) µg prod./bee
72	> 13.0 µg a.s./bee	0.242 (0.080-0.352) µg prod./bee

Conclusions:

In a laboratory study to determine the toxicity of mesosulfuron-methyl (Hoe 130060 00 ZC96 0002), technical substance, to *Apis mellifera*, the 72 hour LD_{50} was determined to be greater than 13.0 µg a.s./bee.

Table CA 8.3.1.1.2- 2: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study. Deviations conclusion about its reliability
M-143107-01-1 KCA 8.3.1.1.2 /01	EPPO No. 170 (1992)	OECD No. 214 (1998)	a) Toxic standard b) climatic conditions	a) Dry methoath should be used but was triazophos b) Temperature should be 25±2°C but was 26.2-28.5°C. Relative humidity should be 50-70% but was 64-83%.
	EPA- 540/9-85-002 (1985) Subdivision L Series 141-1, 1982	(not EU-relevant)	N/A	N/A

CA 8.3.1.2 Chronic toxicity to bees

Study with mesosulfuron-methyl

Report:	[redacted];2014;M-485655-01
Title:	Mesosulfuron-methyl (tech.) - Assessment of chronic effects to the honeybee, Apis mellifera L. in a 10 days continuous laboratory feeding limit test
Report No.:	S13-00143
Document No:	M-485655-01-1
Guidelines:	**not applicable;not applicable**
GLP/GEP:	**yes**

Executive summary:

The chronic effects of the test item mesosulfuron-methyl (tech.) on the honey bee, *Apis mellifera* L.,were assessed in a 10 days continuous feeding test in the laboratory. The continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item mesosulfuron-methyl (tech.) at the treatment level of 120 mg a.s./kg caused no adverse effect regarding mortality, sub-lethal effects and behaviour. No repellent effect of the test item at the treatment level of 120 mg a.s./kg was observed.

The NOEC for mortality was determined at the end of the test period to be 120 mg a.s./kg (nominal). The LC_{50} was determined to be >120 mg a.s./kg (nominal).

Material and methods:

Test item:	Name:	Mesosulfuron-methyl (tech.)
	Tox No.:	09287-01
	Origin Batch No.:	EFME000144
	Purity:	97.4 % w/w (analysed)

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

Samples of the application (feeding) solutions prepared freshly every day throughout the 10 days continuous feeding period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item.

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

Findings:

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

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

At 120 mg a.s./kg mesosulfuron-methyl (tech.), 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 mesosulfuron-methyl (tech.) at the treatment level of 120 mg a.s./kg was 48.5 µg a.s./bee, the corresponding average daily dose was therefore 4.85 µg a.s./bee.

The overall mean consumption of the aqueous sucrose 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 (40.2 mg/bee at 120 mg a.s./kg, compared to 38.4 mg/bee in the control group).

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

Table CA 8.3.1.2- 1: Mean consumption of application (feeding) solution, mean nominal intake of test item accumulated over all test days, average daily dose, cumulative mortality after ten days of continuous exposure (test end) as well as the LC_{50} and NOEC

Treatment Level	Control[1]	Mesosulfuron-methyl 120 mg a.s./kg (nominal)[2]
Cumulative mortality after ten days of continuous exposure [%]	0.0	0.0
Overall mean daily consumption of application (feeding) solution [mg/bee] [3]	38.4	40.2
Mean nominal intake accumulated over ten test days [µg a.s./bee/10 d]	-	48.48
Average daily dose (nominal) throughout ten days of continuous exposure [µg a.s./bee/d]	-	4.85
LC_{50}	> 120 mg a.s./kg (nominal)	
NOEC[4]	120 mg a.s./kg (nominal)	

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

Conclusions:

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

The overall mean daily consumption of the aqueous sucrose 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. Therefore, it can be concluded that there was no repellent effect of the test item at the treatment level of 120 mg a.s./kg.

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

The LC_{50} was determined to be > 120 mg a.s./kg (nominal).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	[redacted], 2013;M-465325-01
Title:	Mesosulfuron-methyl WG 75 - A honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, Apis mellifera L. (Hymenoptera: Apidae)
Report No:	20110174
Document No:	M-465325-01-1
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 the honey bee brood feeding study was to evaluate potential effects of Mesosulfuron-methyl WG 75 administered together with the herbicide safener Mefenpyr-diethyl WG 15 W on brood development and mortality of adult worker honey bees, *Apis mellifera*.
The test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.050 g formulated test item/L (=0.0375 g mesosulfuron-methyl/L) + 0.712 g formulated herbicide safener/L (0.1125 g mefenpyr-diethyl/L) per colony in summer 2012. No adverse effects on any endpoints assessed were observed (i.e. on the survival of adult bees, eggs, larvae and pupae, brood development, behaviour, colony strength and colony conditions). The method of investigating the development of the honey bee brood is based on the method of [redacted] *et al.* (1992).
The administration of Mesosulfuron-methyl WG 75 + the herbicide safener Mefenpyr-diethyl WG 15 W to honey colonies caused no adverse effects on honeybee colonies and brood development. No adverse effects on any endpoints assessed were observed (i.e. on the survival of adult bees, eggs, larvae and pupae, brood development, behaviour, colony strength and colony conditions).

Materials and Methods:

Test item: Mesosulfuron-methyl WG 75; Sample code: 12005180; Specification no.: 102000027087; Batch no.: 2012-001670; Sample description: A.12000302; CAS no.: 208465-21-8; Content of active ingredient (nominal): 750 g/kg; Content of active ingredient (analysed): 748 g/kg.

Herbicide safener: Mefenpyr-diethyl WG 15 W; Sample code: 12006070; Specification no.: 102000027139; Batch no.: 2012-002193; Sample description: A.12000401; CAS no.: 1322-93-6; Content of active ingredient (nominal): 150 g/kg; Content of active ingredient (analysed): 158 g/kg.

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.

Test condition:
Natural field conditions with two different periods:

- Pre-treatment phase: In general stable conditions with some rain; overall favourable conditions during bee activity on all days.
- Exposure phase: In general favourable conditions for bee activity with some rain.

Treatments:
Test item: 0.0375 g mesosulfuron-methyl a.i./L, corresponding to 0.050 g[4] Mesosulfuron-methyl WG 75 in 1 L 50% (w/v) aqueous sucrose solution.
Safener: 0.1125 g mefenpyr-diethyl a.i./L, corresponding to 0.712 g[1] Mefenpyr-diethyl WG 15 W in 1 L 50% (w/v) aqueous sucrose solution.
The test item and the herbicide safener were mixed and set up together.
Reference item: 0.75 g fenoxycarb a.s./L, corresponding to 3.0 g[5] Insegar® 25 WG in 1 L 50% (w/v) aqueous sucrose solution.

4 Calculation based on the analysed active ingredient

5 Calculation based on the nominal active ingredient

Control: Untreated 50% (w/v) aqueous sucrose solution, 1 L per colony.

The feeding solutions were prepared 3 hours before administration to the honey bee colonies (1 L per colony). Due to rainy weather and low flight activity of the honey bees, the treatments were administered in the afternoon simultaneously to all hives 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 criteria:
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 honey bees had taken place and thus the suitability of the test system to detect potential effects on the bee brood. The daily mean mortality of adult honey bees (11.2 bees/colony) and pupae (0.5 pupae/colony) 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 CA 8.3.1.3- 1: Effects of mesosulfuron-methyl WG 75 (+ mefenpyr-diethyl WG 15 W) on Honey bee mortality and honey bee brood development

Test item	Mesosulfuron-methyl WG 75 (+ Mefenpyr-diethyl WG 15 W)		
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 ± SD		
Pre-application(DAT -3 to 0)	22.8 ± 6.5	27.8 ± 11.7	31.0 ± 16.9
Post-application(DAT 1 to 21)	11.2 ± 0.9	12.0 ± 2.8	23.6 ± 7.4[a]
	Mean mortality of pupae/colony		
Pre-application(DAT -3 to 0)	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.3
Post-application(DAT 1 to 21)	0.5 ± 0.2	0.2 ± 0.2	34.8 ± 17.9[a]
	Mean values of brood development (eggs)		
Brood termination rate (%) at BFD 21 (DAT 20)	41.1 ± 33.2	14.7 ± 5.6	85.4 ± 10.9[b]
Brood index at BFD 21 (DAT 20)	3.0 ± 1.7	4.3 ± 0.2	0.7 ± 0.5
Compensation index at BFD 21 (DAT 20)	3.8 ± 0.7	4.4 ± 0.3	1.0 ± 0.8
	Mean values of brood development (young larvae)		
Brood termination rate (%) at BFD 21 (DAT 20)	7.7 ± 4.5	7.3 ± 4.2	43.9 ± 35.6[b]
Brood index at BFD 21 (DAT 20)	4.6 ± 0.2	4.6 ± 0.2	2.8 ± 1.7
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.1	4.7 ± 0.2	2.9 ± 1.8
	Mean values of brood development (old larvae)		
Brood termination rate (%) at BFD 21 (DAT 20)	5.0 ± 4.3	6.7 ± 5.5	51.8 ± 13.4[b]
Brood index at BFD 21 (DAT 20)	4.7 ± 0.2	4.7 ± 0.3	2.4 ± 0.7[c]
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.2	4.8 ± 0.2	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 Broodarea 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 31 bees per colony per day), indicating well adapted colonies. The overall daily mean bee mortality after application of all treatments was 11.2, 12.0 and 23.6 in the control, test item and reference item treatment, respectively. Only the reference item treatment was statistically

significantly greater when compared to the control. Furthermore, the mean mortality was statistically significantly increased 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, 0.2 and 34.8 in the control, test item and reference item treatment, respectively. Only the reference item treatment was statistically significantly greater when compared to the control. Furthermore, statistically significant increased mean pupae mortality was observed on DAT 10 to 21 (6.7 to 105 pupae per colony) in the reference item treatment. 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, 17767 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%, -8% and -27%, respectively and was at study termination 16617, 16267 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 increase of -13%, 11% and -41%, respectively. The brood nest in both the control and the test item treatment remained similar when compared to the pre-treatment values, whereas the reference item showed a distinct decrease when compared to the control and the pre-treatment assessment.

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%, 12% and 1%, respectively. Thus, stores remained similar in all treatments during the course of the study.

Brood termination rate

In the test item treatment, the brood termination was not statistically significantly different in all brood stages when compared to the control. In contrast, the reference item treatment was statistically significantly higher in all selected brood stages (eggs, young and old larvae) when compared to the control. The performance of the reference item treatment 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. 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 and was statistically significantly smaller for the old larvae.

Brood compensation index
Generally the brood compensation indices of all treatments 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. 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 and was statistically significantly smaller for the old larvae

Conclusions:

The administration of Mesosulfuron-methyl WG 75 + the herbicide safener Mefenpyr-diethyl WG 15 W to honey colonies caused no adverse effects on honey bee colonies and brood development. No adverse effects on any endpoints assessed were observed (i.e. on the survival of adult bees, eggs, larvae and pupae, brood development, behaviour, colony strength and colony conditions).

Report:	[redacted] 1; [redacted] ;2015;M-510060-01
Title:	Expert statement upon sponsor request - Mesosulfuron-methyl WG 75 - A honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, Apis mellifera L. (Hymenoptera: Apidae)
Report No:	20110174
Document No:	M-510060-01-1
Guidelines:	**not applicable;not applicable**
GLP/GEP:	**n.a.**

Executive summary:

In 2012, a honey bee brood feeding study ([redacted], 2013; M-465325-01-1; KCA 8.3.1.3/01) was performed with Mesosulfuron-methyl WG 75 to evaluate potential effects of Mesosulfuron-methyl WG 75 administered together with the herbicide safener Mefenpyr-diethyl WG 15 W on brood development and mortality of adult worker honey bees, *Apis mellifera*.
The present re-evaluation solely concerns statistical revisions of the electronically documented data gathered during digital brood assessments, i.e. brood termination rates, brood indices and brood compensation indices. Upon Sponsor request, only the subset of the data of selected eggs was inspected under exclusion of control colony replicate no. 3 (that exhibited comparatively high brood termination rates for selected eggs). The aim of the re-evaluation is to determine if the findings on statistically significant differences for the test item treatment would change when brood termination rate, brood index and compensation index are determined without considering the data from the control replicate performing comparatively weakest.

Material and Methods:

In order to re-assess potential treatment effects on brood development, specifically selected eggs, one of the three replicates of the control group (replicate no. 3) has been excluded from the original data subset in the present data re-evaluation. The rationale of this approach is that control replicate no. 3 exhibited the highest brood termination rate for eggs out of all control replicates (no. 1: 39.7%, no. 2: 8.6%, no 3: 75%).

Comparisons of treatment-specific brood termination rates with the control were performed for all consecutive BFD assessments using Fisher's exact tests (count data for each colony replicate).

Assumptions of population normality of the data were tested using the Shapiro-Wilk test and found being fulfilled throughout. Accordingly, brood indices and brood compensation indices of the test item and reference item treatments were analysed for individual BFD assessments using Welsh two sample t-tests in comparison to the control.

For the Fisher's exact test the test direction was one-sided greater and for t-tests the test direction was one-sided smaller. For all tests the significance level was α = 0.05.

All statistical calculations were carried out using R (R Development Core Team, 2012).

The Fisher's exact test used for assessing brood termination rates is based on count data (brood cells including selected eggs) pooled across colony replicates and can be considered relatively robust regarding unbalanced sample sizes.

For all two-sample comparisons regarding brood indices and brood compensation indices using t-tests, however, it has to be noted that statistical power is vastly limited with a sample size of N=2 in the control group. Therefore, the outcomes of these statistical tests should be treated conservatively.

Results:

All revised statistical results regarding brood development of selected eggs are presented in combination with the revised tables and figures. Only statistically significant differences as compared to the control are highlighted, as well as any result diverging from findings presented in the original final report ([redacted], 2013; M-465325-01-1; KCA 8.3.1.3/01).

Table CA 8.3.1.3- 2: Summary table of effects of Mesosulfuron-methyl WG 75 (+ Mefenpyr-diethyl WG 15 W) on honey bee brood development of selected eggs

Assessment	Control n = 2	Test Item n = 3	Reference Item n = 3
Brood termination rate (%) at BFD 21 (DAT 20)	24.2 ± 22.0	14.7 ± 5.6	85.4 ± 10.9*
Brood index at BFD 21 (DAT 20)	3.8 ± 1.1	4.3 ± 0.3	0.7 ± 0.5
Compensation index at BFD 21 (DAT 20)	4.0 ± 0.8	4.4 ± 0.3	1.0 ± 0.8**

* Statistically significantly greater as compared to the control (Fisher`s exact test; P<0.001).
** Statistically significantly smaller as compared to the control (Welsh two sample t-test; t = 4.0452, df = 2.275, P=0.023).

Table CA 8.3.1.3- 3: Brood termination rate (%) of selected eggs recorded during the study

DAT	Control					Test Item						Reference Item					
	Replicate					Replicate						Replicate					
	1	2	Mean	±	SD	1	2	3	Mean	±	SD	1	2	3	Mean	±	SD
6	26.6	6.2	16.4	±	14.4	7.5	11.5	7.5	8.8	±	2.3	36.0	64.8	40.1	47.0	±	15.6*
11	39.2	8.6	23.9	±	21.6	10.0	12.5	20.5	14.3	±	5.5	73.5	88.6	46.7	69.6	±	21.2*
15	39.7	8.6	24.2	±	22.0	10.5	12.5	21.0	14.7	±	5.6	76.5	97.6	82.2	85.4	±	10.9*
21	39.7	8.6	24.2	±	22.0	10.5	12.5	21.0	14.7	±	5.6	76.5	97.6	82.2	85.4	±	10.9*

* Statistically significantly greater as compared to the control (Fisher`s exact test; for all tests P<0.001).
The adjusted dataset had no impact on significant results.

Table CA 8.3.1.3- 4: Brood index of selected eggs

DAT	Control					Test Item					Reference Item				
	Replicate					Replicate					Replicate				
	1	2	Mean	±	SD	1	2	3	Mean	± SD	1	2	3	Mean	± SD
6	2.2	2.6	2.4	±	0.3	2.9	3.1	3.2	3.1	± 0.2	1.6	1.2	2.0	1.6	± 0.4
11	2.4	3.7	3.1	±	0.9	3.6	3.5	3.2	3.4	± 0.2	1.1	0.5	2.1	1.2	± 0.8
15	2.4	3.7	3.1	±	0.9	3.6	3.5	3.2	3.4	± 0.2	0.9	0.1	0.7	0.6	± 0.4
21	3.0	4.6	3.8	±	1.1	4.5	4.4	4.0	4.3	± 0.3	1.2	0.1	0.8	0.7	± 0.6

The adjusted dataset had no impact on significant results.

Table CA 8.3.1.3- 5: Brood compensation index of selected eggs

DAT	Control					Test Item					Reference Item				
	Replicate					Replicate					Replicate				
	1	2	Mean	±	SD	1	2	3	Mean	± SD	1	2	3	Mean	± SD
6	2.2	2.6	2.4	±	0.3	2.9	3.1	3.2	3.1	± 0.2	1.6	1.2	2.0	1.6	± 0.4
11	2.4	3.7	3.1	±	0.9	3.6	3.5	3.2	3.4	± 0.2	1.1	0.5	2.1	1.2	± 0.8
15	2.4	3.7	3.1	±	0.9	3.6	3.5	3.2	3.4	± 0.2	1.0	0.1	0.7	0.6	± 0.5
21	3.4	4.6	4.0	±	0.8	4.6	4.5	4.1	4.4	± 0.3	1.2	0.1	1.7	1.0	± 0.8**

** Statistically significantly smaller as compared to the control (Welsh two sample t-test; t = 4.0452, df = 2.275, *P*=0.023).

For the adjusted dataset statistically significant effects of the reference item were detected on DAT 21, but not anymore on DAT 15 (probably due to reduced power of the respective statistical test).

Report:	[redacted]; [redacted];2015;M-510267-01
Title:	Mesosulfuron-methyl WG 75 W: Effects on honey bee brood (Apis mellifera L.) under semi-field conditions - Tunnel test
Report No.:	87431033
Document No:	M-510267-01-1
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);none**
GLP/GEP:	**yes**

Executive summary:

A higher tier semi-field honey bee brood study (according to the provisions of the OECD Guidance Document 75) was conducted under forced/confined exposure conditions, by applying the rate (20.11 g product in 400 L tap water/ha corresponding to 15 g mesosulfuron-methyl/ha) of Mesosulfuron-methyl WG 75 (750 g/kg) under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia*.

The test was designed as a replicated tunnel study to assess potential effects of mesosulfuron-methyl to honey bee colonies, including a very detailed assessment of brood development. Tunnels (25 m length x 5.0 m width x 2.5 m height) were set up on a ca. 80 m² plot of *Phacelia tanacetifolia* (2 x 40 m²). Small bee colonies were introduced to the tunnels 4 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 crop) 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. Applications were conducted during daytime and during full flowering of the *Phacelia*-crop (BBCH 65), with confined honey bees actively foraging on the crop during application. After foliar (spray) application of the water (control), test item (Mesosulfuron-methyl WG 75 (750 g/kg)) and the reference item (fenoxycarb), 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 one ore more brood combs and taking a digital picture of the brood combs. After saving the file on a computer, 200 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 combs were 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). Statistical evaluation was done for mortality, foraging activity, colony strength, brood termination rate and brood indices using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student t- test (pairwise comparison) or Welch t- test (pairwise comparison, inhomogeneous variance).

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 Mesosulfuron-methyl WG 75 (750 g/kg) does not adversely affect honey bees and honey bee brood when applied at a rate of 20,11 g product in 400 L tap water/ha (corresponding to 15 g mesosulfuron-methyl/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 occurrence and extent, showed that the prevailing test conditions allowed for a profound detection of effects on immature honey bee life stages.

Material and Methods

Test Item:

Mesosulfuron-methyl WG 75 (750 g/kg); Short name: Mesosulfuron-methyl WG 75 W: mesosulfuron-methyl (AE F130060): 74.6 % w/w (746 g/kg) (analysed); Batch No.: 2014-004600; Sample Description: TOX10512-00; Specification No.: 102000027087.

Test Species:

Honey bees (*Apis mellifera carnica* L.); small bee colonies, maintained according to normal beekeeping practice, containing 9 combs with honey, pollen and brood. The preliminary brood check indicated healthy colonies with all brood stages present and a minimum reserve of food (uncontaminated nectar and pollen). The mean strength of the colonies per treatment group, one day before the application ranged between 7605 and 8066 adult bees per colony.

Test Design:

The test was conducted under forced/confined exposure conditions (tunnel), in order to assess potential effects of Mesosulfuron-methyl WG 75 (750 g/kg) to honey bee colonies including brood development under semi-field conditions. Tunnels (25 m length x 5.0 m width x 2.5 m height) were set up on a ca. 80 m² plot of *Phacelia tanacetifolia* (2 x 40 m²). Small bee colonies were introduced to the tunnels 4 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 crop) 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 one or more brood combs and taking a digital picture of the brood combs. After saving the file on a computer, 200 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: 3 days before to 27 days after application (= end of the trial);
- Behavioural abnormalities: 3 days before to 27 days after application (= end of the trial);
- Foraging activity of the bees: 3 days before to 4 days after application;
- Condition of the colonies (food stores, brood status and colony strength): 1 day before and 4, 9, 15, 21 and 27 days after application;
- Bee brood development (eggs): 1 day before (= BFD0) and 4 (= BFD 5), 9 (= BFD 10), 15 (= BFD 16), 21 (= BFD 22) days after the application.

Application Rates:

<u>Control:</u> 400 L tap water/ha,
<u>Test Item:</u> 15 g mesosulfuron-methyl a.s./ha via 400 L spray solution/ha; 20.11 g product in 400 L tap water/ha (corresponding to 0.050 g product/L),
<u>Reference Item:</u> 300 g fenoxycarb a.s. (1200 g product)/ha in 400 L spray solution/ha (corresponding to nominally 3.00 g product/L),

All applied during full flowering of the crop when honey bees were actively foraging on the Phacelia-crop.

Test Conditions:

Natural field conditions. On the application day, due to the warm and sunny weather, there was high honeybee foraging activity on the crop within the tunnels. Mean temperature during the whole experiment was between 16.3 and 27.5°C. First precipitation after application (1 mm) occurred on day 3 (ca. 72 hours following the application). Thereafter rain occurred on day 4 (27 mm), 5 (12 mm), 8 (1 mm), 12 (2 mm), 13 (16 mm), 17 (1 mm), 18 (22 mm), 19 (11 mm), 21 (3 mm), 23 (6 mm), 25 (7 mm) and 27 (4 mm).

Statistics:

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

Dates of work: July 14, 2014 - August 13, 2014

Results:

Mortality of the adult bees (worker bees)

Pre-application phase (day- 3 to day 0 before application):

Mortality of the pre-application phase in the control and test item group was 35.1 and 69.8 dead bees/colony/day, respectively. This was not statistically significantly different compared to the water control (Student t-test, pairwise comparison, two-sided, α = 0.05). The mortality in the reference item group was 75.6 dead bees/colony/day. This was statistically significantly different compared to the water control (Student t-test, pairwise comparison, two-sided, α = 0.05) but can be considered within normal mortality levels under a confined exposure scenario.

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

Mortality in the test item group was statistically significantly increased on day 0, 3 and 4 after the application (Student t-test, pairwise comparison, one-sided greater, α = 0.05). However, the overall comparison of the mean number of dead bees found on the strips and in the traps after the application from day 0 to day 4 did not show a statistical significant difference between the control and the Mesosulfuron-methyl WG 75 (750 g/kg) treatment group. Average control mortality of adult bees during the exposure phase (day 0 to day 4 following the application) was 53.8 dead bees/colony/day. The average mortality in the test item group was higher with 83.9 dead bees/colony/day but not statistically significant different to the control group (Student t-test, pairwise comparison, one-sided greater, α = 0.05). Reference Item mortality was 71.2 dead bees/colony/day (not statistically significantly different to the control, Student t-test, pairwise comparison one-sided greater, α = 0.05).

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 after the application from day 5 to day 27 did not show a statistical significant difference between the control and the Mesosulfuron-methyl WG 75 (750 g/kg) treatment group (Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$). A mean of 16.7 and 14.4 dead bees per day was found in the period from day 5 to day 27 after treatment in the control and test item group, respectively. Neither did the overall evaluation of the post-application period from day 0 to day 27 show a statistical significant difference between the control and the test item treatment (23.4 dead bees/colony/day in the control and 26.8 dead bees/colony/day in the test item group, respectively) (Student t-test, pairwise comparison, α = 0.05, one-sided greater).
Mortality in the reference item group was statistically significantly increased on day 11, 18 and 19 after the application (Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$). But overall the reference item showed no impact on the adult bee mortality.

Mortality of pupae

Pre-application phase (day -3 to day 0 before application):

During the pre-application phase only one dead pupa was found in the control group on day -3 and none in the test item and reference item group, respectively, resulting in a mean of 0.25 dead pupae/day/colony. There was no statistically significant difference between the treatment groups (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):

During the exposure phase in the tunnels in total 3 dead pupae were found in the control and the test item group, respectively. Mean pupae mortality during exposure phase in the control and test item group was both 0.15 dead pupae/day/colony. Accordingly, the test item group was not statistically significantly different to the control group (Student t-test, pairwise comparison, one-sided greater, α = 0.05). In the reference item group a total of 11 dead pupae and a mean of 0.55 dead pupae/day/colony was found (no statistically significant difference compared to the control group, Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$).

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

During the phase outside the tunnels in total 1 and 8 dead pupae were found in the control and the test item group, respectively. Mean pupae mortality from day 5 to day 27 was 0.09 dead pupae/colony/day in the test item group, which was statistically significantly different compared to the control group (0.01 dead pupae/colony/day) (Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$). The overall post-exposure pupae mortality resulted in total in 4 and 11 dead pupae for control and test item, and consequently in an overall mean pupae mortality from day 0 to day 27 of 0.04 and 0.10 dead pupae/colony/day in the control and test item group, respectively. This was not statistically significantly different to the control group (Student t-test, pairwise comparison, one-sided greater, α = 0.05). Pupae mortality was generally low for control and test item throughout this study. When considering that in the control as well as in the test item treatment group 0.15 pupae/day/colony were found during the exposure phase in the tunnels, the value of 0.10 dead pupae/day/colony in the test item group over the entire post-application period is low and must be considered as biological irrelevant. Pupae mortality in the reference item group was increased and statistically significant different to the control group. The reference item induced pupae mortality of in total 430 pupae, being

4.55 dead pupae/colony/day from day 5 to day 27 and 3.84 dead pupae/colony/day from day 0 to day 27 after the day of application. In both cases, this was statistically significantly different to the control group (Student t-test or Welch t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$).

Foraging Activity

Pre-application phase (day -3 to day 0 before application):

The mean foraging activity in the intended test item and reference item groups was comparable or even higher as the control group, resulting in overall daily mean values of 23.3, 26.3 and 24.8 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 item and reference item treatment groups at the overall daily mean comparison of this period (Student´s t-test, $\alpha = 0.05$, two-sided).

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

Overall, the mean foraging activity in the test item and reference item group from day 0 to day 4 after application were comparable to the control values on these days. The daily mean foraging activity from day 0 to day 4 were 19.3 bees/m²/day in the control group, 16.4 bees/m²/day in the test item group and 20.0 bees/m²/day in the reference item group, respectively. This was not statistically significantly different when compared to the control group (Student t-test or Welch t-test, pair-wise comparison, one-sided smaller, $\alpha = 0.05$).

Behavioural abnormalities

After application of Mesosulfuron-methyl WG 75 (750 g/kg) 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 queens and all brood stages (eggs, larvae and closed brood) were found in all colonies as an indication of healthy and queen right colonies. Moreover the amount of food reserves (uncontaminated nectar and pollen) was sufficient to ensure colony viability and brood status but also allowed that enough space was available for exposure of the brood to new food sources.
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.
In the 5 assessments performed after application, no indication of a test item related effect on the condition of the colonies was observed. All colonies exposed to the test item remained vital with increasing bee numbers and healthy brood. There was no indication of any effect 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 significantly (mean of 7605 to 8066 per colony). The subsequent development of the colony strength among the colonies in the control and test item treatment group followed the same pattern. In general, there was an overall increase of colony strength in the control and test item group observable throughout the course of the study. On the last colony strength assessment day +28 the increase in the test item group was even higher (133%) when

compared to the control group (115%). No statistically 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:

Treatment Group	Day -1[1]	Day +4	Day +9	Day +15	Day +21	Day 27
Control	100%	109%	114%	127%	118%	115%
Test Item	100%	116% (n.s.)	122% (n.s.)	125% (n.s.)	116% (n.s.)	133% (n.s.)
Reference Item	100%	115% (n.s.)	133% (n.s.)	127% (n.s.)	115% (n.s.)	118% (n.s.)

[1] in relation to the application
n.s. = not statistically significant to the control, Student t-test, α=0.05; pairwise; one-sided smaller.

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 31.5 % which was comparable to the control group (29.3 %). This slight increase of the Brood Termination Rate in the test item group was not statistically significantly different compared to the control group (Student t-test, pairwise comparison, one-sided greater, α = 0.05).
Treatment with the reference item Insegar (a.s.: fenoxycarb) caused a decrease of brood development of the marked eggs, resulting in a termination rate of 96.1 %. This decrease was statistically significantly different compared to the control group (Student t-test, pairwise comparison, one-sided greater, α = 0.05).

Brood Compensation Index:

The Brood Compensation Index is an indication for recovery and shows the development of the brood at each assessment. A continuous brood development was observed in the test item group as well as in the control group. The Brood Compensation Indices following the labelling of the egg stage up to day 21 after application (BFD+22) were either identical or slightly lower in the test item group compared to the control. Differences in the Brood Compensation Index between the test item and control were not statistically significant. At the end of the assessment period the Brood Compensation Index of the test item group was comparable to the control group (4.0 *vs* 4.2) and no statistical significant difference was detected. The higher brood termination rate of the marked cells after treatment with the reference item Insegar (a.s. fenoxycarb) is also reflected by the statistically significantly lower Brood Compensation Indices in the reference item group when compared to the control.

Treatment Group	BFD +5	BFD +10	BFD +16	BFD +22
Control	1.9	3.1	3.2	4.2
Test Item	1.9 (n.s.)	3.0 (n.s.)	3.1 (n.s.)	4.0 (n.s.)
Reference Item	0.2 (*)	0.6 (*)	1.5 (*)	3.0 (*)

n.s. = not statistically significant to the control, * = statistically significant to the control, Student t-test, α=0.05, pairwise, one-sided smaller

Brood Index:

The Brood Index as an additional indicator for the bee brood development facilitates a comparison between the different treatments. Following the labelling of the egg stage, the Brood Indices of the test item group were either identical or slightly lower compared to the control values. Differences in the Brood Index between the test item and control were not statistically significant. On the last assessment day (BFD+22) the Brood Index of 3.4 in the test item group was comparable to the control group (3.5). The higher brood termination rate of the marked cells after treatment with the reference item Insegar (a.s.: fenoxycarb) is also reflected by the statistically significantly lower Brood Indices in the reference item group when compared to the control.

Treatment Group	BFD +5	BFD +10	BFD +16	BFD +22
Control	1.8	2.8	2.8	3.5
Test Item	1.9 (n.s.)	2.8 (n.s.)	2.7 (n.s.)	3.4 (n.s.)
Reference Item	0.1 (*)	0.2 (*)	0.2 (*)	0.2 (*)

n.s. = not statistically significant to the control, * = statistically significant to the control, Student t-test, α=0.05, pairwise; one-sided smaller

Accordingly, no adverse effects of the test item on brood development have been observed throughout the study, following the labelling of the egg stage up to day 21 after application (BFD+22).

Table CA 8.3.1.3- 6: Effects of Mesosulfuron-methyl WG 75 (750 g/kg) on honey bees under semi-field conditions (Tunnel Test)

Parameter	Treatment group[1]		
	Control	**Test Item**	**Reference Item Insegar [0.3 kg a.s./ha]**
Mean mortality of worker bees / colony / day [n] during pre-application phase [2] exposure phase in the tunnels [2] phase outside the tunnels [3] overall after application	 35.1 ± 15.0 53.8 ± 27.0 16.7 ± 8.1 23.4 ± 19.3	 69.8 ± 21.5 (n.s.) 83.9 ± 51.6 (n.s.) 14.4 ± 7.6 (n.s.) 26.8 ± 34.3 (n.s.)	 75.6 ± 31.2 (*) 71.2 ± 44.0 (n.s.) 17.8 ± 13.1 (n.s.) 27.3 ± 29.3 (n.s.)
Total mortality of pupae [n] during pre-application phase [4] exposure phase in the tunnels [4] phase outside the tunnels [5] overall after application	 1 (0.25 ± 0.50) 3 (0.60 ± 0.89) 1 (0.04 ± 0.21) 4 (0.14 ± 0.45)	 0 (0.0 ± 0.0) 3 (0.60 ± 0.89) 8 (0.35 ± 0.65) 11 (0.39 ± 0.69)	 0 (0.0 ± 0.0) 11 (2.20 ± 1.92) 419 (18.22 ± 31.16) 430 (15.36 ± 28.83)
Daily mean mortality of pupae [n] during pre-application phase [6] exposure phase in the tunnels [6] phase outside the tunnels [7] overall after application	 0.06 ± 0.13 0.15 ± 0.22 0.01 ± 0.05 0.04 ± 0.11	 0.0 ± 0.0 (n.d.) 0.15 ± 0.22 (n.s.) 0.09 ± 0.16 (*B) 0.10 ± 0.17 (n.s.)	 0.0 ± 0.0 (n.d.) 0.55 ± 0.48 (n.s.) 4.55 ± 7.79 (*) 3.84 ± 7.21 (*)
Mean foraging activity / m² / colony / day [n] during pre-application phase exposure phase in the tunnels	 20.7 ± 2.8 19.3 ± 11.7	 21.9 ± 5.5 (n.s.) 16.4 ± 9.4 (n.s.)	 22.1 ± 5.3 (n.s.) 20.0 ± 11.8 (n.s.)
Mean brood termination rate [%] [8]	29.3	31.5 (n.s.)	96.1 (*)

[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] total number of dead pupae found in dead bee traps and on gauze strips in the tunnels (mean values and standard deviation)
[5] total number of dead pupae found in dead bee traps, only (mean values and standard deviation)
[6] mean number of dead pupae per day and colony found in dead bee traps and on gauze strips in the tunnels
[7] mean number of dead pupae per day and colony found in dead bee traps, only
[8] at BFD 22
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);
n.s. = not statistically significant compared to the control; * = statistically significant compared to the control,
n.d. = not determined
*B = statistically significant compared to the control but not biologically relevant

Conclusions:

To assess the potential effects of Mesosulfuron-methyl WG 75 (750 g/kg) on honey bee colonies including brood development, 20.11 g product in 400 L tap water/ha (corresponding to 15 g mesosulfuron-methyl/ha), tap water for the 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 Mesosulfuron-methyl WG 75 (750 g/kg) does not adversely affect honey bees and honey bee brood when applied at a rate of 20.11 g product in

400 L tap water/ha (corresponding to 15 g mesosulfuron-methyl/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 occurrence 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

Since the new representative formulation IMS+MSM+MPR OD 42 is a mixed-type product containing a second active substance, the tests on non-target arthropods other than bees with this formulation are clearly product related data and as such reported in document MCP.

Studies with the previous representative formulation containing mesosulfuron-methyl + safener mefenpyr-diethyl (MSM + MPR OD 120) are reported in the baseline dossier. These studies may be considered to allow for a general conclusion on the absence of relevant effects caused by the active substance mesosulfuron-methyl at its maximum intended use rate of 15 g a.s./ha. In the context of the present application for EU approval renewal of mesosulfuron-methyl, however, these studies are considered 'supportive information', not relevant for risk assessments.

Report:	[redacted]*; [redacted];1999;M-191378-01
Title:	Toxicity to the ground dwelling predator Pardosa spp. (laboratory) according to IOBC Guideline ([redacted] et al. 1998) Code: AE F130060 00 1K12 A703
Report No:	C005107
Document No:	M-191378-01-1
Guidelines:	**ESCORT Recommendation ([redacted] et al. 1994); IOBC: [redacted] et al. 1998;Deviation not specified**
GLP/GEP:	**yes**

Endpoints according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
0 % mortality at the highest concentration tested,
no adverse effects on feeding rate (15 g a.s./ha)

Note: In the context of application for EU approval renewal of mesosulfuron-methyl, these endpoints are ranked supportive information only. For the updated List of Endpoints, only the corresponding data for the new representative formulation IMS+MSM+MPR OD 42 will be considered.

Report:	[redacted]q; [redacted];2000;M-197173-01
Title:	Toxicity to the foliage dwelling predator Chrysoperla carnea Steph. (Neuroptera, Chrysopidae) in the laboratory AE F130060 + AE F107892 oil flowable 30 + 90 g/L Code: AE F130060 01 1K12 A703
Report No:	C200808
Document No:	M-197173-01-1
Guidelines:	**IOBC;;Deviation not specified**
GLP/GEP:	**yes**

Endpoints according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
-2 % mortality at the highest concentration tested, no adverse effects on reproduction (15 g a.s./ha)

Note: In the context of application for EU approval renewal of mesosulfuron-methyl, these endpoints are ranked supportive information only. For the updated List of Endpoints, only the corresponding data for the new representative formulation IMS+MSM+MPR OD 42 will be considered.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

For studies with the new representative formulation IMS+MSM+MPR OD 42 please refer to the MCP.

Report:	[redacted]p; [redacted];1999;M-194264-01
Title:	Effects of AE F130060 01 1K12 A703 on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Aphidiidae) in the laboratory Code: AE F130060 01 1K12 A703
Report No:	C006599
Document No:	M-194264-01-1
Guidelines:	**IOBC/WPRS: 1988;Deviation not specified**
GLP/GEP:	**yes**

Endpoints according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
17.5 % mortality at the highest concentration tested,
no adverse effects on reproduction (15 g a.s./ha)

Note: In the context of application for EU approval renewal of mesosulfuron-methyl, these endpoints are ranked supportive information only. For the updated List of Endpoints, only the corresponding data for the new representative formulation IMS+MSM+MPR OD 42 will be considered.

CA 8.3.2.2 Effects on Typhlodromus pyri

For studies with the new representative formulation IMS+MSM+MPR OD 42 please refer to the MCP.

Report:	[redacted]0; [redacted]1999;M-193755-01
Title:	Effects on the predatory mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory Code: AE F130060 01 1K12 A703
Report No:	C006605
Document No:	M-193755-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Endpoints according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
8.33 % mortality at the highest concentration tested,
no adverse effects on reproduction (15 g a.s./ha)

Note: In the context of application for EU approval renewal of mesosulfuron-methyl, these endpoints are ranked supportive information only. For the updated List of Endpoints, only the corresponding data for the new representative formulation IMS+MSM+MPR OD 42 will be considered.

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. Nevertheless, four acute studies (on the active substance and metabolites AE F154851, AE F160459 and AE F099095) are mentioned here as supportive information, since they are contained in the baseline dossier and in the List of Endpoints from the first EU review.

Mesosulfuron-methyl

Report:	[redacted]8; [redacted];1998;M-142933-01
Title:	Acute toxicity to earthworms (Eisenia fetida) AE F130060 substance, technical Code: AE F130060 00 1C95 0001
Report No:	A59244
Document No:	M-142933-01-1
Guidelines:	**EU (=EEC): 92/69; OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
14 d-LC_{50} > 1000 mg/kg

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked as supportive information, since acute earthworm testing is no longer a data requirement under Regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding chronic earthworm test.

AE F154851

Report:	[redacted]k; [redacted]; [redacted];2002;M-213390-01
Title:	Acute toxicity to earthworms (Eisenia fetida) Mesosulfuron (provisionally approved ISO) substance, pure metabolite of mesosulfuron-methyl (AE F130060) Code: AE F154851 00 1B96 0001
Report No:	C022594
Document No:	M-213390-01-1
Guidelines:	**EU (=EEC): 92/69/EWG; OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
14 d-LC_{50} > 1000 mg/kg

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked as supportive information, since acute earthworm testing is no longer a data requirement under Regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding chronic earthworm test.

AE F160459

Report:	[redacted]i; [redacted]; [redacted];2002;M-214487-01
Title:	Acute toxicity to earthworms (Eisenia fetida) AE F160459 substance, pure metabolite of mesosulfuron-methyl (AE F130060) Code: AE F160459 00 1B97 0001
Report No:	C023169
Document No:	M-214487-01-1
Guidelines:	**EU (=EEC): 92/69/EWG; OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
14 d-LC_{50} > 1000 mg/kg

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked as supportive information, since acute earthworm testing is no longer a data requirement under Regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding chronic earthworm test.

AE F099095

Report:	[redacted]§; [redacted]; [redacted];2002;M-213404-02; Amended: 2011-12-01
Title:	Acute toxicity to earthworms (Eisenia fetida) AE F099095; substance, pure; metabolite of mesosulfuron (AE F130060)
Report No:	CE02/031
Document No:	M-213404-02-1
Guidelines:	**EU (=EEC): 92/69/EWG; OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

14 d-LC_{50} > 1000 mg/kg

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked as supportive information, since acute earthworm testing is no longer a data requirement under Regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding chronic earthworm test.

CA 8.4.1 Earthworm, sub-lethal effects

For mesosulfuron-methyl and its metabolites AE F154851, AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, and AE F147447, reproductive toxicity studies on *Eisenia fetida* were performed. In these studies, no mortality occurred. No-Observable-Effect-levels ranged from 10 mg/kg dws for the metabolite AE F092944 to to 125 mg/kg dws for the parent compound. An overview of all studies is provided in the following table.

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

Test item	Test species, test design	Ecotoxicological endpoint	Reference
Mesosulfuron-methyl (tech.)	Eisenia fetida reproduction, 56 d (10% peat in test soil), test item sprayed on soil surface	NOEC ≥150 g a.s./ha	[redacted] et al. (2000) M-198271-01-1 KCA 8.4.1 /01
Mesosulfuron-methyl (tech.)	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **125 mg a.s./kg dws**	[redacted] (2010) M-392544-01-1 KCA 8.4.1 /02
AE F154851	*Eisenia fetida* reproduction, 56 d (5% peat in test soil), test item mixed into soil	NOEC **≥93.9 mg/kg dws***	[redacted] (2012) M-425013-01-1 KCA 8.4.1 /03
AE F160459	*Eisenia fetida* reproduction, 56 d (5% peat in test soil), test item mixed into soil	NOEC **90 mg/kg dws**	[redacted] (2012) M-429097-01-1 KCA 8.4.1 /04
AE F099095	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100 mg/kg dws**	[redacted] (2013) M-473217-01-1 KCA 8.4.1 /05
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 /06
AE F160460	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100 mg/kg dws**	[redacted] (2013) M-468911-01-1 KCA 8.4.1 /07
AE F140584	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥117 mg/kg dws**	[redacted] (2013) M-468921-01-1 KCA 8.4.1 /08
AE F147447	*Eisenia fetida* reproduction, 56 d (5% peat in test soil), test item mixed into soil	NOEC **90 mg/kg dws**	[redacted] (2012) M-428651-01-1 KCA 8.4.1 /09

dws = dry weight soil; a.s. = active substance
Bold letters: Values considered relevant for risk assessment in the MCP document
* corrected to an analysed purity of 93.9 %

Studies on mesosulfuron-methyl

Report:	[redacted]d;[redacted];[redacted];[redacted];2000;M-198271-01
Title:	Effects on growth and reproduction of earthworms (Eisenia fetida) AE F130060 substance technical Code: AE F130060 00 1C95 0001
Report No:	C009244
Document No(s):	M-198271-01-1
Guidelines:	**BBA: VI, 2-2 (1994);Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):

56 d-NOEC = 150 g a.s./ha

Note: This study was conducted as a limit test with spray application, using two dose levels of 1x and 10x the maximum areal field use rate of mesosulfuron-methyl. A new dose-response study with standard soil incorporation test design is as well available for mesosulfuron-methyl, and resulted in a definite endpoint value, see summarised as KCA 8.4.1/02 below. It is therefore proposed to update the List of Endpoints entry for chronic earthworm toxicity based on the results of this latter test:

$NOEC_{Reproduction}$ = 125 mg test item/kg dry weight artificial soil

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 2000a, KA 8.4.2/1.
- **Test guideline**: BBA VI 2-2 (1994).
- **GLP compliance**: Yes.
- **Methods**: The effects of AE F130060 (technical substance, purity = 92.0%) on the reproduction of earthworms was assessed in a 56 days laboratory test. The test was conducted with at least 2 months old earthworms, in 2.8 l glass jars (10 cm diameter, 19 cm height), containing 550 g of artificial soil, mixed with the test substance at the rate of 0 (control), 15 and 150 g a.s./ha (nominal). The test substance was prepared in water. Ten worms were randomly selected for control and each dose and each of them was repeated 4 times. Exposure of adults lasted for 28 days after which mortality, biomass and intoxication symptoms were recorded. The jars were hold 4 additional weeks in order to assess the possible effects of mesosulfuron-methyl on the development of offspring.
- A slight deviation in the ambient temperature (0.3-0.5°C) was recorded in the test involving the lowest dose, for about 30 hours between day 17 and day 53. This deviation is not expected to have induced bias in the results. For technical reasons, 4 test vessels were transferred to a segrated room for 7 days, which did not affect the ambient temperature over a 20±0.2°C range. Also this deviation is not expected to have induce bias in the results. Finally, the analysis of the stock solution used to prepare the lowest dose soil supports (15 g a.s./ha) revealed a recovery rate of 54.5%. The results recorded at this rate are consistent with the results recorded at a 10 fold higher rate.
- **Results**: There was no mortality among adult worms up to a rate of 150 g a.s./ha. No significant changes in feeding behaviour was recorded. A significant inhibitory effects (-3.7%) was recorded on the increase in body weight in worms exposed to the lowest dose (15 g a.s./ha). No effect on body weight was observed in worms exposed to the highest dose (150 g a.s./ha). The number of offspring was not influenced by the exposure to mesosulfuron-methyl.

  NOEC 56 days = 150 g a.s./ha (nominal dose).
- **Comments (RMS)**: The study is considered acceptable.

Report:	[redacted]n;[redacted];[redacted];2010;M-392544-01
Title:	Mesosulfuron-methyl - Reproduction toxicity to the earthworm Eisenia fetida in an artificial soil test
Report No:	10P30RR
Document No:	M-392544-01-1
Guidelines:	**OECD Guideline No. 222 for the Testing of Chemicals "Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)" adopted April 13, 2004; ISO Guideline 11268-2 "Soil quality - Effects of pollutants on earthworms (Eisenia fetida) Part 2: Determination of effects on reproduction" adopted July 1998.;The soil moisture was at the start of the test at one treatment and at the end of the test at five treatments slightly higher than required by the guideline. At the end of the test the pH-value was in one treatment 0.1 units higher than required by the guideline. However, study results of the test have not been impacted.**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine a NOEC/LOEC and/or an EC$_{50}$ for the effects of mesosulfuron-methyl (AE F130060) on the reproduction (56 days after application), mortality and the biomass development (28 days after application) of the earthworm *Eisenia fetida* (Lumbricidae) by dermal and alimentary uptake using a standardised artificial soil. The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). As deviation from the guideline the soil moisture was at the start of the test at one treatment and at the end of the test at five treatments slightly higher than required by the guideline. At the end of the test the pH-value was in one treatment 0.1 units higher than required by the guideline. However, study results of the test have not been impacted.
Ten *Eisenia fetida* (clitellate adults) per replicate (8 for the control, 4 per test item concentration) were exposed in artificial soil (with 10 % peat content) to an untreated deionised water control and to the test item for 28 days at nominal concentrations of 63, 125, 250, 500 and 1000 mg a.s./ kg dry weight artificial soil. After 28 days of exposure, the adult worms were removed and the cocoons produced by these animals were kept for a further 28 days in the treated artificial soil. Mortality, biomass and morphological and/or behavioural changes of the adult worms were assessed after 28 days. The number of juvenile earthworms was assessed after 56 days.
Based on the biological and statistical significance observed on biomass and reproduction, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 125 mg test item/ kg dry weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 250 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: mesosulfuron-methyl; Batch code: AE F130060-01-02; Origin batch No.: EFME000144; Sample description: TOX 08878-00; Specification No.: 102000013204; purity: 97.4 % w/w; Certificate of analysis No.: AZ 16385.

Ten *Eisenia fetida* (clitellate adults) per replicate (8 for the control, 4 per test item concentration) were exposed for 28 days in artificial soil (with 10 % peat content) to an untreated deionised water control and to mesosulfuron-methyl at nominal concentrations of 63, 125, 250, 500 and 1000 mg test item/kg artificial soil dry weight at 19.1 - 21.0°C and 428 - 788 lx. After 28 days of exposure, the adult worms were removed and the cocoons produced by these animals were kept for a further 28 days in the treated artificial soil. At the end of the test period (i.e. after 56 days) the juvenile worms hatched from

these cocoons were extracted from the artificial soil. Mortality, biomass and morphological and/or behavioural changes of the adult worms were assessed after 28 days. The number of juvenile earthworms was assessed after 56 days.
As deviation from the guideline the soil moisture was at the start of the test at one treatment and at the end of the test at five treatments slightly higher than required by the guideline. At the end of the test the pH-value was in one treatment 0.1 units higher than required by the guideline. However, study results of the test have not been impacted.

Toxic standard: Carbendazim (Derosal 360g/L SC): 1.0, 3.0 and 5.0 mg a.s./kg dry weight artificial soil, control: artificial soil moistened with deionised water, solvent control: none.

Dates of experimental work: July 20, 2010 – September 16, 2010

Results:

Table CA 8.4.1- 2: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	1.25 %
Mean (± sd) number of juveniles per replicate in the control	≥ 30	420.1±69.6
Coefficient of variation for the number of juveniles in the control	≤ 30 %	16.6 %

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

In a separate toxic standard reference study (ECT Study No.: IRR1007, performed from July 28 to September 22, 2010), the $NOEC_{Reproduction}$ value for carbendazim was 1.0 mg a.s./kg dry weight artificial soil. The $LOEC_{Reproduction}$ value for Carbendazim tested as a reference item was 3.0 mg a.s./kg dry weight artificial soil. Significant reduction in reproduction compared to the control was found at the concentrations of 3 and 5 mg a.s./kg dry weight artificial soil. The observed effect is within the expected range from literature. The effects of carbendazim confirm suitable sensitivity of the test system.

Table CA 8.4.1- 3: Effects on mortality, biomass and reproduction on *Eisenia fetida*

Concentration [mg test item/kg soil d.w.]	Adult mortality [%]	Biomass* [% of initial weight]	Number of juveniles** [% of control]
Control	1.25	154.8	100.0
63	0.0	159.5	89.9
125	0.0	149.6	92.3
250	2.5	153.8	83.8#
500	0.0	154.7	83.1#
1000	0.0	150.2	82.6#
LC_{50}/EC_{50} [mg test item/kg soil d.w.]	-	-	-
NOEC [mg test item/kg soil d.w.]	-	≥ 1000	125
LOEC [mg test item/kg soil d.w.]	-	> 1000	250

- not applicable; * After 28 days of exposure; ** After 56 days of exposure; # Significantly different to control (Williams t-test; 1-sided, $p < 0.05$)

Mortality:

At the control 1.25% mortality was observed. No mortality was observed at all concentrations of the test item tested except in the concentration of 250 mg test item/kg dry weight artificial soil in which 2.5% mortality was determined. No statistical analysis was performed.

Biomass:
Statistical analysis showed no significant difference (Williams t-test; 2-sided, $p < 0.05$) concerning the biomass of the adult worms after 28 days in all concentrations of the test item tested compared to the control.
Therefore, the $NOEC_{Biomass}$ was considered to be > 1000 mg test item/kg dry weight artificial soil. The $LOEC_{Biomass}$ could not be determined and was regarded as > 1000 mg test item/kg dry weight artificial soil.

Reproduction:
Concerning the number of juveniles statistical analysis (Williams t-test; 1-sided, $p < 0.05$) showed a significant difference between the control and the concentrations of 250, 500 and 1000 mg test item/kg soil (dw).
Therefore, the $NOEC_{Reproduction}$. was determined as 125 mg test item/kg dry weight artificial soil and accordingly the $LOEC_{Reproduction}$ was determined as 250 mg test item/kg dry weight artificial soil.

Conclusions:

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

Studies on the metabolites of mesosulfuron-methyl

AE F154851

Report:	[redacted];2012;M-425013-01
Title:	AE F154851: Reproduction toxicity to the earthworm Eisenia fetida in an artificial soil test
Report No:	1-P33RR
Document No:	M-425013-01-1
Guidelines:	**OECD Guideline No. 222 for the Testing of Chemicals "Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)" adopted April 13, 2004; ISO Guideline 11268-2 "Soil quality - Effects of pollutants on earthworms (Eisenia fetida) Part 2: Determination of effects on reproduction" adopted July 1998; none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine effects of the test item AE F154851 (metabolite of mesosulfuron-methyl) on the reproduction (56 days after application) of the earthworm *Eisenia fetida* (Lumbricidae) by dermal and alimentary uptake using a standardised artificial soil. The test was performed as a limit test according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).
Ten *Eisenia fetida* (adult worms with clithellum) per replicate (8 for the control, 8 for each test item concentration) were exposed in artificial soil (with 5 % peat content) to the test item at the concentration of 100 mg test item/kg dry weight artificial soil. The test item was applied once at the

beginning of the test. The duration of the test period (exposure of earthworms to the artificial soil containing the test item) was 56 days. The adult worms were removed from the substrate after 28 days. After 28 days mortality and biomass were determined. After 56 days reproduction was determined.
The $NOEC_{Biomass}$ and the $NOEC_{Reproduction}$ were demonstrated to be ≥ 100 mg test item/kg dry weight artificial soil and accordingly the $LOEC_{Biomass}$ and the $LOEC_{Reproduction}$ can be considered as > 100 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 F154851; batch code: AE F154851 00 1B96 0001; origin batch No.: LOR 21029; Certificate of Analysis No.: AZ 16603; purity: 93.9 % w/w.

Ten adults worms *Eisenia fetida* (with clitellum, fresh weight between 250 and 600 mg) per replicate (8 for the control, 8 for each test item concentration) were exposed in artificial soil (with 5 % peat content) to AE F154851 at the concentration of 100 mg test item/kg artificial soil dry weight. The test item was applied once at the beginning of the test. The duration of the test period (exposure of earthworms to the artificial soil containing the test item) was 56 days. The adult worms were removed from the substrate after 28 days. After 28 days mortality and biomass were determined. After 56 days reproduction was determined.
Temperature during the test ranged between 18.1 and 21.4°C (rec. 20 ± 2 °C); the moisture content ranged from 54.4 – 56.0% (study initiation) to 52.4 – 56.7% (study termination); the pH value of the test substrate was 6.3 – 6.4 at test initiation and 6.6 at experimental termination; the photoperiod was 16 hours light : 8 hours dark and the light intensity was between recommended 400 – 800 Lux.

Toxic standard: Carbendazim (Derosal 360 g/L SC): 1.0, 3.0 and 5.0 mg a.s./kg artificial soil dry weight, control: artificial soil, solvent control: none

Dates of experimental work: October 25, 2011 – December 22, 2011

Results:

Table CA 8.4.1- 4: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	1.25 %
Mean (±SD) number of juveniles per replicate in the control	≥ 30	216.0±32.8
Coefficient of variation for the number of juveniles in the control	≤ 30 %	15.2 %

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

In a separate toxic standard reference study (ECT Study No.: IRR1105, performed from May 18 to July 13, 2011), The $LOEC_{Reproduction}$ value for carbendazim tested as a reference item was 1.0 mg a.s./kg artificial soil dry weight. The observed effect is within the expected range from the guideline (1-5 mg Carbendazim/kg soil d.w.) and hence acceptable sensitivity of the test system is assured.

Table CA 8.4.1- 5: Effects of AE F154851 on mortality, biomass and reproduction on *Eisenia fetida*

Concentration [mg test item/kg soil d.w.]	Adult mortality [%]	Biomass* [% of initial weight]	Number of juveniles* [% of control]
Control	1.25	163.0	100.0
100	0.0	167.3	81.5
NOEC [mg test item/kg soil d.w.]	-	≥ 100	≥ 100
LOEC [mg test item/kg soil d.w.]	-	> 100	> 100

In the control 1.25% mortality was observed. No mortality was observed at the only concentration of the test item.

Statistical analysis showed no significant difference concerning biomass development of individual adults over 28 days (Student-t test, 2-sided; $p \leq 0.05$) and concerning the number of juveniles after 56 days between the control and the only concentration of the test item tested.

Conclusions:

The $NOEC_{Biomass}$ and the $NOEC_{Reproduction}$ were demonstrated to be ≥ 100 mg test item/kg artificial soil dry weight and accordingly the $LOEC_{Biomass}$ and the $LOEC_{Reproduction}$ can be considered as > 100 mg test item/kg artificial soil dry weight.

AE F160459

Report:	[redacted]ö;[redacted];[redacted];2012;M-429097-01
Title:	AE F160459: Reproduction toxicity to the earthworm Eisenia fetida in an artificial soil test
Report No:	11P32RR
Document No:	M-429097-01-1
Guidelines:	**the OECD Guideline No. 222 for the Testing of Chemicals "Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)" adopted April 13, 2004; ISO Guideline 11268-2 "Soil quality - Effects of pollutants on earthworms (Eisenia fetida) Part 2: Determination of effects on reproduction" adopted July 1998;At few short time intervals the temperature dropped down to 17.2°C in the second test run and was therefore, slightly below the range required by the guideline. However, study results of the test have not been impacted.**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine effects of AE F160459 (metabolite of mesosulfuron-methyl) on reproduction (56 days after application) of the earthworm *Eisenia fetida* (Lumbricidae) by dermal and alimentary uptake using a standardised artificial soil. The study was performed as a limit test (1st test run) and a full dose-response test (2nd test run) according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). As deviation from the guideline the temperature was slightly below the range required by the guideline at few short time intervals in the second test run. However, the study results of the test have not been impacted.

In the first run adult *Eisenia fetida* (8 × 10 replicates for each treatment level) were exposed in artificial soil (with 5 % peat content) to the concentration of 100 mg test item/kg artificial soil dry weight.

In the second run adult *Eisenia fetida* (8 × 10 replicates for the control group and 4 × 10 replicates for the treatment groups) were exposed in artificial soil (with 5 % peat content) to the concentrations of 9,

16, 28, 51 and 90 mg test item/ kg dry weight artificial soil. The test item was mixed into the soil at the beginning of each test run. Mortality and biomass was determined after 28 days. After 56 days, reproduction was determined.
Considering both test runs together, the No-Observed-Effect-Concentration (NOEC) for reproduction was 90 mg test item/ kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction could be assumed to be 100 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 F160459; Batch code: AE F160459 00 1B96 0001; Origin batch No.: 2ER0125; Certificate of analysis No.: AZ 16306; Purity: 95.4 % w/w.

Adult earthworms (*Eisenia fetida*, with clitellum, fresh weight between 250 and 600 mg, at least 2 months old) were exposed in an artificial soil in two test runs.
In the first run adults of *Eisenia fetida* (8 × 10 replicates for the control group and 8 × 10 replicates for the treatment group) were exposed in artificial soil (with 5 % peat content) to AE F160459 at the concentration of 100 mg test item/kg artificial soil dry weight. Temperature during the first test run ranged between 18.1 and 21.4°C (rec. 20 ± 2 °C); the moisture content ranged from 53.8 – 54.6% (study initiation) to 55.4 – 57.3% (study termination); the pH value of the test substrate was 6.1 – 6.4 at test initiation and was 6.5 at experimental termination; the photoperiod was 16 hours light : 8 hours dark and the light intensity was between recommended 400 – 800 Lux.
In the first test run statistically significant effects on the number of juveniles have been observed and it could not be demonstrated that the NOEC for reproduction is greater than the limit concentration. Therefore, a second test run was performed using five lower concentrations.
In the second run adult *Eisenia fetida* (8 × 10 replicates for the control group and 4 × 10 replicates for the treatment groups) were exposed in artificial soil (with 5 % peat content) to AE F160459 at the concentrations of 9, 16, 28, 51 and 90 mg test item/ kg dry weight artificial soil. Temperature during the second test run ranged between 17.2 and 21.4°C (rec. 20 ± 2 °C); the moisture content ranged from 53.9 – 56.7% (study initiation) to 52.3 – 62.9% (study termination); the pH value of the test substrate was 6.1 – 6.5 at test initiation and 6.9 – 7.0 at experimental termination; the photoperiod was 16 hours light : 8 hours dark and the light intensity was between recommended 400 – 800 Lux.
The test item was applied once at the beginning of each test run. The duration of the test period (exposure of earthworms to the artificial soil containing the test item) was 56 days. The adult worms were removed from the substrate after 28 days. After 28 days mortality and biomass were determined. After 56 days reproduction was determined.
As deviation from the guideline the temperature dropped down to 17.2°C at few short time intervals in the second test run and was therefore, slightly below the range required by the guideline. However, study results of the test have not been impacted.

Toxic standard: Carbendazim (Derosal 360 g/L SC): 1.0, 3.0 and 5.0 mg a.s./kg dry weight artificial soil, control: artificial soil, solvent control: none.

Dates of exposure period: October 25, 2011 – December 20, 2011 (first run)
January 17, 2012 – March 13, 2012 (second run)

Results:

Table CA 8.4.1- 6: Validity criteria

Validity criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mortality of adults in the control	≤ 10 %	2.5 %	0 %
Mean number of juveniles in the control (± standard deviation)	≥ 30	136.4 ± 28.5	178.6 ± 42.8
Coefficient of variance for the number of juveniles in the control	≤ 30 %	20.9 %	23.8 %

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

In a separate toxic standard reference study (ECT Study No.: IRR1105, performed from May 18 - July 13, 2011), the $LOEC_{Reproduction}$ value for carbendazim was determined as 1.0 mg/kg artificial soil dry weight. The $NOEC_{Reproduction}$ was considered as < 1.0 mg/kg artificial soil dry weight. These observed effects are within the range expected from the guideline (1-5 mg carbendazim/kg artificial soil dry weight) and hence acceptable sensitivity of the test system is assured.

First test run:
2.5 % mortality was observed in the control and at the limit concentration of 100 mg test item/kg dry weight artificial soil.

No statistically significant difference (Student-t test; 2-sided, $p \leq 0.05$) concerning biomass development of individual adults over 28 days between the control and the limit concentration of the test item was determined.

Statistical analysis (Student-t test; 1-sided, $p \leq 0.05$) showed a significant difference concerning the number of juveniles between the control and the limit concentration of the test item.

Therefore, with this first test run it could not be demonstrated that the NOEC for reproduction is greater than the limit concentration of 100 mg test item/kg dry weight artificial soil.

Table CA 8.4.1- 7: First test run: Effects of AE F160459 on mortality, biomass and reproduction of *Eisenia fetida*

Concentration [mg test item/kg soil d.w.]	Adult mortality [%]	Biomass [% of initial weight]	Number of juveniles [% of control]
Control	2.5	160.7	100.0
100	2.5	164.3	77.9#

significantly different to control (Student-t test; 1-sided, $p \leq 0.05$)

Second test run:
No mortality was observed in the control and in the concentrations of 16, 28 and 90 mg test item/ kg dry weight artificial soil and 2.5 % mortality at the concentrations of 9 and 51 mg test item/ kg dry weight artificial soil.

No statistically significant differences (Williams-t test; 2-sided, $p \leq 0.05$) concerning the biomass development of individual adults after 28 days were determined between the control and all concentrations of the test item tested.

Statistical analysis (Welch-t test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the number of juveniles between the control and all concentrations of the test item tested.

Therefore, it could be demonstrated that the NOEC for reproduction is greater than the highest concentration (90 mg test item/kg dry weight artificial soil) of the test item tested in this second test run.

Table CA 8.4.1- 8: Second test run: Effects of AE F160459 on mortality, biomass and reproduction of *Eisenia fetida*

Concentration [mg test item/kg soil d.w.]	Adult mortality [%]	Biomass [% of initial weight]	Number of juveniles [% of control]
Control	0.0	178.6	100.0
9	2.5	174.1	98.3
16	0.0	179.3	92.4
28	0.0	181.0	97.0
51	2.5	177.4	95.5
90	0.0	180.9	99.0

Conclusions:

If considering both test runs together it can be concluded that the $NOEC_{Reproduction}$ is 90 mg test item/kg dry weight artificial soil and accordingly the $LOEC_{Reproduction}$ could be assumed to be 100 mg test item/kg dry weight artificial soil.

AE F099095

Report:	[redacted];2013;M-473217-01
Title:	AE F099095 (BCS-AB40283): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	kra/Rg-R-158/13
Document No:	M-473217-01-1
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 F099095 (metabolite of mesosulfuron-methyl) on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with five different test concentrations.
Adult *Eisenia fetida* (approx. 8 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentrations of 10, 18, 32, 56 and 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 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 ≥ 100 mg test item/ kg dry weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be

> 100 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 F099095; BCS code: BCS-AB40283; Batch code: AE F099095 00 1B98 0001; LIMS No.: 1035243; Origin Batch No. 2ER0131; purity: 97.7 %w/w.

Adult *Eisenia fetida* (approx. 8 months old, 8 × 10 replicates for the control group and 4 × 10 replicates per test concentration of the treatment group) were exposed in an artificial soil (with 10 % peat content) to the nominal test concentrations of 10, 18, 32, 56 and 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 dry weight artificial soil (corresponds to 3.94 – 7.89 – 15.78 mg test item/kg dry weight artificial soil); control: quartz sand, solvent control: none.

Dates of experimental work: September 04, 2013 – November 08, 2013

Results:

Table CA 8.4.1- 9: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	0 %
Mean number of juveniles in the control	≥ 30	270, 232, 253, 236, 202, 202, 231, 236
Coefficient of variance for the number of juveniles in the control	≤ 30 %	9.9%

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

In a most recent 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 CA 8.4.1- 10: Effects of AE F099095 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***					
Test item	**Control**	**AE F099095 (BCS-AB40283)**				
mg test item/kg dry weight artificial soil	---	10	18	32	56	100
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	27.55	30.60	28.97	34.64	24.74	32.20
Standard Deviation	5.07	6.99	3.30	4.29	6.70	4.22
Mean number of offspring per test vessel after 56 days **	232.8	206.5	203.0	189.5	215.8	214.8
Standard Deviation	23.0	19.3	36.6	35.7	35.9	12.7
Coefficient of variance (%)	9.9	9.4	18.0	18.8	16.7	5.9
% of control	---	88.7	87.2	81.4	92.7	92.3

	Reproduction
NOEC (mg test item/kg dry weight soil)	**≥ 100**
EC_{10} (mg test item/kg dry weight soil [1)]) (95% confidence limits)	**n. d.**
EC_{20} (mg test item/kg dry weight soil [1)]) (95% confidence limits)	**n. d.**

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

** no statistical significance compared to the control (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05)

1) Probit analysis

n. d. not determined due to mathematical reasons

Mortality:

After 28 days of exposure no worm died in the control group 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 not observed.

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

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 values for the number of juveniles per test vessel relative to the control were observed.

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

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.

AE F092944

Report:	[redacted]n; [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 determine effects of AE F092944 (metabolite of mesosulfuron-methyl) on survival, growth and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the 1st run and 5 different test concentrations in the 2nd run.

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 × 10 replicates for control and treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal concentration of 100 mg test item/kg artificial soil dry weight.

In the second run adult *Eisenia fetida* (approx. 5 months old, 8 × 10 replicates for the control group and 4 × 10 replicates per test concentration of the treatment group) were exposed in artificial soil (with 10 % peat content) to nominal 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 of the effects 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; LIMS No.: 1034970; Content of a.s. analysed: 99.8 %w/w; Certificate No.: AZ 17077.

In the 1st test run adult *Eisenia fetida* (approx. 6 months old, 8 × 10 replicates for control and treatment group) were exposed in an artificial soil (10 % peat content) to the nominal test concentration of 100 mg test item/kg dry weight artificial soil.
In the 2nd test run adult *Eisenia fetida* (approx. 5 months old, 8 × 10 replicates for the control group and 4 × 10 replicates per test concentration of the treatment group) were exposed in an artificial soil (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, 2013 (first run)
April 12, 2013 – June 14, 2013 (second run)

Results:

Table CA 8.4.1- 11: 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 study (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} values for reproduction were calculated to be 3.06, 3.22 or 3.54 mg a.s./kg dry weight artificial soil, respectively. Confidence limits (95 %) could not be calculated. The results of the reference test indicated that the test system was sensitive to the reference test item.

Table CA 8.4.1- 12: Effects of AE F092944 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	39.40*	13.17	6.14*	9.75	17.29	14.30	12.72
Standard Deviation	4.05	7.13	3.31	6.27	2.82	5.52	8.29	2.81
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
NOEC (mg test item/kg dry weight soil)	**10**
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 mult. sequent. t-test, two-sided, $\alpha = 0.05$)
** statistical significance compared to the control (1st run: Student t-test; 2nd run: Williams mult. sequent. 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 F160460

Report:	[redacted];[redacted];2013;M-468911-01
Title:	Mesosulfuron-methyl-AE F160460: Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	kra/Rg-R-156/13
Document No:	M-468911-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable; ISO 11268-2: 1998(E); OECD 222: April 13, 2004;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F160460 (metabolite of mesosulfuron-methyl) on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with five different test concentrations.
Adult *Eisenia fetida* (approx. 4 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 10, 18, 32, 56 and 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 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 ≥ 100 mg test item/ kg dry weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 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 F160460 (BCS-AU84908); Customer order no.: TOX-No. 09538-00; Batch code: AE F160460-01-02; Origin Batch No. SES 11562-12-4; Purity: 96.7 %w/w; Certificate No.: AZ 17577.

Adult *Eisenia fetida* (approx. 4 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 10, 18, 32, 56 and 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 dry weight artificial soil (corresponds to 3.94 – 7.89 – 15.78 mg test item/ kg dry weight artificial soil); control: quartz sand, solvent control: none.

Dates of experimental work: July 04, 2013 – September 06, 2013

Results:

Table CA 8.4.1- 13: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	1.25 %
Mean number of juveniles in the control	≥ 30	217, 176, 139, 173, 192, 181, 179, 168
Coefficient of variance for the number of juveniles in the control	≤ 30 %	12.3 %

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

In a most recent 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 CA 8.4.1- 14: Effects of AE F160460 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***					
Test item	**Control**	**AE F160460**				
mg test item/kg dry weight artificial soil	---	10	18	32	56	100
Mortality of adult earthworms [%] after 28 days	1.25	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	38.77	37.92	37.03	36.43	37.73	37.42
Standard Deviation	9.23	2.52	5.24	4.37	4.53	2.74
Mean number of offspring per test vessel after 56 days **	178.1	186.5	186.3	141.3	184.5	167.3
Standard Deviation	22.0	11.9	14.5	25.6	25.6	20.1
Coefficient of variance (%)	12.3	6.4	7.8	18.1	13.9	12.0
% of control	---	104.7	104.6	79.3	103.6	93.9
					Reproduction	
NOEC (mg test item/kg dry weight soil)					**≥ 100**	
EC_{10} (mg test item/kg dry weight soil [1)]) (95% confidence limits)					**n. d.**	
EC_{20} (mg test item/kg dry weight soil [1)]) (95% confidence limits)					**n. d.**	

* no statistical significance compared to the control (Williams Multiple Sequential t-test, two-sided, α = 0.05)
** no statistical significance compared to the control (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05)
1) Probit analysis
n. d. not determined due to mathematical reasons or inappropriate data

Mortality:
After 28 days of exposure no worm died in the control group 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 not observed.

Therefore, based on biological and statistical significance (for both test runs):
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 values for the number of juveniles per test vessel relative to the control were observed.

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

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.

AE F140584

Report:	[redacted]; [redacted];2013;M-468921-01
Title:	Mesosulfuron-methyl-AE F140584 (BCS-AU66443): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	kra/Rg-R-155/13
Document No:	M-468921-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable; ISO 11268-2: 1998(E); OECD 222: April 13, 2004;For the three highest application rates 422.55 g dry weight artificial soil were used and at test end soil moisture was above 60 percent of WHC max**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F140584 (BCS-AU66443; metabolite of mesosulfuron-methyl) on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with five different test concentrations.

Adult *Eisenia fetida* (approx. 7 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 10, 18, 37, 66 and 117 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). As deviation from the guideline 422.55 g dry weight artificial soil were used for the three highest application rates and soil moisture was above 60 of WHC_{max} at test end.

Based on the biological and statistical significance observed on growth and reproduction, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 117 mg test item/ kg dry weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 117 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 F140584 (BCS-AU66443); Batch code: AE F140584 00 1B96 0001; Origin Batch No.: LOR 21036; CAS No.: 393509-80-3; LIMS No.: 1213360; purity: 95.5 %w/w; Certificate No.: AZ 18036.

Adult *Eisenia fetida* (approx. 7 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 10, 18, 37, 66 and 117 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). As deviation from the guideline 422.55 g dry weight artificial soil were used for the three highest application rates and soil moisture was above 60 of WHC_{max} at test end.

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

Dates of experimental work: July 29, 2013 – September 30, 2013

Results:

Table CA 8.4.1- 15: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	0%
Mean number of juveniles in the control	≥ 30	313, 244, 250, 278, 321, 272, 274, 300
Coefficient of variance for the number of juveniles in the control	≤ 30 %	9.9%

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

In a most recent 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 CA 8.4.1- 16: Effects of AE F140584 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***					
Test item	**Control**	**AE F140584 (BCS-AU66443)**				
mg test item/kg dry weight artificial soil	---	10	18	37***	66	117
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	1.40	4.61	1.68	5.89	2.88	4.19
Standard Deviation	4.17	5.14	1.73	5.37	5.94	1.75
Mean number of offspring per test vessel after 56 days **	281.5	275.3	241.8	287.3	264.3	253.5
Standard Deviation	27.9	18.5	38.4	25.7	20.8	44.8
Coefficient of variance (%)	9.9	6.7	15.9	8.9	7.9	17.5
% of control	-	97.8	85.9	102.1	93.9	90.8
					Reproduction	
EC_{10} (mg test item/kg dry weight soil [1]) (95% confidence limits)					**n. d.**	
EC_{20} (mg test item/kg dry weight soil [1]) (95% confidence limits)					**n. d.**	

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

** no statistical significance compared to the control (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05)

*** three replicates instead of four were tested

1) Probit analysis

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

Mortality:

After 28 days of exposure no worm died in the control group 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 not observed.

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

NOEC related to growth: ≥ 117 mg test item/kg dry weight artificial soil

LOEC related to growth: > 117 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.

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

NOEC related to reproduction:	≥ 117 mg test item/kg dry weight artificial soil
LOEC related to reproduction:	> 117 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 ≥ 117 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be > 117 mg test item/kg dry weight artificial soil.

AE F147447

Report:	[redacted]6;[redacted];[redacted];2012;M-428651-01
Title:	AE F147447: Reproduction toxicity to the earthworm Eisenia fetida in an artificial soil test
Report No:	11P34RR
Document No:	M-428651-01-1
Guidelines:	**the OECD Guideline No. 222 for the Testing of Chemicals "Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)" adopted April 13, 2004; the International Standard ISO 11268-2 Part 2 (1998) "Soil Quality - Effects of Pollutants on Earthworms (Eisenia fetida) - Part 2: Determination of Effects on Reproduction";At few short time intervals the temperature dropped down to 17.2°C in the second test run and was therefore, slightly below the range required by the guideline. However, study results of the test have not been impacted.**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine effects of AE F147447 (metabolite of mesosulfuron-methyl) on the reproduction (56 days after application) of the earthworm *Eisenia fetida* (Lumbricidae) by dermal and alimentary uptake using a standardised artificial soil. The study was performed as a limit test (1st test run) and a full dose-response test (2nd test run) according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). As deviation from the guideline the temperature was slightly below the range required by the guideline at few short time intervals in the second test run. However, study results of the test have not been impacted.

In the first run adult *Eisenia fetida* (8 × 10 replicates for each treatment level) were exposed in artificial soil in artificial soil (with 5 % peat content) to the concentration of 100 mg test item/kg artificial soil dry weight.

In the second run adult *Eisenia fetida* (8 × 10 replicates for the control group and 4 × 10 replicates for the treatment groups) were exposed in artificial soil (with 5 % peat content) to the concentrations of 9, 16, 28, 51 and 90 mg test item/ kg dry weight artificial soil. Mortality and biomass were assessed after 28 days. The number of juvenile earthworms was assessed after 56 days.

The No-Observed-Effect-Concentration (NOEC) was determined to be 90 mg test item/ kg dry weight artificial soil. The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 100 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 F147447; Batch code: AE F147447 00 1B98 0001; Origin batch No.: 33400-93/2; Certificate of analysis No.: AZ 16208; purity: 98.1 % w/w.

Adult earthworms (*Eisenia fetida*, with clitellum, fresh weight between 250 and 600 mg, at least 2 months old) were exposed in an artificial soil in two test runs.
In the first run adults of *Eisenia fetida* (8 × 10 replicates for the control group and 8 × 10 replicates for the treatment group) were exposed in artificial soil (with 5 % peat content) to AE F147447 at the concentration of 100 mg test item/kg artificial soil dry weight. Temperature during the first test run ranged between 18.1 and 21.4°C (rec. 20 ± 2 °C); the moisture content ranged from 55.0 – 57.1% (study initiation) to 58.3 – 58.5% (study termination); the pH value of the test substrate was 6.3 at test initiation and 6.7 at experimental termination; the photoperiod was 16 hours light : 8 hours dark and the light intensity was between recommended 400 – 800 Lux.
In the first test run statistically significant effects on the number of juveniles have been observed and it could not be demonstrated that the NOEC for reproduction is greater than the limit concentration. Therefore, a second test run was performed using five lower concentrations.
In the second run adult *Eisenia fetida* (8 × 10 replicates for the control group and 4 × 10 replicates for the treatment groups) were exposed in artificial soil (with 5 % peat content) to AE F147447 at the concentrations of 9, 16, 28, 51 and 90 mg test item/ kg dry weight artificial soil. Temperature during the second test run ranged between 17.2 and 21.4°C (rec. 20 ± 2 °C); the moisture content ranged from 54.9 – 58.5% (study initiation) to 47.4 – 54.6% (study termination); the pH value of the test substrate was 6.3 – 6.4 at test initiation and 7.0 – 7.2 at experimental termination; the photoperiod was 16 hours light : 8 hours dark and the light intensity was between recommended 400 – 800 Lux.
The test item was applied once at the beginning of each test run. The duration of the test period (exposure of earthworms to the artificial soil containing the test item) was 56 days. The adult worms were removed from the substrate after 28 days. After 28 days mortality and biomass were determined. After 56 days reproduction was determined.
As deviation from the guideline the temperature dropped down to 17.2°C at few short time intervals in the second test run and was therefore, slightly below the range required by the guideline. However, study results of the test have not been impacted.

Toxic standard: Carbendazim (Derosal 360 g/L SC): 1.0, 3.0 and 5.0 mg a.s./kg dry weight artificial soil, control: artificial soil, solvent control: none.

Dates of exposure period: October 27, 2011 – December 22, 2011 (first run)
January 18, 2012 – March 14, 2012 (second run)

Results:

Table CA 8.4.1- 17: Validity criteria

Validity criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mortality of adults in the control	≤ 10 %	0 %	7.5 %
Mean number of juveniles per replicate in the control (± standard deviation)	≥ 30	200.4 ± 29.8	136.0 ± 39.4
Coefficient of variance for the number of juveniles in the control	≤ 30 %	14.6 %	29.0 %

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

In a separate toxic standard reference study (ECT Study No.: IRR1105, performed from May 18 to July 13, 2011), the $LOEC_{Reproduction}$ value for carbendazim was determined as 1.0 mg/kg dry weight artificial soil. The $NOEC_{Reproduction}$ was considered as < 1.0 mg/kg dry weight artificial soil. These

observed effects are within the range expected from the guideline (1-5 mg carbendazim/kg soil dry weight) and hence acceptable sensitivity of the test system is assured.

First test run:
No mortality was observed in the control and 3.8% at the concentration of 100 mg test item/kg dry weight artificial soil.

A statistically significant difference (Student-t test; 2-sided, $p \leq 0.05$) concerning biomass development of individual adults over 28 days between the control and the limit concentration of the test item was determined.

Statistical analysis (Student-t test; 1-sided, $p \leq 0.05$) showed a significant difference concerning the number of juveniles between the control and the limit concentration of the test item.

Therefore, with this first test run it could not be demonstrated that the NOEC is greater than the limit concentration of 100 mg test item/kg dry weight artificial soil.

Table CA 8.4.1- 18: First test run: Effects of AE F147447 on mortality, biomass and reproduction of *Eisenia fetida*

Concentration [mg test item/kg soil d.w.]	Adult mortality [%]	Biomass [% of initial weight]	Number of juveniles [% of control]
Control	0.0	163.9	100.0
100	3.8	177.7#	73.8#

significantly different to control (Student-t test; 2-sided for biomass, 1-sided for reproduction, $p \leq 0.05$)

Second test run:
7.5% mortality was observed in the control, 2.5% at the concentrations of 51 mg test item/ kg dry weight artificial soil and no mortality at all other concentrations of the test item tested.

No statistically significant differences (Williams-t test; 2-sided, $p \leq 0.05$) concerning the biomass development of individual adults after 28 days were determined between the control and all concentrations of the test item tested.

Statistical analysis (Williams-t test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the number of juveniles between the control and all concentrations of the test item tested.

Therefore, it could be demonstrated that the NOEC for reproduction is greater than the highest concentration (90 mg test item/kg dry weight artificial soil) of the test item tested in this second test run.

Table CA 8.4.1- 19: Second test run: Effects of AE F147447 on mortality, biomass and reproduction of *Eisenia fetida*

Concentration [mg test item/kg soil d.w.]	Adult mortality [%]	Biomass [% of initial weight]	Number of juveniles [% of control]
Control	7.5	172.5	100.0
9	0.0	176.4	111.8
16	0.0	179.9	105.7
28	0.0	174.9	82.2
51	2.5	183.9	75.0
90	0.0	182.2	102.1

Conclusions:

If considering both test runs together it can be concluded that the $NOEC_{Reproduction}$ is 90 mg test item/kg dry weight artificial soil and accordingly the $LOEC_{Reproduction}$ could be assumed 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 mesosulfuron-methyl and its metabolites AE F154851, AE F160459, AE F092944 and AE F147447 reproductive toxicity studies on *Folsomia candida* were performed. In addition, for mesosulfuron-methyl and its terminal metabolite AE F092944 reproductive toxicity studies on *Hypoaspis aculifer* were performed.

In the tests with the collembolan species *Folsomia candida* and 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 mesosulfuron-methyl and ≥ 100 mg/kg dws for the soil metabolites. Details of all studies are provided in the following table.

Based on the consistent absence of effect observed in all studies covering the parent active substance, its initial and terminal metabolites, it was deemed justified to conclude absence of relevant toxicity to non-target soil meso and macrofauna (other than earthworms) as well for the transient intermediate components in the soil metabolic pathway of mesosulfuron-methyl. No further testing was therefore considered necessary.

Table CA 8.4.2.1- 1: Reproductive toxicity data of mesosulfuron-methyl and metabolites to other non-target macro-organisms presented in this chapter

Test substance	Test species	Endpoint	Reference
Mesosulfuron-methyl	*Hypoaspis aculeifer*	NOEC **≥1000 mg a.s./kg dws**	[redacted], 2012 M-429376-01-1 KCA 8.4.2.1/01
	Folsomia candida	NOEC **≥1000 mg a.s./kg dws**	[redacted], 2012 M-426538-01-1 KCA 8.4.2.1/02
AE F154851	*Folsomia candida*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-462785-01-1 KCA 8.4.2.1/03
AE F160459	*Folsomia candida*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-462786-01-1 KCA 8.4.2.1/04
AE F092944	*Hypoaspis aculeifer*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-454043-01-1 KCA 8.4.2.1/05
	Folsomia candida	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-451142-01-1 KCA 8.4.2.1/06
AE F147447	*Folsomia candida*	NOEC **≥ 100 mg/kg dws**	[redacted], 2013 M-462782-01-1 KCA 8.4.2.1/07

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

<u>Studies on mesosulfuron-methyl</u>

Report:	[redacted]y;[redacted];2012;M-429376-01
Title:	Mesosulfuron-methyl (AE F130060): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-67/12
Document No:	M-429376-01-1
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 effect of active substance mesosulfuron-methyl (AE F130060) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in an artificial soil comparing control and treatment. Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 1000 mg test item/kg dry weight artificial soil was 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_x-values could not be calculated. All validity criteria (for the untreated controls) according to the guideline were met.

Materials and Methods:

Test item: mesosulfuron-methyl (AE F130060); Batch code: AE F130060-01-02; Origin Batch No.: EFME000144; LIMS No.: 1101337; Specification No.: 102000013204; Certificate No.: AZ 17171, Customer order No.: Tox-No. 09287-00; purity: 97.4 %w/w.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and to the concentration of one treatment. The concentration of 1000 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% sphagnum peat, air dried and finely ground, 20 % kaolin clay and approximately 0.2 % calcium carbonate ($CaCO_3$).
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen Apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water, 2 g detergent/L fixing solution were added). All Hypoaspis aculeifer were counted under a binocular.

Toxic reference: (Dimethoate EC 400E G): 0.990 – 1.780 – 3.156 – 5.517 – 9.853 mg a.s./kg dry weight artificial soil; control: quartz sand with deionised water, solvent control: none.

Dates of experimental work: January 06, 2012 – January 26, 2012

Results:

Table CA 8.4.2.1- 2: Validity criteria

Validity criteria (control values)	Recommended	Obtained
Mean adult female mortality	≤ 20 %	0 %
Mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	283.9
Coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	13.7 %

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

In a separate non-GLP study ([redacted], kra/HR-O-10/11, March 21, 2011) the LC_{50} (mortality) of the reference item dimethoate was calculated to be 4.051 mg a.s./kg dry weight artificial soil. The $NOEC_{reproduction}$ was calculated to be 3.156 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.517 mg a.s./kg dry weight artificial soil. Dimethoate showed a EC_{50} (reproduction) of 6.445 mg a.s./kg dry weight artificial soil. This shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1- 3: Effects on mortality and reproduction of Hypoaspis aculeifer

Test item	Mesosulfuron-methyl (AE F130060) a.s.		
Test object	*Hypoaspis aculeifer*		
Exposure	Artificial soil		
mg test item/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**
Control	0	283.9 ± 38.8	-
1000	2.5	311.9 ± 34.7	109.9
NOEC (mg test item/kg dry weight artificial soil)			≥1000
LOEC (mg test item/kg dry weight artificial soil)			>1000

No statistical significance (Student t-test for homogeneous variances, one-sided smaller, α = 0.05) was found

Mortality

In the control group 0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The LC50 could not be calculated.

Reproduction

Concerning the number of juveniles statistical analysis (Student t-test for homogeneous variances, one-sided smaller, α = 0.05) revealed no significant difference between control and the concentration of 1000 mg test item/kg dry weight artificial soil. 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. ECx-values could not be calculated.

Conclusions:

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

Report:	[redacted];[redacted];2012;M-426538-01
Title:	Mesosulfuron-methyl (AE F130060) a.s.: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-138/12
Document No:	M-426538-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of the active substance mesosulfuron-methyl (AE F130060) 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 (11 - 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 ≥ 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. All validity criteria for the untreated control of the study according to the OECD Guideline 232 have been fulfilled.

Materials and Methods:

Test item: mesosulfuron-methyl (AE F130060) a.s.; analytical findings: 97.4 % w/w; origin batch no.: EFME000144; customer order no.: TOX-No. 09287-00; specification no.: 102000013204; LIMS no.: 1101337; batch code: AE F130060-01-02.

10 collembolans (11 - 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 74.8 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and 0.2 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C, 400 - 800 lux, with a photoperiod: light : dark = 16 h : 8 h. 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 reference: 44 - 67 - 100 - 150 - 225 mg boric acid//kg soil dry weight; control: quartz sand moistened with deionised water, solvent control: none.

Dates of experimental work: December 16, 2011– January 19, 2012

Results:

Table CA 8.4.2.1- 4: Validity criteria

Validity criteria (untreated control)	Recommended	Obtained
Mean adult mortality	≤ 20 %	20 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	977
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	14.6 %

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

In a separate most recent non-GLP study (ERM-Coll-Ref-15/11, [redacted], March 08, 2011) the EC_{50} of the reference item boric acid was calculated to be 91 mg test item/kg artificial soil dry weight for reproduction. The $NOEC_{reproduction}$ was calculated to be 44 mg test item/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg test item /kg artificial soil dry weight. This shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1- 5: Effects of mesosulfuron-methyl (AE F130060) on mortality and reproduction of *Folsomia candida*

Test item **Test object** **Exposure**	**Mesosulfuron-methyl (AE F130060) a.s.** ***Folsomia candida*** **Artificial soil**			
mg test item/kg soil dry weight **nominal concentration**	**Adult mortality (%)**	**Mean number of juveniles±SD**		**Reproduction (% of control)**
Control	20.0	977.3	± 142.6	-
100	10.0	1260.0	± 131.4	128.9 n.s.
178	20.0	1127.5	± 165.8	115.4 n.s.
316	12.5	1086	± 192.4	111.1 n.s.
562	30.0	1179	± 169.7	120.6 n.s.
1000	22.5	1085.8	± 81.5	111.1 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)				**≥1000**
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)				**>1000**

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

Mortality

In the control group 20 % of the adult *Folsomia candida* died and therefore met the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg artificial soil dry weight.

Reproduction

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) 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 artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >1000 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 1000 mg test item/kg artificial soil dry weight.

Conclusions:

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

Studies on the metabolites of mesosulfuron-methyl

AE F154851

Report:	[redacted]u; [redacted];2013;M-462785-01
Title:	Mesosulfuron-methyl-AE F154851 (BCS-AU80405): Effects on the reproduction of the collembolan Folsomia candida
Report No:	13 lu 48 104 S
Document No:	M-462785-01-1
Guidelines:	**OECD 232 (2009), ISO 11267 (1999);none**
GLP/GLP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of the metabolite AE F154851 (BCS-AU80405; metabolite of mesosulfuron-methyl) 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 juveniles and surviving parental collembolans were 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, and 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 F154851, BCS-code: BCS-AU80405, Batch code: AE F154851-01-01, Origin Batch No.: SES 11372-3-4, LIMS No.: 1310285, Customer order No.: TOX-No: 09197-01, analysed purity: 97.1 % w/w.

10 *Collembola* (9-12 days old) were exposed to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % CaCO3, 18.1 – 20.6°C and a photoperiod: light : dark = 16 h : 8 h (530 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 dry weight; control: quartz sand, solvent control: none.

Dates of experimental work: May 07, 2013 – June 04, 2013

Results:

Table CA 8.4.2.1- 6: Validity criteria

Validity criteria (control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	6.3 %
Mean number of juveniles per replicate	≥ 100	1146
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	10.3 %

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

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 3.6 %.

In the most recent study (BioChem, project No. R 13 10 48 004 S, dated July 16, 2013) the EC_{50} of the reference item boric acid was determined to be 108 mg a.s./kg soil dry weight. The LC_{50} was determined to be 192 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg a.s./kg soil dry weight, respectively.

The EC_{50} value for the reproduction was close to the value of 100 mg a.s./kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

Table CA 8.4.2.1- 7: Effects of AE F154851 on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	**AE F154851** *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 -

Table CA 8.4.2.1- 8: Effects of AE F154851 on mortality of parental collembolans and on number of juvenile collembolans

Endpoint	**AE F154851** **(mg test item/kg soil d.w.)**	
	control	**100**
Mortality of parental collembolans after 4 weeks (%)	6.3	5.0
Mean number of juveniles after 4 weeks	1146	1205
CV %	10.3	13.6
Reproduction (% to control)	100	105

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test, α = 0.05, one-sided greater) and reproduction (Student-t-test, α = 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 5.0 % parental mortality at a concentration of 100 mg test item/kg soil dry weight. 6.3 % 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 1146 in the control and 1205 at 100 mg test item/kg soil dry weight. 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 dry weight.
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dry weight.

Conclusions:

AE F154851 (BCS-AU80405) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at 100 mg test item/kg dry weight.

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

AE F160459

Report:	[redacted];2013;M-462786-01
Title:	Mesosulfuron-methyl-AE F160459 (BCS-AU84907): Effects on the reproduction of the collembolan Folsomia candida
Report No:	13 10 48 103 S
Document No:	M-462786-01-1
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 metabolite AE F160459 (BCS-AU84907; metabolite of mesosulfuron-methyl) 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 juveniles and surviving parental collembolans were 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, and 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 F160459, BCS-code: BCS-AU84907, Batch code: AE F160459 00 1B96 0001, Origin Batch No.: 2ER0125, LIMS No.: 0936149, analysed purity: 95.4 % w/w, Certificate of analysis: AZ 16306.

10 *Collembola* (9-12 days old) were exposed to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, 18.5 – 20.9 °C and a photoperiod: light : dark = 16 h : 8 h (540 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 dry weight; control: quartz sand, solvent control: none.

Dates of experimental work: April 11, 2013 – May 09, 2013

Results:

Table CA 8.4.2.1- 9: Validity criteria

Validity criteria (control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	926
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	13.9 %

All validity criteria for the study were met. Therefore this study is valid.
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 2.5 %.

In the most recent study (BioChem, project No. R 13 10 48 004 S, dated July 16, 2013) the EC_{50} of the reference item boric acid was determined to be 108 mg a.s./kg soil dry weight. The LC_{50} was determined to be 192 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg a.s./kg soil dry weight, respectively.
The EC_{50} value for the reproduction was close to the value of 100 mg a.s./kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

Table CA 8.4.2.1- 10: Effects of AE F160459 on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE F160459 *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 -

Table CA 8.4.2.1- 11: Effects of AE F160459 on mortality of parental collembolans and on number of juvenile collembolans

Endpoint	AE F160459 (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	926	930
CV %	13.9	9.4
Reproduction (% to control)	100	100

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 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 926 in the control and 930 at 100 mg test item/kg soil dry weight. 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 dry weight.
The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dry weight.

Conclusions:

AE F160459 (BCS-AU84907) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* 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.

AE F092944

Report:	[redacted]6; [redacted];2013;M-454043-01
Title:	AE F092944 (BCS-AA25052): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No:	13 10 48044 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 (metabolite of mesosulfuron-methyl) 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 CA 8.4.2.1- 12: 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 CA 8.4.2.1- 13: 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	-	-

Table CA 8.4.2.1- 14: Effects of AE F092944 on mortality of parental collembolans and on number of juvenile collembolans

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];[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 AE F092944 (metabolite of mesosulfuron-methyl) 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 17077.

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 CA 8.4.2.1- 15: 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 CA 8.4.2.1- 16: Effects of AE F092944 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 -

Table CA 8.4.2.1- 17: Effects of AE F092944 on mortality of parental collembolans and on number of juvenile collembolans

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, α = 0.05, one-sided greater) and reproduction (Student-t-test, α = 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 F147447

Report:	[redacted]b; [redacted];2013;M-462782-01
Title:	Mesosulfuron-methyl-AE F147447 (BCS-AU73625): Effects on the reproduction of the collembolan Folsomia candida
Report No:	13 10 48 105 S
Document No:	M-462782-01-1
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 metabolite AE F147447 (BCS-AU73625; metabolite of mesosulfuron-methyl) 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 juveniles and surviving parental collembolans were 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, and 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 F147447, BCS-code: BCS-AU73625, Batch code: AE F147447-01-01, Origin Batch No.: SES 10681-2-3, ICIMS No.: 1310201, Customer order No.: TOX No: 09196-01, analysed purity: 98.9 % w/w.

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 18.1 – 20.6 °C and a photoperiod: light : dark = 16 h : 8 h (530 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: May 07, 2013 – June 04, 2013

Results:

Table CA 8.4.2.1- 18: Validity criteria

Validity criteria (control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	6.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1146
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	10.3 %

All validity criteria for the study were met. Therefore this study is valid.
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 3.6 %.

In the most recent study (BioChem, project No. R 13 10 48 004 S, dated July 16, 2013) the EC_{50} (reproduction) of the reference item boric acid was calculated to be 108 mg a.s./kg soil dry weight. The LC_{50} was determined to be 192 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg a.s./kg soil dry weight, respectively.
The EC_{50} value for the reproduction was close to the value of 100 mg a.s./kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

Table CA 8.4.2.1- 19: Effects of AE F147447 on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE F147447 *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 -

Table CA 8.4.2.1- 20: Effects of AE F147447 on mortality of parental collembolans and on number of juvenile collembolans

Endpoint	AE F147447 (mg test item/kg soil d.w.)	
	control	100
Mortality of parental collembolans after 4 weeks (%)	6.3	5.0
Mean number of juveniles after 4 weeks	1146	1173
CV %	10.3	10.2
Reproduction (% to control)	100	102

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 5.0 % parental mortality at a concentration of 100 mg test item/kg soil dry weight. 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 1146 in the control and 1173 at 100 mg test item/kg soil dry weight. 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 dry weight.
Therefore the No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight.

Conclusions:

AE F147447 (BCS-AU73625) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* 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.

CA 8.5 Effects on soil nitrogen transformation

For mesosulfuron-methyl and its metabolites AE F154851, AE F160459, AE F099095, AE F092944, and AE F147447, 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 0.057 mg/kg dws to 0.137 mg/kg dws. Details of all studies are provided in the following table.

Based on the consistent absence of toxicity observed in all studies covering the parent active substance, its initial and terminal metabolites, it was deemed justified to conclude absence of relevant

effect on soil nitrogen transformation as well for the transient intermediate components in the soil metabolic pathway of mesosulfuron-methyl. No further testing was therefore considered necessary.

Table CA 8.5- 1: Toxicity data of mesosulfuron-methyl and metabolites to soil non-target micro-organisms presented in this chapter

Test item	Test design	Ecotoxicological endpoint	Reference
N-transformation			
Mesosulfuron-methyl (tech.)	28 d	**no unacceptable effects** **≥0.1 mg a.s./kg dws**	[redacted] (1998) M-143358-01-1 KCA 8.5/01
AE F154851	28 d	**no unacceptable effects** **≥0.1 mg/kg dws**	[redacted] (2002) M-214090-01-1 KCA 8.5/02
AE F160459	42 d	**no unacceptable effects** **≥0.1 mg/kg dws**	[redacted] (2002) M-214086-01-1 KCA 8.5/03
AE F099095	28 d	**no unacceptable effects** **≥0.1 mg/kg dws**	[redacted] (2002) M-214088-01-1 KCA 8.5/04
AE F092944	28 d	**no unacceptable effects** **≥0.137 mg/kg dws**	[redacted] (2013) M-453511-01-1 KCA 8.5/09
AE F147447	28 d	**no unacceptable effects** **≥0.057 mg/kg dws**	[redacted] (2013) M-460668-01-1 KCA 8.5/10

dws = dry weight soil

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

Studies on mesosulfuron-methyl

Report:	[redacted]; [redacted]; 1998; M-143358-01
Title:	ORE F130060; Substance, technical; Code: AE F130060 00 1C96 0002 - Effects on soil microbial activity (nitrogen turn-over)
Report No:	A59696
Document No:	M-143358-01-1
Guidelines:	**BBA; VI, 1-1; Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
Deviation of less than 25 % for application rate up to 75 g a.s./ha
(equivalent to ≥0.10 mg a.s./kg dws).

Study summary and RMS evaluation copied from the original Monograph:

- **Reference**: [redacted] 1998b, 8.5.1/1.
- **Test guideline**: BBA guideline VI, 1-1 (1990).
- **GLP compliance**: Yes.

- **Methods**: The influence of AE F130060 (technical substance, purity = 96.0%) on soil microflora nitrogen turn was assessed in a laboratory test. The test was conducted in 2 L stainless steel containers (15 cm diameter × 10 cm height). The effects were measured at day 0, 14 and 28 after test initiation in a silty sand and a loamy silt mixed with the test substance at the rate of 0 (control), 15 and 75 g a.s./ha (prepared in water, nominal). Three replicates were made for each dose and control. Parameters measured were ammonium and nitrate soil concentrations on 40 g soil aliquots.

- **Results**: Mesosulfuron-methyl applied at 1 (15 g/ha) and 5 (75 g/ha) the field rate did not cause any statistically significant effects greater than ± 25% with respect to control values on soil nitrogen transformations.

- **Comments (RMS):** the study is acceptable.

Further study information supplementing the original Monograph summary :

Analytical findings:
The study was conducted in a controlled environment room at 20°C ± 2°C.
The water content of the soil substrate was maintained throughout the test by reweighing the test containers weekly and replenishing lost water by adding deionized water.
The pH values on day 28 were 7.0 and 7.9 for the controls, respectively.

Biological findings:
AE F1 30060 00 1 C96 0002, when applied at field rate (15 g/ha) and at the 5 times field rate (75 g/ha) had a negligible effect on nitrogen turn-over (< ± 25 % deviation of the control treatment) on day 28 after treatment in the silty sand soil 1 and loamy silt (soil 2). Highest deviation of total mineral nitrogen compared to the control was + 9.1%. This difference to the control was statistically not significant.

Table CA 8.5.2: Effects of AE F130060 00 1C96 0002 on nitrogen transformation

Soil	Treatment	Deviation of total mineral nitrogen (N-min) from the control at different times (days) after application of AE F130060 00 1C96 0002 to soil [%]		
		day 0	day 14	day 28
Soil 1 silty sand	15 g/ha	0	- 1.4	+ 0.8
	75 g/ha	+ 5.8	- 3.8	- 2.1
Soil 2 loamy silt	15 g/ha	- 0.5	- 5.3	- 1.5
	75 g/ha	+ 1.5	+ 7.1	+ 9.1

Conclusions:

Application of AE F130060 00 1C96 0002, when applied at a rate equivalent to the maximum recommended (15 g/ha) and at a rate equivalent to 5 times the maximum recommended (75 g/ha) had a negligible effect on the nitrogen turn-over in a silty sand and a loamy silt soil.

Table CA 8.5- 3: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-143358-01-1 KCA 8.5 /01	BBA guideline VI, 1-1 (1990)	OECD Guideline No. 216 (2000)	evaluation to be based on nitrate formation rates (mg NO_3-N/kg per time interval); 3 h, 7 d, 14 d, 28 d, …)	no critical issues. The results are in line with the current guideline

Studies on the metabolites of mesosulfuron-methyl

AE F154851

Report:	[redacted]3;[redacted];[redacted];2002;M-214090-01
Title:	Soil microorganisms: Nitrogen transformation test Code: AE F154851 00 1B96 0001
Report No:	C027822
Document No:	M-214090-01-1
Guidelines:	**OECD: 216;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). The study is filed in this document on the "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report".

Study summary and RMS evaluation copied from the original Monograph:

None available; Study is filed in "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report" Appendix III to EU review report for mesosulfuron-methyl (SANCO/10298/2003-Final).

Further study information supplementing the original Monograph summary :

Executive Summary:

The purpose of this study was to determine the effects of AE F154851 (metabolite of mesosulfuron-methyl) 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 sandy loam soil was exposed for 28 days to concentrations of 15 and 75 g test item/ha dry weight which refer to the one-fold and five-fold recommended use rate of parent active substance mesosulfuron-methyl. Assuming a uniform soil incorporation depth of 5 cm and a soil bulk density of 1.5 g/cm³, the 15 and 75 g test item/ha application rates were equivalent to 0.02 and 0.10 mg test item/kg soil dry weight, respectively.
The reference soil was dosed with a substance (Nitrification Inhibitor Formula 2533™) known to inhibit nitrification at a concentration of 100 mg/kg dry weight.
Powdered alfalfa (*Medicago Sativa*) was added to sieved soil and this mixture was then be divided into appropriate batches for the control, reference and treatment groups. After 0, 5, 14 and 28 days of incubation samples of treated and control soils were extracted and the quantities of nitrate in the extracts were determined. The amount of nitrate formed in the treated soil samples was compared to that in the control soil samples and the percentage of deviation from the control was calculated.

No long-term influence of AE F154851 on nitrogen transformation in soil (difference to control < 25%, OECD 216) could be observed in both test concentrations (0.02 mg/kg dry soil and 0.10 mg/kg dry soil) after 28 days. Differences from the control of +13.3% (test concentration 0.02 mg/kg dry soil) and 6.7% (test concentration 0.10 mg /kg dry soil) were measured at the end of the 28-day incubation period (day 28).

Material and methods:
Test item: AE F154851 (AE F154851 00 1B96 0001, metabolite of mesosulfuron-methyl); Batch No: LOR 21029; Analysed purity: 96.1% w/w.

A sandy loam soil was exposed for 28 days to 0.02 and 0.10 mg test item/kg soil dry weight. Application rates were equivalent to 15 and 75 g test item/ha which refers to the one-fold and five-fold recommended use rate of the parent active substance mesosulfuron-methyl. In addition, an untreated control and a reference toxicant group was tested. Each group contained three replicates.
Determination of the nitrogen transformation (nitrate formation, expressed in mg nitrate/kg dry weight soil/day) in soil enriched with alfalfa powder (concentration in soil 5 g/kg soil dry weight) was conducted at different sampling intervals (0, 5, 14 and 28 days after treatment). In addition, samples of each soil were removed at each sampling interval for percent moisture determinations.
The samples of the test soils were extracted, centrifuged and the liquid phase was analysed for nitrates using High Performance Liquid Chromatography (HPLC). The data was statistically analysed using SAS institute, Inc. (SAS), NC ANOVA procedure. An F-test was used to determine if there were significant differences between the untreated control and the treatments replicates.

Dates of experimental work: July 25, 2002 – September 10, 2002

Results:
Validity Criteria:
Criterion: Coefficients of variation in the control should not deviate more than 15%. The coefficients of variation in the control (nitrate formation) ranged from 4.3 % to 5.8%. On day 14, the maximum CV of the three control replicates was 17.6% which slightly exceeded the demanded range (≤ 15%).However, on day 28 the deviation was only 5.6%. The reference toxicant caused an effect on nitrogen transformation of approx. +26.7% at 100 mg reference item per kg soil dry weight, 28 days after application

Nitrogen transformation:
No long-term influence of AE F154851 on nitrogen transformation in soil could be observed in both test concentrations (0.02 mg/kg dry soil and 0.10 mg/kg dry soil) after 28 days. Differences from the control of +13.3% (test concentration 0.02 mg/kg dry soil) and -6.7% (test concentration 0.10 mg/kg dry soil) were measured at the end of the 28-day incubation period (day 28). AE F154851 had no long-term influence (difference to control< 25%, OECD 216) on the soil nitrogen transformation (measured as nitrate production) at the end of the 28-day incubation period.

Table CA 8.5- 4: Effects on nitrogen transformation in soil after treatment with AE F154851

Sample date (Day)	Control			0.02 mg test item/kg soil dw, equivalent to 15 g/ha dw				0.10 mg test item/kg soil dw, equivalent to 75 g/ha dw				100 mg reference item/kg soil dw			
	Nitrate [1]			Nitrate [1]			% deviation to control	Nitrate [1]			% deviation to control	Nitrate [1]			% deviation to control
5	1.68	±	0.05	1.38	±	0.22	+17.9	1.30	±	0.15	+22.6	0.95	±	0.04	+43.5
14	0.73	±	0.25	0.80	±	0.19	+9.6	0.68	±	0.14	+6.8	0.68	±	0.54	+6.8
28	0.45	±	0.05	0.39	±	0.05	+13.3 ns	0.48	±	0.05	-6.7 ns	0.33	±	0.08	+26.7 ns

[1] Soil nitrate formation rate, in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation
ns = statistically not significantly different from control (rate differences judged at the 0.05 significance level)

Conclusions:
AE F154851 had no long-term influence (difference in the rates of nitrate formation between the treatment and the control <25%, OECD 216) on nitrogen transformation in soils (measured as nitrate production) at the end of the 28-day incubation period. The study was performed in a field soil at a

concentration of up to 0.10 mg test item/kg soil dry weight, which is equivalent to an application rate of up to 75 g test item/ha.

Table CA 8.5- 5: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-214090-01-1 KCA 8.5 /02	OECD Guideline No. 216 (2000)	OECD Guideline No. 216 (2000)	none	The study results are in line with the current guideline.

AE F160459

Report:	[redacted]ö; [redacted]; [redacted];2002;M-214086-01
Title:	Soil microorganisms: Nitrogen transformation test; Code: AE F160459 00 1B97 0901
Report No:	C027820
Document No:	M-214086-01-1
Guidelines:	**OECD: 216; Deviation not specified**
GLP/GEP:	**yes**

Study endpoint: Deviation of less than 25 % for application rate up to 75 g p.m./ha (equivalent to ≤ 0.10 mg p.m./kg dws).

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). The study is filed in this document on the "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report".

Study summary and RMS evaluation (copied from the original Monograph):

None available; Studies filed in "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report" - Appendix III to EU review report for mesosulfuron-methyl (SANCO/10298/2003-Final).

Further study information supplementing the original Monograph summary :

Executive Summary

The purpose of this study was to determine the effects of AE F160459 (metabolite of mesosulfuron-methyl) 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 sandy loam soil was exposed for 42 days to concentrations of 15 and 75 g test item/ha dry weight which refers to the one-fold and five-fold recommended use rate. Assuming a uniform soil incorporation depth of 5 cm and a soil bulk density of 1.5 g/cm^3, the 15 and 75 g a.s./ha application rates were equivalent to 0.02 and 0.10 mg a.s./kg soil dry weight, respectively.
The reference soil was dosed with a substance (Nitrification Inhibitor Formula 2533™) known to inhibit nitrification at a concentration 100 mg/kg dry weight.
Powdered alfalfa (*Medicago sativa*) was added to sieved soil and this mixture was then be divided into appropriate batches for the control, reference and treatment groups. After 0, 5, 14, 28 and 42 days of incubation, samples of treated and control soils were extracted and the quantities of nitrate in the

extracts were determined. The amount of nitrate formed in the treated soil samples was compared to that of the control soil samples and the percentage of deviation from the control was calculated.

No long-term influence of AE F160459 on nitrogen transformation in soil (difference to control < 25%, OECD 216) could be observed in both test concentrations (0.02 mg/kg dry soil and 0.10 mg/kg dry soil) after 42 days. Differences from the control of -9.5% (test concentration 0.02 mg/kg dry soil) and -9.5% (test concentration 0.10 mg /kg dry soil) were measured at the end of the 42-day incubation period (day 42).

Material and methods:
Test item. AE F160459 (AE F160459 00 1B97 0001; metabolite of mesosulfuron-methyl); Batch No 25117/2; Analysed purity: 96.8% w/w.

A sandy loam soil was exposed for 42 days to 0.02 and 0.10 mg test item/kg soil dry weight . Application rates were equivalent to 15 and 75 g test item/ha which refers to the one-fold and five-fold recommended use rate. In addition, an untreated control and a reference toxicant group was tested. Each group contained three replicates.
Determination of the nitrogen transformation (nitrate formation, expressed in mg nitrate/kg dry weight soil/day) in soil enriched with alfalfa powder (concentration in soil 5 g/kg soil dry weight) was conducted at different sampling intervals (0, 5, 14, 28 and 42 days after treatment). In addition, samples of each soil were removed at each sampling interval for percent moisture determinations.
The samples of the test soils were extracted, centrifuged and the liquid phase was analysed for nitrates using High Performance Liquid Chromatography (HPLC). The data was statistically analysed using SAS institute, Inc. (SAS) NC, ANOVA procedure. An F-test was used to determine if there were significant differences between the untreated control and the treatment replicates. In addition, the untreated control and reference toxicant group was statistically analysed using a t-test to determine if there were significant differences between the untreated control and the reference toxicant replicates.

Dates of experimental work: August 26, 2002 – October 15, 2002

Results:
Validity criteria:
Criterion: Coefficients of variation in the control should not deviate more than 15%. The CV in this study slightly exceeds 15%. However, no adverse effects for the test item were proven within this study indicating no concern on the N-transformation. The reference toxicant caused an effect on nitrogen transformation of approx. (5 - +75)% at 100 mg reference item per kg soil dry weight, 5 -42 days after application.

Nitrogen transformation:
No long-term influence of AE F160459 on nitrogen transformation in soil could be observed in both test concentrations (0.02 mg/kg dry soil and 0.10 mg/kg dry soil) after 42 days. Differences from the control of -9.5% (test concentration 0.02 mg/kg dry soil) and -9.5% (test concentration 0.10 mg /kg dry soil) were measured at the end of the 42-day incubation period (on day 42). AE F160459 had no long-term influence (difference to control <25%, OECD 216) on the soil nitrogen transformation (measured as nitrate production) at the end of the 42-day incubation period.

Table CA 8.6.6: Effects on nitrogen transformation in soil after treatment with AE F160459 00 1B97 0001

Sample date (Day)	Control	0.02 mg test item/kg soil dry weight equivalent to 15 g test item/ha		0.10 mg test item/kg soil dry weight equivalent to 75 g test item/ha		100 mg reference item/kg soil dry weight	
	Nitrate [1)]	Nitrate [1)]	% deviat. to control	Nitrate [1)]	% deviat. to control	Nitrate [1)]	% deviat. to control

5	0.61*	±	0.01	0.44	±	0.23	+27.9	0.68	±	0.37	-11.5	0.13	±	0.07	+78.7
14	0.37*	±	0.01	0.24	±	0.12	+35.1	0.26	±	0.14	+29.7	0.15	±	0.15	+59.5
28	0.17*	±	0.00	0.14	±	0.15	+17.6	0.23	±	0.00	+35.3	0.15	±	0.06	+118
42	0.21	±	0.02	0.11	±	0.05	-9.5 ns	0.23	±	0.03	-9.5 ns	0.21	±	0.02	0.0 ns

[1)] Soil nitrate formation rate, in mg/kg soil dry weight/day, mean of 3 replicates and standard deviation
* One of the control replicates (no.3) was excluded of the mean / standard deviation calculation. Excluded rates were determined to be statistically different from other replicates.
ns = statistically not significantly different from control (rate differences judged at the 0.05 significance level)

Conclusions:
AE F160459 had no long-term influence (difference to control <25%, OECD 216) on the soil nitrogen transformation (measured as nitrate production) at the end of the 42-day incubation period. The study was performed in a field soil at a concentration of up to 0.10 mg a.s./kg soil dry weight, which is equivalent to an application rate of up to 75 g a.s./ha.

Table CA 8.5- 7: Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations conclusion about its Reliability
M-214086-01-1 KCA 8.5 /03	OECD Guideline No. 216 (2000)	OECD Guideline No. 216 (2000)	none	The study results are in line with the current guideline

AE F099095

Report:	[redacted]u; [redacted]; [redacted];2002;M-214088-01
Title:	Soil microorganisms: Nitrogen transformation test Code: AE F099095 00 1B99 0001
Report No:	C027821
Document No:	M-214088-01-1
Guidelines:	**OECD: 216;Deviation not specified**
GLP/GEP:	yes

Study endpoint: Deviation of less than 25 % for application rate up to 75 g p.m./ha (equivalent to ≥0.10 mg p.m./kg dws).

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). The study is filed in this document on the "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report".

Study summary and RMS evaluation copied from the original Monograph:

None available. Study is filed in "List of studies which were submitted during the evaluation process and were not cited in draft assessment report" - Appendix III to EU review report for mesosulfuron-methyl (SANCO/10298/2003-Final).

Further study information supplementing the original Monograph summary :

Executive Summary:

The purpose of this study was to determine the effects of AE F099095 (metabolite of mesosulfuron-methyl) 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 sandy loam soil was exposed for 28 days to concentrations of 15 and 75 g test item/ha dry weight which refers to the one-fold and five-fold recommended use rate. Assuming a uniform soil incorporation depth of 5 cm and a soil bulk density of 1.5 g/cm^3 the 15 and 75 g test item/ha application rates were equivalent to 0.02 and 0.10 mg test item/kg soil dry weight, respectively.
The reference soil was dosed with a substance (Nitrification Inhibitor Formula 2533 W) known to inhibit nitrification at a concentration 100 mg/kg dry weight.
Powdered alfalfa (*Medicago sativa*) was added to sieved soil and this mixture was then be divided into appropriate batches for the control, reference and treatment groups. After 0, 5, 14 and 28 days of incubation, samples of treated and control soils were extracted and the quantities of nitrate in the extracts were determined. The amount of nitrate formed in the treated soil samples was compared to that of the control soil samples and the percentage of deviation from the control was calculated.

No long-term influence of AE F099095 00 1B99 0001 on nitrogen transformation in soil (difference to control < 25%, OECD 216) could be observed in both test concentrations (0.02 mg/kg dry soil and 0.10 mg/kg dry soil) after 28 days. Differences from the control of +17.0% (test concentration 0.02 mg/kg dry soil) and -5.9% (test concentration 0.10 mg /kg dry soil) were measured at the end of the 28-day incubation period (day 28).

Material and methods:
Test item: AE F099095 (AE F099095 00 1B99 0001, metabolite of mesosulfuron-methyl); Batch No: KR363/364; Analysed purity: 99.1% w/w

A sandy loam soil was exposed for 28 days to 0.02 and 0.10 mg test item/kg soil dry weight. Application rates were equivalent to 15 and 75 g a.s./ha which refers to the one-fold and five-fold recommended use rate. In addition, an untreated control and a reference toxicant group was tested. Each group contained three replicates.
Determination of the nitrogen transformation (nitrate formation expressed in mg nitrate/kg dry weight soil/day) in soil enriched with alfalfa powder (concentration in soil 5 g/kg soil dry weight) was conducted at different sampling intervals (0, 5, 14 and 28 days after treatment). In addition, samples of each soil were removed at each sampling interval for percent moisture determinations. The samples of the test soils were extracted, centrifuged and the liquid phase was analysed for nitrates using High Performance Liquid Chromatography (HPLC). The data was statistically analysed using SAS institute, Inc. (SAS) NC ANOVA procedure. An F test was used to determine if there were significant differences between the untreated control and the treatment replicates. In addition, the untreated control and reference toxicant group was statistically analysed using a t-test to determine if there were significant differences between the untreated control and the reference toxicant replicates.

Dates of experimental work: August 26, 2002 – October 01, 2002

Results:
Validity Criteria:
Criterion: Coefficients of variation in the control should not deviate more than 15%. The CV in this study slightly exceeds 15%. However, no adverse effects for the test item were proven within this study indicating no concern on the N-Transformation.
The reference toxicant caused an effect on nitrogen transformation of approx. +11.8% at 100 mg reference item per kg soil dry weight, 28 days after application

Nitrogen transformation:
No long-term influence of AE F099095 on nitrogen transformation in soil could be observed in both test concentrations (0.02 mg/kg dry soil and 0.10 mg/kg dry soil) after 28 days. Differences from the control of +17.6% (test concentration 0.02 mg/kg dry soil) and -5.9% (test concentration 0.10 mg /kg dry soil) were measured at the end of the 28-day incubation period (on day 28). AE F099095 had no long-term influence (difference to control <25%, OECD 216) on the soil nitrogen transformation (measured as nitrate production) at the end of the 28-day incubation period.

Table CA 8.5- 8: Effects on nitrogen transformation in soil after treatment with AE F099095 00 B99001

Sample date (Day)	Control			0.02 mg test item/kg soil dry weight equivalent to 15 g test item/ha				0.10 mg test item/kg soil dry weight equivalent to 75 g test item/ha				100 mg reference item/kg soil dry weight			
	Nitrate [1]			Nitrate [1]			% diff. to control	Nitrate [1]			% diff. to control	Nitrate [1]			% diff. to control
5	0.61*	±	0.01	0.75	±	0.28	+23.0	0.58	±	0.10	+4.9	0.43	±	0.07	+47
14	0.36*	±	0.01	0.10	±	0.02	+75.0	0.06	±	0.03	+81.1	0.15	±	0.05	+59.5
28	0.17*	±	0.00	0.07	±	0.07	+17.6 ns	0.18	±	0.07	-5.9 ns	0.05	±	0.06	+11.8 ns

[1] Soil nitrate formation rate, in mg/kg soil dry weight/day, mean and (2 for the control) replicates and standard deviation
* Mean of two replicates. Control replicate 3 was statistically determined to be significant outlier from replicates 1 & 2 and was omitted from the calculation of the mean and standard deviations.
ns = statistically not significantly different from control (rate differences judged at the 0.05 significance level)

Conclusions:
AE F099095 had no long-term influence (difference in the rates of nitrate formation between the treatment and the control <25%, OECD 216) on the soil nitrogen transformation (measured as nitrate production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.10 mg test item/kg soil dry weight, which are equivalent to application rates up to 75 g test item/ha.

Table CA 8.5-9: Summary table

Reference	Followed guidance	Guidance currently in force	Deviations	Critical assessment of the study / Deviations / conclusion about its Reliability
M-214088-01-1 KCA 8.5 /04	OECD Guideline No. 216 (2000)	OECD Guideline No. 216 (2000)	none	The results are in line with the current guideline.

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 48018 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 (metabolite of mesosulfuron-methyl) 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 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
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 CA 8.5- 10: 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 F147447

Report:	[redacted]§; [redacted]§; 2013; M-460668-01
Title:	Mesosulfuron-methyl-AE F147447 (BCS-AU73625): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 076 N
Document No:	M-460668-01-1
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 F147447 (metabolite of mesosulfuron-methyl) 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 days to 0.012 and 0.057 mg test item/kg soil dry weight. Application rates were equivalent to 0.009 and 0.043 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.3 %) to stimulate nitrogen transformation. The test item AE F147447 caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.057 mg/kg at time interval 7-14 days after application. However, no adverse effects of AE F147447 on nitrogen transformation in soil could be observed at both test concentrations (0.012 mg/kg dry soil and 0.057 mg/kg dry soil) at the end of the 28-day experiment. Differences from the control of -7.7 % (test concentration 0.012 mg/kg dry soil) and +7.0 % (test concentration 0.057 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Materials and Methods:

Test item. AE F147447; BCS-code: BCS- AU73625; Batch code: AE F147447-01-01; Origin Batch No.: SES 10681-2-3; Customer order No.: TOX-No.: 09196-01; LIMS No.: 1310201; Certificate No.: AZ 18638, analysed purity: 98.9 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.012 and 0.057 mg test item/kg soil dry weight. Application rates were equivalent to 0.009 and 0.043 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of work: May 31, 2013 – June 28, 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, carried out from 04.01. to 01.02.2013) caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % (required ≥25 %) 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 AE F147447 caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.057 mg/kg at time interval 7-14 days after application. However, no adverse effects of AE F147447 on nitrogen transformation in soil could be observed at both test concentrations (0.012 mg/kg dry soil and 0.057 mg/kg dry soil) at the end of the 28-day experiment. Differences from the control of -7.7 % (test concentration 0.012 mg/kg dry soil) and +7.0 % (test concentration 0.057 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5-11: Effects on nitrogen transformation in soil after treatment with AE F147447

Time Interval (days)	Control			0.012 mg test item/kg soil dry weight equivalent to 0.009 kg test item/ha				0.057 mg test item/kg soil dry weight equivalent to 0.043 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.53	±	0.37	4.13	±	0.46	**+17.0** [n.s.]	4.29	±	0.09	**+21.3** [*s.]
7-14	2.30	±	0.39	2.07	±	0.31	**-10.3** [n.s.]	1.10	±	0.55	**-52.1** [*s]
14-28	1.26	±	0.11	11.7	±	0.15	**-7.7** [n.s.]	1.35	±	0.32	**+7.0** [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
[*s] = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
[n.s.] = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusion:

AE F147447 caused no adverse effects (difference to control < 25 %, OECD 216) in a field soil at concentrations up to 0.057 mg test item/kg soil dry weight on the soil nitrogen transformation (expressed as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.057 mg test item/kg soil dry weight.

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Supportive information: In the new European dossier format/data requirements there is no data point that corresponds to soil carbon transformation studies. Nevertheless, four studies (on the active substance and metabolites AE F154851, AE F160459, and AE F099095) are mentioned here as supportive information, since they are contained in the baseline dossier and in the List of Endpoints from the first EU review.

Studies on mesosulfuron-methyl

Report:	[redacted]j;[redacted];1998;M-143357-01
Title:	AE F130060; substance, technical; Code: AE F130060 00 1C96 0008 - Effects on soil microbial activity (short-term respiration)
Report No:	A59695
Document No:	M-143357-01-1
Guidelines:	**BBA: VI, 1-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final):
Deviation of less than 25 % for application rate up to 75 g a.s./ha
(equivalent to ≥0.10 mg a.s./kg dws).

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked supportive information as soil carbon transformation testing is no longer a data requirement under regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding soil nitrogen transformation test.

Studies on the metabolites of mesosulfuron-methyl

AE F154851

Report:	[redacted]n;[redacted];[redacted];2002;M-214092-01
Title:	Soil microorganisms: Carbon transformation test Code: AE F154851 00 1B96 0001
Report No:	C027823
Document No:	M-214092-01-1
Guidelines:	**OECD: 217;Deviation not specified**
GLP/GEP:	**yes**

Study endpoint: Deviation of less than 25 % for application rate up to 75 g p.m./ha
(equivalent to ≥0.10 mg p.m./kg dws).

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). The study is filed in this document on the "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report".

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked supportive information as soil carbon transformation testing is no longer a data requirement under regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding soil nitrogen transformation test.

AE F160459

Report:	[redacted]5; [redacted]; [redacted]; 2002; M-212102-01
Title:	Soil microorganisms: carbon transformation test Code: AE F160459 00 1H97 0001
Report No:	C026800
Document No:	M-212102-01-1
Guidelines:	**OECD: 217;Deviation not specified**
GLP/GEP:	**yes**

Study endpoint: Deviation of less than 25 % for application rate up to 75 g p.m./ha (equivalent to ≤0.10 mg p.m./kg dws).

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). The study is filed in this document on the "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report".

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked supportive information as soil carbon transformation testing is no longer a data requirement under regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding soil nitrogen transformation test.

AE F099095

Report:	[redacted]6; [redacted]; [redacted]; 2002; M-212100-01
Title:	Soil microorganisms: carbon transformation test Code: AE F099095 00 1B99 0001
Report No:	C026799
Document No:	M-212100-01-1
Guidelines:	**OECD: 217;Deviation not specified**
GLP/GEP:	**yes**

Study endpoint: Deviation of less than 25 % for application rate up to 75 g p.m./ha (equivalent to ≤0.10 mg p.m./kg dws).

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). The study is filed in this document on the "List of studies which were submitted during the evaluation process and were not cited in the draft assessment report".

Note: In context of application for EU approval renewal of mesosulfuron-methyl, this endpoint is ranked supportive information as soil carbon transformation testing is no longer a data requirement under regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding soil nitrogen transformation test.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

For mesosulfuron-methyl, greenhouse screening was performed on a number of higher plant species including crops, broadleaf and grass weeds (KCA 8.6.1 /01). As expected for a sulfonyl urea herbicide, the compound showed significant herbicidal activity to several plants, in both pre- and post-emergence applications. The tests indicated excellent control in particular of grass weeds of high economical importance.

For BCS-CV14885, a component identified in a specific non-guideline test for the retrospective structure assignment to polar leachate radioactivity observed in lysimeter studies (KCA 7.1.4.2 /05), absence of herbicidal activity was confirmed in comparative tests with parent active substance (KCA 8.6.1 /02: pre-emergence application, KCA 8.6.1 /03: post-emergence application). Information from these tests is required for assessing the potential relevance in groundwater of component BCS-CV14885, cf. Document N4.

Soil metabolites AE F154851, AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447 were screened for herbicidal activity in greenhouse assays, at highly exaggerated test rates, with dosing pre- and/or post-emergence. None of the components revealed a pronounced herbicidal effect comparable to that of the parent active substance. For metabolites AE F160459 and AE F147447, this information is required for assessing the potential relevance of these metabolites in groundwater, cf. Document N4.

Details of all studies are provided in the following table.

Table CA 8.6.1- 1: Screening data for effect of mesosulfuron-methyl and selected metabolites to higher terrestrial plants

Test design	Test species	Ecotoxicological endpoint	Reference
Mesosulfuron-methyl, formulated as WP20			
Greenhouse, seedling emergence and growth, 26-28 d	Crop plants (6[1]-8[2] species) Broadleaf plants (13[2]-14[1]- species) Grass plants (13[1,2] species)	Mesosulfuron-methyl is active pre-emergence as well as post-emergence to grass plants and broadleaf plants. At lower use rates below 20 grams, many plants are not susceptible to this herbicide particulary among the broadleaves and more so after post-emergence use. Mesosulfuron-methyl is characterised by its good activity against some grass plants, especially the ones which are worldwide economically important weeds in cereal crops.	[redacted], 1999, M-186436-01-1 KCA 8.6.1 /01
BCS-CV14885, formulated as WP20			
Greenhouse, seedling emergence and growth, 28 d	Weed species (12 species)	After pre-emergence application, BCS-CV14885 showed no biological activity on the range of weeds tested	[redacted], 2013 M-460393-01-1 KCA 8.6.1 /02
Greenhouse, seedling emergence and growth, 21 d	Weed species (12 species)	After post-emergence application, BCS-CV14885 showed no biological activity on the range of weeds tested	[redacted], 2013 M-460647-01-1 KCA 8.6.1 /03
AE F154851, AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447			
Greenhouse, seedling emergence and growth	Grass and broad leaved plants (22 species)	AE F154851: no significant herbicidal activity at 80 g / ha in pre-emergence or post-emergence tests against a wide range of grass and broad-leaved plant species. AE F160459 and AE F160460: only slight to moderate effects pre-emergence at highly exaggerate dose rate of 330 g / ha. This level of activity is 10-100 fold less than that of AE F130060 itself. AE F099095: Primary pre-emergence glasshouse screening found that it has no herbicidal activity at 3000 g/ha. AE F092944: almost herbicidally inactive at 2500 g/ha. AE F140584: In primary pre- and post-emergence screening, this metabolite was herbicidally inactive at rates between 1250 and 5000 g/ha. AE F147447: In primary pre- and post-emergence screening, both it was herbicidally inactive at 1250 g/ha.	[redacted], 1999 M-185253-01-1 KCA 3.6/03

[1] post-emergent application of the test item
[2] pre-emergent application of the test item

Studies on mesosulfuron-methyl

Report:	[redacted]0; [redacted];1999;M-186436-01
Title:	Effectivity of the herbicide AE F130060 on higher plant species as applied under greenhouse conditions
Report No:	C003598
Document No:	M-186436-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). A study evaluation is however available in the Monograph (B.9.9.1.1-).

Studies on the metabolites of mesosulfuron-methyl

BCS-CV14885

Report:	[redacted]u; [redacted];2013;M-460393-01
Title:	Evaluation of the pre-emergence biological activity of mesosulfuron and its metabolite BCS-CV14885
Report No:	FFS13-005
Document No:	M-460393-01-1
Guidelines:	**not applicable;not applicable**
GLP/GEP:	**no**

Executive Summary:

This pre-emergence test was conducted to determine differences in the biological activity of mesosulfuron-methyl and its degradate BCS-CV14885. The study was conducted under standardized glasshouse conditions using WP20 formulations of both mesosulfuron-methyl and its degradate BCS-CV14885. Seeds of the weed species (EPPO code) *Zea mays* (ZEAMA), *Triticum aestivum* (TRZAS), *Triticum aestivum* (TRZAW), *Hordeum vulgare* (HORVS), *Secale cereale* ((SECCW), *Lolium multiflorum* (LOLMU), *Beta vulgaris* (BEAVA), *Brassica napus* (BRSNS), *Helianthus annuus* (HELAN), *Linum usitatissimum* (LIUUT), *Phaseolus vulgaris* (PHSVN) and *Pisum sativum* (PIBST) were planted in pots and pre-emergence applications of mesosulfuron-methyl and BCS-CV14885 were applied at rates of 15, 7.5, 3.75 and 1.875 g a.s./ha. Furthermore, "Blindformulierung WP20" [blind formulation containing no active substance] was applied at rates of 60, 30, 15 and 7.5 g a.s./ha. Effects were assessed visually two and four weeks after application. BCS-CV14885 showed no biological activity on the range of weeds tested.

Materials and Methods:

Test material: 2013-000561 mesosulfuron-methyl; 2013-000563 BCS-CV14885; Blindformulierung [english translation: blind formulation] WP20.

Test species: 12 weed species (EPPO code): *Zea mays* (ZEAMA), *Triticum aestivum* (TRZAS), *Triticum aestivum* (TRZAW), *Hordeum vulgare* (HORVS), *Secale cereale* ((SECCW), *Lolium multiflorum* (LOLMU); *Beta vulgaris* (BEAVA), *Brassica napus* (BRSNS), *Helianthus annuus* (HELAN), *Linum usitatissimum* (LIUUT), *Phaseolus vulgaris* (PHSVN), *Pisum sativum* (PIBST).

Jiffy pots (7 cm diameter) were filled to within 2 cm of the top with a silt-loam soil (20% sand, 57% silt, 23% clay, pH 6.8 and 1.4% organic matter). Seeds of the weed species were sown into these pots and covered with 0.5 to 1 cm of the same soil mixed 1 to 1 with sharp sand. The sowing density was selected based on prior experience to provide approximately 60-70% soil cover by the plants at application timing. After sowing the pots were watered slightly.
All compounds used in the test were dissolved in deionized water and diluted to obtain the required dose rates. The pre-emergence application (06 February 2013) was made using a track-sprayer with a spray volume of 300 L/ha via a flat fan nozzle (TeeJet 8001). Mesosulfuron-methyl and BCS-CV 14885 were applied at rates of 15, 7.5, 3.75 and 1.875 g a.s./ha. "Blindformulierung WP20" was applied at rates of 60, 30, 15 and 7.5 g a.s./ha.
After application, the pots were placed into a glasshouse set 21°C+/-2°C day and 12°C+/-2°C night and watered according to need. High pressure sodium lamps (400W) were used to augment daylight during cloudy conditions and to extend the day length to 14 hours.
Two weeks and four weeks after application, the treated plants were visually assessed for injury compared with the untreated control plants. The assessments were on a percentage basis (0 = no effects, 100 = complete kill).

Dates of experimental work: February 6, 2013 to March 6, 2013

Results:

The results of the visual assessments are presented as means from the 2 replicates in the following tables:

Table CA 8.6.1- 2: Results of the first assessment (14 DAT)

	[g a.s./ha]	ZEAMA	TRZAS	TRZAW	HORVS	SECCW	BEAVA	BRSNS	HELAN	LIUUT	PHSVN	PIBST	LOLMU
2013-000561 Mesosulfuron-methyl	15	0	55	60	0	80	55	60	75	55	55	90	85
	7.5	0	0	35	0	40	25	10	50	0	25	90	55
	3.75	0	0	0	0	10	0	0	10	0	0	63	0
	1.875	0	0	0	0	0	0	0	0	0	0	20	0
2013-000563 BCS-CV 14885	15	0	0	0	0	0	0	0	0	0	0	0	0
	7.5	0	0	0	0	0	0	0	0	0	0	0	0
	3.75	0	0	0	0	0	0	0	5	0	0	0	0
	1.875	0	0	0	0	0	0	0	0	0	0	0	0
Blindformulierung WP 20	60	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0
	7.5	0	0	0	0	0	0	0	0	0	0	0	0

(selectivity/ efficacy)	excellent +++ (0-5/95-100)	good ++ (6-10/90-94)	sufficient + (11-15/80-89)	Side effects +- (16-20/60-79)	Not sufficient/ gaps - (>20/<60)

Table CA 8.6.1- 3: Results of the second assessment (28 DAT)

	[g a.s./ ha]	ZEA MA	TRZ AS	TRZ AW	HOR VS	SEC CW	BEA VA	BRS NS	HEL AN	LIU UT	PHS VN	PIB ST	LOL MU
2013-000561 Mesosulfuron-methyl	15	0	50	55	45	70	90	65	90	50	30	98	92
	7.5	0	0	5	0	25	30	10	43	0	10	93	75
	3.75	0	0	0	0	0	0	0	0	0	0	45	28
	1.875	0	0	0	0	0	0	0	0	0	0	35	0
2013-000563 BCS-CV 14885	15	0	0	0	0	0	0	0	0	0	0	0	0
	7.5	0	0	0	0	0	0	0	0	0	0	0	0
	3.75	0	0	0	0	0	0	0	5	0	0	0	0
	1.875	0	0	0	0	0	0	0	0	0	0	0	0
Blindformulierung WP 20	60	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0
	7.5	0	0	0	0	0	0	0	0	0	0	0	0

(selectivity/ efficacy)	excellent	good	sufficient	Side effects	Not sufficient/ gaps
	+++ (0-5/95-100)	++ (6-10/90-94)	+ (11-15/80-89)	± (16-20/60-79)	- (>20/<60)

Conclusion:

In a direct comparison study under highly sensitive glasshouse screening conditions, the mesosulfuron-methyl degradate BCS-CV14885 showed no biological activity on the range of weeds tested.

Report:	[redacted] 1[redacted];2013;M-460647-01
Title:	Evaluation of the post-emergence biological activity of mesosulfuron and its metabolite BCS-CV 14885
Report No:	FFS135004
Document No:	M-460647-01-1
Guidelines:	**not applicable;not applicable**
GLP/GEP:	**no**

Executive Summary:

This post-emergence test was conducted to determine differences in the biological activity of mesosulfuron-methyl and its degradate BCS-CV14885. The study was conducted under standardized glasshouse conditions using WP20 formulations of both mesosulfuron-methyl and its degradate BCS-CV14885. Seeds of the weed species (EPPO code) *Zea mays* (ZEAMA), *Triticum aestivum* (TRZAS), *Triticum aestivum* (TRZAW), *Hordeum vulgare* (HORVS), *Secale cereale* ((SECCW), *Lolium multiflorum* (LOLMU); *Beta vulgaris* (BEAVA), *Brassica napus* (BRSNS), *Helianthus annuus* (HELAN), *Linum usitatissimum* (LIUUT), *Phaseolus vulgaris* (PHSVN) and *Pisum sativum* (PIBST) were planted in pots and post-emergence applications of mesosulfuron-methyl and BCS-CV14885 were made at rates of 15, 7.5, 3.75 and 1.875 g a.s./ha. Furthermore, "Blindformulierung WP20" [blind formulation not containing active substance] was applied at rates of

60, 30, 15 and 7.5 g a.s./ha. Effects were assessed visually one and three weeks after application. BCS-CV14885 showed no biological activity on the range of weeds tested.

Materials and Methods:

Test materials: 2013-000561 mesosulfuron-methyl; 2013-000563 BCS-CV14885; Blindformulierung WP20.

Test species: 12 weed species (EPPO code): *Zea mays* (ZEAMA), *Triticum aestivum* (TRZAS), *Triticum aestivum* (TRZAW), *Hordeum vulgare* (HORVS), *Secale cereale* ((SECCW), *Lolium multiflorum* (LOLMU); *Beta vulgaris* (BEAVA), *Brassica napus* (BRSNS), *Helianthus annuus* (HELAN), *Linum usitatissimum* (LIUUT), *Phaseolus vulgaris* (PHSVN), *Pisum sativum* (PIBST).

Jiffy pots (7 cm diameter) were filled to within 2 cm of the top with a silt-loam soil (20% sand, 57% silt, 23% clay, pH 6.8 and 1.4% organic matter). Seeds of the weed species (listed above) were sown into these pots and covered with 0.5 to 1 cm of the same soil mixed 1 to 1 with sharp sand. The sowing density was selected based on prior experience to provide approximately 60-70% soil cover by the plants at application timing. After sowing the pots were placed into a glasshouse set 16°C+/-2°C at day and 12°C+/-2°C at night and watered according to need. High pressure sodium lamps (400W) were used to augment daylight during cloudy conditions and to extend the day length to 14 hours.
All compounds used in the test were dissolved in deionized water and diluted to obtain the required dose rates. The post-emergence application (06 February 2013) was made using a track-sprayer with a spray volume of 300 L/ha via a flat fan nozzle (TeeJet 8001). Mesosulfuron-methyl and BCS-CV 14885 were applied at rates of 15, 7.5, 3.75 and 1.875 g a.s./ha. "Blindformulierung WP20" was applied at rates of 60, 30, 15 and 7.5 g a.s./ha.
One week and three weeks after application, the treated plants were visually assessed for injury compared with the untreated control plants. The assessments were on a percentage basis (0 = no effects, 100 = complete kill).

Dates of experimental work: February 6, 2013 to February 28, 2013

Results:

The results of the visual assessments are presented as means from the 2 replicates in the following tables:

Table CA 8.6.1- 4: Results of the first assessment (7 DAT)

	[g a.s./ ha]	ZEA MA	TRZ AS	TRZ AW	HOR VS	SEC CW	BEA VA	BRS NS	HEL AN	LIU UT	PHS VN	PIB ST	LOL MU
2013-000561 Mesosulfuron-methyl	15	3	0	0	0	0	70	25	73	0	20	20	10
	7.5	0	0	0	0	0	70	15	70	0	10	20	10
	3.75	0	0	0	0	0	50	0	70	0	10	20	10
	1.875	0	0	0	0	0	30	0	70	0	10	20	10
2013-000563 BCS-CV 14885	15	0	0	0	0	0	0	0	30	0	0	0	0
	7.5	0	0	0	0	0	0	0	10	0	0	0	0
	3.75	0	0	0	0	0	0	0	5	0	0	0	0
	1.875	0	0	0	0	0	0	0	0	0	0	0	0
Blindformulierung WP 20	60	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0
	7.5	0	0	0	0	0	0	0	0	0	0	0	0

(selectivity/ efficacy)	excellent +++ (0-5/95-100)	good ++ (6-10/90-94)	sufficient + (11-15/80-89)	Side effects +- (16-20/60-79)	Not sufficient/ gaps - (>20/<60)

Table CA 8.6.1- 5: Results of the second assessment (21 DAT)

	[g a.s./ ha]	ZEA MA	TRZ AS	TRZ AW	HOR VS	SEC CW	BEA VA	BRS NS	HEL AN	LIU UT	PHS VN	PIB ST	LOL MU
2013-000561 Mesosulfuron-methyl	15	8	3	0	0	0	63	35	80	0	45	35	55
	7.5	0	0	0	0	0	60	20	75	0	33	20	40
	3.75	0	0	0	0	0	40	0	50	0	25	20	10
	1.875	0	0	0	0	0	20	0	40	0	15	10	0
2013-000563 BCS-CV 14885	15	0	0	0	0	0	0	10	20	0	0	0	0
	7.5	0	0	0	0	0	0	0	8	0	0	0	0
	3.75	0	0	0	0	0	0	0	3	0	0	0	0
	1.875	0	0	0	0	0	0	0	0	0	0	0	0
Blindformulierung WP 20	60	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0
	7.5	0	0	0	0	0	0	0	0	0	0	0	0

(selectivity/ efficacy)	excellent +++ (0-5/95-100)	good ++ (6-10/90-94)	sufficient + (11-15/80-89)	Side effects +- (16-20/60-79)	Not sufficient/ gaps - (>20/<60)

Conclusion:

In a direct comparison study under highly sensitive glasshouse screening conditions, the mesosulfuron-methyl degradate BCS-CV14885 showed no biological activity on the range of weeds tested.

AE F154851, AE F160459, AE F099095, AE F092944, AE F160460, AE F140584, AE F147447

A study (M-185253-01-1) was submitted in the original EU dossier, under the efficacy section. It can be found under point KCA 3.6/03 of this dossier.

The endpoints from this study were not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final). A study evaluation is however available in the Monograph (B.3.1.5.3.1), concluding that none of the identified metabolites showed significant herbicidal activity as compared to the active substance.

The test included the following components assayed for herbicidal activity against a range of grass and broad leaved plant species:

AE F154851
AE F160459
AE F099095
AE F092944
AE F160460
AE F140584
AE F147447

AE F118772 (not an environmental metabolite)
AE F151015 (not an environmental metabolite)

CA 8.6.2 Testing on non-target plants

Test results of studies on non-target plants are product related data and as such reported in document MCP. Since the representative formulation IMS+MSM+MRR OD42 is a mixed-type product containing a second herbicide active substance, no conclusion specifically relvant to the individual substance mesosulfuron-methyl can be drawn from the results of these tests.

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

For mesosulfuron-methyl a screening study on entomology species was performed. Details of the study are provided in the following table.

Table CA 8.7- 1: Effect data of mesosulfuron-methyl WG 20 to entomology screening species presented in this chapter

Test design	Test species	Ecotoxicological endpoint	Reference
Mesosulfuron-methyl, formulated as WG 20			
different treated stages (eggs, larvae, all stages), 6 d; additional root systemicity test on *Aphis fabae*,	*Spodoptera littoralis, Heliothis virescens, Aphis fabae, Nilaparvata lugens, Diabrotica undecimpunctata, Meloidogyne incognita, Tetranychus urticae, Vicia, faba, Aphis fabae* (root systemic activity)	The test item is not effective on any tested species.	[redacted] 2000, M-198522-01-1, KCA 8.8.1/01

Report:	[redacted];[redacted];2000;M-198522-01
Title:	Effectivity of the herbicide mesosulfuron-methyl (provisionally approved ISO) on entomology screening species Code: AE F130060
Report No:	C010159
Document No:	M-198522-01-1
Guidelines:	**EU(=EEC): 91/414;Deviation not specified**
GLP/GEP:	**no**

Study endpoint according to the Monograph evaluation (B.9.9.2):
Mesosulfuron-methyl was not effective on any tested species.

The endpoint from this study was not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final).

CA 8.8 Effects on biological methods for sewage treatment

For mesosulfuron-methyl, studies with activated sludge and *Pseudomonas putida* have been conducted. An overview on both studies is provided in the following table. Based on these test results, the Monograph evaluation (B9.10.3) concluded no impact of mesosulfuron-methyl on sewage treatment processes.

Table CA 8.8- 1: Effect data of mesosulfuron-methyl to activated sludge presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Mesosulfuron-methyl			
Activated sludge	Respiration inhibition, 3 h, static (OECD 209)	Activated sludge, inhibition of respiratory activity: EC_{20} > 1000 mg/L EC_{50} > 1000 mg/L EC_{80} > 1000 mg/L	[redacted], 1996 M-141355-01-1 KCA 8.8. /01
Pseudomonas putida	Cell multiplication inhibition test, 16.5 h (DIN/EN/ISO 10712 (1996) (DEV-L8)	*Pseudomonas putida*, inhibitory effect of water-soluble test substances: EC_{10} 75 mg/L (Confidence interval: 12-144 mg/L, P = 95 %) EC_{50} 298 mg/L (Confidence interval: 158-699 mg/L, P = 95 %) The EC_{50} of the test item is in the range of >100 mg/L to 1000 mg/L	[redacted], 1997 M-142498-01-1 KCA 8.8. /02

Report:	[redacted] <; [redacted] ;1996;M-141355-01
Title:	Testing of respiration inhibition to activated sludge of Hoe 120060, substance technical (Code: Hoe 120060 00 ZC96 0002)
Report No:	A57673
Document No:	M-141355-01-1
Guidelines:	**EU (=EEC): 88/302/C; OECD: 209 Deviation not specified**
GLP/GEP:	**yes**

Study endpoint according to the Monograph evaluation (B.9.10.1):

EC_{50} 3 h > 1,000 mg a.s./l; nominal concentration

The results from this study were not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final).

Report:	[redacted] l; [redacted] ;1997;M-142498-01
Title:	Determination of the inhibitory effect of water constituents on bacteria (Pseudomonas cell multiplication inhibition test)
Report No:	A58780
Document No:	M-142498-01-1
Guidelines:	**DIN 10712; ISO 10712; Deviation not specified**
GLP/GEP:	**yes**

Study endpoint according to the Monograph evaluation (B.9.10.2):

EC_{50} 16.5 h = 298 mg a.s./l; nominal concentration, 95% CI = [158-699] mg/l.

The results from this study were not mentioned in the Review Report for mesosulfuron-methyl (SANCO/10298/2003-Final).

CA 8.9 Monitoring data

No monitoring data have been created by the notifier since no additional data was deemed necessary to complete risk assessments. No relevant and reliable monitoring studies were found in the required literature searches of the peer reviewed open literature.