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Document Title

Summary of the ecotoxicological studies for Iodosulfuron-methyl-sodium

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Section 8: Ecotoxicological studies

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

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Version history

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

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Ecotoxicological properties of iodosulfuron-methyl-sodium (AE F115008) in soil, water and air were submitted and evaluated within the original EU Dossier which resulted in the Annex I inclusion in 2003. The evaluation of the studies evaluated at that time was published in the form of a Monograph and its amendments. These studies are presented in this document in grey boxes. Copies of the study reports are included in the electronic dossier (Baseline Dossier). The numbering and the headlines correspond to latest EU requirements. No detailed summary of these data are presented in this update. In the Supplemental Dossier for Annex I Renewal presented here, only those ecotoxicological studies are described, which were not submitted within the baseline dossier.

For a better overview, study endpoints resulting from the evaluation process of Annex I inclusion are presented in this document, together with the information whether or not this endpoint was listed in the List of Endpoints in the Review Report (SANCO/10166/2003-Final).

Due to changes in triggers for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table 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 for risk assessment*

Compartment	Compound / Code
Soil, Groundwater	Iodosulfuron-methyl-sodium AE F075736 AE F145741 AE F145740 AE 0002166 AE F161778 BCS-CW81253 AE 0000119 AE F059411
Surface water	Iodosulfuron-methyl-sodium AE F075736 AE F145741 AE F145740 AE 0002166 AE F161778 BCS-CW81253 AE 0000119 AE F059411 AE 0014966 AE 1234964 AE F154781 AE F159737 AE 0034855
Plant material	Iodosulfuron-methyl-sodium

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

Metabolite testing for aquatic organisms

Compared to the toxicity to other aquatic organisms iodosulfuron-methyl-sodium show higher toxicity to the alga *Pseudokirchneriella subcapitata*. But *Lemna* is by far the most sensitive standard aquatic organism to the parent compound. Therefore, the metabolite testing was confined to these two species in most cases, with four exceptions: AE F075736, AE F059411, AE 1234964 and AE F159737. These are common metabolites with one or more other sulfonyl urea herbicides. Tests with further aquatic species have been performed in context of risk assessments for other parent compounds. Although for the risk assessment of iodosulfuron-methyl-sodium these studies on further species are not considered essential, they are provided here for sake of completeness.

Metabolite testing for soil organisms

As earthworms for the active ingredient iodosulfuron-methyl-sodium and the soil mite *Hypoaspis aculifer* for the representative formulation are the most sensitive species, the metabolites AE F145741, AE F145740 and AE 0002166, whose chemical structures are very similar to the parent compound, were tested for these two species only. However, for the metabolites BCS-CW81253, AE 0000119 and AE F059411 the full data package is provided as their chemical structures are very different to that of the parent compound.

For AE F075736 due to its occurrence of 88.5% in soil and its herbicidal activity, data for all species (earthworms, soil mites and springtails) are provided.

No studies for earthworms, soil mites and springtails were performed for the metabolite AE F161778, as data for earthworms and soil mites from AE F145741 as preceding metabolite and data for all test species from BCS-CW81253 as succeeding metabolite are available and do not show any toxicity (NOEC ≥100 mg/kg dws).

N-transformation-studies were done for the parent and all metabolites.

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

Three acute studies on different bird species, bobwhite quail, mallard duck and Japanese quail, were performed. The highest tested dose level in all studies was 2000 mg/kg bw. No mortality occurred in any of these studies. Details of the studies are provided in the following table.

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

Test species	Test design	Ecotoxicological endpoint			Reference
Bobwhite quail	acute, oral	LD_{50} LD_{50}	**> 2000** 1) 2) 3776 3)	mg as/kg bw	XXXXX 1998 M-181334-01-1 KCA 8.1.1.1 /02
Mallard duck	acute, oral	LD_{50} LD_{50}	**> 2000** 1) 3776 3)	mg as/kg bw	XXXXX, 1997 M-142450-01-1 KCA 8.1.1.1 /03
Japanese quail	acute, oral	LD_{50} LD_{50}	**> 2000** 1) 3776 3)	mg as/kg bw	XXXXX, 1996 M-140780-01-1 KCA 8.1.1.1 /01

1) Endpoint based on 10 birds per group; no mortality occurred during study
2) Endpoint (LD_{50}) listed in Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final)
3) LD_{50} extrapolated according EFSA GD B&M 2009
Bold letters: Value considered relevant for risk assessment in the MCP document

Studies on iodosulfuron-methyl-sodium

Report:	[redacted]; [redacted];1996;M-140780-01
Title:	Acute oral toxicity in the male and female Japanese quail (Coturnix coturnix japonica) Hoe 115008 substance, technical Code: Hoe 115008 00 ZC89 0001
Report No:	A57019
Document No(s):	M-140780-01-1
Guidelines:	**OECD: Draft (1992); USEPA (=EPA): 71-1;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final).

Report:	[redacted]; [redacted];1998;M-181334-01
Title:	Bobwhite quail acute oral toxicity test AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	C000842
Document No(s):	M-181334-01-1
Guidelines:	**OECD: Draft from 1992; USEPA (=EPA): §71-1, 540/9-82-024;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

LD_{50} > 2000 mg as/kg bw*

* No mortality was observed at the highest test level. Therefore an LD50 can be extrapolated according EFSA Guidance Document Birds &Mammals 2009.

Report:	[redacted];[redacted];1997;M-142450-01
Title:	Acute oral toxicity in the male and female mallard duck (Anas platyrhynchos) Hoe 115008 substance, technical Code: Hoe 115008 00 ZC89 0001
Report No:	A58728
Document No(s):	M-142450-01-1
Guidelines:	**OECD: Draft; USEPA (=EPA): FE 71-1;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final).

CA 8.1.1.2 - Short-term dietary toxicity to birds

Three short-term dietary studies on different bird species, bobwhite quail, mallard duck and Japanese quail, were performed. The lowest LC_{50} was determined to be > 5000 ppm. Details of the studies are provided in the following table.

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

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	5-day dietary	LC_{50} ≥ 5000 [1) 2)] ppm LDD_{50} 800 mg as/kg bw/d	[redacted], 1998 M-181275-01-1 KCA 8.1.1.2 /02
Mallard duck	5-day dietary	LC_{50} ≥ 5000 ppm LDD_{50} 1600 mg as/kg bw/d	[redacted], 1996 M-141346-01-1 KCA 8.1.1.2 /03
Japanese quail	5-day dietary	LC_{50} ≥5000 [1)] ppm LDD_{50} ≥ 1100 mg as/kg bw/d	[redacted], 1996 M-141824-01-1 KCA 8.1.1.2 /01

[1)] 10 birds per group; no mortality occurred during study
[2)] Endpoint listed in Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final)

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1996;M-141824-01
Title:	Avian dietary LC 50 test in the Japanese quail Hoe 115008 substance, technical Code: Hoe 115008 00 ZC89 0001
Report No:	A58711
Document No(s):	M-141824-01-1
Guidelines:	**OECD: 205;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final).

Report:	[redacted];1998;M-181275-01
Title:	Bobwhite quail dietary LC50 study Hoe 115008 substance, technical Code: Hoe 115008 00 ZC89 0001
Report No:	C000806
Document No(s):	M-181275-01-1
Guidelines:	**OECD: 205; USEPA (=EPA): §71-2, PB83-153908;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

LC_{50} > 5000 ppm*

* This endpoint corresponds to the endpoint of the conclusion in the study report.

Report:	[redacted];1996;M-141346-01
Title:	Avian dietary LC50 test in the mallard duck (Anas platyrhynchos) Hoe 115008 substance, technical Code: Hoe 115008 00 ZC89 0001
Report No:	A57664
Document No(s):	M-141346-01-1
Guidelines:	**OECD: 205;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final).

CA 8.1.1.3 - Sub-chronic and reproductive toxicity to birds

Four reproductive studies on different bird species, bobwhite quail, japanese quail and mallard duck were performed. The NOAEL was determined to be ≥ 78 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 iodosulfuron-methyl-sodium presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Bobwhite quail	6-weeks feeding chronic, reproduction	NOAEC ≡ NOAEL	≥ 1000 [1)] ≥ 93	ppm mg as/kg bw/d	[redacted], 1998 M-181277-01-1 KCA 8.1.1.3 /02
Bobwhite quail	23-weeks feeding chronic, reproduction	NOAEC ≡ NOAEL	≥ 1077 **≥ 78**	ppm mg as/kg bw/d	[redacted], 2004 M-242537-01-1 KCA 8.1.1.3 /04
Japanese quail	22-weeks feeding chronic, reproduction	NOAEC ≡ NOAEL	≥ 1000 ≥ 104	ppm mg as/kg bw/d	[redacted], 1998 M-181284-01-1 KCA 8.1.1.3 /01
Mallard duck	22-weeks feeding chronic, reproduction	NOAEC ≡ NOAEL	≥ 1000 ≥ 125	ppm mg as/kg bw/d	[redacted] 1999 M-191367-01-1 KCA 8.1.1.3 /03

1) Endpoint listed in review report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final)
Bold letters: Value considered relevant for risk assessment in the MCP document

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1998;M-181284-01
Title:	Reproduction toxicity study in the Japanese quail (Coturnix coturnix japonica) Hoe 115008 substance technical Code: Hoe 115008 00 ZC89 0001
Report No:	C000812
Document No(s):	M-181284-01-1
Guidelines:	**OECD: 206, 4-Apr-1984; USEPA (=EPA): §71-4, Oct. 1982;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final).

Report:	[redacted];[redacted];1998;M-181277-01
Title:	Bobwhite quail 6-week dietary reproduction study - Limit-Test Hoe 115008 substance technical Code: Hoe 115008 00 ZC89 0001
Report No:	C000807
Document No(s):	M-181277-01-1
Guidelines:	**OECD: 206, Draft 1997; USEPA (=EPA): §71-4;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

NOEC = 1000 ppm*

* In the review report the term NOEL was used. However, the value corresponds to the NOAEC reported in the conclusion in the study report.

Two new studies have been performed since the Annex I inclusion and are submitted within this supplemental dossier for the iodosulfuron-methyl-sodium Annex I Renewal

Report:	[redacted];[redacted];[redacted];1999;M-191367-01
Title:	Mallard duck dietary reproduction toxicity study AE F115008 substance technical Code: AE F115008 00 1C89 0001
Report No:	C005102
Document No:	M-191367-01-1
Guidelines:	**OECD: 206; USEPA (=EPA): §71-4;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The objective of this study was to assess the effects of continuous dietary exposure to iodosulfuron-methyl-sodium (code: AE F115008) on the reproductive performance of mallard ducks (*Anas platyrhynchos*) according to USEPA (FIFRA) guidelines. The study was comprised of three treated groups and one control group. Each group contained 16 pairs of birds with one male and one female per pen. Dietary concentrations of 0, 40, 200 and 1000 ppm of technical AE F115008 (equivalent to an achieved daily intake of 0, 5.4, 23.7 and about 125 mg test item/kg body weight/day) were fed to groups of adult birds for ten weeks prior to egg laying and during 11 weeks of egg production. The no observed effect concentration for mallard ducks treated with AE F115008 in the diet during this reproduction study was 1000 ppm test concentration (equivalent to an achieved daily intake of 125 mg AE F115008 /kg body weight/day).

Material and Methods:

Test item: technical AE F115008; Code: AE F115008 00 1C89 0001; Batch No.: 21436/02/950601, Purity: 87.4% (w/w); No. of certificate: AZ 06501.

The study was comprised of three treated groups and one control group. Each group consisted of 16 pens with one male and one female per pen. Dietary concentrations of 0, 40, 200 and 1000 ppm of technical AE F115008 were fed to groups of adult birds for ten weeks prior to egg laying and during 11 weeks of egg production.
Adult birds were observed for mortality, clinical signs of toxicity, body weight, feed consumption and egg production. Each bird was subjected to a gross postmortem examination.
Eggs were collected, incubated and subsequently examined for fertility, embryoviability and hatchability. Hatched offspring were reared for 14 days on untreated diet and monitored for survival and body weight. Egg shell thickness was measured in a representative proportion of eggs.

Dates of experimental work: July 22, 1998 to January 28, 1999

Results:

Mallard ducks exposed to AE F115008 at dietary concentrations of 0, 40, 200 or 1000 ppm for 22 weeks received daily intakes of 0, 5.4, 23.7 and about 125 mg AE F115008 /kg body weight/day, respectively.

Mortalities
No mortalities occurred during the test.

Clinical observations
No clinical signs of toxicity were seen at any of the concentrations tested. Incidental clinical observations such as foot lesions, an unkempt appearance and feather loss, normally associated with pen wear or interactions among penmates, were observed. Other clinical signs such as lameness, wing droop and a thin appearance occasionally were noted, 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.

Body weight:
There were no treatment-related effects upon adult body weight at any of the concentrations tested. Any differences in body weight between the control group and each of the treatment groups were not statistically significant at any of the body weight intervals.

Feed consumption:
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 were increases in feed consumption that were statistically significant at $p < 0.01$ during week 8 and at $p < 0.05$ during Weeks 9, 11, 12 and 18. Since the increases were not consistent over the test period and were not concentration-dependent, the observed differences were not considered to be treatment-related. Any other differences in feed consumption between the control and treatment groups were slight, not statistically significant, and not consistent over the test period.

The estimated test substance intake for mallard ducks during the test was 5.4, 23.7 and 125 mg AE F115008 /Kg body weight/day for the 40, 200 and 1000 ppm treatment groups, respectively.

<u>Necropsy findings:</u>
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.

<u>Egg production, embryo viability, hatching and offspring survival:</u>
There were no treatment-related effects upon reproductive performance in the 40, 200 or 1000 ppm treatment groups. There was a slight increase in hatchlings as a percentage of live three-week embryos at the 40 ppm test concentration. Although the difference from the control value was statistically significant at $p < 0.05$, it represented an improvement and was considered not to be treatment-related. Any other differences from the control group were not statistically significant for any of the reproductive parameters measured.
There was also a slight, but statistically significant ($p < 0.01$) decrease in live three-week embryos as a percentage of viable, embryos at the 200 ppm test concentration; however, in the absence of a confirmatory finding at the 1000 ppm concentration, this minimal change was not considered to be treatment-related, but due primarily to the exceptional high performance (100%) by the control group.

<u>Egg shell trhickness:</u>
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.

<u>Offspring body weights:</u>
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 from the control group were not statistically significant.

<u>Test Diet Analysis</u>
Samples of test diets fed to mallards were analyzed for AE Fl 15008. Diet samples were collected from the 40, 200 and 1000 ppm test concentrations and analyzed to evaluate the homogeneity and stability of the test substance in the diet, and also the achieved concentrations. The achieved concentration, stability and homogeneous distribution of the test substance in the diet for seven days at room temperature were confirmed as acceptable, i.e. in the range of 85-105% of nominal.

<u>Validity criteria:</u>
This study was of excellent technical quality as evidenced by a very high reproductive performance of the control pairs, which was in 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-week (≥ 10), egg shell thickness (>0.34 mm) and adult mortality (≤ 10%) were clearly met by the control group.

Conclusions:
The no observed effect concentration for mallard ducks treated with AE F115008 in the diet during this reproduction study was 1000 ppm test concentration (equivalent to an achieved daily intake of 125 mg AE F115008 /kg body weight/day).

Report:	[redacted];[redacted];2004;M-242537-01
Title:	Effect of technical Iodosulfuron methyl sodium on northern bobwhite reproduction
Report No:	EBIMX013
Document No(s):	M-242537-01-1
Guidelines:	**FIFRA 71-4 Avian Reproduction;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of Iodosulfuron-methyl-sodium (code: AE F115008; purity 92.3%) on the reproduction of Northern Bobwhite Quail (*Colinus virginianus*) after dietary uptake.
Colinus virginianus were exposed to treated feed during a period of 23 weeks. Eggshell thickness, embryo viability and survival, survival of hatchlings and their body weight were observed thereafter for another 14 days while fed with untreated feed. Concentrations in feed were 100, 333 and 1000 ppm which corresponded to daily uptake doses of 7.4, 25 and 78 mg a.s./kg b.w./day. In addition untreated diet as negative control was tested. Mortality, signs of intoxication, food consumption, body weight, reproduction parameters and gross necropsy were used to determine the endpoints. The NOEC was determined to be 1077 ppm, which corresponds to an NOEL of 78 mg/kg b.w./day.

Materials and Methods:

Test item: Iodosulfuron-methyl-sodium, substance, technical; identification code: AE F115008; batch no.: AAIR03011; analysed purity: 92.3% w/w.

90 pairs of young northern bobwhites (*Colinus virginianus*, 16 weeks old at receipt) were acclimated to the lab for 4 weeks. Eighteen pairs were dosed for 23 weeks at each treatment level. The mean measured dietary concentrations were control (<10), 103, 372 and 1077 mg a.s./kg feed. The corresponding daily dietary dose was 0, 7, 25 and 78 mg a.s./kg bw/day at these treatment levels, respectively. The exposure period was divided into a pre-photostimulation period of 10 weeks, including a pre-egglaying period of 7 weeks and an egglaying period of 15 weeks. Within the consecutive post-adult termination period of 14 days survival and body weight of the F1-generation was observed up to two weeks after hatch. Each cage served as one replicate containing 1 male and 1 female. The test was conducted with 18 replicates per dose. Per dose a total amount of 11 kg feed was prepared weekly. Mortality and signs of intoxications were assessed daily. Body weight measurements were conducted at week 1, 3, 5, 7 and 9 and after birds were sacrificed. The food consumption was calculated from weighing the residual food weekly throughout the study. Egg incubation was initiated weekly (after start of reproduction). Some eggs were retained for measurements of shell thickness. Candling in order to assess embryo viability and survival was done on day 11 of incubation and on day 18 of incubation, respectively. Body weight of hatchlings was measured after completion of hatching and after 14 days.
Food was analysed in order to verify the homogeneity and the concentrations of the test item and its ambient stability in the feeder.

Dates of experimental work: October 01, 2003 – May 10, 2004

Results:

Validity Criteria:
The control mortality was less than 10%. Measured concentrations of test item in the feed were above 80% of nominal. The shell thickness of eggs from the controls was above the species-specific threshold. The average number of 14-day-old survivors per hen in the controls was above the species-specific threshold.

Analytical findings:
Results from analytical measurements are summarized as follows:

Table CA 8.1.1.3-2: Homogeneity and analytical verification of concentrations of AE F115008

	Homogeneity in diet		Verification of concentrations in diet	
	mean and % cv (1st measurement)	**mean and % cv (2nd measurement)**	**range**	**mean percent of day 0**
100 ppm	116 (2%)	102 (3%)	95 - 109 ppm	103%
333 ppm	n.d.	n.d.	364 - 381 ppm	112%
1000 ppm	1169 (4%)	1048 (4%)	1034 - 1101 ppm	108%

Table CA 8.1.1.3-3: Stability of AE F115008 during a seven-day period in the freezer and the feeder

	freezer		feeder	
day 0 concentration	**mean measured (ppm a.i.)**	**mean percent of nominal**	**mean measured (ppm a.i.)**	**mean percent of day 0**
116 ppm	95	82	107	92
1169 ppm	1108	95	1153	99

Biological findings:
Six adult females and three males died during the study. There was no significant difference in adult mortality as compared to the control at any treatment level. Symptoms (bloody intestinal tracts and pericardial sacs associated with small lesions) were not treatment related. No overt signs of intoxication were observed during the study in any adult test group. Occurrences of feather loss, abrasions, etc., all associated with normal laboratory cage housing, were observed in the control and all treatment levels.
The female treatment birds and the controls had very similar weight gains over the exposure period. Although the male birds at the 1077 mg a.s./kg treatment level had a slower weight gain than the controls, the measured body weight of the male birds at all treatment levels was very similar.

Table CA 8.1.1.3-4: Effect of AE F115008 on body weight and food consumption of *Colinus virginianus*

nominal test concentration	over-all (weeks 1 to 23)	
	mean food consumption (g/bird/day)	dietary dose (mg/kg/day)
untreated control	21	
100	22	7.4
333	21	25.0
1000	22	78.0

Table CA 8.1.1.3-5: Effect of AE F115008 on mean body weight (g) of *Colinus virginianus*

sex	week 1	week 3	week 5	week 7	week 9	termination	difference
Control							
Male	271	277	278	287	287	309	36
Female	281	288	293	298	299	341	62
100 ppm							
Male	277	284	286	294	295	316	39
Female	280	285	286	294	296	345	64
333 ppm							
Male	285	282	282	290	292	320	33
Female	279	282	284	290	291	348	63
1000 ppm							
Male	281	283	285	291	293	308	28
Female	281	283	286	293	295	352	69

Table CA 8.1.1.3-6: Summary of reproductive performance of *Colinus virginianus* treated with AE F115008

	control	100 ppm	333 ppm	1000 ppm
Total eggs laid	746	706	769	725
Eggs cracked	13	11	8	5
Eggs set	637	630	701	658
Live 3-Week Embryos	592	539	595	625
Hatchlings	534	514	557	596
14-Day Old Survivors	530	505	543	589
Eggs Laid/Hen	41	39	43	40
14-Day Old Survivors/Hen	29	28	30	33
Eggs Not Cracked/Eggs Laid (%)	98	96	99	99
Viable Embryos / Fertile Eggs (%)	99	98	99	99
Hatchlings/Eggs Laid per Hen (%)	71	67	73	81
14-Day Old Survivors/Eggs Set (%)	82	78	77	88

Table CA 8.1.1.3-7: Egg shell thickness of *Colinus virginianus* treated with AE F115008

	# eggs measured	shell thickness (mean ± SD) mm
control	14	0.21± 0.02
100 ppm	16	0.21± 0.01
333 ppm	14	0.21± 0.01
1000 ppm	16	0.21± 0.02

Table CA 8.1.1.3-8: Body weight of hatchlings of *Colinus virginianus* treated with AE F115008

	hatchlings	14 day old survivors
	body weight (mean ± SD) g	body weight (mean ± SD) g
control	8.0 ± 0.7	46.9 ± 2.9
100 ppm	8.0 ± 0.5	47.4 ± 2.8
333 ppm	7.8 ± 0.6	46.0 ± 2.2
1000 ppm	8.0 ± 0.5	46.4 ± 2.8

There were no compound related adverse effects noted during the 23-week exposure.

Conclusions:

Based on all parameters, the lowest-observed-effect level (LOEL) for adult Northern Bobwhite Quail (*Colinus virginianus*) exposed to technical Iodosulfuron-methyl-sodium in the diet was greater than 1077 mg a.s./kg feed or 78 mg a.s./kg bw/day and the NOEL was 1077 mg a.s./kg feed or 78 mg a.s./kg bw/day.

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 2678 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 iodosulfuron-methyl-sodium presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Rat	acute, oral	LD_{50}	**2678**	mg as/kg bw	[redacted], 1993 M-132162-01-1 KCA 5.2.1 /01

1) Mean of male and female
Bold letters: Value used for risk assessment

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

LD_{50} = 2678 mg/kg bw

CA 8.1.2.2 - Long-term and reproduction toxicity to mammals

A two-generation feeding reproduction study on rats was performed. The NOAEC was determined to be 500 ppm. Details of the studies are provided in the following table.

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

Test species	Test design	Ecotoxicological endpoint			Reference
Rat	Two-generation feeding-reproduction	NOAEC* = NOAEL	500 **≥ 50**	ppm mg as/kg bw/d	[redacted], 1998 M-182825-01-1 KCA 5.6.1 /02

*This endpoint was presented as NOEL/NOAEL in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final))
Bold letters: Value used for risk assessment

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

NOEC = 500 ppm

NOAEC = 500 ppm is stated in chapter 3 "Ecotoxicology" of SANCO/10166/2003-Final (2003). According to chapter 1 "Toxicology and metabolism" of SANCO/10166/2003-Final (2003), the NOAEL = 500 ppm corresponds to a NOAEL of 50 mg as/kg bw/d.

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

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

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

Since iodosulfuron-methyl-sodium 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 iodosulfuron-methyl-sodium to wildlife presented below.

Wild Mammals:
Potential endocrine activity and potential population relevant effects of iodosulfuron-methyl-sodium 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 iodosulfuron-methyl-sodium is not an endocrine disrupter.

Birds:
The population relevant effects of iodosulfuron-methyl-sodium on birds were studied in reproductive toxicity studies on Japanese quail, bobwhite quail and mallard ducks. For all three 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 any of the species, it is concluded that there are no population relevant adverse effects of iodosulfuron-methyl-sodium. No additional studies seem necessary.

[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.

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

Conclusion:

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

CA 8.2 - Effects on aquatic organisms

Aquatic organisms have been tested with the active ingredient and the metabolites included in the residue definition for aquatic risk assessment (see MCA Section CA 7.4.1).
Compared to the toxicity to other aquatic organisms iodosulfuron-methyl-sodium show higher toxicity to the alga *Pseudokirchneriella subcapitata*. But *Lemna* is by far the most sensitive standard aquatic organism to the parent compound. Therefore, the metabolite testing was confined to these two species in most cases, with four exceptions: AE F075736, AE F059411, AE 1234964 and AE F159737. These are common metabolites with one or more sulfonyl urea herbicides. Tests with further aquatic species have been performed in context of risk assessments for other parent compounds. Although for the risk assessment of iodosulfuron-methyl-sodium these studies on further species are not considered essential, they are provided here for sake of completeness.

CA 8.2.1 - Acute toxicity to fish

For iodosulfuron-methyl-sodium three acute toxicity studies on three different fish species were performed. The tested dose level in all studies was 100 mg a.s./L. No sublethal effects and only intoxication symptoms of one individual fish in one concentration (in one study only) were observed in the treatment, resulting in an LC_{50} of > 100 mg a.s./L.
For the metabolites AE 1234964 and AE F159737 acute studies on rainbow trout were conducted with test doses of 100 mg/L. The 96-hour-LC_{50} of both studies was > 100 mg/L.
Details of all studies are provided in the following table.

Table CA 8.2.1-1: Acute toxicity data of iodosulfuron-methyl-sodium and metabolites to fish presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Iodosulfuron-methyl-sodium				
Oncorhynchus mykiss (rainbow trout)	Acute, static	96 h	LC_{50} > 100	[redacted], 1998 CE96/098 M-143096-01-1 KCA 8.2.1 /01
Lepomis macrochirus (bluegill sunfish)	Acute, static	96 h	**LC_{50} > 100**	[redacted], 1998 CE96/098 M-143095-01-1 KCA 8.2.1 /02
Cyprinodon variegates (sheepshead minnow)	Acute, static	96 h	LC_{50} > 100	[redacted], 2000 M-238449-02-1 KCA 8.2.1 /03
AE 1234964				
Oncorhynchus mykiss (rainbow trout)	Acute, static	96 h	LC_{50} > 100	[redacted], 2006 M-278097-01-1 KCA 8.2.1 /04
AE F159737				
Oncorhynchus mykiss (rainbow trout)	Acute, static	96 h	LC_{50} > 100	[redacted], 2006 M-278099-01-1 KCA 8.2.1 /05

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

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1998;M-143096-01
Title:	Acute toxicity to rainbow trout (Oncorhynchus mykiss) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59423
Document No:	M-143096-01-1
Guidelines:	**EU (=EEC): 92/69 C.1; OECD: No. 203; USEPA (=EPA): E § 72-1;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted];[redacted];1998;M-143095-01
Title:	Acute toxicity to bluegill sunfish (Lepomis macrochirus) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59422
Document No:	M-143095-01-1
Guidelines:	**EU (=EEC): 92/69 C.1; OECD: No. 203; USEPA (=EPA): E § 72-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

EC_{50} > 100 mg/L

Report:	[redacted];[redacted];2000;M-238449-02; Amended: 2000-02-28
Title:	96 hour acute toxicity to the sheepshead minnow, Cyprinodon variegatus, in a static renewal system: AE F115008 technical 89.6% w/w: AE F115008 00 1C89 0001
Report No:	B002715
Document No(s):	M-238449-02-1
Guidelines:	**EU (=EEC): Annex II Point 8.2.1; USEPA (=EPA): 72-3;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary

The aim of the study was to determine the acute effects of Iodosulfuron-methyl-sodium (code: AE F115008 00 1C89 0001; purity 86.9% w/w) to sheepshead minnow (*Cyprinodon variegatus*). *Cyprinodon variegatus* were exposed in a semi-static system over a period of 96 hours to the nominal concentration of 100 mg a.s./L (limit test). 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 > 100 mg a.s./L, the 96-hour-NOEC was determined to be 100 mg a.s./L.

Material and Methods

Test item: Iodosulfuron-methyl-sodium, technical; Batch No.: CR21436/02/950601; Code No.: AE F115008 00 1C89 0001; CAS Reg. No.: 144550-36-7; Analysed purity: 86.9% w/w; Certificate of Analysis: AZ 07987.

Juvenile sheepshead minnows (*Cyprinodon variegatus*) were exposed to Iodosulfuron-methyl-sodium in a semi-static system over a period of 96 hours to a nominal concentration of 100 mg a.s./L in synthetic sea water (mena temperature of 21.9°C). In addition a water control was tested. Each vessel (glass aquaria; 20 L) served as one replicate filled with 15 L synthetic sea water. 10 fish were used per replicate. Length of fish at test start was mean 2.5 cm (range 2.1 to 3.0 cm). Body weight of fish at test start was mean 0.480 (range 0.238 to 0.717 g). The static biological loading was 0.320 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 day 0 and day 4. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: October 18, 1999 – October 22, 1999

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 99% of nominal calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table CA 8.2.1-2: Nominal and measured concentrations of AE F115008 00 1C89 0001

Nominal concentration in mg test item / L	Measured Concentration in mg a.s./L (average of 2 detections)		
	on day 0	on day 4	Mean
Control	< LOQ	< LOQ	< LOQ
100	104.2	95.6	99.87

Biological findings:
No mortality was observed in any replicate. No sublethal behavioural changes were observed.

Conclusion

The acute effect of Iodosulfuron-methyl-sodium (AE F115008 00 1C89 0001) on sheepshead minnow (*Cyprinodon variegatus*) can be quantified as a 96-hour-LC_{50} of >100 mg a.s./L. The highest concentration with no observed mortality and no sublethal behavioural effects can be set to 100 mg a.s./L.

Studies on the metabolites of iodosulfuron-methyl-sodium

AE 1234964

Report:	[redacted];[redacted];2006;M-278097-01
Title:	Acute toxicity of MKH 6561-sulfonamide acid to rainbow trout (Oncorhynchus mykiss) in a 96-hour static test - limit test -
Report No:	30183230
Document No:	M-278097-01-1
Guidelines:	**Commission Directive 92/69/EEC, Annex Part C, C.1:"Acute Toxicity for Fish", Official Journal of the European Communities No. L 383 A, dated December 29, 1992 OECD Guideline for Testing of Chemicals, Section 2, No. 203: "Fish, Acute Toxicity Test", adopted July 17, 1992;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to evaluate the acute toxicity of AE 1234964 (also called: MKH 6561-sulfonamide acid) to rainbow trout (*Oncorhynchus mykiss*). Juvenile Rainbow trout were exposed in a static test over a period of 96 hours to the nominal concentration of 100 mg test item/L and to control (test water only) under defined conditions (limit test). The method is recommended by the test guidelines, and also rainbow trout is one of the fish species recommended by the international test guidelines of the OECD and EEC.
The recorded effects were the mortality and visible abnormalities of the fish. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour NOLEC value was ≥ 100 mg test item/L, therefore the LC50 was greater than 100 mg test item/L.

Material and Methods:

Test item: MKH 6561-sulfonamide acid; Batch code: AE 1234964-PU-01; Origin Batch No.: M00102; Content of active ingredient: 99 % w/w; Certificate No.: AZ 13380.

Juvenile *Oncorhynchus mykiss* were exposed to AE 1234964 in a static system over a period of 96 hours to the nominal concentration of 100 mg test item/L. In addition a reconstituted water control was tested. 20-Litre glass aquaria filled with 18 L test medium were used as test units. The test was

performed with one replicate for the test concentration and the control. 10 fishes were used per replicate. The test media was slightly aerated during the test. The water hardness was 2.5 mmol/L (= 250.0 mg/L) as $CaCO_3$. The mean length of the fish in the test was 5.12 cm (mean of ten fishes). The mean body wet weight was 1.16 g (mean of ten fishes). The maximum loading rate was 1 g fish/L test water. The test fish were observed after approximately 2, 24, 48, 72 and 96 hours test duration for visible abnormalities (e.g. apathy, convulsions, strong ventilation, tumbling etc.) and mortality. The quantification of the test item was performed using liquid chromatography (HPLC-method).

Dates of experimental work: May 22, 2006 to May 26, 2006 (biological part)
May 26, 2006 (date of analysis)

Results:

Validity criteria:
The experiment is valid because no fish died in the control and oxygen saturation was always > 60 %.

Analytical results:
At the start of the test just before introduction of the fish 99 % of the nominal test concentration was found. After 96 hours test duration 102 % of the nominal value was determined. Thus, during the test period of 96 hours the fish were exposed to a mean of 100 % of nominal. Therefore, all reported results are related to nominal concentration of the test item.

Table CA 8.2.1-3: Summary of analytical results

Sample description [mg test item/L]	% of nominal[1]	RSD
control	n.a.	n.a.
100	100	2

[1] mean value of all measured samples per treatment group (start and end)
RSD: relative standard deviation per treatment group
n.a.: not applicable

Biological results:
The 96-hour NOEC (highest concentration tested without toxic effects after the exposure period of 96 hours), respectively the 96-hour NOLEC (maximum concentration which did not cause any mortality within the period of test) of AE 1234964 to Rainbow trout was determined to be at least 100 mg test item/L. The NOEC and the NOLEC might even be higher than this concentration, but concentrations in excess of 100 mg test item/L have not been tested. The 96-hour LOEC, the 96-hour LC_{50} and the 100% mortality were higher than 100 mg test item/L. These values could not be quantified due to the absence of toxicity of AE 1234964 up to the tested concentration.

Conclusions:

The toxic effect of the test item, AE 1234964 to Rainbow Trout (*Oncorhynchus mykiss*) was assessed in a static, limit test. The 96-hour NOLEC value was ≥ 100 mg test item/L, therefore the LC_{50} was greater than 100 mg test item/L.

AE F159737

Report:	[redacted];[redacted];2006;M-278099-01
Title:	Acute toxicity of MKH 6561-saccharine to rainbow trout (Oncorhynchus mykiss) in a 96-hour static test - limit test -
Report No:	30193230
Document No:	M-278099-01-1
Guidelines:	**Commission Directive 92/69/EEC, Annex Part C, C.1: "Acute Toxicity for Fish", Official Journal of the European Communities No. L 383 A, dated December 29, 1992** **OECD Guideline for Testing of Chemicals, Section 2, No. 203: "Fish, Acute Toxicity Test", adopted July 17, 1992;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to evaluate the acute toxicity of AE F159737 (also called: MKH 6561-saccharine) to rainbow trout (*Oncorhynchus mykiss*). Juvenile Rainbow trout were exposed in a static test over a period of 96 hours to the nominal concentration of 100 mg test item/L and to control (test water only) under defined conditions (limit test). The method is recommended by the test guidelines, and also Rainbow trout is one of the fish species recommended by the international test guidelines of the OECD and EEC.
The recorded effects were the mortality and visible abnormalities of the fish. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour NOLEC value was ≥ 100 mg test item/L, therefore the LC50 was greater than 100 mg test item/L.

Material and Methods:

Test item: MKH 6561-Saccharine; Product code: AE F159737 00 1B99 0002; Batch No.: M00402; Content of active ingredient: 99.9 % w/w; Certificate No.: AZ 11460.

Juvenile *Oncorhynchus mykiss* were exposed to AE F159737 in a static system over a period of 96 hours to the nominal concentration of 100 mg test item/L. In addition a reconstituted water control was tested. 20-Litre glass aquaria filled with 18 L test medium were used as test units. The test was performed with one replicate for the test concentration and the control. 10 fishes were used per replicate. The test media was slightly aerated during the test. The water hardness was 2.5 mmol/L (= 250.0 mg/L) as $CaCO_3$. The mean length of the fish in the test was 5.12 cm (mean of ten fishes). The mean body wet weight was 1.16 g (mean of ten fishes). The maximum loading rate was 1 g fish/L test water. The test fish were observed after approximately 2, 24, 48, 72 and 96 hours test duration for visible abnormalities (e.g. apathy, convulsions, strong ventilation, tumbling etc.) and mortality. The quantification of the test item was performed using liquid chromatography (HPLC-method).

Dates of experimental work: May 22, 2006 to May 26, 2006 (biological part)
May 26, 2006 to May 27, 2006 (date of analysis)

Results:

Validity criteria:
The experiment is valid because no fish died in the control and oxygen saturation was always > 60 %.

Analytical results:
At the start of the test just before introduction of the fish 107 % of the nominal test concentration was found. After 96 hours test duration 106 % of the nominal value was determined. Thus, during the test period of 96 hours the fish were exposed to a mean of 106 % of nominal. Therefore, all reported results are related to nominal concentration of the test item.

Table CA 8.2.1-4: Summary of analytical results

Sample description [mg test item/L]	% of nominal[1]	RSD
control	n.a.	n.a.
100	106	1

[1] mean value of all mesured samples per treatment group (start and end)
RSD: relative standard deviation per treatment group
n.a.: not applicable

Biological results:
The 96-hour NOEC (highest concentration tested without toxic effects after the exposure period of 96 hours), respectively the 96-hour NOLEC (maximum concentration which did not cause any mortality within the period of test) of AE F159737 to Rainbow trout was determined to be at least 100 mg test item/L. The NOEC and the NOLEC might even be higher than this concentration, but concentrations in excess of 100 mg test item/L have not been tested. The 96-hour LOEC, the 96-hour LC_{50} and the 100% mortality were higher than 100 mg test item/L. These values could not be quantified due to the absence of toxicity of AE F159737 up to the tested concentration.

Conclusions:

The toxic effect of the test item, AE F159737 to Rainbow trout (*Oncorhynchus mykiss*) was assessed in a static, limit test. The 96-hour NOLEC value was ≥ 100 mg test item/L, therefore the LC_{50} was greater than 100 mg test item/L.

CA 8.2.2 - Long-term and chronic toxicity to fish

CA 8.2.2.1 - Fish early life stage toxicity test

Two chronic studies on different fish species were performed. The maximum tested dose levels were 100 mg a.s./L in the chronic study with rainbow trout, and 10.2 mg a.s./L in the study on early life stage exposure with fathead minnow. In the chronic study slight effects on length increase of fish were observed during the time of study, resulting in a NOEC of 10 mg a.s./L. In the study on early life stage exposure no treatment related effects were observed at the maximum dose level, resulting in a NOEC of 10.2 mg a.s./L.
Details of the studies are provided in the following table.

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

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Iodosulfuron-methyl-sodium				
Oncorhynchus mykiss (rainbow trout)	Juvenile growth	28d	**NOEC 10**	[redacted] [redacted] 1998 CE96/100 M-143097-01-1 KCA 8.2.2.1/01
Pimephales promelas (fathead minnow)	Early Life Stage flow-through	35 d	NOEC 10.2	[redacted] 2004 201022 M-240261-01-1 KCA 8.2.2.1 /02

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

Studies on iodosulfuron-methyl-sodium

Report:	KCA 8.2.2.1 /01; [redacted] 1998;M-143097-01
Title:	Effects on juvenile growth of rainbow trout (Oncorhynchus mykiss) in a 28 days flow-through study AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59424
Document No:	M-143097-01-1
Guidelines:	**ISO: 10229; OECD: 204, Draft;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

NOEC = 10 mg/L

Report:	[redacted];[redacted];2004;M-240261-01
Title:	Early life stage toxicity of AE F115008 Iodosulfuron-methyl-sodium technical to the fathead minnow (Pimephales promelas) under flow-through conditions
Report No:	201022
Document No:	M-240261-01-1
Guidelines:	**FIFRA Guideline 72-4 OPPTS Guideline 850.1400 OECD Guideline 210;Test solution volume tuinover rate and dissolved oxygen were below ranges specified in protocol for a brief period (see 4.0 Results and Discussion); IK) other significant deviations.**
GLP/GEP:	**no**

Executive Summary

The aim of the study was to determine the effects of Iodosulfuron-methyl-sodium (code: AE F115008; purity 92.3%) to early life stages of fathead minnow (*Pimephales promelas*) in a flow-through toxicity test.

Eggs and fry of *Pimephales promelas* were exposed in a flow through system over a period of 35 days to nominal concentrations of 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L (corresponding to analytically verified concentrations of 0.62, 1.16, 2.53, 4.90 and 10.2 mg a.s./L (95 to 119% of nominal)). In addition a water control was tested.

The volume turnover rate of 5 to 10 total volume turnovers per day was not archieved on Days 17, 19 and 20 due to diluter malfunction. The turnover rates were calculated to be 2.4, 4.5 and 2.9 turnovers

per day, respectively. Due to the stability of the material the reduction of turnover rate did not have an impact on the concentration of the test material and did not negatively impact the interpretation of the study results. On Day 22 an error with a toxicant delivery pump had occurred. The initial sample recoveries, taken after observing the error, ranged from 52 to 65% of nominal concentrations. On Day 25 it was determined that the replacement syringe pump failed. Confirmation samples were taken on Day 26 resulting in recoveries between 98 and 112% of nominal. On Days 14 and 20 reduction in dissolved oxygen had occurred. It is not likely that these malfunctions had an impact on the toxicity information collected or on the survival of fish within the replicates.
Hatching rates, sublethal symptoms, survival and growth (length, wet and dry weight) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as mean measured figures. The NOEC was 10.2 mg a.s./L and the LOEC was > 10.2 mg a.s./L.

Materials and Methods:

Test material: Iodosulfuron-methyl-sodium, technical; Batch number: AAIR03011, code: AE F115008, purity: 92.3%
Fathead Minnow (*Pimephales promelas*) eggs starting at < 24 hours old were exposed to iodosulfuron-methyl-sodium in a flow through system over a period of 35 days. Test vessels were dosed via a modified proportional diluter with a renewal rate of approximately seven turnovers/day. Nominal concentrations were 0.63, 1.25, 2.5, 5.0 and 10.0 mg a.s./L. In addition a water control was tested. Each vessel (glass aquaria; 8.4 L) served as one replicate containing one egg cup and filled with 7 L of water (blended spring and reverse osmosis waters). 35 eggs at initiation (thinned to 20 alevin after hatching phase) were used per replicate. The test was conducted with 4 replicates per treatment level. Thinning of surplus alevin took place at day 5, the post-hatch phase started at day 6. In this phase observations of abnormal behavior, abnormal physical changes and were recorded daily. Mortality was assessed on day 5 and on day 35. At study termination (day 35) fish were sacrificed and their wet and dry weight and length was determined.
For analytical verification of the test item concentrations samples were taken at on days 0, 7, 14, 21, 26 and 35. Additionally, single samples were taken on Days 11, 17, 19, 20, 22 and 25 to confirm exposure concentrations. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: October 29, 2003 – December 03, 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 0.62, 1.16, 2.53, 4.90 and 10.2 mg a.s./L (95 to 119% of 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 F115008

Nominal concentration (mg a.s./L)	**Mean measured (mg a.s./L)**		
	Mean	SD	Percent of nominal
Control	-	-	-
0.63	0.62	0.17	99.2
1.25	1.16	0.33	92.8
2.50	2.53	0.68	101.1
5.0	4.9	1.38	98.0
10.0	10.2	2.67	102.1

SD = Standard Deviation

Biological findings:
Mortality and hatching success was observed as listed below:

Table CA 8.2.2.1-3: Effect of AE F115008 on hatching success and mortality of *Pimephales promelas*

Mean measured concentration (mg a.i./L)	**% hatch**	**% survival**		**Mean length (mm)**	**Mean dry weight (mg)**
	Day 5	**Day 5**	**Day 35**	**Day 35**	**Day 35**
Control	93.6	93	83	22.1	40.0
0.62	92.1	92	94	21.8	41.5
1.16	92.9	93	95	21.4	40.9
2.53	93.6	94	91	21.9	43.3
4.9	95.0	95	93	22.4	46.5
10.2	91.4	91	88	21.8	47.2

Some newly hatched alevin were pale in colour and near the bottom of test vessels and some fry had bent spine (scoliosis). The observation was made in all test levels including the controls and followed no dose-response relationship. Observations were within background frequencies for control fish.

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

Test substance	Iodosulfuron-methyl-sodium technical			
Test object	Fathead Minnow			
Exposure	35 Day, flow-through (ELS)			
Fry survival (Day 5 & 35):	NOEC	10.2 mg a.s./L	LOEC	> 10.2 mg a.s./L
Percent hatch:	NOEC	10.2 mg a.s./L	LOEC	> 10.2 mg a.s./L
Time to hatch:	NOEC	10.2 mg a.s./L	LOEC	> 10.2 mg a.s./L
Growth (length & weight):	NOEC	10.2 mg a.s./L	LOEC	> 10.2 mg a.s./L
Morphological & behavioral effects:	NOEC	10.2 mg a.s./L	LOEC	> 10.2 mg a.s./L
MATC (geometric mean of lowest NOEC & LOEC)	> 10.2 mg a.s./L			

Conclusion:

No treatment related effects occurred in the early life stage exposure of the fathead minnow to Iodosulfuron-methyl-sodium technical to 10 mg a.s./L. The NOEC was 10.2 mg a.s./L and the LOEC was >10.2 mg a.s./L for all endpoints.

CA 8.2.2.2 - Fish full life cycle test

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

Fish

Population relevant effects of Iodosulfuron-methyl-sodium on fish were studied in an early life-stage test (ELS). No effects were seen at the highest tested concentration of 10 mg/L.
No further testing is indicated to evaluate the endocrine disrupter potential of Iodosulfuron-methyl-sodium 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 iodosulfuron-methyl-sodium one acute study on *Daphnia magna* was performed. The tested dose level ranged from 10 to 100 mg a.s./L. No intoxication symptoms were observed during the time of study. Very low immobilisation occurred in the concentrations of 56 mg a.s./L and 100 mg a.s./L, resulting in a NOEC of 32 mg a.s./L and an EC_{50} >100 mg a.s./L.
For the metabolite AE F059411 one acute study on *Daphnia magna* was conducted. No immobilisation and no intoxication symptoms occurred at the tested dose level of 100 mg/L, resulting in a NOEC of 100 mg/L and an EC_{50} >100 mg/L.
For the metabolite AE 1234964 one acute study on *Daphnia magna* was conducted. No significant effect occurred at the tested dose level of 100 mg/L, resulting in a NOEC of 100 mg /L and an EC_{50} >100 mg /L.
For the metabolite AE F159737 one acute study on *Daphnia magna* was conducted. 10% of the Daphnia were immobile after 48 hours test duration at the tested dose level of 100 mg/L, resulting in a NOEC of < 100 mg/L and an EC_{50} >100 mg/L.
Details of all studies are provided in the following table.

Table CA 8.2.4.1-1: Acute toxicity data of iodosulfuron-methyl-sodium and metabolite to *Daphnia magna* presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Iodosulfuron-methyl-sodium				
Daphnia magna (water flea)	Acute, static	48 h	EC_{50} **>100**	[redacted], 1998 CE96/101 M-143098-01-1 KCA 8.2.4.1 /01
AE F059411				
Daphnia magna (water flea)	Acute, static	48 h	EC_{50} **>100**	[redacted] et al., 1998 CE98/088 M-181330-01-1 KCA 8.2.4.1 /02
AE 1234964				
Daphnia magna (water flea)	Acute, static	48 h	EC_{50} **>100***	[redacted] & [redacted], 2006 30182220 M-278971-01-1 KCA 8.2.4.1 /03
AE F159737				
Daphnia magna (water flea)	Acute, static	48 h	EC_{50} **>100**	[redacted] & [redacted], 2006 30192220 M-278973-01-1 KCA 8.2.4.1 /04

*The study was conducted as a limit test. Only a NOEC =100 mg/L is mentioned in the report.
Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1998;M-143098-01
Title:	Acute toxicity to Daphnia magna (waterflea) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59425
Document No:	M-143098-01-1
Guidelines:	**EU (=EEC): 92/69 C.2; OECD: 202; USEPA (=EPA): E 72-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

EC_{50} > 100 mg/L

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F059411

Report:	[redacted];[redacted];[redacted];1998;M-181330-01
Title:	Acute toxicity to Daphnia magna (waterflea) AE F059411 substance, technical Metabolite of AE F115008 Code: AE F059411 00 1C99 0001
Report No:	C000840
Document No:	M-181330-01-1
Guidelines:	**EU (=EEC): 92/69 C.2; OECD: 202; USEPA (=EPA): E § 72-2; Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the acute effects of AE F059411 to *Daphnia magna*. *Daphnia magna* (< 24 hour old neonates) were exposed in a static system over a period of 48 hours to a nominal concentration of 100 mg /L (corresponding to analytically verified concentrations of 99.1% to 103.7% and 98.8% to 100.9% of nominal at test start and test end, respectively; mean measured concentrations 99.0% to 102.3% of the nominal values). In addition a water 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 > 100 mg/L, the 48-hour-NOEC was determined to be 100 mg/L.

Materials and Methods:

Test item: AE F059411 (metabolite of iodosulfuron-methyl), technical;
Code: AE F059411 00 1C99 0001 purity: 99.6 % w/w; Certificate No.: AZ 07411.

Daphnia magna (< 24 hour old neonates) were exposed to the test item in a static system over a period of 48 hours. The nominal concentration was 100 mg/L (limit test). In addition a water control was tested. Each vessel (glass beaker; 300 mL) served as one replicate filled with 200 mL artificial mineral medium modified M4 (Elendt 1990). 20 daphnids were used per replicate. Biological loading rate was 10 mL/animal. The test was conducted with 6 replicates at 100 mg/L. In the controls 2 replicates were tested. Immobilisation of daphnids, intoxication symptoms were assessed after 24 and 48 hours.
For analytical verification of the test item concentrations samples were taken at 0 and 48 hours from all concentrations. AE F059411 00 1B99 0001 (99.3% (w/w) served as analytical standard. HPLC was used as analytical method. The LOD and LOQ were 2.28 mg/L and 3.80 mg/L in the aqueous sample, respectively. The range of linearity was 0 to 4.6 mg/L in the analyte solution prepared for HPLC.

Dates of experimental work: August 18, 1998 to August 20, 1998

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 99.1% to 103.7% and 98.8% to 100.9% of nominal at test start and test end, respectively; mean measured concentrations

99.0% to 102.3% of the nominal values calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table CA 8.2.4.1-2: Nominal and measured concentrations of AE F059411 00 1C 99 0001

Nominal Concentration (mg /L)	Day 0 (New) Measurement			Day 2 (Old) Measurement		
	1	2	3	1	2	3
Control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
100	98.7	103.3	102.5	98.4	100.5	100.5

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

Table CA 8.2.4.1-3: Effect of AE F059411 00 1C 99 0001 on *Daphnia magna*

Nominal Test Concentration (mg/L)	Exposed Daphnids (n)	Immobilised Daphnids 24 h. (n)	48 h. (n)
Control	40	0	0
100	120	0	0

No sublethal behavioural changes were observed.

Conclusions:

In a static-acute toxicity test to determine the effects of AE F059411 to *Daphnia magna* (Waterflea) the concentration estimated to immobilise 50% of the test animals (EC_{50}) after 24 and 48 hours test duration was >100 mg/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.

AE 1234964

Report:	[redacted];[redacted];[redacted];2006;M-278971-01
Title:	Acute toxicity of MKH 6561-sulfonamide acid to Daphnia magna in a 48-hour immobilization test
Report No:	30182220
Document No:	M-278971-01-1
Guidelines:	**Commission Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for Daphnia", Official Journal of the European Communities No. L 383 A, dated December 29, 1992 - OECD Guideline for Testing of Chemicals 202: "Daphnia sp., Acute Immobilisation Test adopted April 13, 2004.;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to evaluate the influence of the test item AE 1234964 (also called: MKH 6561-sulfonamide acid) on the immobilisation (survival) of *Daphnia magna*. [redacted] female *Daphnia* (< 24 hours old) were exposed for 48 hours in a static test at a concentration of 100 mg test item/L as a limit test. In addition a water control was tested. Immobility or mortality and

sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. No significant effect was determined at 100 mg test item/L after 48 hours test duration.

Materials and Methods:

Test item: MKH 6561-sulfonamide acid (metabolite of iodosulfuron-methyl-sodium); Batch code: AE 1234964 PU 01; Origin Batch No.: M00102; Purity: 99 % w/w; Certificate No.: AZ 13380.

Female *Daphnia magna* (< 24 hour old) were exposed to the test item in a static system over a period of 48 hours. The nominal concentration was 100 mg/L (limit test). In addition a water control was tested. Each vessel (glass beaker; 100 mL) served as one replicate filled with 80 mL test medium. 30 *Daphnia* per control and test concentration, divided into 6 groups of 5 animals were used. Each group was in 80 mL test medium. Immobility or mortality and behaviour of daphnids were assessed after 24 and 48 hours. For analytical verification of the test item concentrations duplicate samples were taken at 0 and 48 hours from the test concentration and control. HPLC was used as analytical method.

Dates of experimental work: June 06, 2006 to June 23, 2006 (biological part)
June 23, 2006 to June 24, 2006 (date of analysis)

Results:

Validity Criteria:
The experiment is valid because the immobilisation of *Daphnia magna* in the control was 0.0% and only 1 *Daphnia* was trapped at the water surface. According the OECD guideline not more than 10% of the control daphnids should show immobilisation or unusual behaviour such as trapping at surface of water. At the end of the test the dissolved oxygen concentration in the test media was ≥ 8.5 mg O_2/L in the control and test vessels.

Analytical findings:
At the start of the test just before introduction of the *Daphnia* 102 % of the nominal test concentration was found. After 48 hours test duration 103% of the nominal value was determined. Thus, during the test period of 48 hours the *Daphnia* were exposed to a mean of 103 % of nominal. Therefore, all reported results are related to nominal concentrations of the test item.

Table CA 8.2.4.1-4: Summary of analytical results

Sample description [mg/L]	% of nominal[1]	RSD [%]
control	n.a.	n.a.
100	103	1

[1] mean value of all measured samples per treatment group (start and end)
RSD Relative standard deviation per treatment group
n.a. not applicable

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

Table CA 8.2.4.1-5: Effect of AE 1234964 on *Daphnia magna*

Nominal Test Concentration [mg/L]	Exposed Daphnids	No. of immobilised *Daphnia*		% of immobilised *Daphnia*	
		24 h	48 h	24 h	48 h
Control	30	0	0*	0	0
100	30	0+	0+*	0	0

*:1 daphnia (control) and 2 daphnia (at 100 mg test item/L) showed unusual behaviour (trapping at surface of water)
+: test item particles on the antennae of all daphnids

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

24-hour-figures:
NOEC 100 mg test item/L
48-hour-figures:
NOEC 100 mg test item/L

Conclusions:

No significant effect was determined at 100 mg/L AE 1234964 after 48 hours test duration.

AE F159737

Report:	[redacted]; 2006; M-278973-01
Title:	Acute toxicity of MKH 6561-saccharine to Daphnia magna in a 48-hour immobilization test
Report No:	30192220
Document No:	M-278973-01-1
Guidelines:	**Commission Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for Daphnia", Official Journal of the European Communities No. L 383 A, dated December 29, 1992 - OECD Guideline for Testing of Chemicals 202: "Daphnia sp., Acute Immobilisation Test adopted April 13, 2004.;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to evaluate the influence of the test item AE F159737 (also called: MKH 6561-saccharine) on the immobilisation (survival) of *Daphnia magna*. Young female *Daphnia* (< 24 hours old) were exposed for 48 hours in a static test at a concentration of 100 mg test item/L as a limit test. In addition a water control was tested. Immobility or mortality 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} value was higher than 100 mg test item/L.

Materials and Methods:

Test item: MKH 6561-saccharine (metabolite of iodosulfuron-methyl-sodium); Product code: AE F159737 00 1B99 0002; Batch No.: M00402; Purity: 99.9 % w/w; Certificate No.: AZ 11460.

Female *Daphnia magna* (< 24 hour old) were exposed to the test item in a static system over a period of 48 hours. The nominal concentration was 100 mg/L (limit test). In addition a water control was tested. Each vessel (glass beaker; 100 mL) served as one replicate filled with 80 mL test medium. 30

Daphnia per control and test concentration, divided into 6 groups of 5 animals were used. Each group was in 80 mL test medium. Immobility or mortality and behaviour of daphnids were assessed after 24 and 48 hours. For analytical verification of the test item concentrations duplicate samples were taken at 0 and 48 hours from the test concentration and control. HPLC was used as analytical method.

Dates of experimental work: June 06, 2006 to June 23, 2006 (biological part)
June 23, 2006 to June 24, 2006 (date of analysis)

Results:

Validity Criteria:
The experiment is valid because the immobilisation of *Daphnia magna* in the control was 0.0% and only 1 *Daphnia* was trapped at the water surface. According the OECD guideline not more than 10% of the control daphnids should show immobilisation or unusual behaviour such as trapping at surface of water. At the end of the test the dissolved oxygen concentration in the test media was ≥ 8.5 mg O_2/L in the control and test vessels.

Analytical findings:
At the start of the test just before introduction of the *Daphnia* 105% of the nominal test concentration was found. After 48 hours test duration 103% of the nominal value was determined. Thus, during the test period of 48 hours the *Daphnia* were exposed to a mean of 104 % of nominal. Therefore, all reported results are related to nominal concentrations of the test item.

Table CA 8.2.4.1-6: Summary of analytical results

Sample description [mg/L]	% of nominal[1]	RSD [%]
control	n.a.	n.a.
100	104	1

[1] mean value of all measured samples per treatment group (start and end)
RSD Relative standard deviation per treatment group
n.a. not applicable

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

Table CA 8.2.4.1-7: Effect of AE F159737 on *Daphnia magna*

Nominal Test Concentration [mg/L]	Exposed Daphnids	No. of immobilised *Daphnia*		% of immobilised *Daphnia*	
		24 h	48 h	24 h	48 h
Control	30	0	0*	0	0
100	30	1	3	3	10

*:1 daphnia showed unusual behaviour (trapping at surface of water)

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

24-hour-figures:

EC_{50}:	> 100 mg test item/L
0% immobility	< 100 mg test item/L
100% immobility	> 100 mg test item/L
NOEC	< 100 mg test item/L

48-hour-figures:

EC_{50}:	> 100 mg test item/L
0% immobility	< 100 mg test item/L
100% immobility	> 100 mg test item/L
NOEC	< 100 mg test item/L

Conclusions:

The 48-hour EC_{50} value was higher than 100 mg/L AE F159737.

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 iodosulfuron-methyl-sodium to *Mysidopsis bahia* presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Iodosulfuron-methyl-sodium				
Mysidopsis bahia (mysid shrimp)	static acute	96 h	**LC_{50} >100**	[redacted], 2000 B002713 M-238447-02-1 KCA 8.2.4.2 /01

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

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];2000;M-238447-02; Amended: 2000-02-28
Title:	96 hour acute toxicity to the Mysid shrimp, Mysidopsis bahia, in a static renewal system: AE F115008 technical 89.6 percent w/w: AE F115008 00 1C89 0001
Report No:	B002713
Document No(s):	M-238447-02-1
Guidelines:	**USEPA (=EPA): 72-3;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary

The aim of the study was to determine the acute effects of Iodosulfuron-methyl-sodium to *Americamysis bahia* (formerly *Mysidopsis bahia*).
Americamysis bahia (< 24 hours old) were exposed in a static system over a period of 96 hours to nominal concentrations of 100 mg a.s./L (limit test) (corresponding to an analytically verified

concentration of 99.2 mg a.s./L). In addition a water control was tested. All treatments had 10 mysids per test vessel (i.e., 30 mysids per treatment level). Test solutions were not renewed. Immobilisation 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-EC_{50} was >100 mg/L, the 96-hour-NOEC was determined to be 100 mg/L.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium, technical; Code No.: AE F115008 00 1C89 0001; Batch No.: CR21436/02/950601; Sample No.: ZBA438; CAS Reg. No.: 144550-36-7; Assay: 86.9 % w/w; Certificate of Analysis: AZ 07987
Americamysis bahia (formerly *Mysidopsis bahia*) (< 24 hours old) were exposed to Iodosulfuron-methyl-sodium in a static system over a period of 96 hours. Nominal concentration was 100 mg a.s./L (limit test). In addition a water control was tested. Each vessel (Pyrex® beakers; 1 L) served as one replicate filled with 0.8 L synthetic sea water. 10 mysids were used per replicate. The test was conducted with 3 replicates per treatment level.
For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. AEF115008 00 1B97 0003 served as analytical standard. High-performance liquid chromatography (HPLC) was used as analytical method. The limit of quantification (LOQ) was 0.005 mg/L. Immobilisation of mysids, intoxication symptoms and physical-chemical water parameters were assessed as indicated below in the result section.

Dates of experimental work: 30 October 1999 - 03 November 1999

Results:

Analytical findings:
Analytical verification of test solutions revealed measured concentrations of 99.2 mg a.s./L calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table CA 8.2.4.2-2: Nominal and measured concentrations of AE F115008 00 1C89 0001

Nominal Concentration (mg ai/L)	Day 0 (New)		Day 4 (Old)		Mean Measured (mg a.i./L)	Mean Percent of Nominal
	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal		
100	96.3		102.2		99.2	99%

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

Table CA 8.2.4.2-3: Effect of AE F115008 00 1C89 0001 on immobilisation of *Americamysis bahia*

mg/L	No. of organisms	Observation period	
		96 hours	
		# immob.	% mort.
Control			
Replicate #1	10	0	0.00%
Replicate #2	10	0	0.00%
Replicate #3	10	0	0.00%
100 mg/L			
Replicate #1	10	0	0.00%
Replicate #2	10	0	0.00%
Replicate #3	10	0	0.00%

No sublethal behavioural changes were observed.

Conclusions:

The 96 hour LC_{50} of AE F115008 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. Based on effects on reproduction, the overall NOEC was 10 mg/L. Details of the study are provided in the following table.

Table CA 8.2.5.1-1: Reproductive toxicity data of iodosulfuron-methyl-sodium to *Daphnia magna* presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Iodosulfuron-methyl-sodium				
Daphnia magna (water flea)	Static renewal	21 d	NOEC **10**	[redacted] & [redacted], 1998 CE96/102 M-143099-01-1 KCA 8.2.5.1 /01

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

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];[redacted];1998;M-143099-01
Title:	Effect on growth and reproduction of Daphnia magna AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59426
Document No:	M-143099-01-1
Guidelines:	**OECD: 202; USEPA (=EPA): E § 72-4;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

NOEC 10 mg/L

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

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

CA 8.2.5.3 - Development and emergence in *Chironomus* species

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

CA 8.2.5.4 - Sediment dwelling organisms

Iodosulfuron-methyl-sodium is highly water soluble and does not accumulate in the sediment. No testing with sediment dwelling organisms is triggered. Moreover, the chronic NOEC of 10 mg/L for Daphnia does not indicate any risk to aquatic invertebrates in general.

CA 8.2.6 - Effects on algal growth

Potential effects of iodosulfuron-methyl-sodium on algal growth were investigated with four different algae species, a green alga, a blue-green alga and a freshwater diatom and a marine diatom. The green alga *Pseudokirchneriella subcapitata* was found to be the most sensitive algae species. The ErC_{50} of iodosulfuron-methyl-sodium for this species is 0.152 mg a.s./L.

For metabolites AE F075736, AE F145741, AE F145740, AE 0002166, AE F161778, BCS-CW81253, AE F154781, AE F059411, AE 0014966, AE 0000119, AE 0034855, AE 1234964 and AE F159737 studies were performed with green algae. The lowest E_rC_{50} was determined to be >0.56 mg/L for the metabolite AE F075736.

For this metabolite a study with freshwater diatom was additionally performed where the EC_{50} was above the highest tested dose level (EC_{50} >100 mg/L).

Table CA 8.2.6-1: Growth effect data of iodosulfuron-methyl-sodium and its metabolites to algae presented in this chapter
(Since the new aquatic GD[3] focusses on endpoints based on growth rates the old E_bC_{50} figures were omitted from the table above.)

Test organism	Test system	Test duration	Endpoint [mg/L]		Reference
Iodosulfuron-methyl-sodium					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	0.178	[redacted], 1998 CE96/097 M-143094-01-1 KCA 8.2.6.1 /01
		96 h	E_rC_{50}	**0.152**	
Navicula pelliculosa (diatom)	growth inhibition	72 h / 96 h	E_rC_{50}	>100	[redacted] & [redacted], 1998 CE97/108 M-143100-01-1 KCA 8.2.6.2 /01

[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

Test organism	Test system	Test duration	Endpoint [mg/L]		Reference
Navicula pelliculosa (diatom)	growth inhibition	72 h / 96 h	E_rC_{50}	>100	[redacted], [redacted] & [redacted], 2000 C005665 M-192458-01-1 KCA 8.2.6.2 /03
Anabaena flos-aquae (blue-green alga)	growth inhibition	72 h	E_rC_{50}	2.0	[redacted] & [redacted], 2000 B002714 M-238448-01-2 KCA 8.2.6.2 /04
		96 h	E_rC_{50}	1.7	
Skeletonema costatum (marine diatom)	growth inhibition	24 h	E_rC_{50}	43	[redacted] & [redacted], 2000 BY98W506 M-238456-01-2 KCA 8.2.6.2 /05
		48 h	E_rC_{50}	54	
		72 h	E_rC_{50}	68	
		96 h	E_rC_{50}	79	
AE F075736					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h / 96 h	E_rC_{50}	**>0.56**	[redacted], [redacted] & [redacted], 1998 CE98/093 M-181569-01-1 KCA 8.2.6.1 /03
Navicula pelliculosa (diatom)	growth inhibition	72 h / 96 h	E_rC_{50}	>100	[redacted] et al. 1998 CE98/094 M-181581-01-1 KCA 8.2.6.2 /02
AE F145741					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**10.9**	[redacted], 2013 EBIML037 M-470687-01-1 KCA 8.2.6.1 /04
AE F145740					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>10**	[redacted], 2013 EBIMN062 M-465388-01-1 KCA 8.2.6.1 /05
AE 0002166					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>10**	[redacted], 2013 EBIML035 M-470669-01-1 KCA 8.2.6.1 /06
AE F161778					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>10**	[redacted], 2013 EBIML036 M-468872-01-1 KCA 8.2.6.1 /07
BCS-CW81253					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>10**	[redacted], 2013 EBIMN061 M-465389-01-1 KCA 8.2.6.1 /08
AE 0000119					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h / 96 h	E_rC_{50}	**>100**	[redacted] & [redacted], 2002 CE01/066 M-205698-01-1 KCA 8.2.6.1 /09

Test organism	Test system	Test duration	Endpoint [mg/L]		Reference
AE F059411					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h / 96 h	E_rC_{50}	**>100**	[redacted]; [redacted] & [redacted]; 1998 CE98/087 M-181379-01-1 KCA 8.2.6.1 /02
AE 0014966					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	48.0	[redacted] & [redacted], 2002 CE01/067 M-203681-01-1 KCA 8.2.6.1 /10
		96 h	E_rC_{50}	**47.5**	
AE 0034855					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h / 96 h	E_rC_{50}	**>109**	[redacted] & [redacted], 2002 CE01/071 M-210624-01-1 KCA 8.2.6.1 /11
AE 1234964					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>100**	[redacted] & [redacted], 2006 30181210 M-293396-01-1 KCA 8.2.6.1 /12
AE F159737					
Pseudokirchnerilla subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>100**	[redacted] & [redacted], 2006 30191210 M-281243-01-1 KCA 8.2.6.1 /13
AE F154781					
Pseudokirchneriella subcapitata (green alga)	growth inhibition	72 h	E_rC_{50}	**>10**	[redacted], 2013 EBIMN105 M-476160-01-1 KCA 8.2.6.1 /14

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

CA 8.2.6.1 - Effects on growth of green algae

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];1998;M-143094-01
Title:	Algal growth inhibition (Pseudokirchneriella subcapitata) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59421
Document No:	M-143094-01-1
Guidelines:	**EU (=EEC): C.3; OECD: 201; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

$$EC_{50} = 0.070 \text{ mg/L*}$$

* This endpoint corresponds to the E_bC_{50} after 72 hours. The respective E_rC_{50} is 0.178 mg/L.

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F059411

Report:	[redacted];[redacted];[redacted];[redacted];1998;M-181379-01
Title:	Algal growth inhibition (Pseudokirchneriella subcapitata) AE F059411 substance, technical Metabolite of AE F115008 Code: AE F059411 00 1C99 0001
Report No:	C000867
Document No:	M-181379-01-1
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 iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

EC_{50} >100 mg/L

AE F075736

Report:	[redacted];[redacted];[redacted];1998;M-181569-01
Title:	Algal growth inhibition - Pseudokirchneriella subcapitata AE F075736 (metsulfuron-methyl) metabolite of AE F115008 substance, technical Code: AE F075736 00 1C92 0001
Report No:	C000975
Document No:	M-181569-01-1
Guidelines:	**EU (=EEC): C.3; OECD: 201; USEPA (=EPA): J 123-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

EC_{50} = 0.123 mg/L*

* Presented in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final) as endpoint of the metabolite AE F059411 (M 4). The EC_{50} corresponds to the E_bC_{50} after 72 hours of 0.123 mg/L of the conclusions in the study report. The respective E_rC_{50} is >0.56 mg/L.

AE F145741

Report:	[redacted];2013;M-470687-01
Title:	Pseudokirchneriella subcapitata - Growth inhibition test with BCS-AU71532 - limit test
Report No:	E 201 4592-3
Document No:	M-470687-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009;none**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the influence of metabolite AE F145741 (other code: BCS-AU71532) on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_x for growth rate of algae biomass (cells per volume). The study was designed to meet OECD criteria. Algae were exposed in a static test system for 3 days to nominal concentrations of 0.625, 1.25,

2.50, 5.00 and 10.0 mg pure metabolite (p.m.)/L and a 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, 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 AE F145741 was determined as 10.9 mg p.m./L and the NOE_rC as < 0.625 mg p.m./L

Material and methods:

Test item: BCS-AU71532 (metabolite of iodosulfuron-methyl-sodium); Origin batch No.: GSE 61191-8-6; Customer order No.: TOX10009200; LIMS No.: 1323754; Purity: 97.8% w/w.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as Selenastrum capricornutum) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0.625, 1.25, 2.50, 5.00 and 10.0 mg pure metabolite/L in comparison to an untreated control. The test volume was 150 mL test medium per replicate. 3 replicate vessels per test level and 6 replicate vessels per control were used during the test. The pH values ranged from 8.0 to 8.1 in the controls and the incubation temperature ranged from 21.7 °C to 22.4 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6081 lux (mean value).
Quantitative amounts of the test item were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period. HPLC was used as analytical method.

Dates of experimental work: September 20, 2013 to October 7, 2013

Results:

Validity criteria:
The study conditions met all validity criteria, requested by the mentioned guideline: Biomass increased in the control by more than 16-fold within the evaluation period (ca. 80-fold). The mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35%. The percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical findings:
The analytical findings of AE F145741 in the treatment levels found on day 0 and 3 were 113% to 116% of nominal (average 115%). Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

Table CA 8.2.6.1-1: Summary of analytical results

Nominal concentration [mg p.m./L]	Actual concentration (mg p.m./L)							
	Day 0				Day 3			
	Determination		Average	%	Determination		Average	%
	1.	2.			1.	2.		
control	< 0.050	< 0.050	< 0.050	--	< 0. 050	< 0.050	< 0.050	--
0.625	0.726	0.720	0.723	116	0.728	0.713	0.720	115
1.25	1.44	1.46	1.45	116	1.45	1.46	1.45	116
2.50	2.86	2.86	2.86	114	2.87	2.88	2.88	115
5.00	5.68	5.72	5.70	114	5.65	5.70	5.67	113
10.0	11.3	11.2	11.3	113	11.4	11.3	11.4	114
			Mean	115			**Mean**	115

Biological findings:
Observations are listed as follows:

Table CA 8.2.6.1-2: Effects of the static 72 hour algae growth inhibition test

Nominal concentration [mg p.m./L]	Cell number after 72 h (means) per mL	72 h average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
control	805 000	1.462	--
0.625	604 000	1.366*	6.5
1.25	493 000	1.299*	11.1
2.50	309 000	1.143*	21.8
5.00	168 000	0.941*	35.6
10.0	102 000	0.775*	47.0

test initiation with 10,000 cells/mL
* significantly (α=0.05, one-sided smaller) reduced, based on Williams' multiple sequential t-test procedure

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

Conclusions:

After 72 hours the E_rC_{50} for AE F145741 was 10.9 mg p.m./L (95% CI: 10.1 – 12.0 mg p.m./L) and the NOE_rC was < 0.625 mg p.m./L.

AE F145740

Report:	[redacted];[redacted];2013;M-465388-01
Title:	Pseudokirchneriella subcapitata growth inhibition test with BCS-AU71533 - limit test
Report No:	EBIMN062
Document No:	M-465388-01-1
Guidelines:	**EU Directive 91/414/EEC** **Regulation (EC) No. 1107/2009** **OECD Guideline 201;none**
GLP/GEP:	**yes**

Executive Summary:

The objective of this 72 hour growth inhibition test is, to verify the assumption that the metabolite AE F145740 (other code: BCS-AU1533) will cause no relevant adverse effects on the growth of the green algae *Pseudokirchneriella subcapitata* at the limit test item concentration of 10.0 mg pure

metabolite/L. 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 concentration of 10.0 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 alterations in algae cells that might influence extinction measurements such as unusual cell size, 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 > 10.0 mg p.m./L and the (0 – 72 h)-NOE_rC was determined to be ≥ 10.0 mg p.m./L.

Material and Methods:

Test item. BCS-AU71533; Analysed purity: 97.5 % w/w; Origin batch No: GSE 61082-3-3; Sample description: Customer order no.: TOX09988-00; LIMS No.: 1301958.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions a nominal concentration of 10.0 mg pure metabolite/L in comparison to untreated control. The limit concentration of 10.0 mg pure metabolite/L was chosen to demonstrate that the test item was less toxic towards *Pseudokirchneriella subcapitata* than the active substance. The test volume was 150 mL test medium per replicate. 3 replicate vessels per test level and 6 replicate vessels per control were used during the test. The pH values ranged from 8.0 to 8.7 in the control and the incubation temperature ranged from 21.7°C to 22.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6424 lux (mean value).
Quantitative amounts of AE F145740 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: May 31, 2013 to July 11, 2013

Results:

Validity criteria:
The study conditions met all validity criteria requested by the mentioned guideline(s). Biomass increased in the control by more than 16-fold within the evaluation period. 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%. Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical findings:
The analytical findings of AE F145740 (BCS-AU71533) in the treatment level found on day 0 and day 3 were 104 % of nominal. Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

Table CA 8.2.6.1-3: Concentrations of AE F145740 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg p.m./L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<1.00	<1.00	<1.00	--
10.0	10.4	10.3	10.4	104

Table CA 8.2.6.1-4: Concentrations of AE F145740 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg p.m./L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<1.00	<1.00	<1.00	--
10.0	10.3	10.4	10.4	104

Biological findings:

Observations on growth rates are listed as follows:

Table CA 8.2.6.1-5: The static 72 hour algae growth inhibition test provided the following tabulated effects

nominal concentration [mg p.m./L]	cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	inhibition of average specific growth rate [%]
control	1 105 000	1.568	--
10.0	1 107 000	1.569	0.0

Test initiation with 10,000 cells/mL

Conclusions:

The (0 - 72h)-E_rC_{50} for AE F145740 is >10.0 mg p.m./L and the (0 - 72h)-NOE_rC is ≥10.0 mg p.m./L.

AE 0002166

Report:	[redacted];2013;M-470669-01
Title:	Pseudokirchneriella subcapitata - Growth inhibition test with BCS-AW35544 - limit test
Report No:	E 201 4589-9
Document No:	M-470669-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009;none**
GLP/GEP:	**yes**

Executive Summary:

The objective of this 72 hour growth inhibition test was to verify the assumption that the metabolite od iodosulfuron-methyl-sodium AE 0002166 (other code: BCS-AW35544) causes no relevant adverse effects on the growth of the green algae *Pseudokirchneriella subcapitata* at the limit test item concentration of 10.0 mg pure metabolite (p.m.)/L. 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 concentration of 10.0 mg p.m./L in comparison to an untreated

control. Six 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, samples were examined under a microscope. The limit test concentration of 10.0 mg p.m./L caused a statistically significant inhibition of 9.9 %. However, this inhibition was not considered to be relevant since the (0 - 72h)-E_rC_{50} for AE 0002166 was clearly > 10.0 mg p.m./L.

Material and methods:

Test item: BCS-AW35544 (AE 0002166; metabolite of iodosulfuron-methyl-sodium); Origin batch No.: GSE 61266-1-3; TOX No.: 10007-00; LIMS No.: 1319418; Analysed purity: 95.2% w/w.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to a nominal concentration of 10.0 mg pure metabolite/L in comparison to an untreated control. The limit concentration of 10.0 mg pure metabolite/L was chosen to demonstrate that the test item was less toxic towards *Pseudokirchneriella subcapitata* than the active substance. The test volume was 150 mL test medium per replicate. Six replicate vessels per test level and six replicate vessels per control were used during the test. The pH values ranged from 8.0 to 8.4 in the controls and the incubation temperature ranged from 21.8°C to 22.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6233 lux (mean value). Quantitative amounts of AE 0002166 were measured in the treatment group and in the control on day 0 and day 3 of the exposure period. HPLC was used as analytical method.

Dates of experimental work: September 13, 2013 to September 24, 2013

Results:

Validity criteria:
The study conditions met all validity criteria, requested by the mentioned guideline: Biomass increased in the control by more than 16-fold within the evaluation period (ca. 92-fold). The mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35%. The percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical findings:
The analytical finding of AE 0002166 (BCS-AW35544) in the treatment level found on day 0 was 114 % of nominal. On day 3 analytical findings of 115% of nominal were found.
Based on analytical findings all results are given as nominal concentration of the test item in the test medium.

Table CA 8.2.6.1-6: Summary of analytical results

Nominal concentration [mg p.m./L]	Actual concentration (mg p.m./L)							
	Day 0				Day 3			
	Determination		Average	%	Determination		Average	%
	1.	2.			1.	2.		
control	< 0.200	< 0.200	< 0.200	--	< 0.200	< 0.200	< 0.200	--
10.0	11.4	11.4	11.4	114	11.5	11.5	11.5	115

Biological findings:
Observations are listed as follows:

Table CA 8.2.6.1-7: Effects of the static 72 hour algae growth inhibition test

Nominal concentration [mg p.m./L]	Cell number after 72 h (means) per mL	72 h average specific growth rates [days-1]	Inhibition of average specific growth rate [%]
control	921 000	1.592	--
10.0	572 000	1.433*	9.9

test initiation with 10,000 cells/mL
* significantly (α=0.05, one-sided smaller) reduced, based on Student's t-test for homogeneous variances

Conclusions:

The limit test concentration of 10.0 mg p.m./L caused a statistically significant inhibition of 9.9 %. However, this inhibition was not considered to be relevant since the (0 - 72h)-E_rC_{50} for AE 0002166 was clearly > 10.0 mg p.m./L.

AE F161778

Report:	[redacted];2013;M-468872-01
Title:	Pseudokirchneriella subcapitata - Growth inhibition test with BCS-AU85549 - limit test
Report No:	EBIML036
Document No:	M-468872-01-1
Guidelines:	**OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006); EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009;none**
GLP/GEP:	**yes**

Executive Summary:

The objective of this 72 hour growth inhibition test is, to verify the assumption that the metabolite of iodosulfuron-methyl-sodium AE F161778 (other code: BCS-AU85549) will cause no relevant adverse effects on the growth of the green algae *Pseudokirchneriella subcapitata* at the limit test item concentration of 10.0 mg pure metabolite (p.m.)/L. 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 concentration 10.0 mg p.m./L in comparison to untreated control. Six 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, samples were examined under a microscope. The limit test concentration of 10.0 mg p.m./L caused a statistically significant inhibition of 7.8 %. However, this

inhibition is not considered to be relevant since the (0 - 72h)-E_rC_{50} for AE F161778 is clearly >10.0 mg p.m./L.

Material and Methods:

Test item: BCS-AU85549; Analysed purity: 96.3 % w/w; Origin batch No.: GSE 61201-4-3; Customer order No.: TOX10008-00; LIMS no.: 1307990.

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 10.0 mg pure metabolite/L in comparison to untreated control. The limit concentration of 10.0 mg pure metabolite/L was chosen to demonstrate that the test item was less toxic towards *Pseudokirchneriella subcapitata* than the active substance. The test volume was 150 mL test medium per replicate. Six replicate vessels per test level and six replicate vessels per control were used during the test. The pH values ranged from 7.9 to 8.2 in the control and the incubation temperature ranged from 22.4°C to 22.9°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6163 lux (mean value).
Quantitative amounts of AE F161778 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: May 31, 2013 to July 16, 2013

Results:

Validity criteria:
The study conditions met all validity criteria, requested by the mentioned guideline(s).The biomass increased in the control by more than 16-fold within the evaluation period. 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%. Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical findings:
The analytical findings of AE F161778 (BCS-AU85549) in the treatment levels found on day 0 and day 3 were 112 % of nominal.
Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

Table CA 8.2.6.1-8: Concentrations of AE F161778 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	Actual Concentration (mg p.m./L)			
	1. Determination	2. Determination	Average	%
Control	<1.00	<1.00	<1.00	--
10.0	11.2	11.2	11.2	112

Table CA 8.2.6.1-9: Concentrations of AE F161778 in the test solutions at day 3

Nominal Concentration in mg p.m./L	Day 3			
	Actual Concentration (mg p.m./L)			
	1. Determination	2. Determination	Average	%
Control	<1.00	<1.00	<1.00	--
10.0	11.1	11.2	11.2	112

Biological findings:

Observations on growth rates are listed as follows:

Table CA 8.2.6.1-10: The static 72 hour algae growth inhibition test provided the following tabulated effects

nominal concentration [mg p.m./L]	cell number after 72 h (means) per mL	(0-72h) average specific growth rates [days^{-1}]	inhibition of average specific growth rate [%]
control	924 000	1.508	--
10.0	649 000	1.391	7.8*

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 adjustment

Conclusions:

The limit test concentration of 10.0 mg p.m./L caused a statistically significant inhibition of 7.8 %. However, this inhibition is not considered to be relevant since the (0 - 72h) E_rC_{50} for AE F161778 is clearly >10.0 mg p.m./L.

BCS-CW81253

Report:	[redacted];[redacted];2013;M-465389-01
Title:	Pseudokirchneriella subcapitata - Growth inhibition test with BCS-CW81253 - Limit test
Report No:	EBIMN061
Document No:	M-465389-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; OECD Guideline 201;not specified**
GLP/GEP:	**yes**

Executive Summary:

The objective of this 72 hour growth inhibition test is, to verify the assumption that the metabolite of iodosulfuron-methyl-sodium, BCS-CW81253 will cause no relevant adverse effects on the growth of the green algae *Pseudokirchneriella subcapitata* at the limit test item concentration of 10.0 mg pure metabolite/L. 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 concentration 10.0 mg pure metabolite (p.m.)/L in comparison to 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, samples were examined under a microscope. Based on analytical findings, the

biological endpoints are reported as nominal figures. The (0 - 72h)-E_rC_{50} for BCS-CW81253 is > 10.0 mg p.m./L and the (0 - 72h) - NOE_rC is ≥ 10.0 mg p.m./L.

Material and Methods:

Test item. BCS-CW81253; Analysed content: 99.0 % w/w; Batch code: BCS-CW81253-PU-01; Origin batch No.: GSE61145-5-3; Sample description: TOX09918-00; LIMS no.:1306024.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentration of 10.0 mg /L in comparison to untreated control. The limit concentration of 10.0 mg pure metabolite/L was chosen to demonstrate that the test item was less toxic towards *Pseudokirchneriella subcapitata* than the active substance. The test volume was 150 mL test medium per replicate. 3 replicate vessels per test level and 6 replicate vessels per control were used during the test. The pH values ranged from 8.0 to 8.7 in the controls and the incubation temperature ranged from 21.7°C to 22.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6424 lux (mean value).
Quantitative amounts of BCS-CW81253 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: May 31, 2013 to July 04, 2013

Results:

Validity criteria:
The study conditions met all validity criteria, requested by the mentioned guideline(s).The biomass increased in the control by more than 16-fold within the evaluation period. 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%. Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical findings:
The analytical findings of BCS-CW81253 in the treatment levels found on day 0 and on day 3 were 102 % of nominal.
Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

Table CA 8.2.6.1-11: Concentrations of BCS-CW81253 in the test solutions at day 0

Nominal Concentration in mg p.m./L	Day 0			
	Actual Concentration (mg p.m./L)			
	1. Determination	2. Determination	Average	%
Control	<1.00	<1.00	<1.00	--
10.0	10.1	10.3	10.2	102

Table CA 8.2.6.1-12: Concentrations of BCS-CW81253 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	**Actual Concentration (mg p.m./L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<1.00	<1.00	<1.00	--
10.0	10.2	10.1	10.2	102

Biological findings:

Observations on growth rates are listed as follows:

Table CA 8.2.6.1-13: The static 72 hour algae growth inhibition test provided the following tabulated effects

nominal concentration [mg p.m./L]	cell number after 72 h (means) per mL	(0-72h) average specific growth rates [days^{-1}]	inhibition of average specific growth rate [%]
control	1 105 000	1.568	--
10.0	1 128 000	1.575	-0.4

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

Conclusions:

The (0-72h)-E_rC_{50} for BCS-CW81253 is >10.0 mg p.m./L and the (0-72h)-NOE_rC is ≥10.0 mg p.m./L.

AE 0000119

Report:	[redacted];[redacted];[redacted];2002;M-205698-01
Title:	Algal growth inhibition - Pseudokirchneriella subcapitata AE 0000119 substance, pure Code: AE 0000119 00 1B98 0001
Report No:	C018210
Document No:	M-205698-01-1
Guidelines:	**OECD guideline 201, US-EPA Pesticide Assessment Guidelines J § 123-2 and according to EU guidelines under GLP; Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE 0000119 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0000119 00 1B98 0001; purity 97.8% (w/w)) to *Pseudokirchneriella subcapitata*. Cultures of *Pseudokirchneriella subcapitata* with an initial cell density of 10000 cells/mL were exposed in a static system over a period of 96 hours to nominal concentrations of 10, 18, 32, 56 and 100 mg/L. In addition a water control was tested.
72 and 96 hour growth rate based on cell density and visual assessment of potential cell deformations were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour-E_rC_{50} was > 100 mg a.s./L, the 96-hour-NOAEC was determined to be 100 mg a.s./L. The 96-hour-NOEC was 18 mg a.s./L.

Material and Methods:

Test item: AE 0000119 (metabolite of iodosulfuron-methyl-sodium); code: AE 0000119 00 1B98 0001; purity 97.8% w/w; Analytical certificate No.: AZ 08376.

Green alga (*Pseudokirchneriella subcapitata*) were exposed to AE 0000119 in a static system over a period of 96 hours. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer flasks; 300 mL) served as one replicate filled with 100 mL test solution with an initial pH of 7.5. At test initiation the cell density was 10000 cells/mL. The test was conducted with 3 replicates per treatment level. In the controls 6 replicates were tested. Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72 and 96 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber.
For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 0.44 mg/L in the aqueous sample and 0.74 mg/L in the aqueous sample, respectively. The range of linearity was 0.13 to 10.6 mg/L in the analyte solution prepared for HPLC.

Dates of experimental work: November 22, 2001 to November 29, 2001

Results:

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

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

Table CA 8.2.6.1-14: Nominal and measured concentrations of AE 0000119

nominal concentration (mg a.s./L)	… based on purity of test substance	measured day 0		measured day 4		Mean measured mg a.s./L	Mean Percent of Nominal
		mg a.s./L	% nominal	mg a.s./L	% nominal		
Control	0	< LOQ		< LOQ			
10	9.78	9.65	98.7	10.99	112.4	10.32	**105.6**
18	17.6	18.33	104.1	19.62	111.5	18.98	**107.8**
32	31.3	32.06	102.4	32.51	103.9	32.28	**103.2**
56	54.77	55.56	101.4	57.48	105	56.52	**103.2**
100	97.8	100.2	102.5	108.21	110.6	104.2	**106.5**

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

Table CA 8.2.6.1-15: Effect of AE 0000119 on growth-inhibition of *Pseudokirchneriella subcapitata*

Nominal concentration (mg a.s./L)	Percentual inhibition according to mean area under the growth curve after 72 h	Percentual inhibition according to mean growth rate after 72 h	Percentual inhibition according to mean area under the growth curve after 96 h	Percentual inhibition according to mean growth rate after 96 h
control	0	0	0	0
10	18.66	5.49	14.3	2.66
18	11.61	1.68	1.99	-0.65
32	-25.7	-9.72	-30.74 *	-5.84
56	-22.62	-9.67	-27.75 *	-5.34
100	-29.2 *	-12.52	-41.3 *	-7.88 *

* Statistically significant difference from control (Duncan's test; $p < 0.05$)

No cell abnormalities were observed.

Conclusions:

The effect of AE 0000119 (metabolite of iodosulfuron-methyl-sodium) on *Pseudokirchneriella subcapitata* can be quantified as a 96-hour-E_rC_{50} of > 100 mg a.s./L. The highest concentration with no observed growth inhibition and no cell deformations can be set to 100 mg a.s./L.

<u>AE 0014966</u>

Report:	[redacted];[redacted];[redacted];2002;M-203681-01
Title:	Algal growth inhibition - Pseudokirchneriella subcapitata AE 0014966 substance, pure Code: AE 0014966 00 1B98 0001
Report No:	C017113
Document No:	M-203681-01-1
Guidelines:	**EU (=EEC): C.3; OECD: 201; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE 0014966 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0014966 00 1B98 0001; purity 97.6% w/w) to *Pseudokirchneriella subcapitata*. Cultures of *Pseudokirchneriella subcapitata* with an initial cell density of 10000 cells/mL were exposed in a static system over a period of 96 hours to nominal concentrations of 10, 18, 32, 56, and 100 mg/L. In addition a water control was tested.
72 and 96 hour growth rate based on cell density and visual assessment of potential cell deformations were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour-E_rC_{50} was 47.5 mg a.s./L (95% confidence limits 32 - 56 mg a.s./L), the 96-hour-NOEC was determined to be 10 mg/L.

Material and Methods:

Test item: AE 0014966 (metabolite of iodosulfuron-methyl-sodium); code: AE 0014966 00 1B98 0001; purity 97.6% w/w; Analytical certificate No.: AZ 08006.

Green alga (*Pseudokirchneriella subcapitata*) were exposed to AE 0014966 in a static system over a period of 96 hours. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer flasks; 300 mL) served as one replicate filled with 100 mL test solution with an initial pH of 7.5. At test initiation the cell density was 10000 cells/mL. The test was conducted with 3 replicates per treatment level. In the controls 6 replicates were tested. Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72 and 96 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber.
For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. AE 0014966 00 1B98 0001 served as analytical standard. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 1.85 mg/L in the aqueous sample and 3.08 mg/L in the aqueous sample, respectively. The range of linearity was 20 to 410 µg/L in the analyte solution prepared for HPLC.

Dates of experimental work: October 22, 2001 to October 26, 2001

Results:

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

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

Table CA 8.2.6.1-16: Nominal and measured concentrations of AE 0014966

nominal concentration (mg a.s./L)	… based on purity of test substance	measured day 0		measured day 4		Mean measured mg a.s./L	Mean Percent of Nominal
		mg a.s./L	% nominal	mg a.s./L	% nominal		
control	0	< LOQ		< LOQ			
10	9.76	10.03	102.7	9.65	98.8	9.84	**100.8**
18	17.57	18.74	106.7	18.98	108.1	18.86	**107.4**
32	31.23	30.06	96.2	34.76	111.3	32.41	**103.8**
56	54.66	55.89	102.3	44.02	80.5	49.96	**91.4**
100	97.6	96.42	98.8	105.11	107.7	100.77	**103.2**

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

Table CA 8.2.6.1-17: Effect of AE 0014966 on growth-inhibition of *Pseudokirchneriella subcapitata*

Nominal concentration (mg a.s./L)	Percentual inhibition according to mean area under the growth curve after 72 h	Percentual inhibition according to mean growth rate after 72 h	Percentual inhibition according to mean area under the growth curve after 96 h	Percentual inhibition according to mean growth rate after 96 h
control	0	0	0	0
10	3	-0.27	-1.09	-0.58
18	13.52 *	3.3	21.41 *	5.65 *
32	22.07 *	6.06 *	31.57 *	8.47 *
56	88.21 *	69.81 *	95.41 *	69.89 *
100	89.07 *	66.47 *	95.73 *	74.42 *

* Statistically significant difference from control (Duncan's test; $p < 0.05$)

Conclusions:

The nominal concentration of AE 0014966 inhibiting the growth and the resulting E_bC_{50} (concentration for a 50% reduction of growth based on a comparison of areas under the growth curves) in comparison with the untreated control 72 and 96 hours test duration were 40.1 mg test item /L and 36.8 mg test item /L respectively.
The nominal concentration of AE 0014966 inhibiting the growth and the resulting E_rC_{50} (concentration for a 50% reduction of specific growth rate based on a comparison of slopes of the growth curves) in comparison with the untreated control after 72 and 96 hours test duration were 48.0 mg test item /L and 47.5 mg test item /L respectively.
The no observed effect concentration (NOEC), defined as the concentration which had no significant effect on growth inhibition or cell morphology after 96 h, was 10 mg/L.

AE 0034855

Report:	[redacted];[redacted];2002;M-210624-01
Title:	Algal growth inhibition - Pseudokirchneriella subcapitata AE 0034855 substance, pure Code: AE 0034855 00 1B99 0001
Report No:	C021124
Document No:	M-210624-01-1
Guidelines:	**EU (=EEC): C.3; OECD: 201; USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE 0034855 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0034855 00 1B99 0001; purity 98.5 % w/w) to *Pseudokirchneriella subcapitata*. Cultures of Pseudokirchneriella subcapitata with an initial cell density of 10000 cells/mL were exposed in a static system over a period of 96 hours to nominal concentrations of 10, 18, 32, 56, and 100 mg/L (corresponding to analytically verified concentrations of 75.5% to 111.4% and 73.3% to 106.9% of nominal values in freshly prepared and aged test solutions, respectively, mean measured values 74.4% to 109.2%). In addition a water control was tested.
72 and 96 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 mean measured figures. The 96-hour-E_rC_{50} was >109 µg a.s./L, the 96-hour-NOEC was determined to be 109 mg a.s./L (with recovery from transient effect at 109 mg/L after 72 hours).

Material and Methods:

The test item was identified as AE 0034855, substance, pure; Code: AE 0034855 00 1B99 0001; purity: 98.5 % (w/w); Analytical certificate No.: AZ 08468.

Green alga (*Pseudokirchneriella subcapitata*) were exposed to AE 0034855 in a static system over a period of 96 hours. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer flasks, 300 mL) served as one replicate filled with 100 mL test solution with an initial pH of 7.5. At test initiation the cell density was 10000 cells/mL. The test was conducted with 3 replicates per treatment level. In the controls 6 replicates were tested. Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72 and 96 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber.
For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 1.449 mg/L in the aqueous sample and 2.416 mg/L in the aqueous sample, respectively. The range of linearity was approx. 1 to 70 mg/L in the analyte solution prepared for HPLC.

Dates of experimental work: January 21, 2002 to January 25, 2002

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 75.5% to 111.4% and 73.3% to 106.9% of nominal values in freshly prepared and aged test solutions, respectively, mean measured values 74.4% to 109.2% calculated as arithmetic mean. Biological results are reported as mean measured. The mean measured concentrations were 7.3, 13.7, 26.4, 47.3 and 107.5 mg/L. Detailed analytical results are presented in the following table:

Table CA 8.2.6.1-18: Nominal and measured concentrations of AE 0034855

nominal concentration (µg a.s./L)	… based on purity of test substance	measured day 0		measured day 4		Mean Measured	Mean Percent of Nominal
		µg a.s./L	% nominal	µg a.s./L	% nominal		
control		< LOQ		< LOQ			
10	9.85	7.44	75.5	7.22	73.3	7.33	74.4
18	17.73	13.73	77.4	13.59	76.6	13.66	77.0
32	31.52	25.68	81.5	27.08	85.9	26.38	83.7
56	55.16	49.29	89.4	45.3	82.1	47.3	85.7
100	98.5	109.77	111.4	105.27	106.9	107.52	109.2

Biological findings:

Observations on growth rates are listed as follows:

Table CA 8.2.6.1-19: Effect of AE 0034855 on growth-inhibition of *Pseudokirchneriella subcapitata*

Nominal concentration (µg a.i./L)	% inhibition according to mean area under the growth curve	% inhibition according to mean growth rate	% inhibition according to mean area under the growth curve	% inhibition according to mean growth rate
	after 72 h	after 72 h	after 96 h	after 96 h
control	0	0	0	0
7.4	5.15	0.99	3.82	0.68
13.9	-5.05	-2.87	-5.53	-1.1
26.8	1.03	-2.21	-6.99	-1.76
48	17.19	5.73	8.02	0.83
109	35.93 *	14.51 *	18.89	2.14

* Statistically significant difference from control (Duncan's test; $p < 0.05$)

No cell abnormalities were observed.

Conclusions:

The effect of AE 0034855 (metabolite of iodosulfuron-methyl-sodium) (AE 0034855 00 1B99 0001) on *Pseudokirchneriella subcapitata* can be quantified as 96-hour-E_rC_{50} of >109 µg a.s./L and 96-hour-E_bC_{50} of >109 µg a.s./L. The highest concentration with no observed growth inhibition and no cell deformations can be set to 109 mg a.s./L (with recovery from transient effect at 109 mg/L after 72 hours).

AE 1234964

Report:	[redacted];[redacted];[redacted];2006;M-293396-01
Title:	Toxicity of MKH 6561-sulfonamide acid to Pseudokirchneriella subcapitata in an algal growth inhibition test
Report No:	30181210
Document No:	M-293396-01-1
Guidelines:	**Commission Directive 92/69/EEC, Annex Part C, C.3: "Algal Inhibition Test", Official Journal of the European Communities No. L 383 A, dated December 29, 1992. - OECD Guideline for Testing of Chemicals, Section 2, No. 201: "Alga, Growth Inhibition Test", adopted June 7, 1984. - OECD Guideline for Testing of Chemicals, No. 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition", draft revised October 22, 2004.;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this test was to determine the inhibitory effect of the metabolite of iodosulfuron-methyl-sodium AE 1234964 (other code: MKH 6561-sulfonamide acid) on the growth of the freshwater green algal species *Pseudokirchneriella subcapitata*. Exponentially growing cultures of this unicellular algal species were exposed to a geometric series of concentrations of the test item (100, 32, 10, 3.2 and 1.0 mg test item/L) and a control (pure reconstituted water) under defined conditions. The test was performed with three replicates per test concentration and six replicates in the control. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations. The test method and the test species *Pseudokirchneriella subcapitata* were recommended by the test guidelines. The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium. The test solutions were analysed after 0 and 72 hours of exposure. The 72 hours E_rC_{50} value was > 100 mg test item/L for growth rate.

Material and methods:

Test item: MKH6561-sulfonamide acid (metabolite of iodosulfuron-methyl-sodium); Batch code: AE 1234964 PU 01; Origin batch No.: M00102; Content of active ingredient: 99% w/w; Certificate No.: AZ 13380.

Green algae (*Pseudokirchneriella subcapitata*) were exposed to the test item AE 1234946 in a static system over a period of 72 hours. Nominal concentrations were 100, 32, 10, 3.2 and 1.0 mg test item/L, and a control (pure reconstituted water). Erlenmeyer flasks of 50 mL volume with 30 mL test medium were used as test units. The test was performed with three replicates per test concentration and six replicates in the control. The water hardness was 0.24 mmol/L (= 24 mg/L) as $CaCO_3$. Defined volumes of the algae suspensions from all replicates were sampled after 24, 48 and 72 hours of exposure and the cell densities in the samples were determined by spectrophotometrical measurement. For the determination of an influence of the test item on the algal cells, from the test concentration of 100 mg test item/L a sample was microscopically examined after the test period of 72 hours. For analytical verification of the test item concentrations duplicate samples were taken at 0 and 72 hours from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of work: June 19, 2006 to June 22, 2006 (biological part)
June 23, 2006 to June 24, 2006 (date of analysis)

Results:

Validity criteria:
The experiment is valid, because the cell density in the control cultures increased by a factor of 391 within 72 hours, the coefficient of variation on the sectional (daily) growth rates in the control cultures during the course of the test was 20.8 % and the coefficient of variation of average growth in replicate control cultures was 5.5 %.

Analytical findings:
At the start of the test 90% of the nominal test concentrations were found (average for all test concentrations). After 72 hours test duration 92% of the nominal values were determined (average for all test concentrations). Thus, during the test period of 72 hours the algae were exposed to a mean of 91% of nominal. Therefore, all reported results are related to nominal concentration of the test item.
In the lowest test concentration a mean value of 74% of nominal was found. Considering the mean recovery rate of 83% of the respective fortification level, it can be assumed, that this slightly reduced value is not result of wrong preparation of this test concentration or loss of test item. Additionally this test concentration is below the NOEC determined in this test.

Table CA 8.2.6.1-20: Summary of analytical results

Sample description [mg test item/L]	% of nominal[1]	RSD
control	n.a.	n.a.
1.0	74	9
3.2	89	5
10	94	3
32	100	2
100	100	1

[1] mean value of all mesured samples per treatment (start and end)
RSD: relative standard deviation per treatment group
n.a.: not applicable

Biological findings:
Observations are listed as follows:

Table CA 8.2.6.1-21: Influence of AE T234964 on the growth of *Pseudokirchneriella subcapitata*

Nominal concentration [mg test item/L]	72 h cell density (x 10000/mL)	72 h Growth Rate (μ)	72 h % Inhibition	72 h Area Under The Curve (A)	72 h % Inhibition
Control	195.330	1.988	0.0	133.346	0.0
1.0	194.142	1.981	0.4	131.572	1.3
3.2	195.231	1.989	0.0	134.208	-0.6
10	188.826	1.978	0.5	129.034	3.2
32	203.917	2.001	-0.7	139.678	-4.7
100	195.675	1.989	-0.1	133.494	-0.1

Table CA 8.2.6.1-22: Summary of biological results

Parameter (0 - 72 h)	Growth rate µ [mg test item/L]
72-hour E_rC_{50}	> 100
72-hour E_rC_{10}	> 100
72-hour NOE_rC	≥ 100
72-hour LOE_rC	> 100

No significant inhibition was observed at all test concentrations.

Conclusions:

The 72 hours E_rC_{50} value of AE 1234964 was > 100 mg test item/L for growth rate.

AE F159737

Report:	[redacted];2006;M-281243-01
Title:	Toxicity of MKH 6561-Saccharine to Pseudokirchneriella subcapitata in an algal growth inhibition test
Report No:	30191210
Document No:	M-281243-01-1
Guidelines:	**Commission Directive 92/69/EEC, Annex Part C, C.3: "Algal Inhibition Test", Official Journal of the European Communities No. L 383 A, dated December 29, 1992 ; OECD Guideline for Testing of Chemicals, Section 2, No. 201: "Alga, Growth Inhibition Test", adopted June 7, 1984 ; OECD Guideline for Testing of Chemicals, No. 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition", draft revised October 22, 2004.;none**
GLP/GEP:	**yes**

Executive summary:

The purpose of this test was to determine the inhibitory effect of the test item AE F159737 (other code: MKH 6561-Saccharine) on the growth of the freshwater green algal species *Pseudokirchneriella subcapitata*.
Exponentially growing cultures of this unicellular algal species were exposed to the nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg test item/L and to a control under defined conditions. The test was performed with three replicates per test concentration and six replicates in the control. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations. The test method and the test species *Pseudokirchneriella subcapitata* were recommended by the test guidelines.
The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium. The test solutions were analysed after 0 and 72 hours of exposure. The 72 hours E_rC_{50} value was >100 mg test item/L for growth rate.

Material and Methods:

Test item: AE F159737 (MKH 6561-Saccharine); Batch No.: M00402; Common name: 1,2-benzisothiazol-3(2H)-one 1,1-dioxide; Product code: AE F159737 00 1B99 0002; purity: 99.9 % w/w; Certificate No.: AZ 11460.

The freshwater green algal *Pseudokirchneriella subcapitata* was exposed during 72 hours to 100, 32, 10, 3.2 and 1.0 mg test item/L, and a control (pure reconstituted water without addition of the test item). Three replicates per test concentration and six replicates in the control were used during the test. The pH ranged from 7.9 to 8.2 at test start and from 8.7 to 9.2 at test end. The water temperature was 23 to 24°C over the whole period of testing at a continuous illumination of 7088 lux (mean value, range from 6590 to 7600 lux). The water hardness was 0.24 mmol/L (= 24 mg/L) as $CaCO_3$. Quantitative amounts of AE F159737 were analysed in duplicate test media samples from all test concentrations and both sampling times (0 and 72 hours) using liquid chromatography (HPLC-method). From the control samples only one of the duplicate samples was analysed from each of both sampling times.

Dates of experimental work: June 19, 2006 to June 22, 2006 (biological part)
June 12, 2006 (analytical part)
June 23, 2006 to June 24, 2006 (date of analysis)

Results:

Validity criteria:
The experiment is valid because:
- The cell density in the control cultures increased by a factor of 391 within 72 hours.
- The coefficient of variation on the sectional (daily) growth rates in the control cultures during the course of the test was 20.8 %.
- The coefficient of variation of average growth in replicate control cultures was 5.5 %.

Analytical findings:
At the start of the test 106 % of the nominal test concentrations were found (average for all test concentrations). After 72 hours test duration 106 % of the nominal values were determined (average for all test concentrations). Thus, during the test period of 72 hours the algae were exposed to a mean of 106 % of nominal. Therefore, all reported results are related to nominal concentration of the test item.

Table CA 8.2.6.1-23: Summary of analytical results

Sample description [mg test item/L]	% of nominal[1]	RSD
Control	n.a.	n.a.
1.0	115	1
3.2	106	1
10	105	1
32	100	3
100	105	2

[1] Mean value of measured samples per treatment group (start and end)
RSD relative standard deviation per treatment group
n.a. not applicable

Biological findings:
No significant inhibition of the growth rate was observed at test concentrations of 1.0, 3.2, 32 and 100 mg/L. The significant inhibition of 2.6% at 10 mg test item/L after 72 hours of exposure is considered to be coincidently and not test item related.

Table CA 8.2.6.1-24: Influence of AE F159737 on the growth of *Pseudokirchneriella subcapitata*

Parameter (0 - 72 h)	Growth rate µ [mg test item/L]
72-hour E_rC_{50}	>100
72-hour E_rC_{10}	>100
72-hour NOE_rC	≥100
72-hour LOE_rC	>100

Conclusions:

The inhibitory effect of the test item AE F159737 on the growth of the freshwater green algal species *Pseudokirchneriella subcapitata* was assessed over a test period of 72 hours. The 72 hours E_rC_{50} value was >100 mg test item/L for growth rate.

AE F154781

Report:	[redacted]; [redacted]; 2013; M-476160-01
Title:	Pseudokirchneriella subcapitata - Growth inhibition test with AE F154781 - limit test
Report No:	EBMN103
Document No:	M-476160-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009;none**
GLP/GEP:	**yes**

Executive Summary:

The objective of this 72 hour growth inhibition test is, to verify the assumption that the test item AE F154781 will cause no relevant adverse effects on the growth of the green algae *Pseudokirchneriella subcapitata* at the limit test item concentration of 10.0 mg pure metabolite (p.m.)/L. 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 concentration of 10.0 mg p.m./L in comparison to 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 alterations in algae cells that might influence extinction measurements, such as unusual cell size, 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 > 10.0 mg p.m./L and the (0 – 72 h)-NOE_rC was determined to be ≥ 10.0 mg p.m./L.

Material and Methods:

Test item. AE F154781; Analysed purity: 91 % w/w; Origin batch No: 0201893-ACB; Certificate No.: AZ 18907; LIMS No.: 1324968.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions a nominal concentration of 10.0 mg pure metabolite/L in comparison to untreated control. The limit concentration of 10.0 mg pure metabolite/L was chosen to demonstrate that the test item was less toxic towards *Pseudokirchneriella subcapitata* than the active substance. The test volume was 150 mL test medium per replicate. 3 replicate vessels per test level and 6 replicate vessels per control were used during the test. The pH values ranged from 8.0 to 8.4 in the controls and the incubation temperature ranged from 21.8°C to 22.2°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6233 lux (mean value).
Quantitative amounts of AE F154781 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: September 09, 2013 to September 26, 2013

Results:

Validity criteria:
The study conditions met all validity criteria, requested by the mentioned guideline(s). Biomass increased in the control by more than 16-fold within the evaluation period. 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%. Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical findings:
The analytical finding of AE F154781 in the treatment level found on day 0 was 111 % of nominal. On day 3 analytical findings of 111 % of nominal were found. Based on the analytical findings all results are given as nominal concentrations of the test item in the test medium.

Table CA 8.2.6.1-25: Concentrations of AE F154781 in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	Actual Concentration (mg p.m./L)			
	1. Determination	2. Determination	Average	%
Control	<0.578	<0.578	<0.578	--
10.0	11.1	11.0	11.1	111

Table CA 8.2.6.1-26: Concentrations of AE F154781 in the test solutions at day 3

Day 3				
Nominal Concentration in mg p.m./L	Actual Concentration (mg p.m./L)			
	1. Determination	2. Determination	Average	%
Control	<0.578	<0.578	<0.578	--
10.0	11.1	11.1	11.1	111

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

Table CA 8.2.6.1-27: The static 72 hour algae growth inhibition test provided the following tabulated effects

nominal concentration [mg p.m./L]	cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	inhibition of average specific growth rate [%]
control	921 000	1.592	--
10.0	892 000	1.582	0.6

Test initiation with 8,000 cells/mL

Conclusions:

The (0-72h)-E_rC_{50} for AE F154781 is >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

Studies on iodosulfuron-methyl-dosium

Report:	[redacted];[redacted];[redacted];1998;M-143100-01
Title:	Algal growth inhibition (Navicula pelliculosa) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59427
Document No:	M-143100-01-1
Guidelines:	**EU (=EEC): 92/69 C.3; OECD: 201; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted];[redacted];[redacted];[redacted];2000;M-192458-01
Title:	Algal growth inhibition - Navicula pelliculosa Iodosulfuron (prov. approved ISO) substance, technical Code: AE F115008 00 1C89 0001
Report No:	C005665
Document No:	M-192458-01-1
Guidelines:	**OECD: No. 201; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to determine the growth effects of Iodosulfuron-methyl-sodium to the diatom *Navicula pelliculosa* (*Bacillariophyceae*) under static conditions.
Triplicate cultures of *Navicula pelliculosa* with an initial cell density of 10 000 cells/mL were exposed in a synthetic medium at 25 ± 1°C for 96 hours to nominal concentrations of 10, 18, 32, 56 and 100 mg test item/L with four replicates each. In addition an untreated water control with eight replicates was tested. The Fe-citrate stock solution was adjusted in deviation from recommendations of the guidelines and $FeSO_4$*7 H_2O/Na_2EDTA*2 H_2O stock solution was added in order to support growth of the algal species.
Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72 and 96 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber. Chemical analysis of the freshly prepared and the aged (96 hours old) test solutions was perfomed for the active ingredient using High Performance Liquid Chromatography with ultraviolet detection (HPLC/UV).

Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour-E_rC_{50} (growth rate) and E_bC_{50} (area under the growth curve) were > 100 mg test item/L and the no observed adverse effect concentration (NOAEC) was determined to be 100 mg test item/L.

Material and methods:

Test item: Iodosulfuron-methyl-sodium (AE F115008); code: AE F115008 00 1C89 0001, purity: 86.9% w/w; Analytical certificate No.: AZ 07987.

Triplicate algal cultures with an initial cell density of 10 000 algal cells/mL were incubated in a synthetic medium at 25 + 1°C for 96 hours. Nominal test item concentrations were 10, 18, 32, 56 and 100 mg test item /L with four replicates each, together with an untreated control with eight replicates. Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72 and 96 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber.
Chemical analysis of the freshly prepared and aged (96 hours old) test solutions was performed using High Performance Liquid Chromatography with ultraviolet detection (HPLC/UV).

Dates of experimental work: August 13, 1999 – September 24, 1999

Results:

Analytical findings:
Analyses of freshly prepared water determined for the active substance resulted in test item concentrations ranging from 95.2% to 102.5% of nominal values. Analyses of aged water (96 h) at experimental termination resulted in test item concentrations ranging from 93.9% to 98.6% of nominal values. The mean measured values over the time of exposure ranged from 94.6% to 100.6% of the nominal values. As all analysed concentrations were above 80% of nominal, nominal values were used for reporting the results. Detailed analytical results are presented in the following table:

Table CA 8.2.6.2-1: Nominal and measured concentrations of AE F115008

Nominal concentration of the test substance [mg/L]	Nominal concentration of active ingredient [mg/L]	Day 0		Day 4		Mean	
		Measured active ingredient [mg/L]	Nominal active ingredient [%]	Measured active ingredient [mg/L]	Nominal active ingredient [%]	Measured active ingredient [mg/L]	Nominal active ingredient [%]
0.00	0.00	0.00	-	0.00	-	0.00	-
10.00	8.69	8.91	102.5	8.57	98.6	8.74	100.6
18.00	15.64	15.14	96.8	14.87	95.1	15.00	95.9
32.00	27.81	26.48	95.2	26.35	94.8	26.41	95.0
56.00	48.66	46.31	95.2	46.18	94.9	46.24	95.0
100.00	86.90	82.75	95.2	81.64	93.9	82.19	94.6

Biological findings:
The E_rC_{50} (growth rate) and E_bC_{50} (area under the growth curve) are summarised below:

	EC-values after 72 and 96 hours	
	E_bC_{50}	E_rC_{50}
EC_{50} mg/L	> 100	> 100

Significant inhibition of the growth rate and the area under the growth curve (significance level of alpha = 0.05) was not observed in any of the nominal concentrations. The highest tested concentration of 100 mg/L led to higher cell concentrations after 72 hours in comparison to the untreated control. Observations on growth rates are listed as follows:

Table CA 8.2.6.2-2: Effect of AE F1 15008 on growth-inhibition of *Navicula pelliculosa*

Nominal treatment level (mg/L)	Mean area under the growth curve (10^4/mL*h)		Mean growth rate (h^{-1})		Percentual inhibition according to mean area under the growth curve		Percentual inhibition according to mean growth rate	
	after 72 h	after 96 h	after 72 h	after 96 h	after 72 h	after 96 h	after 72 h	after 96 h
control	367.42	1034.90	0.03699	0.03836	0.00	0.00	0.00	0.00
10	208.92	840.84	0.03395	0.03908	41.55	18.75	8.22	-1.88
18	211.47	948.24	0.03367	0.04096	40.83	8.37	8.99	-6.79
32	193.62	829.71	0.03004	0.03957	45.83	19.83	18.79	-3.16
56	166.68	755.31	0.03008	0.03884	53.37	27.02	18.68	-1.25
100	742.71	2038.47	0.05587	0.04145	-107.80	-96.97	-51.04	-8.07

No cell abnormalities were observed.

Conclusions:

In a growth inhibition test to Navicula pelliculosa all treatments leads to nearly the same or higher cell concentrations in comparison with the untreated control. For the concentration of 100 mg/L the test item seemed to be useful for the algal growth. 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 observed adverse growth inhibition and no cell deformation was 100 mg test item/L.

Report:	[redacted]; [redacted]; [redacted];2000;M-238448-01
Title:	Effect to Anabaena flos-aquae (blue-green alga) in a growth inhibition test: AE F115008 technical 86.9% w/w; AE F115008 00 1C89 0001
Report No:	B002714
Document No(s):	M-238448-01-2
Guidelines:	**EU (=EEC): Annex II Point 8.2.6; OECD: 201; USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of Iodosulfuron-methyl-sodium to the blue-green algae, *Anabaena flos-aquae*, in a static system over a 96 hour exposure period.

Triplicate cultures of *Anabaena flos-aquae* with an initial cell density of 10 000 cells/mL were exposed in a static system over a period of 96 hours to nominal concentrations of 0.6, 1.1, 1.8, 3.0 and 5.0 mg a.s./L (corresponding to mean measured concentrations of 0.6, 1.1, 1.8, 3.0 and 5.0 mg a.s./L) in AAP (Algal Assay Procedure) algal media for a 96 hour period. In addition six replicate algal cultures were cultured without test substance as the control treatment.
At study initiation all treatments had a pH of 7.1 to 7.9, which is outside of the protocol specifications of 7.2 ± 0.3. This effect is believed to have had no appreciable impact on the results or quality of the study.
Cell density of each culture was counted under a microscope using a hemacytometer 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. Discrete measurements of temperature, pH, dissolved oxygen and conductivity were obtained at test initiation and at test termination.
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 3.1 mg/L and 2.6 mg/L. The E_rC_{50} (growth rate) values for 72 and 96 hours were calculated as 2.0 mg/L and 1.7 mg/L. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were 0.6 mg/L and 1.1 mg/L, respectively.

Material and methods:

Test material: Iodosulfuron-methyl-sodium, technical; Code No.: AE F115008 00 1C89 0001; Batch No.: CR21436/02/950601; Content: 86.9% w/w, Certificate of Analysis: AZ 07987

Triplicate algal cultures with an initial nominal cell count of approximately 1 x 104 cells/ml were exposed to the nominal concentration of 0.6, 1.1, 1.8, 3.0, and 5.0 mg/L of the test substance in AAP algal 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 algae. Samples at test termination were taken as composite samples and centrifuged at 1000 g for 15 minutes prior to analysis to remove algal cells and any undissolved particulates. All samples were analyzed by High Performance Liquid Chromatography (HPLC) with Ultraviolet Detection (UV) for quantification of AE F115008.
Test methodology was in agreement with OECD 201 and USEPA 123-2 guidelines.

Dates of experimental work: November 15, 1999 – November 19, 1999

Results:

Analytical findings:
The method efficiency of samples fortified with AE F115008 ranged from 96 to 105 % with a mean percent recovery of 100 % (SD = 4.2%). Analytical verification of test solutions revealed mean measured concentrations of 0.6, 1.1, 1.8, 3.0, and 5.0 mg/L (99 to 103% of nominal). There were no residues of AE F115008 found in the dilution water or control samples. Biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table CA 8.2.6.2-3: Nominal and measured concentrations of AE F1 15008

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	NF	NF	NF	--
Control	0	NF	NF	NF	--
0.6	0.6	0.6296	0.602	0.6158	103%
1.1	1.1	1.1395	1 .0756	1.1076	101%
1.8	1.8	1.8761	1.7569	1.8165	101%
3.0	3.0	3.1197	2.866	2.9929	100%
5.0	5.0	4.9287	4.983	4.9559	99%

NF = Not found

Biological findings:
Algal cell numbers in the control increased by a factor of 11 from test initiation to 96 hours.
Observations on growth rates at 72 and 96 hours are listed as follows:

Table CA 8.2.6.2-4: Effect of AE F1 15008 on growth-inhibition of *Anabaena flos-aquae*

Nominal concentration (mg a.i./L)	Cell density (cells*10^4/mL)		Specific Growth Rate (μ)		% Inhibition		Area Under The Curve (cells*10^5/mL)		% Inhibition	
	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Control	5.5	11.2	0.0227	0.0249	--	--	8.37	14.7	--	--
0.6	5.3	16.3	0.0230	0.0287	-1	-15	7.44	13.4	11	9
1.1	4.9	16.2	0.0217	0.0183	5	27*	9.00	16.3	-18	-11
1.8	1.6	2.4	0.00654	0.00884	71*	64*	5.08	7.28	39	51*
3.0	1.7	1.9	0.00674	0.00631	70*	75*	3.56	5.28	57*	64*
5.0	1.8	1.6	0.00833	0.00511	63*	79*	3.16	4.88	62*	67*

* Statistically significant difference from control (William's test, p # 0.05)

Biological endpoints derived:
From the results presented above the following No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), E_rC_{50} (specific growth rate) and E_bC_{50} (area under the curve) can be derived:

Table CA 8.2.6.2-5: Biological endpoints

Time (hours)	NOEC (mg/L)	LOEC (mg/L)	EC_{50} Method	E_bC_{50} (± 95% CL) (mg/L)	E_rC_{50} (± 95% CL) (mg/L)
72	1.1	1.8	Nonlinear Regression	3.1 (1.6 to 6.1)	2.0 (1.1 to 3.8)
96	0.6	1.1	Nonlinear Regression	2.6 (1.3 to 5.1)	1.7 (1.2 to 2.4)

Conclusions:

The E_bC_{50} (biomass) values for 72 and 96 hours, as determined by nonlinear regression, were calculated as 3.1 mg/L (95% CL = 1.6 to 6.1 mg/L) and 2.6 mg/L (95% CL = 1.3 to 5.1 mg/L), respectively. The E_rC_{50} (growth rate) values for 72 and 96, as determined by nonlinear regression, were calculated as 2.0 mg/L (95% CL = 1.1 to 3.8 mg/L) and 1.7 mg/L (95% CL = 1.2 to 2.4 mg/L), respectively. The no observed effect concentration (NOEC) was 0.6 mg/L and the lowest observed effect concentration (LOEC) was 1.1 mg/L under the conditions of this study.

Report:	[redacted];[redacted];[redacted];2000;M-238456-01
Title:	Effect to Skeletonema costatum (marine diatom) in a growth inhibition test: AE F115008 technical 86.9% w/w: AE F115008 00 1C89 0001
Report No:	B002722
Document No(s):	M-238456-01-2
Guidelines:	**EU (=EEC): Annex II Point 8.2.6; OECD: 201; USEPA (=EPA): 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to determine the effects of Iodosulfuron-methyl-sodium to the marine diatom, *Skeletonema costatum*, in a static system over a 96 hour exposure period.
Triplicate Cultures of Skeletonema costatum with an initial cell density of 10 000 cells/mL were exposed to nominal concentrations of 6.3, 13, 25, 50 and 100 mg a.s./L (corresponding to analytically verified concentrations of 6.2, 13, 25, 50, and 102 mg a.s./L) in Marine Algal Assay (MAA) media for a 96 hour period. In addition six MAA medium control replicates were tested.
The age of the stock culture used to inoculate treatment flasks at study initiation exceeded with 7 days old the protocol specific age of 4 to 6 days old. The pH at study termination deviated by a maximum of 1.2 pH units from initial values. This change in pH is attributed to the rapid growth of diatoms. These protocol deviations are thought to have had no appreciable impact on the results or quality of the study.
At 24 hour intervals, the cell density of each culture was counted under a microscope using a hemacytometer. Average specific growth rate and biomass were 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 and at test termination. All samples were analysed by High Performance Liquid Chromatography (HPLC) with ultraviolet detection (UV) for quantification of the test item. 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 36 mg/L and 41 mg/L. The E_rC_{50} (growth rate) values for 72 and 96 hours were calculated as 68 mg/L and 79 mg/L. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for 96 hours were 13 mg/L and 25 mg/L, respectively.

Materials and Methods:

Test material: Iodosulfuron-methyl-sodium, technical; Code No.: AE F115008 00 1C89 0001; Batch No.: CR21436/02/950601, Sample No.: ZBA438; CAS Reg. No.: 144550-36-7; purity: 86.9% w/w; Certificate of Analysis: AZ 07987.

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 6.3, 13, 25, 50, and 100 mg/L of the test substance in Marine

Algal Assay (MAA) media for a 96 hour period. Six replicate diatom cultures were cultured without test substance as the control treatment. At 24 hour intervals, the cell density (cells/mL) of each culture was counted under a microscope using a hemacytometer. 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 F115008.
Test methodology was in agreement with OECD 201 and USEPA 123-2 guidelines.

Dates of experimental work: October 25, 1999 – October 30, 1999

Results:

Analytical findings:
The method efficiency of samples fortified with AE F115008 ranged from 96 to 99 % with a mean percent recovery of 98 % (SD = 1.3). Analytical verification of test solutions revealed mean measured concentrations of 6.2, 13, 25, 50, and 102 mg a.i./L calculated as arithmetic mean (99 – 102 % of nominal). There were no residues of AE F115008 in the dilution water and control samples greater than the limit of quantification (0.005 mg/L). The measured concentration of AE F115008 indicated that the nominal concentration was achieved at test initiation and remained stable throughout the study. Therefore, biological results are reported as nominal. Detailed analytical results are presented in the following table:

Table CA 8.2.6.2-6: Nominal and measured concentrations of AE F115008

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	NF	< LOQ	< LOQ	-
Control	0	NF	< LOQ	< LOQ	-
6.3	6.3	6.3827	6.0493	6.2160	99%
13	13	12.9142	12.7044	12.8093	99%
25	25	25.0476	24.3519	24.6998	99%
50	50	49.4644	50.5526	50.0085	100%
100	100	101.7723	102.2833	102.0278	102%

NF = not found, LOQ = Limit of Quantification (0.005 mg/L)

Biological findings:
Diatom cell numbers in the control increased by a factor of at least 16 from test initiation to 48 hours. Observations on growth rates at 72 and 96 hours are listed as follows:

Table CA 8.2.6.2-7: Effect of AE F115008 on growth-inhibition of *Skeletonema costatum*

Nominal concentration (mg a.s./L)	Cell density (cells*10⁴/mL)		Specific Growth Rate (µ)				Area Under The Curve (cells*10⁵/mL)			
					% Inhibition				% Inhibition	
	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h	72 h	96 h
Control	44.4	69.7	0.0526	0.0442	--	--	96.5	231	--	--
6.3	45.9	69.0	0.0531	0.0441	-1	0	96.0	232	1	0
13	45.8	67.6	0.0531	0.0439	-1	1	91.1	225	6	3
25	36.4	61.2	0.0498	0.0428	5	3	71.8	186	26*	19*
50	14.3	33.5	0.0370	0.0366	30*	17*	26.6	81.6	72*	65*
100	2.7	3.7	0.0140	0.0135	73*	70*	3.3	8.58	97*	96*

* Statistically significant difference from control (Bonferroni t-test, $p \leq 0.05$)

Biological endpoints derived:

From the results presented above the following No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) can be derived:

Table CA 8.2.6.2-8: Biological endpoints

Time (hours)	Specific growth rate (µ)		Specific Growth Rate (µ)	
	NOEC (mg/L)	LOEC (mg/L)	NOEC (mg/L)	LOEC (mg/L)
24	25	50	25	50
48	13	25	6.3	13
72	13	25	13	25
96	25	50	13	25

The E_rC_{50} (specific growth rate) and E_bC_{50} (area under the curve) are presented in the following table:

Table CA 8.2.6.2-9: E_rC_{50} (specific growth rate) and E_bC_{50} (area under the curve)

Time (hours)	EC_{50} Method	E_rC_{50} (± 95% CL) (mg/L)	E_bC_{50} (± 95% CL) (mg/L)
24	Nonlinear Regression	43 (37 to 51)	39 (31 to 47)
48	Nonlinear Regression	54 (51 to 57)	33 (30 to 37)
72	Nonlinear Regression	68 (66 to 70)	36 (34 to 39)
96	Nonlinear Regression	79 (77 to 81)	41 (38 to 43)

Conclusion:

The E_bC_{50} (biomass) values for 72 and 96 hours, as determined by nonlinear regression, were calculated as 36 mg/L (95% CL = 34 to 39 mg/L) and 41 mg/L (95% CL = 38 to 43 mg/L), respectively. The E_rC_{50} (growth rate) values for 72 and 96 hours, as determined by nonlinear regression, were calculated as 68 mg/L (95% CL = 66 to 70 mg/L) and 79 mg/L (95% CL = 77 to 81 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 13 mg/L and 25 mg/L, respectively.

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F075736

Report:	[redacted];[redacted];[redacted];[redacted];1998;M-181581-01
Title:	Algal growth inhibition (Navicula pelliculosa) AE F075736 (Metsulfuron-methyl) Metabolite of AE F115008 substance, technical Code: AE F075736 00 1C92 0001
Report No:	C000982
Document No:	M-181581-01-1
Guidelines:	**EU (=EEC): 92/69 C.3; OECD: 201; USEPA (=EPA): J, § 123-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

CA 8.2.7 - Effects on aquatic macrophytes

For iodosulfuron-methyl-sodium, toxicity studies on different aquatic macrophytes were performed. Besides *Lemna gibba*, also *Myriophyllum spicatum* and *Elodea canadensis* were tested under laboratory conditions as additional macrophyte species. In addition, an outdoor growth inhibition study was performed with a total of nine species representing different taxonomic groups. Since *Lemna gibba* turned out to be the most sensitive species to iodosulfuron-methyl-sodium, a higher-tier study (long-term exposure) was performed with this species.
Studies investigating the toxicity to *Lemna gibba* were also performed for all metabolites of the residue definition for risk assessment in surface water. It was found that one metabolite, AE F075736, has a similar activity to *Lemna* as the parent compound, while all other metabolites turned out to be non-toxic to these organisms.
Details of all studies are provided in the following table.

Table CA 8.2.7-1: Effect data of iodosulfuron-methyl-sodium and metabolites to aquatic macrophytes presented in this chapter (Since the new aquatic GD[4] focusses on endpoints based on growth rates the old E_bC_{50} figures were omitted from the table above.)

Test organism	Test system	Test duration	Endpoint [mg/L]		Reference
Iodosulfuron-methyl-sodium					
Lemna gibba (duck weed)	Growth inhibition	14 d	7 d EC_{50} 14 d EC_{50}	0.00079 0.00083	[redacted] & [redacted], 1997 BY97W501A M-141441-02-1 KCA 8.2.7 /01
Lemna gibba (duck weed)	growth inhibition, mimicking exposure of outdoor study	7 d	E_rC_{50} (frond number) E_rC_{50} (frond area)	**0.00108** 0.00112	[redacted], 2013 E 412 3763 – 6 M-469584-02-1 KCA 8.2.7 /07
		42 d	E_rC_{50} NOEC	**0.000609** **0.0004**	
Macrophytes in outdoor ponds 9 macrophytes	Growth inhibition	6 weeks	NOEC	0.00027	[redacted], 2011 13798.6259 M-407716-01-1 KCA 8.2.7 /06
	Growth inhibition + recovery	2 d + 5.5 weeks	NOEC	**0.00072**	
Myriophyllum spicatum *Elodea canadensis* (aquatic plant)	growth inhibition	10 d	NOEC NOEC	0.001 0.00046	[redacted] & [redacted]-[redacted], 1998 C000104 M-180262-01-1 KCA 8.2.7 /04
Myriophyllum spicatum (aquatic plant)	growth inhibition	14 d	E_yC_{50} NOEC	0.00203 0.00089	[redacted] et al., 2012 EBIML032 M-431705-01-1 KCA 8.2.7 /08
AE F075736					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**0.000511** 0.000169	[redacted] & [redacted], 1998 CE98/095 M-182336-01-1 KCA 8.2.7 /03
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	0.00112 0.00032	[redacted] & [redacted], 2001 C015669; M-200947-01-1 KCA 8.2.7 /09
AE F145741					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**3.84** 0.76	[redacted], 2013 EBIML041 M-462128-01-1 KCA 8.2.7 /10

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

Test organism	Test system	Test duration	Endpoint [mg/L]		Reference
AE F145740					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**>10.0** >10.0	[redacted], 2013 EBIMN063 M-462121-02-1 KCA 8.2.7 /11
AE 0002166					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**0.023** 0.00769	[redacted], 2002 C018083 M-205481-01-1 KCA 8.2.7 /12
AE F161778					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**0.0281** 0.010	[redacted], 2001 C008628 M-197639-01-1 KCA 8.2.7 /13
BCS-CW81253					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**>10.0** >10.0	[redacted], 2013 EBIMN060 M-462125-01-1 KCA 8.2.7 /14
AE 0000119					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**>100** 100	[redacted], 2002 C020878 M-210320-01-1 KCA 8.2.7 /15
AE F059411					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**>100** 32	[redacted], 2002 C017092 M-203638-01-1 KCA 8.2.7 /16
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	>100 56	[redacted] & [redacted], 1998 CE98/089 M-181177-01-1 KCA 8.2.7 /02
AE 0014966					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**0.575** 0.18	[redacted], 2002 C003832 M-186853-01-1 KCA 8.2.7 /17
AE 0034855					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	>**100** 100	[redacted], 2002 C020876 M-210318-01-1 KCA 8.2.7 /18
AE 1234964					
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} NOEC	**>100** 0.32	[redacted], 2006 30184240 M-281240-01-1 KCA 8.2.7 /19

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
AE F159737				
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} **>100** NOEC 0.32	[redacted], 2006 30194240 M-281250-01-1 KCA 8.2.7 /20
AE F154781				
Lemna gibba (duck weed)	Growth inhibition, static	7 d	E_rC_{50} **>10** NOEC ≥10	[redacted], 2013 EBIMN106 M-470494-01-1 KCA 8.2.7 /21

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

<u>Studies on iodosulfuron-methyl-sodium</u>

Report:	[redacted];[redacted];[redacted];1997;M-141441-02; Amended: 1998-01-19
Title:	Toxicity to duckweed (Lemna gibba), in a static system AE F115008 technical 87.4% w/w Code: AE F115008 00 1C89 0001
Report No:	A57770, BY97W50, BY97W50 1a
Document No(s):	M-141441-02-1
Guidelines:	**USEPA (=EPA): 122-2;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

$$EC_{50} = 0.00083 \text{ mg/L}$$

Report:	[redacted];[redacted];2014;M-479697-01
Title:	Iodosulfuron-methyl-sodium rationale for the replacement of the old 14-day Lemna growth inhibition study ([redacted] & [redacted] 1997; M-141441-02) with the 7-day endpoints from the Lemna study ([redacted] 2013; M-469584-01-1)
Document No(s):	M-479697-01-1
Guidelines:	M-479697-01-1
GLP/GEP:	**not specified;not specified**

Two *Lemna*-studies have been conducted with iodosulfuron-methyl-sodium tech. a.s. (see Table CA 8.2.7-1; KCA 8.2.7 /01 and KCA 8.2.7 /07). The first one is a 14-day study conducted in 1997 by [redacted] & [redacted] according to EPA Guideline 122-2. In this study only frond number was determined on days 2, 5, 7, 9, 11 and 14. A second endpoint like frond dry weight or frond area, which is mandatory according to OECD 221 (2006), has not been determined. Moreover, inhibition percentages were calculated by using the absolute frond counts in the treatments compared to the control, while nowadays a 7-day E_rC_{50} based on growth rate inhibition is used for risk assessments.

The second study ([redacted] 2013) was performed according to the currently valid guideline OECD 221 (2006) measuring two endpoints, frond number and frond area. This study can be considered as fully valid study without restrictions. This 6-week study was designed to mimic the exposure of an outdoor-pond study and to obtain 6-week effect data for *Lemna* – a species that could not be kept in outdoor

ponds. Beside the 6-week endpoints, effect data were calculated on a weekly basis. The endpoints obtained from the first 7-day period can be used for tier-1 risk assessments.
The NOEC determined by [redacted] & [redacted] was 0.4 µg/L. In the new *Lemna*-study 7-day E_rC_{10}-figures were 0.449 and 0.501 µg/L for frond counts and frond area, respectively.
The new *Lemna* study ([redacted] 2013; M-469584-02-1) shall replace the old study mentioned above for the following reasons:

1. In the new study two endpoints, frond number and frond area, where measured.
2. The new study has been conducted on the currently valid guideline OECD 221 (2006).
3. The growth rate related endpoints have been used already in the past but a lot of regulators were using the biomass related values because they are lower. Nevertheless the scientific community in Europe was already convinced since a long time that the focus should be on the growth rate related endpoints. This is as well reflected in the current versions of the OECD guidelines for algae and *Lemna*. In these guidelines it is stated that the growth rate related endpoints are preferred. Within a risk assessment sensitivities of different plant species are compared. As their growth, the test durations and the test designs are different a comparison of sensitivities only makes sense when growth rate related endpoints are used. This endpoint cannot be determined from the old study.
4. The fact, that the NOEC from the old study is very close to the 7-day E_rC_{10}-figures from the new study indicates, that the test organisms were of equal sensitivity.

Overall, it can be concluded that the new fully valid and according to current state of the science performed 7-day *Lemna*-study supersedes the old 14-day Lemna study, based on frond counts solely. **Consequently the EU-agreed endpoint of 0.83 µg/L, based on frond counts shall be replaced by the new 7-day E_rC_{50} of 1.08 µg a.s./L based on growth rate.**
The OECD 221 test guideline states that even though the results based on yield or frond counts are often lower than the endpoints based on the average specific growth rate "this should not be interpreted as differences in sensitivity between the two response variables" but is "due to the mathematical basis of the respective approaches".

Report:	[redacted];[redacted];[redacted];1998;M-180262-01
Title:	Impact of AE F115008 substance, technical on the aquatic macrophytes Myriophyllum spicatum and Elodea canadensis
Report No:	C000104
Document No(s):	M-180262-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted];[redacted];2011;M-407716-01
Title:	Outdoor growth inhibition and recovery of aquatic plants exposed to iodosulfuron-methyl-sodium WG50
Report No:	13798.6259
Document No:	M-407716-01-1
Guidelines:	**Not applicable to higher tier evaluation;none**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of Iodosulfuron-methyl-sodium on the growth of a selection of nine species of aquatic macrophytes in artificial ponds under outdoor conditions. Following a two to four week acclimation phase the ponds were dosed once with nominal concentrations of 0.10, 0.25, 0.63, 1.6, 3.9, 9.8, 24 or 61 µg a.s./L. In addition a water control was tested. During the test duration of six weeks a continuous dissipation of the test substance was observed in the ponds that can be regarded as static water-sediment systems. In additional ponds treated with 0.25 and 0.63 µg a.s./L the water was renewed after two days mimicking a short-term peak exposure. As a tenth species *Lemna gibba* was tested in bioassays using samples of pond water enriched with nutrient medium.
Biological endpoints were calculated as nominal and mean initial measured concentrations of 0.11, 0.27, 0.68, 1.8, 4.1, 10, 25 and 61 µg a.s./L. The lowest NOEC was nominal 0.25 (0.27 initially measured) µg a.s./L for mean shoot dry weight of *Potamogeton pectinatus*. The lowest EC_{50} was nominal 0.50 (0.54 initially measured) µg a.s./L for leaf dry weight of *Salvinia minima*. A short-term peak exposure to 0.72 µg a.s./L followed by a 5.5 week recovery period had no effect on the macrophytes in the static fresh water test ponds.

Materials and Methods:

Test item: Iodosulfuron-methyl-sodium WG 50; Batch number: 2010-003463, purity: 50.5% w/w.

Test species:
Monocotyledon: Elodea (*Elodea canadensis*), Sago Pondweed (*Potamogeton pectinatus*), Reed Sweetgrass (*Glyceria maxima*) and Arrowhead weed (*Sagittaria latifolia*);
Dicotyledon: Water Lily (*Nymphaea odorata*), Coontail weed (*Ceratophyllum demersum*), Variable milfoil (*Myriophyllum heterophyllum*), Water Mint (*Mentha aquatica*), Fanwort (*Cabomba caroliniana*); Fern: Water fern (*Salvinia minima*); Laboratory Exposure: Duckweed (*Lemna gibba*).
The selected plant species were chosen because they represent a wide range of freshwater aquatic habitats and they represent both monocotyledon and dicotyledon plants and one fern. From an ecological perspective they represent submerged species as well as floating species and those emerging from the water surface. *Glyceria maxima* was removed from the study on exposure day 29 due to generally poor health in all treated and control ponds. Data of this species were not evaluated.

Thirty-four, square, 3000-L, outdoor, freshwater ponds (inside dimensions 230 cm × 230 cm × 60 cm deep) were constructed by stacking 15 cm x 15 cm x 240 cm pressure-treated timbers. The frames were lined with liners designed for use in aquatic horticulture. Each pond contained a 5-cm layer of sandy loam soil to serve as sediment. The percent sand:silt:clay of the soil was determined to be 75:19:6%, respectively, the percent organic matter was 5.2% and the pH was 6.9. Each pond was filled with approximately 1850 liters (35-cm depth) of unchlorinated well water and fortified in hardness to approximately 160 mg/L as $CaCO_3$. The ponds received full sunlight throughout the day. The covers were temporarily installed over the ponds when heavy rain was forecast, in order to prevent major dilution of the test solutions.

The test species were planted separately in pots of different size applicable for the expected growth of the particular species. Pots with *Mentha* and *Glyceria* were elevated close to the water surface, as these species grow in shallow water. Pots were filled to approximately 75% full with the soil mixture and 0.40 to 0.50 g Scotts Osmocote slow-release fertilizer pellets were evenly dispersed and pushed into the mid-depth of the soil. The soil surface was then covered with approximately 2 cm of masonry sand.

There were two exceptions: The rootless *Ceratophyllum* plants were exposed in submerged mesh bags and the floating *Salvinia* plants were confined to corrals in order to avoid them spreading over the whole pond surface. Plants were placed in the ponds for a 2 to 4 week acclimation period prior to exposure to the test substance, as follows:

Table CA 8.2.7-2: Survey of species-specific characteristics of methods

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

Duckweed was tested in parallel in water samples from the ponds fortified with nutrients. Preceding experiences had revealed that duck weed does not grow well in corrals in the artificial ponds. For the *Lemna*-bioassay one day after dosing and on a weekly base afterwards approximately 0.2-L of whole water column samples were collected from each pond, excluding the peak dose ponds. An analytical trial was conducted prior to the exposure phase to examine if filtration removed dissolved test substance from pond water. Since it was found not to decrease the test substance concentration, the water samples were filtered through a 0.45 micron filter in an attempt to remove competing algal cells. The filtered solutions were then fortified with 20X AAP medium nutrients to enrich the water samples. The samples were equilibrated in temperature to 24 ± 2 °C prior to use.

Sterile 270-mL crystallizing dishes served as the test vessels and were conditioned prior to use by rinsing with the appropriate test solution. One-hundred milliliters of the appropriate nutrified test solution was then placed in each replicate vessel. Then 15 fronds were transferred into each test vessel in order to run a standard seven-day *Lemna*-test. After 7 days again water samples were taken from the ponds, filtered as described above and enriched with nutrients. 15 fronds from the preceding 7-day period were transferred into the new samples for the next 7-day test. This procedure was repeated until start of week 5. As a consequence the *Lemna*-cultures from the last week had been exposed continuously over a period of six weeks like the plants in the outdoor ponds.

For analytical verification of the test item concentrations and its main metabolite AE F075736 (metsulfuron-methyl) samples were taken during weeks 2, 4 and 6 from all ponds. A liquid chromatography/mass spectrometry (LC/MS/MS) was used as analytical method. Iodosulfuron-methyl (purity 98.2%) and metsulfuron-methyl (AE F075736; purity 98.4%) served as analytical standards. The method validation study was conducted prior to the initiation of the test and established an average recovery of 90.1% ± 4.16% for iodosulfuron-methyl-sodium WG50 and 107% ± 11.6% for AE F075736 from microcosm pond water.

At the outdoor ponds health observations were performed on submerged, emergent and floating plants during weeks 2, 4 and 6. Due to its rapid growth rate *Salvinia* was observed on a weekly basis and leaves were counted. In addition, the plants in the peak dose ponds were also observed on exposure days 2 and 7. Visual observations such as chlorosis, leaf curl and reduced biomass were recorded. Effects observed were rated as percentage effect against the control plants. The number of *Nymphaea odorata* leaves emerged from the water surface was counted during the health observations on weeks 2, 4 and 6. Additionally algal blooms and water turbidity was noted.
After test termination above ground plant material was harvested and shoot length and dry weight were determined and the respective growth rates were calculated. In case of *Lemna* and *Salvinia* frond and leaf numbers, respectively were assessed instead of shoot length.

Dates of exposure (outdoor ponds, ten aquatic plants): June 01, 2010 – July 16, 2010

Dates of exposure (laboratory exposure, *Lemna gibba*): June 02, 2010 – July 17, 2010

Results:

Environmental conditions:
The pH of the water in the outdoor ponds ranged from 7.8 to 9.2. Continuous temperature monitoring established that the temperature ranged from 26.4 to 27.6 °C during the test period. Natural sunlight was used for illumination. Dissolved oxygen concentrations ranged from 7.93 mg/L to 9.17 mg/L. The range of hardness values were 169 to 191 mg/L as $CaCO_3$. The environmental conditions maintained throughout the test period were within acceptable limits for the growth and survival of the test species. Total rainfall during the exposure period was 11 cm. Due to the use of covers, approximately 0.80 cm of rainfall was prevented from entering the ponds on 1 to 2 June 2010. The remaining rainfall entering the ponds (e.g., 10 cm) generally replenished water evaporated during the study. Water levels were maintained within 10 % of the initial depth (e.g., 35 cm).
The pH of the *Lemna*-test and control solutions ranged from 8.0 to 8.1 at test initiation and 9.5 to 10 at termination of the exposure period. Solution pH generally increases as the solutions age due to the use of inorganic carbon by the test organisms for photosynthesis. Continuous temperature monitoring established that the temperature ranged from 22 to 27 oC during the test period (see Protocol Deviation). Light intensity of the test area ranged from 380 to 740 footcandles (4100 to 7900 lux). The photosynthetically active radiation (PAR) of the test area at test initiation ranged from 87 to 130 µE/m2/s. These conditions were within acceptable limits for the growth and survival of the test organism.

Analytical findings:
Initial measured concentrations ranged from 100 to 110% of nominal concentrations and defined the treatment levels as 0.11, 0.27, 0.68, 1.8, 4.1, 10, 25 and 61 µg iodosulfuron-methyl-sodium/L. Initial

measured concentrations of the Peak 0.25 and Peak 0.63 µg a.s./L treatments were both 110% of nominal concentrations and defined the treatment levels as 0.27 and 0.72 µg a.s./L.

Table CA 8.2.7-3: Mean measured concentrations of iodosulfuron-methyl-sodium (µg a.s./L) in ponds with static exposure over 6-weeks

Nominal Conc. (µg a.s./L)	Day 0	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
0.1	0.11	**110**	0.07625	**76.25**	0.05625	**56.25**	0.0335	**33.5**
0.25	0.27	**110**	0.19	**77.5**	0.14	**55.75**	0.08275	**33.25**
0.63	0.68	**110**	0.5	**80.0**	0.34	**54.667**	0.2167	**34.33**
1.6	1.8	**110**	1.2667	**78.667**	0.9100	**56.667**	0.5267	**32.67**
3.9	4.1	**110**	3.05	**77.5**	2.1	**54**	1.25	**32.5**
9.8	10	**100**	7.45	**76.0**	4.95	**50.5**	3.05	**31.0**
24	25	**110**	18.5	**76.5**	13.5	**55.5**	7.5	**31.5**
61	61	**100**	46.5	**76.5**	33.5	**55.0**	20.5	**34.0**

Table CA 8.2.7-4: Mean measured concentrations of AE F075736 (µg/L) in ponds with static exposure over 6-weeks

Nominal Conc. (µg a.s./L)	Day 0	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
0.1	<0.0018	NA	<0.0019	NA	<0.0022	NA	<0.0020	NA
0.25	<0.0018	NA	<0.0019	NA	0.04025	**16.25**	0.045	**18.0**
0.63	<0.0018	NA	0.0463	**7.367**	0.0733	**16.667**	0.1267	**20.0**
1.6	<0.0019	NA	0.1100	**6.967**	0.2533	**15.667**	0.290	**18.0**
3.9	<0.0019	NA	0.265	**6.75**	5.9	**15.5**	7.2	**18.5**
9.8	<0.0019	NA	0.645	**6.55**	1.45	**14.5**	1.6	**16.5**
24	<0.0019	NA	1.5	**6.25**	3.8	**16**	4.3	**18.0**
61	<0.0019	NA	2.9	**4.85**	7.5	**12**	9.1	**15.0**

Table CA 8.2.7-5: Mean measured concentrations of iodosulfuron-methyl-sodium (µg a.s./L) in ponds with peak exposure over 2-days

Nominal Conc. (µg a.s./L)	Day 0	% Nom.	Day 3	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
Peak 0.25	0.27	**110**	<0.0020	NA	<0.018	NA	<0.019	NA	<0.021	NA
Peak 0.63	0.72	**110**	0.024	3.9	<0.018	NA	<0.022	NA	<0.023	NA

Biological results are based on mean initial measured concentrations of 0.11, 0.27, 0.68, 1.8, 4.1, 10, 25 and 61 and µg a.s./L.

Biological findings:

Glyceria maxima was removed from the study on exposure day 29 due to generally poor health in all treated and control ponds.

Growth inhibition of all other species was observed as listed below.

Table CA 8.2.7-6: 6-week NOEC and EC50-figures (µg a.s./L) for nine aquatic macrophytes tested in the outdoor ponds, based on initial measured concentrations

	Week 6 Mean Shoot Length		Week 6 Growth Rate Based on Mean Shoot Length		Week 6 Mean Shoot Dry Weight		Week 6 Growth Rate Based on Dry Weight	
	NOEC	EC_{50} (95% CL[a])	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Elodea canadensis	NC[b]	NC	NC	NC	0.68	1.4 (0.058-4.3)	0.68	1.4 (0.058-4.3)
Potamogeton pectinatus	NC[b]	NC	NC	NC	0.27	1.7 (1.2-2.2)	0.27	1.5 (1.0-2.3)
Sagittaria latifolia	1.8[c]	36 (32-38)	1.8	2.9 (2.3-4.1)	4.1	8.8 (8.2-9.1)	4.1	7.9 (7.4-8.0)
Nymphaea odorata	61	>61 (NA)[d]	61	>61 (NA)	10	16 (5.8-22)	10	14 (5.4-21)
Ceratophyllum demersum	NC	NC	NC	NC	61	7.4 (NA-39)	61	6.2 (NA-11)
Myriophyllum heterophyllum	1.8	17 (13-19)	1.8	3.1 (2.3-3.8)	4.1	9.9 (4.2-15)	4.1	6.7 (3.1-13)
Mentha aquatica	4.1	59 (29-NA)	4.1	7.6 (2.8-11)	4.1	11 (2.8-14)	4.1	6.0 (NA-9.0)
Cabomba caroliniana	4.1	>61 (NA)	4.1	26 (15-NA)	61	>61 (NA)	61	>61 (NA)
	Week 6 Mean Leaf Density		Week 6 Growth Rate Based on Leaf Density		Week 6 Mean Leaf Dry Weight		Week 6 Growth Rate Based on Leaf Dry Weight	
	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Salvinia minima	1.8	0.58 (0.032-NA)	0.68	>0.68 (NA)	1.8	0.54 (0.046-0.64)	1.8	0.54 (0.046-0.64)

a CL = Confidence level.
b NC = Not calculated. Due to the constant branching and the fact that stems could not be associated with an individual plant, plant lengths were not measured.
c The highest treatment level was not statistically analysed since all plants in one replicate were dead at test termination. Since 12 and 16% reduction was observed in the two highest treatment levels, the NOEC was empirically estimated to be the fourth highest treatment level.
d NA = Not applicable. Corresponding 95% confidence interval could not be calculated.

For all species exposed in the outdoor ponds and all biological endpoints measured, there were no significant differences when the 2-day peak exposures (e.g., 0.27 and 0.72 µg a.s./L initial measured concentrations) were compared to the untreated controls. Moreover, there were no differences when the 2-day peak exposures were compared to the respective treatment levels with 6-week exposure. From these findings, it can be concluded that an exposure to the 0.72 µg a.s./L initial measured concentration followed by a 5.5 week recovery period had no effect on the macrophytes in the static fresh water test ponds.

Table CA 8.2.7-7: Results of weekly *Lemna*-bioassays with water samples from the outdoor ponds. The endpoints are expressed as initial mean measured concentrations in the outdoor ponds.

Exposure Period	43-Day Frond Density		0-43-Day Growth Rate Based on Frond Density		43-Day Dry Weight[a]	
	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)	NOEC	EC_{50} (95% CL)
Day 8	0.27	1.1 (0.66-1.4)	0.68	1.7 (1.4-2.3)		
Day 15	<0.11	3.0 (2.0-3.7)	0.27	3.2 (2.3-4.1)		
Day 22	1.8	5.4 (2.6-8.0)	1.8	4.2 (2.7-6.5)		
Day 29	0.68	1.3 (0.027-2.9)	0.68	1.4 (0.088-2.8)		
Day36	<0.11	0.68 (NA[bc]-1.9)	0.68	1.8 (NA-2.1)		
Day 43	0.68	2.9 (2.0 - 4.4)	1.8	2.6 (1.9 - 4.4)	61	>61 (NA)[bd]

a Dry weight was only analysed at test termination (day 43).
b NA =Not Applicable.
c Lower confidence limit could not be calculated.
d EC value was empirically estimated; therefore confidence limits could not be calculated.

Conclusions:

The most sensitive macrophyte species in terms of the lowest NOEC was *Potamogeton pectinatus* with a 6-week-NOEC of 0.27 µg a.s./L. The lowest 6-week EC_{50} of 0.54 µg a.s./L was obtained for *Salvinia minima*. In case of the 48-hour peak exposure the overall-NOEC is 0.72 µg a.s./L. The 6-week NOEC and EC_{50} for the growth rate of fronds in *Lemna gibba* are 1.8 and 2.6 µg a.s./L, respectively.

The results from the *Lemna*-bioassays have to be treated with care, since the *Lemna*-cultures were infested with algae although the samples from the pond water had been filtered. Therefore it was decided to run the following 6-week *Lemna*-study under sterile laboratory conditions while mimicking the decreasing concentration of iodosulfuron-methyl and the increase of AE F075736 on a weekly basis:

Report:	KCA 8.2.7 /07; [redacted], E.; 2013; M-469584-02
Title:	Lemna gibba G3 - Prolonged growth inhibition test with iodosulfuron-methyl-sodium (AE F115008) with stepwise decreasing concentrations and metsulfuron-methyl (AE F075736) with stepwise increasing concentrations over a 6 week test duration
Report No:	E 412 3763 - 6
Document No:	M-469584-02-1
Guidelines:	**OECD - 221 (March 23, 2006)** **EU Directive 91/414/EEC** **Regulation (EC) No. 1107/2009** **US EPA OCSPP 850.4400;none**
GLP/GEP:	**no**

Executive Summary:

The aim of the study was to determine the long-term influence over a total period of six weeks of the test item 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.

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for six weeks under static exposure conditions to the nominal concentrations of 0.10, 0.20, 0.40, 0.80 and 1.60 µg/L. The test concentrations were derived based the analytical findings of a multi species outdoor pond-study investigating several macrophyte species. Over the six week exposure period decreasing concentrations of iodosulfuron-methyl-sodium over time were tested. In addition to the decreasing iodosulfuron-methyl-sodium concentrations increasing concentrations of metsulfuron-methyl were used. 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 every 7 day period. Growth and growth inhibition were calculated of those. The concentration which inhibited the growth of *Lemna gibba* by 50 percent (EC_{50}) was determined. Over the test period of 6 weeks the *Lemna* plants were transferred in new test solutions every 7 days using 12 fronds from the respective treatment level of the preceding week. At the start of each 7-day period the initial concentrations of iodosulfuron-methyl-sodium were reduced in order to simulate the exposure pattern observed in an outdoor pond study.
Based on initial nominal concentrations the following 6-week endpoints can be derived:

6-week end point	mean growth rate	
	effect on frond no. [µg a.s./L]	effect on total frond area of plants [µg a.s./L]
EC_{50} (CI 95%)	0.679 (0.246 – 0.771)	0.609 (0.590 – 0.626)
EC_{20} (CI 95%)	0.533 (0.0350 – 0.650)	0.505 (0.481 – 0.526)
EC_{10} (CI 95%)	0.469 (0.0124 – 0.604	0.457 (0.432 – 0.480)
LOEC	0.800	0.800
NOEC	0.400	0.400

Material and Methods:

Test item: iodosulfuron-methyl-sodium; analysed content of active substance: 93.0 % w/w; origin batch no: ELIR003050; specification number 102000000739; Tox No.: 09144-01.

Test item: metsulfuron-methyl (AE F075736); analysed content of test item: 98.6 % w/w; origin batch no: 33074-238; batch code: AE F075736 001B98 0002; analysis sample ID: AZ 16744.

3 x 12 fronds of *Lemna gibba G3* per test concentration were exposed in a chronic multi-generation test for six weeks under static exposure conditions to the following nominal concentrations. The test concentrations were derived based the analytical findings of a multi species outdoor pond-study investigating several macrophyte species. *Lemna gibba* was not growing under the pond study conditions, and the Lemna-bioassays that ran in parallel to the outdoor macrophyte study with samples of pond water were heavily infested with algae. For that reason *Lemna gibba* was exposed to the exposure regime described in the table below, mimicking the outdoor-concentrations under laboratory conditions.

Over the six week exposure period decreasing concentrations of iodosulfuron-methyl-sodium over time were tested. The respective nominal concentration patterns are presented in the following table.

Table CA 8.2.7-8: Nominal concentration patterns of iodosulfuron-methyl-sodium over the six week exposure period

nominal **initial** test levels iodosulfuron-methyl-sodium [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	90.6	77.5	65.3	55.1	42.6
0.10	0.10	0.091	0.077	0.065	0.055	0.043
0.20	0.20	0.181	0.155	0.131	0.110	0.085
0.40	0.40	0.362	0.310	0.261	0.220	0.171
0.80	0.80	0.725	0.620	0.523	0.441	0.341
1.60	1.60	1.45	1.24	1.05	0.881	0.682
pH	7.6 – 9.1	7.4 – 9.0	7.5 – 8.9	7.5 – 8.9	7.5 – 9.0	7.5 – 9.1
temperature range	23.8 – 25.3	24.9 – 25.3	24.9 – 25.3	24.9 – 25.2	24.9 – 25.2	24.7 – 25.1
light intensity (lux)	6814	6577	6746	6646	6601	6580

AE F075736 is the primary metabolite of iodosulfuron-methyl-sodium in soil and water/sediment systems. Therefore, in addition to the decreasing iodosulfuron-methyl-sodium concentrations increasing concentrations of AE F075736 were used. The respective concentrations are described in the following table.

Table CA 8.2.7-9: Nominal concentration patterns of metsulfuron-methyl over the six week exposure period

nominal **initial** test levels iodosulfuron-methyl-sodium [µg /L]	Nominal concentration of AE F075736 [µg/L] week 1	Nominal concentration of AE F075736 [µg/L] week 2	Nominal concentration of AE F075736 [µg/L] week 3	Nominal concentration of AE F075736 [µg/L] week 4	Nominal concentration of AE F075736 [µg/L] week 5	Nominal concentration of AE F075736 [µg/L] week 6
0.10	0	0.00013	0.007	0.011	0.016	0.017
0.20	0	0.00026	0.014	0.021	0.032	0.034
0.40	0	0.00052	0.028	0.042	0.064	0.069
0.80	0	0.00104	0.056	0.084	0.127	0.138
1.60	0	0.00208	0.112	0.168	0.254	0.275

Dates of experimental work: September 17, 2012 to August 12, 2013

Results:

Validity Criteria:

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

Analytical findings:

Table CA 8.2.7-10: Analytical findings of iodosulfuron-methyl-sodium based on nominal concentrations.

	day 0			day 7		
	min [%]	max [%]	average [%]	min [%]	max [%]	average [%]
week1	71	97	84.5	73	108	97.6
week 2	98	111	104	107	351	168
week 3	99	178	120	95	110	105
week 4	81	112	104	80	143	119
week 5	105	136	116	115	161	129
week 6	112	126	119	126	142	135

Table CA 8.2.7-11: Analytical findings of AE F075736 based on nominal concentrations.

	day 0			day 7		
	min [%]	max [%]	average [%]	min [%]	max [%]	average [%]
week1	-	-	-	-	-	-
week 2	135	17567	8627	137	18401	9390
week 3	103	7657	2321	96	119	104
week 4	104	115	110	107	143	125
week 5	102	144	112	106	138	116
week 6	101	106	103	110	128	117

During the study analytical measurements were performed to verify the nominal test item concentrations of the active ingredient iodosulfuron-methyl-sodium. From the second week onwards the concentrations of the metabolite AE F075736 were analyzed as well.
In case of AE F075736 increasing concentrations were added over the six week testing period. In the first week of the study AE F075736 was not added to the test solutions. In the second week of the study AE F075736 was added for the first time. The concentrations were very low and nominally below the LOQ of 2.1 ng/L. The nominal AE F075736 concentrations ranged between 0.13 and 2.08 ng/L. For these extremely low concentrations the chemical analysis revealed recoveries in the range of 135 and 18401%. The reason for these high recoveries cannot be explained. In the third week of the study the fresh test solutions resulted again in high recoveries for AE F075736 ranging between 103 and 7657% of nominal. At the end of the third week the content of AE F075736, measured in the aged test solution, resulted in recoveries between 96 and 119%. As the metabolite is known to be stable the reason for the different values for fresh and aged test solutions remain unclear. When evaluating the analytical findings it has to be considered that the concentrations resulting in very high recoveries were in the lower ng/L range.
The endpoints are based on nominal iodosulfuron-methyl-sodium concentrations. The exceedance of the nominal concentrations for metsulfuron-methyl, as analytically determined, can be considered as a worst case. Therefore these findings do not affect the overall integrity of the study. The growth

inhibition in the first weeks is clearly driven by iodosulfuron-methyl-sodium. The impact of AE F075736 is negligible. The analytically determined concentrations of AE F075736 can be seen as a worst case and cannot lead to an underestimation of a potential risk by iodosulfuron-methyl-sodium and its metabolite AE F075736 towards *Lemna gibba*.

Biological results:
Growth inhibition was observed as listed below.

Table CA 8.2.7-12: Derived inhibitions of growth rate of frond numbers

nominal **initial** test levels	% inhibition of mean growth rate of **frond numbers**					
iodosulfuron-methyl-sodium [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	90.6	77.5	65.3	55.1	42.6
control	--	--	--	--	--	--
0.10	4.1	1.2	55.4	1.6	1.7	-1.7
0.20	4.7	2.1	50.6	20.7	9.6	1.4
0.40	3.7	6.5	51.8	25.4	15.9	2.8
0.80	35.1	56.2	69.0	83.6	83.1	71.7
1.60	70.5	85.4	88.4	91.4	93.4	91.6
NOEC	>=1.6	0.200	<0.100	0.100	0.200	0.400
LOEC	>1.6	0.400	<=0.100	0.200	0.400	0.800
EC_{10}	0.449	0.384	n.d.	0.211	0.358	0.496
EC_{20}	0.607	0.490	n.d.	0.284	0.420	0.533
EC_{50}	1.08	0.781	0.134	0.504	0.570	0.679

negative value shows growth stimulation

Table CA 8.2.7-13: Derived inhibitions of growth rate of frond area

nominal **initial** test levels	% inhibition of mean growth rate of **frond area**					
iodosulfuron-methyl-sodium [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	90.6	77.5	65.3	55.1	42.6
control	--	--	--	--	--	--
0.10	4.6	1.1	54.3	4.9	0.5	0.9
0.20	4.8	2.5	45.3	28.1	10.4	0.6
0.40	3.5	5.3	46.0	31.5	14.8	3.0
0.80	30.2	55.3	81.1	88.6	87.2	88.9
1.60	71.3	91.9	93.0	90.5	91.1	100.3
NOEC	0.400	0.400	<0.100	0.100	0.200	0.400
LOEC	0.800	0.800	<=0.100	0.200	0.400	0.800
EC_{10}	0.501	0.429	n.d.	0.147	0.369	0.457
EC_{20}	0.660	0.523	n.d.	0.213	0.426	0.505
EC_{50}	1.12	0.767	0.169	0.432	0558	0.609

Observed visual effects:

Observed visual effects on *Lemna gibba* are listed in the table below.

Table CA 8.2.7-14: Survey of visual effects.

nominal **initial** test levels iodosulfuron-methyl-sodium [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
control	-	-	-	-	-	-
0.10	-	-	7	-	-	-
0.20	-	-	7	7	-	-
0.40	-	-	7	7	-	-
0.80	7	7	7	1,3,7	1,2,7	1,2,7
1.60	7	7	7,2	1,3,7	1,2,7	1,2,7

1. Yellow fronds
2. Fronds necrotic
3. Smaller size of fronds
4. Detached roots
5. Curly roots
6. Curved fronds
7. Overlapping fronds
8. Reduced root growth
9. Long connections between the fronds

- no visual effects

Conclusions:

The effects of iodosulfuron-methyl-sodium to growth inhibition of *Lemna gibba* during a 6-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 8.2.7-15: Endpoints 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%)	0-7	**1.08** (0.901 – 1.33)	**1.12** (0.953 – 1.34)
EC_{50} (CI 95%)	7-14	0.781 (0.635 – 0.962)	0.767 (0.695 – 0.844)
EC_{50} (CI 95%)	14-21	0.134 (n.d.)	0.169 (n.d.)
EC_{50} (CI 95%)	21-28	0.504 (0.136 – 1.90)	0.432 (0.0235 – 8.74)
EC_{50} (CI 95%)	28-35	0.570 (0.432 – 0.747)	0.558 (0.391 – 0.831)
EC_{50} (CI 95%)	35-42	0.679 (0.246 – 0.771)	0.609 (0.590 – 0.626)

Bold values: The study of [redacted] & [redacted] (1997; M-141441-02-1) was not conducted to the recent OECD Guideline, Therefore the day 0-7 EC_{50} values of the study of [redacted] (2013; M-469584-02-1) should be used as standard endpoints for risk assessment in the MCP instead of the E_rC_{50} of 0.00083 mg/L from the study of [redacted] & [redacted] (1997). A rationale is given by [redacted] (2014; M-479697-01-1, KCA 8.2.7 /05)

The six week exposure of *Lemna gibba* to iodosulfuron-methyl-sodium leads to increasing effects when the dissipation of iodosulfuron-methyl-sodium and the increasing concentration of its metabolite metsulfuron-methyl in a static water-sediment system is mimicked.
Based on initial nominal concentrations the following 6-week endpoints can be derived:

Table CA 8.2.7-16: Derived 6-week endpoints based on initial nominal concentrations

6-week end point	mean growth rate	
	effect on frond no. [µg a.s./L]	effect on total frond area of plants [µg a.s./L]
EC_{50} (CI 95%)	0.679 (0.246 – 0.771)	0.609 (0.590 – 0.626)
EC_{20} (CI 95%)	0.533 (0.0350 – 0.650)	0.505 (0.481 – 0.526)
EC_{10} (CI 95%)	0.469 (0.0124 – 0.604	0.457 (0.432 – 0.480)
LOEC	0.800	0.800
NOEC	0.400	0.400

Report:	[redacted];[redacted];[redacted];[redacted];2012;M-431705-01
Title:	Toxicity of Iodosulfuron-methyl-sodium technical to the aquatic macrophyte, Myriophyllum spicatum
Report No:	EBIML032
Document No:	M-431705-01-1
Guidelines:	**OCSPP Guideline Number 850.SUPP;none**
GLP/GEP:	**yes**

Executive Summary

The objective of this study was to determine the dose-response effect of Iodosulfuron-methyl-sodium to the rooted aquatic macrophyte, *Myriophyllum spicatum*, over an exposure period of 14 days. *Myriophyllum spicatum* shoots were exposed to nominal (mean measured) concentrations of control (<LOQ), 0.10 (0.11), 0.30 (0.31), 0.90 (0.89), 2.7 (2.70), and 8.1 (8.45) µg a.s./L. Effects on yield for total shoot length, total plant wet weight and total plant dry weight were determined on a per plant basis, based on the growth of each plant during the 14 day growth intervals. Mean measured concentrations are determined based on results of the recoveries from days 0, 7, and 14 and ranged from 99 to 112% of the nominal concentration. The toxicity values were calculated based on mean measured concentrations. The statistical NOEC, LOEC and E_yC_{50} for the most sensitive endpoint (shoot length yield) was 0.89, 2.70 and 2.03 µg a.s./L, respectively.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium, technical; Batch No.: ELIR003050; CAS No.: 144550-36-7; Purity: 93.0%.

Data from a preliminary rangefind study was used to set the definitive test concentrations. Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed for 14 days under static conditions to nominal (mean measured) concentrations of control (<LOQ), 0.10 (0.11), 0.30 (0.31), 0.90 (0.89), 2.7 (2.70), and 8.1 (8.45) µg a.s./L. The test system consisted of three replicate test vessels per treatment group. Each replicate contained five plants for a total of 15 plants

per group. Following a 7 day acclimation period, the five shoots in each replicate were thinned to three uniform appearing shoots. Remaining shoots were then exposed to the test solutions for 14 days. Following the 14 day exposure period plants were sacrificed and measured. All test vessels were contained in an environmentally controlled study area. During the test, a photoperiod of 16 h light : 8 h dark was maintained at a mean light intensity of 9,897 lux. No aeration was used.
For analytical verification samples were taken of all test solutions including control on Day 0, Day 7 and Day 14.

Dates of experimental work: November 03, 2011 – November 17, 2011

Results:

Validity Criteria:
Not applicable, higher tier study.

Analytical findings:
Mean measured concentrations are determined based on results of the recoveries from days 0, 7, and 14 and ranged from 99 to 112% of the nominal concentration. The toxicity values were calculated based on these mean measured concentrations. Detailed analytical results are presented in the following table:

Table CA 8.2.7-17: Measured test concentrations of iodosulfuron-methyl-sodium, technical during the exposure to *Myriophyllum spicatum*

Nominal Conc. (µg a.s./L)	Day 0 Measured Conc. (µg a.s./L)	Day 0 % Nominal[1] (%)	Day 7 Measured Conc. (µg a.s./L)	Day 7 % Nominal[1] (%)	Day 14 Measured Conc. (µg a.s./L)	Day 14 % Nominal[1] (%)	Mean Measured Conc. (µg a.s./L)[1]	Mean measured % Nominal[1] (%)
Control	<0.05	---	<0.05	---	<0.05	---	<0.05	---
0.10	0.14	142	0.10	104	0.09	94%	0.11	112
0.30	0.31	102	0.32	107	0.31	103%	0.31	104
0.90	0.89	99	0.96	107	0.84	93%	0.89	99
2.7	2.58	95	3.00	111	2.55	95%	2.70	100
8.1	9.65	119	7.81	96	8.02	99%	8.45	104

LOQ = 0.05 µg a.s./L
[1]Calculations for mean, standard deviation, and percent of nominal concentration are based on recoveries from Day 0, Day 7 and Day 14.
Calculations were made in Microsoft Excel using unrounded data. Calculations using rounded data may vary slightly

Biological findings:
Active growth of the control plants during the 14 day exposure period was demonstrated by an average total shoot length yield of approximatel 30.2 cm. Plants in the control vessels and all treatment groups appeared normal throughout the study. At study termination roots and shoots appeared normal in the controls and in the 0.11, 0.31, and 0.89 µg a.s./L treatment groups. In the two highest treatment groups, the plant shoots appeared normal, but a reduction in root mass was observed. Growth data for all plants was included in the data analysis.

Total shoot length yield:

Shoot length yield was analyzed at test termination on study day 14. Data analysis showed a statistically significant difference, in comparison to the control data, in the two highest treatment levels. Percent inhibitions as compared to the control group were 2.1, 6.5, 8.4, 66.3 and 92.2% for the 0.11, 0.31, 0.89, 2.70 and 8.45 µg a.s./L test groups, respectively.

Total plant wet weight yield:

Total plant wet weight yield was analyzed at test termination on study day 14. Data analysis showed a statistically significant difference, in comparison to the control data, in the two highest treatment levels. Percent inhibitions as compared to the control group were -5.6, 1.3, -5.7, 55.4, and 93.7% for the 0.11, 0.31, 0.89, 2.70 and 8.45 µg a.s./L test groups, respectively.

Total plant dry weight yield:

Plant dry weight yield was analyzed at test termination on study day 14. Data analysis showed a statistically significant difference, in comparison to the control data, in the highest treatment level. Percent inhibitions as compared to the control group were 2.8, 8.0, 12.2, 19.0, and 47.1% for the 0.11, 0.31, 0.89, 2.70 and 8.45 µg a.s./L test groups, respectively.

Table CA 8.2.7-18: Mean yield for plant shoots and dry weights during the exposure of *Myriophyllum spicatum* to iodosulfuron-methyl-sodium, technical

Mean Measured Concentration (µg a.s./L)	Length Yield (cm)	% Inhibition	Wet Weight Yield (g)	% Inhibition	Dry Weight Yield (g)	% Inhibition
Control	30.2	NA	1.1691	NA	0.1171	NA
0.11	29.5	2.1	1.2340	-5.6	0.1138	2.8
0.31	28.2	6.5	1.1539	1.3	0.1078	8.0
0.89	27.6	8.4	1.2360	-5.7	0.1029	12.2
2.70	10.2	66.3 *	0.5219	55.4 *	0.0949	19.0
8.45	2.4	92.2 *	0.0734	93.7 *	0.0619	47.1 *

*Statistically significant difference from control (Dunnett's one-tailed test; $p \leq 0.05$).
% Inhibition=100-((Treatment group parameter mean/control parameter mean)*100).
These calculations were done in Microsoft Excel, on the unrounded numbers. Manual calculations may vary slightly

Biological endpoints derived:

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

Table CA 8.2.7-19: Toxicity to *Myriophyllum spicatum*

Test Substance	Iodosulfuron-methyl-sodium technical		
Test Object	*Myriophyllum spicatum*		
Exposure	14 Day – Static Exposure		
Endpoint Units	(µg a.s./L)		
Endpoint results	Day 14 Shoot Length Yield	Day 14 Wet Weight Yield	Day 14 Dry Weight Yield
Highest Concentration Without an Effect (NOEC)	0.89	0.89	2.70
Lowest Concentration With an Effect (LOEC)	2.70	2.70	8.45
E_yC_{50}	2.03 (1.76 to 2.29)	2.51 (2.13 to 2.89)	> 8.45

Conclusions:

The most sensitive endpoint in the 14 day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to Iodosulfuron-methyl-sodium technical was shoot length yield. The statistical NOEC, LOEC and E_yC_{50} for this endpoint was 0.89, 2.70 and 2.03 µg a.s./L, respectively

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F075736

Report:	[redacted]; [redacted]; [redacted]; 1998; M-182336-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F075736 (metsulfuron-methyl) metabolite of AE F115009 substance, technical Code: AE F075736 00 1C92 0001
Report No:	E001214
Document No:	M-182336-01-1
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 iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

EC_{50} = 0.000418 mg/L*

Since the new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant, the biomass based endpoint of EC_{50} = 0.000418 mg/L according to the Review Report should to be revised and replaced by 0.000511 mg/L.

* Presented in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final) as endpoint of the metabolite AE F059411 (M 4). The EC_{50} corresponds to the E_bC_{50} (nominal) after 7 days of 0.418 µg/L of the conclusions in the study report.

Report:	[redacted];[redacted];2001;M-200947-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test with recovery phase Metsulfuron-methyl substance, pure (metabolite of AE F115008) Code: AE F075736 00 1B98 0001
Report No:	C015669
Document No:	M-200947-01-1
Guidelines:	**ASTM: E 1415-91; OECD: Draft June 1998; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE F075736 (metabolite of iodosulfuron-methyl-sodium) (code: AE F075736 00 1B98 0001; purity 98.4% w/w) on the growth and recovery potential of duck weed (*Lemna gibba*).
Cultures of *Lemna gibba* with an initial density of 12 fronds per vessel were exposed in a static renewal system over a period of 7 days to nominal concentrations of 0.32, 0.56, 1.0, 1.8, 3.2 and 5.6 µg/L (corresponding to analytically verified concentrations of 95.8% to 115.5% and 94.4% to 108.2% % of nominal values in freshly prepared and aged test solutions, respectively). At day 7 the test continued with untreated nutrient solutions (recovery phase). Again, growth and abnormal appearance of fronds were determined at days 10, 12 and 14. In addition a water control was tested.
Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The NOEC regarding growth inhibition during the recovery phase (day 10 to 14) was 3.2 µg/L.

Material and Methods:

Test item: AE F075736; Common name: metsulfuron-methyl; Code: AE F075736 00 1B98 0001; Analytical certificate No.: AZ 08473; Purity 98.4 % (w/w).

Duck weed (*Lemna gibba*) were exposed to AE F075736 (metabolite of iodosulfuron-methyl-sodium) in a static renewal system over a period of 7 days. Nominal concentrations were 0.32, 0.56, 1.0, 1.8, 3.2 and 5.6 µg/L. In addition a water control was tested. Each vessel (Erlenmeyer-flasks; 300 mL) served as one replicate filled with 150 mL 20xAAP with an initial pH of 7.5+0.1. At test initiation the number of fronds was 12 fronds per vessel. 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 fronds were transferred to pure nutrient medium and the test was prolonged with three replicates but with untreated nutrient solutions for another 7 days (recovery phase between day 7 and 14). Again, growth and abnormal appearance of fronds were determined at days 10, 12 and 14.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 0.010 µg/L in the aqueous sample and 0.016 µg/L in the aqueous sample, respectively. The range of linearity was 18.72 to 748.8 µg/L in the analyte solution prepared for HPLC.

Dates of experimental work: July 27, 2001 to August 10, 2001

Results:

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

Analitical findings:
Analytical verification of test solutions revealed measured concentrations of 95.8% to 115.5% and 94.4% to 108.2% % of nominal values in freshly prepared and aged test solutions, respectively calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table.

Table CA 8.2.7-20: Nominal and measured concentrations of AE F075736 as % of nominal

Nominal treatment level (µg/L)	control	0.32	0.56	1.00	1.80	3.20	5.60
Freshly prepared test solutions							
Nominal a.s. (mg/L)	0.00	0.31	0.55	0.98	1.77	3.15	5.51
Day 0	107.0	99.4	101.6	93.9	105.9	105.3	97.0
Day 3	113.1	125.3	112.7	110.3	101.9	99.7	97.1
Day 5	110.4	121.8	122.7	109.7	98.3	99.9	93.4
Mean a.s.	110.2	115.5	112.4	104.6	102.0	101.6	95.8
Aged test solutions							
Nominal a.s. (mg/L)	0.00	0.31	0.55	0.98	1.77	3.15	5.51
Day 3	110.0	110.1	104.9	97.2	100.0	102.7	90.6
Day 5	108.0	99.4	102.4	105.3	102.6	97.1	98.5
Day 7	104.8	98.3	117.3	113.8	96.3	95.9	94.3
Mean a.s.	107.6	102.6	108.2	105.5	99.6	98.5	94.4

Since plants were transferred from treated test solutions to untreated nutrient medium at start of the recovery phase a contamination of the nutrient medium could not be excluded. The maximum figure obtained was 0.17 µg/L at the nominal treatment level of 1 µg/L. This concentration is below the NOEC obtained from the treatment phase (see below). The contamination of the nutrient medium at start of the recovery phase can therefore be regarded as not relevant.

Biological findings:
Growth inhibition was observed as listed below.

Table CA 8.2.7-21: Effect of AE F075736 on growth-inhibition (frond number and dry weight) during the exposure phase of *Lemna gibba*

	Frond number		biomass	
Treatment level	growth rate	Percentage of inhibition	growth rate	Percentage of inhibition
untreated control	0.384	0	22.25	0
0.32	0.381	0.73	23.45	-5.4
0.56	0.248 *	35.35	19.27 *	13.36
1	0.144 *	62.38	12.35 *	44.48
1.8	0.094 *	75.62	9.71 *	56.36
3.2	0.09 *	76.47	8.27 *	62.81
5.6	0.095 *	75.27	7.28 *	67.29

* significant difference at $p<0.05$

During the treatment phase vaulted and overlapped fronds were observed in concentrations of and above 0.56 µg/L.

Table CA 8.2.7-22: Effect of AE F075736 on growth-inhibition (frond dry weight) during the recovery phase of *Lemna gibba*

treatment level (between day 0 and 7)	mean growth rate (d^{-1})	percentual inhibition of growth rate (frond number)	mean growth rate (d^{-1})	percentual inhibition of growth rate (frond number)	mean increase in biomass (mg)	percentual inhibition of biomass increase day 7 to 14
(µg/L)	day 7 to 14		day 10 to 14		day 7 to 14	
control	0.386	0	0.359	0	20.03	0
0.32	0.383	0.93	0.377	-5.15	20.33	-1.54
0.56	0.395	-2.35	0.455	-26.77	20.33	-1.54
1.0	0.315 *	18.56	0.388	-8.05	14.57 *	27.26
1.8	0.276 *	28.58	0.397	-10.52	11.73 *	41.41
3.2	0.24 *	37.82	0.376	-4.77	9.00 *	55.06
5.6	0.18 *	53.43	0.271 *	-24.63	8.23 *	58.88

* significant difference at $p<0.05$

During the recovery phase the following changes in plant appearance in treatment levels of and above 0.56 µg/L: Fronds were small and vaulted. Fronds from the same plant were spread and partly overlapped. Some fronds had turned to yellow. The roots had poorly developed and/or had the shape of a corkscrew.
The growth at 0.32 and 0.56 µg/L is close to the control. At the three highest treatment level growth was obviously enhanced after day 10 (day 3 of the recovery phase). This is likely to be due to the subsequent dilution of residues of the test item. The slow onset of recovery made it reasonable to evaluate the results twice:

1. Growth rate regarding frond numbers and biomass between day 7 and day 14.
2. Growth rate regarding frond numbers between day 10 and day 14. This approach omits the first three days of the recovery phase during which growth apparently was still retarded.

Conclusions:

During the treatment phase levels of 50% growth inhibition were calculated as follows:

	E_rC_{50}	E_bC_{50}
EC_{50} (µg/L) (95% confidence interval)	1.12 (1.10 - 1.13)	1.31 (1.00 - 1.80)

The no observed effect concentration (NOEC) regarding growth inhibition and changes in plant appearance and development was set to nominal 0.32 µg/L after 7 days test duration.
Since growth was retarded during the first three days of the recovery phase growth rates were considered as relevant for the period between day 10 and 14 only.

During the recovery phase levels of 50% growth inhibition were calculated as follows. The inhibition of the growth regarding biomass (dry weight) increase (Δb) could not be determined for this time interval since no biomass measurements from day 10 are available.

	during recovery phase day 7 to 14		during recovery phase day 10 to 14
	E_rC50	E_bC50	E_rC_{50}
EC_{50} (µg/L) (95% confidence interval)	4.96 (3.20 - 5.60)	2.59 (1.80 - 3.20)	>5.6

Between day 7 and 14 the no observed effect concentration (NOEC) regarding growth inhibition during the 7-day recovery phase is 0.56 µg/L and regarding changes in plant appearance and development was set to nominal 0.32 µg/L.
The no observed effect concentration (NOEC) regarding growth inhibition during the interval between day 3 and day 7 of the recovery phase is 3.2 µg/L in terms of the nominal concentration in the preceding treatment phase. Changes in plant appearance were observed at treatment levels of and above nominal 0.56 µg/L but are regarded as not relevant due to the well developed recovery potential expressed in terms of growth rate.

AE F145741

Report:	[redacted];2013;M-462128-01
Title:	Lemna gibba G3 - Growth inhibition test with AE F145741 (metabolite of iodosulfuron-methyl-sodium) under static conditions
Report No:	EBIML041
Document No:	M-462128-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; A slight deviation of preparing the test medium is explained and discussed within chapter 4 (Method)**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the influence of the test item AE F145741 (metabolite of iodosulfuron-methyl-sodium) on exponentially growing *Lemna gibba G3* expressed as NOEC, LOEC and EC_x for growth rate of the response variables, frond number, biomass (dry weight) and total frond area of plants.

3 x 12 fronds of *Lemna gibba* G3 per test concentration and 6 x 12 fronds per control were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 0.625, 1.25, 2.5, 5.0 and 10 mg pure metabolite/L in comparison to a 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. Additionally, at the end of the test the dry weight of all plants from each vessel was determined. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 % (EC_{50}) was determined where possible. Since the analytical measurements showed results higher than 120 % of nominal the calculated endpoints are based on geometric mean measured concnetrations of the test item.

The most sensitive response variable in this study was total frond area of plants resulting in a (0-7 day) - E_rC_{50} of 3.84 mg p.m./L. The lowest NOE_rC was 0.76 mg p.m./L and was based on statistical data analysis of frond number and the total frond area of plants.

Material and Methods:

Test item: AE F145741 (metabolite of iodosulfuron-methyl-sodium); analysed content: 94.4 %, origin batch No: 25398-52; sample description: AZ No. 16823; batch code: AE F145741 00 1C94 0001.

3 x 12 fronds of *Lemna gibba* G3 per test concentration and 6 x 12 fronds per control were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 0.625, 1.25, 2.5, 5.0 and 10 mg p.m./L in comparison to a control. The pH values ranged from 7.6 to 9.0 in the control and the incubation temperature ranged from 24.7°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6870 lux (average of nine measurements).
Quantitative amounts of AE F145741 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.

As slight deviation from the guideline the final test medium was a mixture of 20 % 20X AAP medium which was prepared on April 12, 2013 and 80 % 20X AAP medium which was prepared on April 16, 2013. The procedure of preparing the test nutrient medium had no effect on Lemna growth as shown in a doubling time clearly below the validity criterion of 2.5 days doubling time.

Dates of experimental work: April 17, 2013 to July 12, 2013

Results:

Validity criteria:
The study met all validity criteria, requested by the mentioned guidelines. The frond number increased in the control by a factor of 16.8 within 7 days corresponding to a doubling time (T_d) of about 2.0 days, respectively.

Analytical findings:
The analytical findings of AE F145741 found in all freshly prepared test levels on day 0 ranged between 114 and 118 % of nominal. In aged test levels on days 7 analytical findings ranged between 117 and 139 % of nominal.
Based on the analytical findings all results are given as geometric mean measured concentrations of the test item in the test medium.

Table CA 8.2.7-23: Measured concentrations of AE F145741 in test solutions

Day	Nominal concentration (geometric mean) [mg p.m./L]	Actual concentration [mg AE F145741/L]			
		Determination 1	Determination 2	Average	%
0	Control	< 0.502	< 0.502	< 0.0502	--
7		< 0.502	< 0.502	< 0.0502	--
0	0.625 (0.76)	0.719	0.722	0.720	115
7		0.804	0.802	0.803	128
0	1.25 (1.60)	1.46	1.47	1.47	117
7		1.74	1.75	1.74	139
0	2.5 (2.93)	2.95	2.95	2.95	118
7		2.93	2.93	2.92	117
0	5.0 (6.10)	5.89	5.91	5.90	118
7		6.31	6.32	6.31	126
0	10 (11.6)	11.4	11.3	11.4	114
7		11.8	11.8	11.8	118
0	**mean**				116
7					126

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

Table CA 8.2.7-24: Survey of biological findings and the derived inhibitions of growth rate

test concentration [mg p.m../L]		final results (replicate means day 7)			% inhibition of mean growth rate		
geometric mean measured	nominal	frond no.	total frond area [mm²]	total biomass [mg dw]	frond no.	total frond area	total biomass
Control	Control	201.8	1464.2	28.2			
0.76	0.625	225.3	1623.0	31.7	-4.0	-4.5	-4.2
1.60	1.25	176.7	1304.0	28.4	4.6	5.4	-0.25
2.93	2.50	63.3	432.0	17.7	41.2	46.9	16.5
6.10	5.00	32.7	214.0	12.3	64.6	72.7	29.2
11.6	10.0	26.0	167.0	10.3	72.9	80.5	35.4

-% inhibition: increase in growth relative to the control

Observed visual effects:
On day 5 and 7 overlapping fronds were observed in the test concentrations 2.93 to 11.6 mg p.m./L. There were no observed visual effects on the test item.

Since the analytical measurements showed results higher than 120 % of nominal the calculated endpoints are based on geometric mean measured concentrations of the test item.

Table CA 8.2.7-25: Survey of 7-day endpoints for AE F145741

end point (0-7 day)	effect on mean growth rate of frond no. [mg p.m./L]	effect on mean growth rate of total frond area of plants [mg p.m./L]	effect on mean growth rate of total biomass of plants [mg p.m./L]
E_rC_{50}	4.69 (2.40 – 10.63)	3.84 (1.97 – 7.88)	> 11.4
LOE_rC	1.60	1.60	2.93
NOE_rC	0.76	0.76	1.60

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

Conclusions:

The most sensitive response variable in this study was total frond area of plants resulting in a (0-7 day) - E_rC_{50} of 3.84 mg p.m./L.
The lowest NOE_rC was 0.76 mg p.m./L and was based on statistical data analysis of frond number and the total frond area of plants.

AE F145740

Report:	[redacted];[redacted];2013;M-462121-02; Amended: 2013-09-09
Title:	Lemna gibba G3 - Growth inhibition test with BCS-AU71533 (metabolite of iodosulfuron-methyl-sodium) under static conditions
Report No:	EBIMN063
Document No:	M-462121-02-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400;not specified**
GLP/GEP:	**yes**

Executive summary:

The objective of this growth inhibition test was, to verify the assumption that the test item AE F145740 (metabolite of iodosulfuron-methyl-sodium, other code: BCS-AU71533) will cause no adverse effects on the growth of *Lemna gibba G3* at the only test item concentration of 10 mg pure metabolite / L.
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 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 % (EC_{50}) was determined where possible. Since the analytical measurements showed results of 80.0 – 120 % of nominal the calculated endpoints are based on nominal concentrations of the test item.
The test item caused no adverse effects on the growth of Lemna gibba G3 up to the limit test item concentration of 10 mg p.m./L. The overall E_rC_{50} for the test item was > 10 mg p.m./L and the NOE_rC was > 10 mg p.m./L.

Material and Methods:

BCS-AU71533 (other code: AE F145740, metabolite of iodosulfuron-methyl-sodium); batch ID: AE F145740-PU-2, origin batch No.: GSE61082-3-3; sample description: TOX09988-00; LIMS No.:1301958; analysed content: 97.5 % w/w.

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a water control. The pH values ranged from 7.6 to 8.9 in the control and the incubation temperature ranged from 23.9°C to 24.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.57 klux.
Quantitative amounts of AE F145740 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: May 22, 2013 – July 11, 2013

Results:

Validity criteria:
The study met all validity criteria, requested by the mentioned guidelines. The frond number increased in the control by a factor of 14.1 within 7 days corresponding to a doubling time (T_d) of about 1.8 days, respectively.

Analytical findings:
The analytical finding of AE F145740 found on day 0 was 103 % of nominal and 109 % of nominal on day 7. All reported results are based on nominal values of the test item.

Table CA 8.2.7-26: Measured concentrations of AE F145740 in test solutions

Day	Nominal concentration [mg p.m./L]	Actual concentration [mg AE F145740/L]			
		Detection 1	Detection 2	Average	% of nominal
0	Control	< 1.00	< 1.00	< 1.00	--
7		< 1.00	< 1.00	< 1.00	--
0	10.0	10.6	10.7	10.7	107
7		10.9	11.0	10.9	109

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

Table CA 8.2.7-27: Survey of biological findings and the derived inhibitions of growth rate

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	169.3	1271.8	--	--
10.0	165.8	1238.3	0.9	0.8

-% inhibition: increase in growth relative to the control

Observed visual effects (fronds):
There were no visual effects observed in the test concentrations.

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

Table CA 8.2.7-28: Survey of 7-day endpoints for AE F145740

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

Conclusions:

AE F145740 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 0002166

Report:	[redacted]; [redacted];2002;M-205481-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE 0002166 (metabolite of AE F115008) substance, technical Code: AE 0002166 00 1C92 0001
Report No:	C018083
Document No:	M-205481-01-1
Guidelines:	**ASTM: E 1415-91;OECD: draft June 1998; USEPA(=EPA): FIFRA § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE 0002166 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0002166 00 1C92 0001; purity 91.8% w/w) on the growth of duck weed (*Lemna gibba*).
Cultures of *Lemna gibba* with an initial density of 12 fronds per vessel were exposed in a static renewal system over a period of 7 days to nominal concentrations of 10, 18, 32, 56 and 100 µg/L corresponding to analytically verified concentrations of 83.6% to 91.8% of nominal values in freshly prepared test solutions and 65.3% to 93.8% of nominal values in aged test solutions. Time-weighted average concentrations for 10, 18, 32, 56 and 100 µg/L were 76.85%, 83.01%, 86.35%, 92.16% and 74.03% of nominal, respectively. In addition a water control was tested.
Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as time weighted average figures. The E_rC_{50} regarding growth inhibition was 23.0 µg/L (95% confidence limits 14.9 - 27.6 µg/L) and E_bC_{50} was 58.3 µg/L (95% confidence limits 51.6 - 74.0 µg/L). The NOEC was determined to be 7.69 µg/L.

Material and Methods:

Test item: AE 0002166 (metabolite of iodosulfuron-methyl-sodium); Code: AE 0002166 00 1C92 0001; Purity: 91.8 % (w/w).

Duck weed (*Lemna gibba*) were exposed to AE 0002166 (metabolite of iodosulfuron-methyl-sodium) in a static renewal system over a period of 7 days. Nominal concentrations were 10, 18, 32, 56 and 100 µg/L. In addition a water control was tested. Each vessel (Erlenmeyer-flasks; 300 mL) served as one replicate filled with 150 mL 20xAAP with an initial pH of 7.5+0.1. At test initiation the number of fronds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level. In the controls 3 replicates were tested. Growth and abnormal appearance of fronds in each replicate were

determined on test days 3, 5 and 7. The physical-chemical water parameters were assessed on test days 0, 3, 5 and 7.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 0.21 µg/L and 0.36 µg/L, respectively. The range of linearity was 6.4 to 1624 µg/L.

Dates of experimental work: October 12, 2001 – October 19, 2001

Results:

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

Analytical findings:
Analytical verification of test solutions revealed measured concentrations of 83.6% to 91.8% of nominal values in freshly prepared test solutions and 63.3% to 93.8% of nominal values in aged test solutions. Time-weighted average concentrations for 10, 18, 32, 56 and 100 µg/L were 76.85%, 83.01%, 86.35%, 92.16% and 74.03% of nominal, respectively. Based on these analytical findings the biological endpoints are reported as time weighted average figures. Detailed analytical results are presented in the following table:

Table CA 8.2.7-29: nominal and measured concentrations of AE 0002166 as % of nominal

nominal treatment level (µg/L)	0.00	10	18	32	56	100
Freshly prepared test solutions						
nominal a.s. (µg/L)	0.00	9.18	16.52	29.38	51.41	91.8
day 0	103.0	64.5	92.7	89.7	90.9	102.0
day 3	96.6	104.4	89.5	87.5	92.6	83.6
day 5	95.2	81.9	89.4	*	*	68.1
mean a.i.	98.2	83.6	90.5	88.6	91.8	84.6
Aged test solutions						
nominal a.s. (µg/L)	0.00	9.18	16.52	29.38	51.41	91.8
day 3	91.3	79.6	*	*	86.6	60.3
day 5	81.6	69.8	64.8	81.2	93.9	62.8
day 7	91.7	69.2	86.8	86.8	100.8	66.7
mean a.i.	88.2	72.8	75.8	84.0	93.8	63.3

*: no value; outlier

Biological findings:
Growth inhibition was observed as listed below.

Table CA 8.2.7-30: Effect of AE 0002166 on growth-inhibition (frond number and dry weight) *of Lemna gibba*

	Frond number		biomass	
Treatment level	growth rate	Percentage of inhibition	growth rate	Percentage of inhibition
untreated control	0.399	0	29.1	0
10	0.384	3.9	27.2	6.4
18	0.276 *	30.8	21.9 *	24.9
32	0.166 *	58.4	15.9 *	45.4
56	0.109 *	72.8	15.3 *	47.3
100	0.071 *	82.3	13.0 *	55.2

* Statistically different from controls (p#0.05)

A significant inhibition at a significance level of alpha = 0.05 of growth both related on frond number and biomass was observed in nominal concentrations of nominal 18 µg/L (14.94 µg/L in terms of time-weighted average) and above.

Intoxication symptoms (fronds yellow coloured, roots shorter) were observed at the concentrations 18 and 32 µg/L.

Conclusions:

The effect of AE 0002166 (metabolite of iodosulfuron-methyl-sodium) (AE 0002166 00 1C92 0001) on growth inhibition of *Lemna gibba* can be quantified as follows: 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 26.9 µg/L (95% confidence limits 18 - 32 µg/L) or 23.0 µg/L (95% confidence limits 14.9 - 27.6 µg/L) in terms of time-weighted average concentrations. 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 68.2 µg/L (95% confidence limit 56-100 µg/L) or 58.3 µg/L (95% confidence limit 51.6-74.0 µg/L) in terms of time-weighted average concentrations. The E_bC_{50} figures have to be treated with care since the highest inhibition rate observed was 55.2%. An inhibition rate of at least 65% at the highest treatment level is required in order to obtain reliable EC_{50}-levels.
The no observed effect concentration (NOEC) defined as no significant growth inhibition and no changes in plant appearance and development was set to nominal 10 µg/L (7.69 µg/L in terms of time weighted average).

AE F161778

Report:	[redacted];[redacted];2001;M-197639-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F161778 (metabolite of AE F115008) substance, technical 93.7% Code: AE F151778 00 1C94 0001
Report No:	C008628
Document No:	M-197639-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): J § 132-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE F161778 (metabolite of iodosulfuron-methyl-sodium) (code: AE F161778 00 1C94 0001; purity 93.7% w/w) on the growth of duck weed (*Lemna gibba*) 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 cultures of *Lemna gibba* with an initial density of 12 fronds per vessel were exposed in a static renewal system over a period of 7 days to nominal concentrations of 10, 18, 32, 56 and 100 µg/L (corresponding to analytically verified concentrations of 95.2 to 102.4% and 99.3 to 108.6% of nominal values in fresh and aged test solution, respectively). In addition a water control was tested. Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The EC_{50} regarding growth inhibition was 28.1 µg/L (95% confidence limits: 18.0 to 32.0 µg/L) and 30.5 µg/L (95% confidence limits: 18.0 to 32.0 µg/L) for frond number and dry weight, respectively. The NOEC was determined to be 10 µg/L.

Material and Methods:

Test item: AE F161778; CAS name: Methyl 2-[3-(4-hydroxy-6-methyl-1,3,5-triazin-2-yl) ureido-sulfonyl] benzoate; substance, technical; Code: AE F161778 00 1C94 0001; Purity: 93.7 % (w/w) AE F161778.

Duck weed (*Lemna gibba*) were exposed to AE F161778 (metabolite of iodosulfuron-methyl-sodium) (code: AE F161778 00 1C94 0001; purity 93.7% w/w) in a static renewal system over a period of 7 days. Nominal concentrations were 10, 18, 32, 56 and 100 µg/L. In addition a water control was tested. Each vessel (Erlenmeyer-flasks; 300 mL) served as one replicate filled with 150 mL 20xAAP with an initial pH of 7.5±0.1. At test initiation the number of fronds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level. In the controls 3 replicates were tested. Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7. The physical-chemical water parameters were assessed on test days 0, 3, 5 and 7.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 2.81 µg/L and 4.22 µg/L, respectively. The range of linearity was 0 to 375 µg/L.

Dates of experimental work: January 14, 2000 to January 21, 2000

Results:

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

Analytical findings:
Analytical verification of test solutions revealed measured concentrations of 95.2 to 102.4% and 99.3 to 108.6% of nominal values in fresh and aged test solution, respectively calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table.

Table CA 8.2.7-31: Nominal and measured concentrations of AE F161778 as % of nominal

Nominal treatment level (µg/L)	control	10.00	18.00	32.00	56.00	100.00
Freshly prepared test solutions						
Nominal a.s. (mg/L)	0.00	9.37	16.87	29.98	52.47	93.70
Day 0	82.0	98.6	94.8	98.2	107.7	102.5
Day 3	86.4	112.7	119.6	111.2	107.7	118.6
Day 5	84.2[1]	80.2	90.1	76.1	85.7	86.0
Mean a.s.	84.2	97.2	101.5	95.2	100.3	102.4
Aged test solutions						
Nominal a.s. (mg/L)	0.00	9.37	16.87	29.98	52.47	93.70
Day 3	83.4[2]	105.3	110.0	121.9	116.4	130.1
Day 5	86.2	94.4	91.0	94.2	87.2	85.7
Day 7	80.7	103.7	97.4	89.8	94.2	110.1
Mean a.s.	83.4	101.2	99.5	101.9	99.3	108.6

[1] Mean recovery rate of day 0 and 3 fresh water

[2] Mean recovery rate of day 5 and 7 aged water

Biological findings:
Growth inhibition was observed as listed below.

Table CA 8.2.7-32: Effect of AE F161778 on growth-inhibition (frond number and dry weight) of *Lemna gibba*

	Frond number		biomass	
Treatment level	growth rate	Percentage of inhibition	growth rate	Percentage of inhibition
untreated control	0.387		19.57	
10	0.386	0.4	19.5	0.3
18	0.335 *	13.5	16.13 *	17.6
32	0.147 *	62	9.13 *	53.3
56	0.112 *	71	6.00 *	69.3
100	0.096 *	75.2	4.63 *	76.3

* Statistically different from controls ($p \leq 0.05$)

No abnormalities were observed.

Conclusions:

The effect of AE F161778 (metabolite of iodosulfuron-methyl-sodium) (AE F161778 00 1C94 0001) on growth inhibition of *Lemna gibba* can be quantified as follows: The E_rC_{50} regarding growth inhibition was 28.1 µg/L (95% confidence limits: 18.0 to 32.0 µg/L) and the E_bC_{50} 30.5 µg/L (95% confidence limits: 18.0 to 32.0 µg/L) for frond number and dry weight, respectively.
The NOEC defined as no significant growth inhibition and no changes in plant appearance and development was determined to be 10 µg/L.

BCS-CW81253

Report:	[redacted];[redacted];2013;M-462125-01
Title:	Lemna gibba G3 - Growth inhibition test with BCS-CW81253 (metabolite of iodosulfuron-methyl-sodium) under static conditions
Report No:	EBIMN060
Document No:	M-462125-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400;not specified**
GLP/GEP:	**yes**

Executive summary:

The objective of this growth inhibition test was, to verify the assumption that the test item BCS-CW81253 (metybolite of iodosulfuron-methyl-sodium) will cause no adverse effects on the growth of *Lemna gibba G3* up to a test item concentration of 10 mg pure metabolite / L under defined conditions for 7 days.
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 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 % (EC_{50}) was determined where possible. Since the analytical measurements showed recoveries from 80.0 to 120 %of nominal, the results are given as nominal concentrations of the test item in the test medium. The (0 – 7 day)-E_rC_{50} was > 10 mg p.m./L, the (0 – 7 d)-NOE_rC was determined to be ≥ 10 mg p.m./L. BCS-CW81253 caused no adverse effects on the growth of *Lemna gibba G3* up to the limit test item concentration of 10 mg pure metabolite/L.

Material and Methods:

Test item: BCS-CW81253; Batch ID: BCS-CW81253-PU-01; Origin batch No.: GSE61145-5-3; Sample description: TOX09918-00; LIMS No.:1306024; analysed content: 99.0 % w/w.

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a water control. The pH values ranged from 7.6 to 8.9 in the control and the incubation temperature ranged from 23.9°C to 24.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.57 klux (mean value).
Quantitative amounts of BCS-CW81253 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: May 22, 2013 – July 10, 2013

Results:

Validity criteria:
For the test to be valid, the following performance criteria should be met. The frond number in the control must increase by a factor of 7 corresponding to a doubling time (T_D) of less than 2.5 days.
The study met all validity criteria, requested by the mentioned guidelines. The frond number increased in the control by a factor of 14.1 within 7 days corresponding to a doubling time (T_d) of about 1.8 days, respectively.

Analytical findings:
The analytical finding of BCS-CW81253 found on day 0 was 107 % of nominal and 114 % of nominal on day 7. All reported results are based on nominal values of the test item.

Table CA 8.2.7-33: Measured concentrations of BCS-CW81253 in test solutions

Day	Nominal concentration [mg p.m./L]	Actual concentration [mg BCS-CW81253/L]			
		Determination 1	Determination 2	Average	%
0	Control	< 1.00	< 1.00	< 1.00	--
7		< 1.00	< 1.00	< 1.00	--
0	10	10.7	10.6	10.7	107
7		11.4	11.4	11.4	114

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

Table CA 8.2.7-34: Survey of biological findings and the derived inhibitions of growth rate

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	169.3	1271.8	--	--
10.0	184.5	1358.8	-3.2	-1.9

-% inhibition: increase in growth relative to the control

Observed visual effects (fronds):
There were no visual effects observed in the test concentrations.

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

Table CA 8.2.7-35: Survey of 7-day endpoints for BCS-CW81253

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

Conclusions:

BCS-CW81253 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 0000119

Report:	[redacted]; [redacted];2002;M-210320-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE 0000119 (metabolite of AE F115008) substance, pure Code: AE 0000119 00 1B98 0001
Report No:	C020878
Document No:	M-210320-01-1
Guidelines:	**OECD guideline, US-EPA Pesticide Guidelines J 123-2 and according to ASTM E 1415-91 guideline under GLP;none**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of AE 0000119 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0000119 00 1B98 0001; purity 97.8% (w/w)) on the growth of duck weed (*Lemna gibba*).
Triplicate cultures of *Lemna gibba* with an initial density of 12 fronds per replicate were exposed under semi-static conditions over a period of 7 days to nominal concentrations of 10, 18, 32, 56 and 100 mg/L (corresponding to analytically verified concentrations of 115.6% to 117.6% and 115.0% to 118.9% of nominal values in freshly prepared and aged test solutions, respectively). In addition a water control was tested.
Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7. Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The EC_{50} regarding growth inhibition was > 100 mg/L for both, frond number and dry weight. The NOEC was determined to be 100 mg/L.

Material and Methods:

Test item. AE 0000119; substance, pure; Code: AE 0000119 00 1B98 0001; Analysed content: 97.8 % (w/w); Analytical certificate No.: AZ 08376.

Duck weed (Lemna gibba) were exposed to AE 0000119 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0000119 00 1B98 0001; purity 97.8% (w/w)) in a static renewal system over a period of 7 days. Nominal concentrations were 10, 18, 32, 56 and 100 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer-flasks; 300 mL) served as one replicate filled with 150 mL 20xAAP with an initial pH of 7.5+0.1. At test initiation the number of fronds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 0.45 mg/L and 0.75 mg/L respectively. The range of linearity was 0.13 to 10.6 mg/L.
Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed as indicated below in the result section.

Dates of experimental work: November 16, 2001 – November 23, 2001

Results:

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

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

Test temperature:	mean 23.8 °C (range: 23.5 to 24.0 °C)
pH:	8.7 to 9.0 (in aged test solutions)
Light source	wide spectrum fluorescent lamps of the universal white-type L25
Hardness:	2.5 mmol Ca^{2+} + Mg^{2+}/L
Acid binding capacity	2.8 mmol HCl/L
Conductivity:	mean: 1633 µS/cm (range: 1602 to 1679 µS/cm)

Analytical findings:
Analytical verification of test solutions revealed measured concentrations of 115.6% to 117.6% and 115.0% to 118.9% of nominal values in freshly prepared and aged test solutions, respectively calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table:

Table CA 8.2.7-36: nominal and measured concentrations of AE 0000119

nominal treatment level (µg/L)	10	18	32	56	100
Freshly prepared test solutions					
nominal a.s. (µg/L)	9.78	17.6	31.3	54.77	97.8
day 0	9.81	18.05	32.3	54.72	101.87
day 3	11.26	21.16	36.76	64.36	114.01
day 5	12.84	22.4	40.36	73.3	129.28
mean a.i.	11.31	20.54	36.47	64.13	115.05
Aged test solutions					
nominal a.s. (µg/L)	9.78	17.6	31.3	54.77	97.8
day 3	11.45	21.12	37.18	64.91	119.09
day 5	12.08	22.51	39.32	70.64	126.07
day 7	10.2	18.28	32.91	59.6	103.67
mean a.s.	11.24	20.63	36.47	65.05	116.28

Biological findings:
Growth inhibition was observed as listed below.

Table CA 8.2.7-37: Effect of AE 0000119 on growth-inhibition (frond number and dry weight) of *Lemna gibba*

	Frond number		biomass	
Treatment level	growth rate	Percentage of inhibition	growth rate	Percentage of inhibition
untreated control	0.39852	0	25.467	0
10	0.39701	0.38	24.667	3.14
18	0.39779	0.18	25.7	-0.92
32	0.39242	1.53	24.767	2.75
56	0.393	1.39	26.2	-2.88
100	0.39729	0.31	25.9	-1.7

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

7-day-figures (growth rate frond number):

highest concentration with no effect (NOE_rC): 100 mg/L

E_rC_{50}: > 100 mg/L

7-day-figures (biomass):

highest concentration with no effect (NOE_bC): 100 mg/L

E_bC_{50}: > 100 mg/L

Conclusions:

In a Growth Inhibition Test (CE01/065-1), method EPA / OECD / ASTM) to determine the effect of AE 0000119; substance, pure; code: AE 0000119 00 1B98 0001 (metabolite of iodosulfuron-methyl-sodium) to L*emna gibba* (Duckweed) the concentration of test item 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 frond number was not observed in any treatment level up to 100 mg/L. A significant inhibition of biomass increase (dry weight) was not observed in any treatment level up to 100 mg/L.
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 F059411

Report:	[redacted];[redacted];[redacted];1998;M-181177-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F059411 metabolite of AE F115008 substance, technical Code: AE F059411 00 1C99 0001
Report No:	C000745
Document No:	M-181177-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): J 123-2;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted];[redacted];2002;M-203638-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F059411 substance, pure (metabolite of AE F115008) Code: AE F059411 00 1B99 0002
Report No:	C017092
Document No:	M-203638-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): E§ 132-2;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the effects of AE F059411 (metabolite of iodosulfuron-methyl-sodium) (code: AE F059411 00 1B99 0002; purity 99.7 % w/w) on the growth of duck weed (*Lemna gibba*).
Triplicate cultures of *Lemna gibba* with an initial density of 12 fronds per vessel were exposed in a static renewal system over a period of 7 days to nominal concentrations of 10, 18, 32, 56 and 100 mg/L (corresponding to analytically verified concentrations of 93.8% to 105.2% and 80.5% to 107.9% of nominal values in freshly prepared and aged test solutions, respectively). In addition a water control was tested.
Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The EC_{50} regarding growth inhibition was > 100 mg/L for both, frond number and dry weight. The NOEC was determined to be 32 mg/L (due to a very small variation of control data) and 100 mg/L for frond number and dry weight, respectively.

Material and methods:

Test item: AE F059411 (metabolite of iodosulfuron-methyl-sodium); code:
AE F059411 00 1B99 0002; certificate No.: AZ 08123; purity: 99.7 % w/w.

Duck weed (*Lemna gibba*) were exposed to AE F059411 in a static renewal system over a period of 7 days. Nominal concentrations were 10, 18, 32, 56 and 100 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer-flasks; 300 mL) served as one replicate filled with 150 mL 20xAAP with an initial pH of 7.5±0.1. At test initiation the number of fronds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level. Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7. The physical-chemical water parameters were assessed on test days 0, 3, 5 and 7.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: October 19, 2001 to October 26, 2001

Results:

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

Analytical findings:

Analytical verification of test solutions revealed measured concentrations of 93.8% to 105.2% and 80.5% to 107.9% of nominal values in freshly prepared and aged test solutions, respectively calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table:

Table CA 8.2.7-38: Nominal and measured concentrations of AE F059411

Nominal treatment level (µg/L)	10	18	32	56	100
Freshly prepared test solutions					
Nominal a.i. (mg/L)	9.97	17.95	31.9	55.83	99.7
Day 0	10.49	17.93	32.26	55.99	100.89
Day 3	9.51	17.52	31.8	55.39	101.56
Day 5	9.35	17.46	31.14	53.94	103.73
Mean a.i.	9.78	17.64	31.73	55.11	102.06
Aged test solutions					
Nominal a.i. (mg/L)	9.97	17.95	31.9	55.83	99.7
Day 3	10.03	17.75	31.87	55.81	100.15
Day 5	8.52	16.60	31.00	55.26	102.83
Day 7	8.03	15.07	26.95	51.00	107.62
Mean a.i.	8.86	16.47	29.94	54.02	103.53

Biological findings:

Growth inhibition was observed as listed below.

Table CA 8.2.7-39: Effect of AE F059411 on growth-inhibition (frond number and dry weight) of *Lemna gibba*

	Frond number		Biomass	
Treatment level	**Growth rate**	**Percentage of inhibition**	**Growth rate**	**Percentage of inhibition**
control	0.3871		26.4	
10	0.3847	0.61	25.6	3.15
18	0.3844	0.68	28.3	-7.19
32	0.3842	0.75	26.7	-0.88
56	0.3795 *	1.95	26.5	-0.13
100	0.3781 *	2.31	25.2	4.67

* Statistically different from controls ($p < 0.05$); (due to a very small variation of control data)

No plant abnormalities were observed.

Conclusions:

The effect of AE F059411 (metabolite of iodosulfuron-methyl-sodium) on growth inhibition of *Lemna gibba* can be quantified as follows: The EC_{50} regarding growth inhibition was > 100 mg/L for both, frond number and dry weight. The NOEC was determined to be 32 mg/L (due to a very small variation of control data) and 100 mg/L for frond number and dry weight, respectively.

<u>**AE 0014966**</u>

Report:	[redacted];[redacted];2002;M-186853-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE 0014966 (metabolite of iodosulfuron AE F115008) substance, technical Code: AE 0014966 00 1B98 0001
Report No:	C003832
Document No:	M-186853-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): J § 123-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE 0014966 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0014966 00 1B98 0001; purity 97.6% w/w) on the growth of duck weed (*Lemna gibba*).
Cultures of *Lemna gibba* with an initial density of 12 fronds per vessel were exposed in a static renewal system over a period of 7 days to nominal concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 mg/L (corresponding to analytically verified concentrations of 101.2 to 107.3% and 95.1 to 103.8% of nominal values in freshly prepared and aged test solutions, respectively). In addition a water control and a solvent control (DMSO) were tested.
Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The E_rC_{50} regarding growth inhibition was 0.575 mg/L (95% confidence limits 0.56 - 1.0 mg/L) and E_bC_{50} was 0.380 mg/L (95% confidence limits 0.32 - 0.56 mg/L) for frond number and dry weight, respectively. The NOEC was determined to be 0.18 mg/L.

Material and Methods:

Test item: AE 0014966; Code: AE 0014966 00 1B98 0001; CAS name: 2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) ureidosulfonyl] benzoic acid; Analytical certificate No.: AZ 06999; purity: 97.6% (w/w).

Duck weed (*Lemna gibba*) were exposed to AE 0014966 (metabolite of iodosulfuron-methyl-sodium) in a static renewal system over a period of 7 days. Nominal concentrations were 0.1, 0.18, 0.32, 0.56 and 1.0 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 150 mL 20xAAP with an initial pH of 7.5±0.1. At test initiation the number of fronds was 12 fronds per vessel. The test was conducted with 3 replicates per treatment level. In the controls 3 replicates were tested. Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 7.8 mg/L and 13.0 µg/L, respectively. The range of linearity was 0 to 410 µg/L.

Dates of experimental work: March 26th, 1999 to April 02nd, 1999

Results:

<u>Validity criteria:</u>
The validity criterion of a doubling time less than 60 hours (2.5 days) in the control is fulfilled.

Analitical findings:
Analytical verification of test solutions revealed measured concentrations of 101.2 to 107.3% and 95.1 to 103.8% of nominal values in freshly prepared and aged test solutions, respectively calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table.

Table CA 8.2.7-40: Nominal and measured concentrations of AE 0014966 as % of nominal

Nominal treatment level (µg/L)	control	100.00	180.00	320.00	560.00	1000.00
Freshly prepared test solutions						
Nominal a.i. (mg/L)	0.00	97.60	175.68	312.32	546.56	976.00
Day 0	105.7	83.9	94.7	86.3	94.2	87.6
Day 3	100.5	114.5	119.4	103.1	104.6	118.2
Day 5	98.0	109.7	110.9	114.2	107.5	105.3
Mean a.i.	101.4	102.7	107.3	101.2	102.1	103.7
Aged test solutions						
Nominal a.i. (mg/L)	0.00	97.60	175.68	312.32	546.56	976.00
Day 3	113.3	87.8	98.8	86.4	93.3	100.1
Day 5	97.1	93.9	97.3	89.6	94.0	105.6
Day 7	100.5	109.6	104.7	109.4	105.5	105.5
Mean a.i.	103.6	97.1	100.3	95.1	97.6	103.8

Biological findings:
Growth inhibition was observed as listed below.

Table CA 8.2.7-41: Effect of AE 0014966 on growth-inhibition (frond number and dry weight) of *Lemna gibba*

	Frond number		biomass	
Treatment level	growth rate	Percentage of inhibition	growth rate	Percentage of inhibition
untreated control	0.383	-0.15	17	0.78
solvent control	0.382	0	17.13	0
0.1	0.382	0.07	17.3	-0.97
0.18	0.377	1.3	16.83	1.75
0.32	0.326 *	14.64	10.7 *	37.55
0.56	0.198 *	48.28	3.97 *	76.85
1	0.068 *	82.19	0.73 *	95.72

* Statistically different from controls (p=0.05)

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

Intoxication symptoms were not observed.

Conclusions:

The effect of AE 0014966 (metabolite of iodosulfuron-methyl-sodium) (AE 0014966 00 1B98 0001) on growth inhibition of *Lemna gibba* can be quantified as follows: 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 0.575 mg/L (95% confidence limits 0.56 – 1.0 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 0.380 mg/L (95% confidence limit 0.32 – 0.56 mg/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 mg/L.

<u>**AE 0034855**</u>

Report:	[redacted];[redacted];2002;M-210318-01
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE 0034855 (metabolite of AE F115008) substance, pure Code: AE 0034855 00 1B99 0001
Report No:	C020876
Document No:	M-210318-01-1
Guidelines:	**ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): E § 132-2;Deviation not specified**
GLP/GEP:	**yes**

Executive summary:

The aim of the study was to determine the effects of AE 0034855 (metabolite of iodosulfuron-methyl-sodium) (code: AE 0034855 00 1B99 0001; purity 98.5 % w/w) on the growth of duck weed (*Lemna gibba*).
Cultures of *Lemna gibba* with an initial density of 12 fronds per vessel were exposed in a static renewal system over a period of 7 days to nominal concentrations of 10, 18, 32, 56 and 100 mg/L (corresponding to analytically verified concentrations of 90.6% to 104.3% of nominal values in freshly prepared test solutions and 80.4% to 108.8% of nominal values in aged solutions). In addition a water control was tested.
Frond numbers at each occasion and total biomass (dry weight) at test termination) were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. 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. 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. The NOEC was determined to be 100 mg/L and for frond number and dry weight, respectively.

Material and Methods:

Test item: AE 0034855 (metabolite of iodosulfuron-methyl-sodium); code: AE 0034855 00 1B99 0001; CAS name: (4-hydroxy-6-methyl-1,3,5-triazin-2-yl)urea; purity: 98.5 % w/w.

Duck weed (*Lemna gibba*) were exposed to AE 0034855 in a static renewal system over a period of 7 days. Nominal concentrations were 10, 18, 32, 56 and 100 mg/L. In addition a water control was tested. Each vessel (Erlenmeyer-flasks; 300 mL) served as one replicate filled with 150 mL 20xAAP with an initial pH of 7.5+0.1. At test initiation the number of fronds was 12 fronds per vessel. The test

was conducted with 3 replicates per treatment level. Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7.
For analytical verification of the test item concentrations samples were taken at day 0 (fresh water), day 3 and 5 (fresh and aged water) and day 7 (aged water) from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method. The LOD and LOQ were 0.71 mg/L and 1.18 mg/L, respectively. The range of linearity was 1 to 70 mg/L.

Dates of experimental work: Jan 25, 2002 to Feb 01, 2002

Results:

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

Analytical Findings
Analytical verification of test solutions revealed measured concentrations of 90.6% to 104.3% of nominal values in freshly prepared test solutions and 80.4% to 108.8% of nominal values in aged solutions calculated as arithmetic mean. Based on these analytical findings the biological endpoints are reported as nominal figures. Detailed analytical results are presented in the following table.

Table CA 8.2.7-42: Nominal and measured concentrations of AE 0034855 as % of nominal

Nominal treatment level (µg/L)	control	10.00	18.00	32.00	56.00	100.00
Freshly prepared test solutions						
Nominal a.s. (mg/L)	0.00	9.85	17.73	31.52	55.16	98.50
Day 0	98.5	91.4	91.4	90.7	90.7	90.6
Day 3	97.3	104.3	102.9	100.5	99.9	100.4
Day 5	97.2	90.8	99.4	99.6	99.3	101.0
Mean a.i.	97.7	95.5	97.9	96.9	96.6	97.3
Aged test solutions						
Nominal a.s. (mg/L)	0.00	9.85	17.73	31.52	55.16	98.50
Day 3	98.2	94.6	91.3	89.8	90.3	91.6
Day 5	98.2	96.1	98.3	98.6	99.7	99.3
Day 7	121.5	108.8	101.2	94.1	80.7	80.4
Mean a.i.	105.9	99.8	96.9	94.2	90.2	90.5

Biological findings:
Growth inhibition was observed as listed below.

Table CA 8.2.7-43: Effect of AE 0034855 on growth-inhibition (frond number and dry weight) of *Lemna gibba*

	Frond number		biomass	
Treatment level	growth rate	Percentage of inhibition	growth rate	Percentage of inhibition
CONTROL	0.38841	0	22.9	0
10	0.3973	-2.29	23.3	-1.75
18	0.38561	0.72	22.03333	3.78
32	0.39107	-0.68	22.86667	0.15
56	0.38656	0.47	23.76667	-3.78
100	0.38642	0.51	24.33333	-6.26

A significant inhibition at a significance level of alpha = 0.05 of growth both related on frond number was not observed in any treatment level up to 100 mg/L. A significant inhibition of biomass increase (dry weight) was not observed in any treatment level up to 100 mg/L.

No cell abnormalities were observed.

Conclusions:

The effect of AE 0034855 (metabolite of iodosulfuron-methyl-sodium) (AE 0034855 00 1B99 0001) on growth inhibition of Lemna gibba can be quantified as follows: 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. 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. The NOEC was determined to be nominal 100 mg/L.

AE 1234964

Report:	[redacted];[redacted];2006;M-281240-01
Title:	Toxicity of MKH 6561-Sulfonamide Acid to the aquatic plant Lemna gibba in a growth inhibition test
Report No:	30184240
Document No:	M-281240-01-1
Guidelines:	**Revised Proposal for a new OECD Guideline 221: "Lemna sp. Growth Inhibition Test", October 22, 2004.;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this test was to determine the inhibitory effect of the test item AE 1234964 (metabolite of iodosulfuron-methyl-sodium, further code: MKH 6561-sulfonamide acid) on the growth of the freshwater aquatic plant *Lemna gibba*. Cultures of *Lemna gibba* were exposed to various concentrations of the test item (0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg test item/L) and a control (reconstituted water) under defined conditions. The inhibition of growth expressed as NOEC, LOEC, and EC_{50} for growth rate of frond number and for growth rate of dry weight of plants in relation to control cultures was determined over a test period of 7 days. Based on analytical findings the biological endpoints are reported as nominal figures. The 7-day EC_{50} was > 100 mg test item/L for

growth rate of frond number and for growth rate of dry weight, respectively. The 7-day NOEC was determined to be 0.32 mg test item/L for growth rate of frond number and growth rate of dry weight, respectively.

Material and methods:

Test item: MKH 6561-sulfonamide acid (AE 1234964, metabolite of iodosulfuron-methyl-sodium); Batch code: AE 1234964-PU-01; Origin batch No.: M00102; Certificate No.: AZ 13380; Content of active ingredient: 99% w/w.

Cultures of Duck weed plants (*Lemna gibba*) were exposed to various concentrations of AE 1234964 (0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg test item/L) and to a control (reconstituted water) in a static system over a period of 7 days. At test initiation the number of fronds was 12 fronds per vessel (glass flasks of 250 mL volume with about 170 mL test medium). The test was conducted with 3 replicates per treatment level and 6 control replicates. At test start frond and colony numbers were recorded and the dry weight of a sample of fronds identical to that used to inoculate the test vessels was determined. On days 3, 5 and 7 frond numbers and appearance of colonies were observed. At the end of the test the dry weight of all plants from each vessel was determined. For analytical verification duplicate samples were taken from test media of all test concentrations at the start and at the end of the test. From the control samples only one of the duplicate samples was analysed from both sampling times. Liquid chromatography (LC-MS/MS-method) was used as analytical method.

Dates of experimental work: June 23, 2006 to June 30, 2006 (biological part)
July 17, 2006 to July 19, 2006 (date of analysis)

Results:

Validity Criteria:
The doubling time of fronds was 1.82, corresponding to an approximately 14.4-fold increase in 7 days, and thus, the validity criterion of a doubling time less than 60 hours (2.5 days) in the control was fulfilled.

Analytical findings:
At the start of the test 94% of the nominal test concentrations were found (average for nominal test concentrations of 0.32 to 100 mg test item/L). After 7 days test duration 95% of the nominal values were determined (average for nominal test concentrations of 0.32 to 100 mg test item/L). Thus, during the test period of 7 days the *Lemna* plants were exposed to a mean of 95% of nominal. Therefore, all reported results are related to nominal concentrations of the test item.

Table CA 8.2.7-44: Summary of analytical results for the test item AE 1234964

Sample description [mg/L]	% of nominal[1]	RSD [%]
control	n.a.	n.a.
0.10	n.d.	n.a.
0.32	96	2
1.0	92	1
3.2	92	3
10	94	1
32	93	7
100	102	4

[1] mean value of all measured samples per treatment group
RSD Relative standard deviation per treatment group
n.d. not determined, since below the NOEC
n.a. not applicable

Biological findings:

The shape of fronds and colonies after the test period of 7 days was not different to those in the control up to and including the nominal test concentrations of 3.2 mg test item/L. At test concentrations of 10 mg test item/L and above colonies were deformed.

The 7-day EC_{50} was 431 and 318 mg test item/L for growth rate of frond number and for growth rate of dry weight, respectively. The 7-day NOEC was determined to be 0.32 mg test item/L. The 7-day LOEC was determined to be 1.0 mg test item/L.

Observations on growth rates and the percentage of inhibition for frond number and biomass are listed in the table below.

Table CA 8.2.7-45: Effect of AE 1234964 on growth-inhibition (mean frond number and dry weight) of *Lemna gibba*

Treatment level [mg/L]	Frond number						Biomass	
	Growth rate			Percentage of inhibition			Growth rate	Percentage of inhibition
	0-3 d	0-5 d	0-7 d	0-3 d	0-5 d	0-7 d		
control	0.361	0.394	0.381	0.0	0.0	0.0	0.411	0.0
0.1	0.368	0.399	0.397	-2.0	-1.3	-4.2	0.425	-3.3
0.32	0.384	0.409	0.391	-6.3	-4.0	-2.8	0.419	-1.9
1.0	0.377	0.381	0.355	-4.5	3.1	6.7	0.365	11.1
3.2	0.374	0.346	0.305	-3.7	12.1	19.8	0.312	24.2
10	0.357	0.342	0.288	1.2	13.2	24.3	0.292	28.9
32	0.323	0.325	0.267	10.5	17.5	29.8	0.273	33.5
100	0.331	0.306	0.253	8.4	22.3	33.6	0.267	34.9

Conclusions:

The 7-days EC_{50} values were > 100 mg test item/L for growth rate of frond number and for growth rate of dry weight.

AE F159737

Report:	[redacted];2006;M-281250-01
Title:	Toxicity of MKH 6561-Saccharine to the aquatic plant Lemna gibba in a growth inhibition test
Report No:	30194240
Document No:	M-281250-01-1
Guidelines:	**Revised Proposal for a new OECD Guideline 221: "Lemna sp. Growth Inhibition Test", October 22, 2004.;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this test was to determine the inhibitory effect of the test item AE F159737 (metabolite of iodosulfuron-methyl, further code: MKH 6561-Saccharine) on the growth of the freshwater aquatic plant *Lemna gibba*. Cultures of *Lemna gibba* were exposed to various concentrations of the test item (0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg test item/L) and a control (reconstituted water) under defined conditions. The inhibition of growth expressed as NOEC, LOEC, and EC_{50} for growth rate of frond number and for growth rate of dry weight of plants in relation to control cultures was determined over a test period of 7 days. Based on analytical findings the biological endpoints are reported as nominal figures. The 7-day EC_{50} was > 100 mg test item/L for growth rate of frond number and for growth rate of dry weight, respectively. The 7-day NOEC was determined to be 0.32 mg test item/L for growth rate of frond number and 10 mg test item/L for growth rate of dry weight.

Material and methods:

Test item: MKH 6561-saccharine (AE F159737, metabolite of iodosulfuron-methyl-sodium); Batch No.: M00402; Product code: AE F159737 00 1B99 0002; Certificate No.: AZ 11460; Content of active ingredient: 99.9 % w/w.

Cultures of Duck weed plants (*Lemna gibba*) were exposed to various concentrations of AE F159737 (0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg test item/L) and to a control (reconstituted water) in a static system over a period of 7 days. At test initiation the number of fronds was 12 fronds per test vessel (glass flasks of 250 mL volume with about 170 mL test medium). The test was conducted with 3 replicates per treatment level and 6 control replicates. At test start frond and colony numbers were recorded and the dry weight of a sample of fronds identical to that used to inoculate the test vessels was determined. On days 3, 5 and 7 frond numbers and appearance of colonies were observed. At the end of the test the dry weight of all plants from each vessel was determined. For analytical verification duplicate samples were taken from test media of all test concentrations and the control at the start and at the end of the test. From the control samples only one of the duplicate samples was analysed from both sampling times. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: August 11, 2006 to August 21, 2006 (biological part)
September 12, 2006 to September 13, 2006 (date of analysis)

Results:

Validity Criteria:
The doubling time of fronds was 1.8, corresponding to an approximately 14.5-fold increase in 7 days, and thus, the validity criterion of a doubling time less than 60 hours (2.5 days) in the control was fulfilled.

Analytical findings:
At the start of the test 95% of the nominal test concentrations were found. After 7 days test duration 100% of the nominal values were determined. Thus, during the test period of 7 days the Lemna were exposed to a mean of 97% of nominal. Therefore, all reported results are related to nominal concentrations of the test item. In the lowest test concentration a mean value of 76% of nominal was found. Considering the mean recovery rate of 91% of the respective fortification level, it can be assumed, that this slightly reduced value is not result of wrong preparation of this test concentration or loss of test item. Additionally this test concentration is below the NOEC determined in this test.

Table CA 8.2.7-46: Summary of analytical results for the test item AE F159737

Sample description [mg/L]	% of nominal[1]	RSD [%]
control	n.a.	n.a.
0.10	76	15
0.32	93	8
1.0	101	3
3.2	101	12
10	98	7
32	107	6
100	107	2

[1] mean value of all measured samples per treatment group
RSD Relative standard deviation per treatment group
n.a. not applicable

Biological findings:
At 32 and 100 mg test item/L necrosis was observed after 5 and 7 days of exposure. The 7-day EC_{50} was > 100 mg test item/L for growth rate of frond number and for growth rate of dry weight, respectively. The 7-day NOEC was determined to be 0.32 mg test item/L for growth rate of frond number and 10 mg test item/L for growth rate of dry weight. The 7-day LOEC was determined to be 1.0 mg test item/L for growth rate of frond number and 32 mg test item/L and for growth rate of dry weight.
Observations on growth rates and the percentage of inhibition for frond number and biomass are listed in the table below.

Table CA 8.2.7-47: Effect of AE F159737 on growth-inhibition (mean frond number and dry weight) of *Lemna gibba*

Treatment level [mg/L]	Frond number						Biomass	
	Growth rate			Percentage of inhibition			Growth rate	Percentage of inhibition
	0-3 d	0-5 d	0-7 d	0-3 d	0-5 d	0-7 d		
control	0.353	0.387	0.379	0.0	0.0	0.0	0.404	0.0
0.1	0.290	0.365	0.345	18.0	9.7	9.1	0.374	7.5
0.32	0.341	0.346	0.349	3.7	10.7	7.9	0.381	5.8
1.0	0.309	0.310	0.310	12.4	19.9	18.3	0.364	9.8
3.2	0.276	0.295	0.274	21.9	23.7	27.8	0.365	9.5
10	0.268	0.276	0.269	24.3	28.6	28.9	0.364	9.8
32	0.230	0.270	0.244	34.8	30.3	35.6	0.334	17.2
100	0.234	0.236	0.240	33.8	39.1	36.7	0.313	22.6

Conclusions:

The 7-days EC_{50} values were > 100 mg test item/L for growth rate of frond number and for growth rate of dry weight.

AE F154781

Report:	[redacted];[redacted];2013;M-470494-01
Title:	Lemna gibba G3 - Growth inhibition test with AE F154781 (metabolite of iodosulfuron-methyl-sodium) under static conditions
Report No:	E 412 4513 - 0
Document No:	M-470494-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400;not specified**
GLP/GEP:	**yes**

Executive Summary:

The objective of this growth inhibition test was to verify the assumption that AE F154781 (metabolite of iodosulfuron-methyl-sodium) causes no adverse effects on the growth of *Lemna gibba G3* up to a test item concentration of 10 mg pure metabolite/L. For this purpose exponentially growing cultures of *Lemna* were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10 mg pure metabolite in comparison to a water control. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 percent (EC_{50}) was determined where possible.
No adverse effects on the growth of *Lemna gibba* where found at the limit test item concentration of 10 mg pure metabolite/L.

Material and methods:

Test item: AE F154781 (metabolite of iodosulfuron-methyl-sodium); Batch code:
AE F 154781-TE-01; Origin batch No.: 0201893-ACB; LIMS No.: 1020598; TOX-No.: AZ 16782;
Analysed content: 89 % w/w.

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 the nominal concentration of 10.0 mg p.m./L in comparison to a control. The pH values ranged from 7.5 to 9.0 in the control and the incubation temperature ranged from 24.5°C to 24.7°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6800 lux (average of nine measurements). 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. Visual observations were made on study days 2, 5, and 7.
Quantitative amounts of AE F154781 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: June 13, 2013 to September 18, 2013

Results:

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

Analytical findings:
The analytical finding of AE F154781 found on day 0 was 111% of nominal and 114% of nominal on day 7. Since the analytical measurements showed results of 80.0 – 120% of nominal the calculated endpoints are based on nominal concentrations of the test item.

Table CA 8.2.7-48: Summary of analytical results

Nominal concentration [mg p.m./L]	Actual concentration (mg pm/L)							
	Day 0				Day 7			
	Determination 1.	Determination 2.	Average	%	Determination 1.	Determination 2.	Average	%
Control	< 0.578	< 0.578	< 0.578	--	< 0.578	< 0.578	< 0.578	--
10.0	11.1	11.1	11.1	111	11.4	11.4	11.4	114

p.m.: pure metabolite

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

Table CA 8.2.7-49: Survey of biological results and derived inhibition percentages based on growth rates

nominal test concentration [mg p.m./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm²]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	172.8	1464.0	--	--
10.0	184.2	1488.5	-2.4	0.3

Observed visual effects:
No visual effects on *Lemna gibba* were observed.

The results based on nominal concentrations of the test item AE F154781 are shown in the table below.

Table CA 8.2.7-50: Survey of 7-day endpoints for AE F154781

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

Conclusions:

AE F 154781 caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

Peer reviewed literature – included in the dossier on request of the rapporteur:

Report:	[redacted];[redacted];[redacted];[redacted];[redacted];2013;M-469998-01
Title:	Influence of pH, light cycle, and temperature on ecotoxicity of four sulfonylurea herbicides towards Lemna gibba
Document No(s):	M-469998-01-1
Guidelines:	M-469998-01-1
GLP/GEP:	**not applicable;not applicable**

EXECUTIVE SUMMARY

The toxicity of metsulfuron-methyl towards *Lemna gibba* was investigated at three pH levels (6, 7.5 and 9), at two temperatures (15 and 24°C) and two light regimes (continuous and 12:12 h light:dark cycle). It is demonstrated that varying test conditions have an influence on the toxicity of metsulfuron-methyl on *L. gibba*. The EC_{10} and EC_{50} values derived from the test carried out according to OECD 211 guideline (OECD 2006) are 0.27 and 0.37 µg/L, respectively.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Not specified
Active substance(s):	Metsulfuron-methyl (CAS 74223-64-6)
Chemical state and description:	Not specified
Source of test item:	[redacted], Switzerland
Batch number:	Not specified
Purity:	90-96% (depending on substance, not further specified)
Storage conditions:	Not specified
Water solubility:	0.548 g/L at pH 5; 2.79 g/L at pH 7
pKa:	3.75
Log Kow at 25°C:	-1.7 at pH 7
Hydrolysis DT_{50}:	22 days at pH 5; > 30 days at pH 6; no degradation at pH 7

2. Test organism(s)

Species:	*Lemna gibba*
Common name:	Duckweed
Source of test species:	Purchased from the [redacted].

3. Breeding of test organism(s)

Housing conditions:	Cultured in Erlenmeyer flasks in 20× AAP medium (OECD 2006) and an initial pH of 7.5 (not adjusted over time). Each week colonies were transferred to freshly prepared medium and new cultures were established the same way.
Temperature:	24 ± 2 °C
Photoperiod:	Continuous white fluorescent light (Philips 30W/33) with intensity of 125±12.5 µE/m²/s
Observations:	Not specified

B. Study design and methods

1. Test procedure

Test system:	Growth inhibition at various test conditions (OECD guideline 221 with modifications listed below)
Test concentrations:	6 concentrations with factor 3 between.
Solvent/Buffer:	Acetone was used with a maximum concentration < 100 µL/L.
Control(s):	3 controls and 3 solvent controls
Number of replicates:	3 replicates, each with 8 fronds (2-3 colonies).
Test medium:	20× APP medium adjusted to the relevant pH with NaOH or HCl
Medium change intervals:	Twice during the test period.
Test duration/conditions:	See table 1
Measurements:	Counting of fronds (counted at start, both media renewals, and end of test).
Statistics:	Growth rate was calculated by linear regression of growth curves in a semi-logarithmic data plot with log(biomass) versus time on the axes. The effect of treatments was tested by ANOVA and where significant dose effect was found, data were fitted to a three parameter log-logistic concentration-response model. A time-weighted mean (TWM; OECD, 1998[5]) of the highest concentration during the test period was calculated and used to calculate lower exposure concentrations for estimation of ECx values in the concentration-response model.

2. Chemical analysis

Guideline/protocol:	Not specified
Method:	LC-MS/MS
Sampling and pre-treatment:	At test initiation, at each renewal, and at the end of the tests, 25 mL test solution of the highest concentrations was sampled. The samples were concentrated using disposable Bond Elut ENV cartridges (500 mg, 6 mL) followed by a heptane wash step prior to drying the cartridges with atmospheric air to push out all water. The dried cartridges were kept in -18°C until they were analyzed by Eurofins Environment Sweden AB.
Conduction:	Not specified
Reference item:	Not specified
Recovery:	Not specified
Limit of detection:	Not specified
Limit of quantification:	0.1 µg/L

Table 1: Overview of the test conditions in the five Test Series

	Test series 1	Test series 2	Test series 3	Test series 4	Test series 5
pH	6.0	7.5	9.0	7.5	7.5
Buffer	10 mM MES	-	10 mM TRIS	-	-
Light regime	Continuous	Continuous	Continuous	12:12h light:dark	Continuous
Temperature	24 ± 2°C	24 ± 2°C	24 ± 2°C	24 ± 2°C	15 ± 2°C
Duration	7 days	7 days	7 days	7 days	11 days

Test Series 2 are the tests carried according to the OECD 211 guideline (OECD 2006)

RESULTS

1. Validity criteria:

For all tests, except the one at 15°C, the doubling time (table 2) was less than 2.5 days which is in accordance with the validity criterion of the OECD standard test (OECD 2006). The increase in pH

[5] OECD, 1998. OECD Guidelines for Testing of Chemicals. *Daphnia magna* Reproduction Test. OECD Guideline 211. Germany for Economic Cooperation and Development, Paris, France.

was in several instances greater than the 1.5 units that OECD 221 advises as the maximum pH drift during the incubation. However, this is not an invalidating factor if the validity criterion on doubling time is met.

Table 2: The doubling time (T2) in the control (days) and the maximum pH drift (pH units) up (+) or down (-) from the initial pH value for all the tests with L. gibba and metsulfuron-methyl

	Test series 1	Test series 2	Test series 3	Test series 4	Test series 5
T2 for controls	1.4	1.5	2.4	1.6	3.8
pH drift controls	+0.2	+1.6	-0.3	+1.6	+1.8
pH drift highest concentration	+0.2	+1.2	+0.2	+1.1	+1.5

2. Chemical analysis:

Results are shown in table 3. In Test Series 5 (the concentrations were not analyzed and therefore nominal calculations were used in the calculation of EC values.

Table 3: The nominal value of the highest concentration, the TWM of the measured (highest) concentrations at the beginning and end of all renewal periods, and the difference between the nominal concentration and TWM in percent ("-": TWM<Nominal)

	Nominal value [µ/L]	TWM [µg/L]	Difference [%]
Test series 1 (pH 6)	12.15	11.8	-3
Test series 2 (pH7.5)	24.3	25.5	+5
Test series 3 (pH 9)	121.5	127	+5
Test series 4 (12:12)	24.3	25.5	+5
Test series 5 (15°C)	24.3	-	-

3. The influence of pH, light cycle and temperature on toxicity:

For metsulfuron-methyl a decrease in EC_{50} was seen when going from pH 6 to 7.5 but it increased significantly from pH 6 to 9 (table 4).

Table 4: EC_{10} and EC_{50} values (µg/L) with 95 % confidence intervals for the growth rate inhibition tests with *Lemna gibba* and metsulfuron-methyl carried out at different test conditions

	EC_{10} (95% C.I.)	EC_{50} (95% C.I.)
Test series 1 (pH 6)	0.012 (0.057-0.18)	0.064 (0.51-0.77)
Test series 2 (pH7.5)	0.27 (0.11-0.42)	0.37 (0.26-0.49)
Test series 3 (pH 9)	0.27 (0.099-0.44)	1.4 (1.0-1.9)
Test series 4 (12:12)	0.16 (0.082-0.25)	0.50 (0.40-0.60)
Test series 5 (15°C)	0.043 (-0.089[a])	0.68 (0.36-1.00)

Tests in Test Series 2 are carried out according to the OECD 211 guideline (OECD 2006)
Calculations of ECx values are based on TWM, except for Test Series 5, which was based on nominal concentrations
[a] Estimation of lower confidence limit not possible

RESULTS SUMMARY

Varying test conditions, i.e. pH, have an influence on the toxicity of metsulfuron-methyl on *Lemna gibba*. The EC_{10} and EC_{50} values derived from the test carried out according to OECD 211 guideline (OECD 2006) are 0.27 and 0.37 µg/L, respectively.

Evaluation by the notifier

For metsulfuron-methyl (AE F075736; metabolite of iodosulfuron-methyl-sodium) the following Lemna-growth inhibition study has been performed by the notifier Bayer CropScience:

Lemna gibba growth inhibiton test	7 days	**ErC50: 0.511 µg/L** EbC50: 0.44 µg/L	NOEC: 0.169 µg/L	[redacted] & [redacted] (1998); M-182336-01-1

The endpoint of 0.511 µg/L calculated from growth rates based on frond counts is regarded as the endpoint to be used for risk assessments according to current regulations. Due to a clear dose-response in that study, the 95% confidence limits are within a narrow range between 0.32 and 0.56 µg/L.

Lemna-endpoint given by [redacted] et al. (2013)

In order to compare the influence of pH, light cycle and temperature [redacted] et al. (2013) performed a test series according to OECD 221 (20x AAP medium; pH 7.5 at test start; light intensity 125 µE/m2/s; temperature 24 plus minus 2 °C). All these parameters are exactly as specified in the current OECD-guideline 221.

The tests were conducted semi-static with two replacements during the test.

Test duration was 7 days. Only the series at 15 °C was done over 11 days, due to reduced growth in the controls.

Even analytical measurements were conducted. (105% of nominal in case of metsulfuron-methyl) (see table 3 in paper).

On page 34 it is mentioned that "The test variable, growth rate based on counting of fronds (counted at start, both media renewals, and end of test), was calculated by linear regression of growth curves in a semi-logarithmic data plot with log(biomass) versus time on the axes." That means, that the EC50-figures presented in this paper are calculated from growth rate figures of frond numbers. A second endpoint (i.e. biomass or frond area) has not been determined.

The authors report an EC50 of 0.37 (95% confidence limits 0.26 – 0.49) µg/L for metsulfuron under standard conditions (table 5). They used this endpoint as a standard in order to compare it with the other test series where the environmental parameters were varied.

Finally, the authors stated in the discussion section: "For SUs Lemna spp. appears to be among the most sensitive species for the endpoints considered ([redacted] et al. 2004; [redacted] and [redacted] 2005a) and it may therefore be suggested that a low assessment factor should be applied to EC50-values when setting the EQS."

The endpoint of 0.517 (95% conf limits 0.32 – 0.56) µg/L from the regulatory valid study submitted by BCS is not contradictory to the findings of [redacted] et al. (2013), since the endpoint of 0.37 is within the 95%-confidence limits of the endpoint from the regulatory study performed by [redacted] & [redacted] (1998; M-182336-01-1). Thus, the paper published by [redacted] et al. (2013) does not contain any new information with regard to the growth inhibition of Lemna under standard conditions required by OECD 221.

On the other hand the quality of the published data – although apparently high – cannot be judged by BCS, as neither the experimental work and reporting was performed under GLP nor is the raw data available for review by BCS.

Due to these reasons this publication is not considered relevant for the risk assessment of iodosulfuron-methyl-sodium.

CA 8.2.8 - Further testing on aquatic organisms

An 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 iodosulfuron-methyl-sodium to further aquatic organisms presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Iodosulfuron-methyl-sodium				
Crassostrea virginica (eastern oyster)	flow-through	96 h	EC_{50} >120 NOEC 72	[redacted], [redacted] & [redacted], 1998 B002674 M-238409-01-2 KCA 8.2.8/01

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];1999;M-238409-01
Title:	Flow-through mollusc shell deposition test: AE F115008
Report No:	B002674
Document No(s):	M-238409-01-2
Guidelines:	**USEPA (=EPA): 72-3(c);Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The aim of the study was to determine the acute effects of iodosulfuron-methyl-sodium to Eastern oyster (*Crassostrea virginica*).
Crassostrea virginica (mean valve height 30 to 50 mm) were exposed in a flow-through system over a period of 96 hours to nominal concentrations of 17, 29, 47, 78, and 130 mg a.s./L (corresponding to analytically verified concentrations of 15.7, 26.0, 42.2, 72.0, and 120 mg/L). In addition a saltwater control 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 > 120 mg a.s./L, the 96-hour-NOEC was determined to be 72.0 mg a.s./L.

Material and methods:

Test item: Iodosulfuron-methyl-sodium, technical (AE F115008); Code No.: AE Fl 15008 00 1C89 0001; Batch No.: CR21436/02/950601, purity: 86.9% w/w.

Crassostrea virginica (mean valve height 30 to 50 mm) were exposed to the test item in a flow-through system over a period of 96 hours. Nominal concentrations were 17, 29, 47, 78, and 130 mg a.s./L (corresponding to analytically verified concentrations of 15.7, 26.0, 42.2, 72.0, and 120 mg/L). In addition a saltwater control was tested. Each vessel (glass aquaria; 20 L) served as one replicate filled with 15 L unfiltered, natural seawater. 10 oysters were used per replicate. Immediately prior to the test initiation, each oyster was ground with a rotary grinder to remove approximately 3 to 5 mm of shell and form a smooth edge. The test was conducted with 2 replicates per treatment level. Mortality and intoxication symptoms of the oysters were determined visually and recorded initially and after 24, 48, 72, and 96 hours. Shell deposition was assessed at the end of the study. Physical and

chemical parameters were measured and recorded daily in each test chamber. The temperature in one vessel was recorded continuously during the test.
For analytical verification of the test item concentrations samples were taken at 0 and 96 hours from all concentrations. High-performance liquid chromatography (HPLC) was used as analytical method.

Dates of experimental work: August 17, 1999 to September 21, 1999

Results:

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

Analytical Findings:
Analytical verification of test solutions revealed measured concentrations of 15.7, 26.0, 42.2, 72.0 and 120 mg/L calculated as arithmetic mean. Biological results are reported as mean measured. Detailed analytical results are presented in the following table.

Table CA 8.2.8-2: Nominal and measured concentrations of iodosulfuron-methyl-sodium

Nominal Concentration (mg a.s./L)	Replicate #	Day 0 measured (mg a.i./L)	Day 4 measured (mg a.i./L)	Mean measured (mg a.i./L)	Standard Deviation	Mean percent of nominal
17	1	15.5	15.7	15.7	0.1	92
	2	15.7	15.7			
29	1	25.5	26.2	26.0	0.4	90
	2	25.7	25.4			
47	1	41.8	42.5	42.2	0.3	90
	2	42.8	42.3			
78	1	71.7	72.5	72.0	0.4	92
	2	71.8	71.9			
130	1	119	120	120	1	92
	2	120	122			

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

Table CA 8.2.8-3: Effect of iodosulfuron-methyl-sodium on shell deposition of *Crassostrea virginica*

Mean measured concentration (mg a.s./L)	Mortality %	Mean shell deposition at the longest finger (mm)	Percent of control
Control	0	2.6	
15.7	0	2.4	92
26.0	0	2.4	92
42.2	0	2.2	85
72.0	0	2.5	96
120	0	2.0 *	77

* Significantly reduced compared to the control, based on Williams' Test ($p < 0.05$)

No sublethal behavioural changes were observed.

Conclusions:

The acute effect of iodosulfuron-methyl-sodium on *Crassostrea virginica* can be quantified as a 96-hour-EC_{50} of > 120 mg a.s./L. The highest concentration with no observed immobilisation and no sublethal behavioural effects can be set to 72.0 mg a.s./L.

CA 8.3 - Effect on arthropods

CA 8.3.1 - Effects on bees

Iodosulfuron-methyl sodium has a low acute toxicity to honey bees, with LD_{50} (oral and contact) above the highest tested dose level (oral: LD_{50} > 107.6 µg a.s./bee, contact: LD_{50} > 100 µg a.s./bee). The calculated Hazard Quotients for iodosulfuron-methyl sodium at the maximum application rate of 10 g a.s./ha are well below the validated trigger value which would indicate the need for a refined risk assessment; no adverse effects on honey bee mortality are to be expected. This conclusion is confirmed by the results of the bee brood feeding study as well as by the results of the semi-field study, which covered the maximum application rates of 10 g iodosulfuron-methyl sodium a.s./ha. The acute laboratory study conducted with bumble bees revealed no sensitivity differences between honey bee and bumble bee foragers.

Regarding potential side effects of iodosulfuron-methyl-sodium on immature honey bee life stages, the conducted bee brood feeding study (Oomen *et al.*, 1992) found slightly-moderately, but statistically significantly increased termination rates of eggs, young and old larvae. Despite of this observation, there was concurrently an identical (better) brood nest development than in the control. In addition, the brood index and brood compensation indices displayed a continuous increase, indicating a successful development of the brood; overall the study revealed no ecologically adverse effects on the survival of adult bees and pupae, behaviour, colony strength and overall colony conditions. Thus, when considering the severity of the exposure situation in this worst-case screening test in combination with the absence of effects on both, colony level parameters and also on the overall development of bee brood, it can be concluded even on the basis of this worst-case screening study that the use of iodosulfuron-methyl sodium as a post-emergence (until early stem elongation) herbicide in cereals - a crop which poses for bees not a profitable feeding and foraging area for nectar and pollen - does not pose an unacceptable risk for adult honey bees, immature honey bee life stages and honey bee colonies.

Nonetheless, in order to clarify whether the conclusions on the basis of lower tiered honey bee studies are correct, iodosulfuron-methyl-sodium was subjected to confined semi-field testing (according to the provisions of OECD Guidance Document No. 75), by applying the maximum rate of Iodosulfuron-methyl sodium + mefenpyr-diethyl OD 400 (100+300 g/L) to full-flowering *Phacelia* during honey bees actively foraging on the crop. This study design, although being conservative for an actual exposure situation of honey bees in cereals, is from an apidological and apicultural point of view more realistic than an in-hive feeding of the test compound via a treated sugar solution, which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration). The results of this higher tier semi-field study confirmed the conclusions made above on the basis of the outcome of the lower-tiered studies, as no adverse direct or delayed effects on mortality of worker bees or pupae, foraging activity, behaviour, nectar- and pollen storage, queen survival, colony strength, colony development as well as the development of bee brood were observed, even under aggravated, forced exposure conditions and by digitally following-up in a very detailed manner the fate of individually marked brood cells (digital photographic assessment) from egg stage until emergence.

Overall, it can be concluded that iodosulfuron-methyl-sodium, when applied at the maximum application rate of 10 g a.s./ha in cereals, even during the flowering period of potentially bee-attractive weeds inside the cereal cropping area, does not pose an unacceptable risk to honey bees and honey bee colonies.

Table CA 8.3.1- 1: Honey bee toxicity of iodosulfuron-methyl-sodium to bees

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity (laboratory) in honey bees			
Iodosulfuron-methyl sodium, tech.	LD_{50}-oral, 48/72 h	LD_{50} > 80 µg a.s./bee	[redacted], 1996 M-141821-01-1 KCA 8.3.1.1 /01
Iodosulfuron-methyl sodium, tech.	LD_{50}-contact, 48/72 h	LD_{50} > 150 µg a.s./bee	[redacted], 1996 M-141225-01-1 KCA 8.3.1.1.2 /01
Iodosulfuron-methyl sodium, tech.	LD_{50}-oral, 48 h LD_{50}-contact, 48 h	**LD_{50} > 107.6 µg a.s./bee** **LD_{50} > 100 µg a.s./bee**	[redacted], 2012 M-436273-01-1 KCA 8.3.1.1 /01
Acute contact toxicity (laboratory) in bumble bees			
Iodosulfuron-methyl sodium, tech.	LD_{50}-contact, 48 h	LD_{50} > 100 µg a.s./bee	[redacted], 2014 M-477331-01-1 KCA 8.3.1.1 /02
Chronic toxicity in adult honey bees (laboratory)			
Iodosulfuron-methyl sodium, tech.	10 d chronic adult feeding study	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted], 2014 M-479336-01-1 KCA 8.3.1.2 /01
Bee brood feeding test			
Iodosulfuron-methyl sodium WG 10 (+Mefenpyr-diethyl WG 15)	Honey bee brood feeding (Oomen *et al.*, 1992)	Slightly, but statistically significantly increased termination rate of eggs, young and old larvae, identical (better) brood nest development than in the control; brood index and brood compensation indices displayed a continuous increase, indicating a successful development of the brood. No ecologically adverse effects on the survival of adult bees and pupae, behaviour, colony strength and overall colony conditions by feeding honey bee colonies sugar syrup at a iodosulfuron-methyl sodium concentration typically present in the spray tank (25 ppm)	[redacted], 2013 M-465335-01-1 KCA 8.3.1.3 /01
Cage and tunnel studies			
Iodosulfuron-methyl sodium + mefenpyr-diethyl OD 400 (100+300 g/L)	Semi-field honey bee brood study (according to OECD 75; forced exposure conditions) in *Phacelia*; application during full bloom and bees actively foraging	No adverse effects on mortality, flight intensity, behaviour, brood development (brood termination rate, brood index, compensation index) as well as on colony vitality at maximum application rate (0.1 L product/ha)	[redacted], 2014 M-477913-01-1 KCA 8.3.1.3 /02

Bold values: endpoints used for risk assessment

CA 8.3.1.1 - Acute toxicity to bees

In addition to the already available acute laboratory studies with technical iodosulfuron-methyl-sodium ([redacted]; 1996, Doc.-No.: M-141821-01-1 and M-141225-01-1; 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 iodosulfuron-methyl sodium according to current guidelines and requirements. Moreover, an acute contact toxicity study in bumble bees has been conducted (KCA 8.3.1.1 /02) in order to benchmark potential sensitivity differences to honey bees.

In addition, a chronic 10 day adult feeding limit test was conducted with Iodosulfuron-methyl-sodium WG 10 (KCA 8.3.1.2 /01). The respective study summaries are presented below.

<u>Studies with technical iodosulfuron-methyl-sodium</u>

Report:	[redacted];[redacted];2004;M-436273-01
Title:	Effects of iodosulfuron-methyl-sodium tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	73071035
Document No:	M-436273-01-1
Guidelines:	**OECD 213 and 214 (1998);none**
GLP/GEP:	**yes**

Executive Summary:

The aim of this study was to determine the acute contact and oral toxicity of iodosulfuron-methyl-sodium to the honey bee (*A. mellifera* L.) under laboratory conditions. For this purpose female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 107.6 µg a.s./bee for feeding (oral limit test, value based on the actual intake of the test item). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.
The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 107.6 µg a.s./bee.

Material and methods:

Test item: Iodosulfuron-methyl-sodium tech.; Origin Batch No.: ELIR003050; LIMS no.: 1024641; Customer order no.: TOX-no: 09144-00; Article no.: 05942802; Specification No.: 102000000739; Content: 93.0 % w/w (analytical).

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 were used per test item dose level, control and reference item dose level, respectively. 50 worker bees (*Apis mellifera*) per dose were exposed under laboratory conditions for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees per dose were exposed for 48 hours for feeding (oral limit test, value based on the actual intake of the test item) to a single dose of 107.6 µg a.s. per bee. For the contact test a single 5 µL droplet of iodosulfuron-methyl-sodium, dissolved in tap water with 0.5 % Adhäsit, was placed on the dorsal bee thorax, likewise for the toxic reference (dimethoate) and the control (tap water). For both oral tests aqueous stock solutions of the test item and reference item were prepared and mixed with ready-to-use sugar syrup (30% sucrose, 31% glucose, 39% fructose) at a concentration of 50% (w/w). For the control, tap water and sugar syrup was used at the same ratio 50% (w/w) tap water, 50% (w/w) ready-to-use sugar syrup. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 1 hour 50 minutes the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh,

untreated food. The number of dead bees was determined after 4 hours (first day); 24 and 48 hours. Behavioural abnormalities (e.g. vomiting, apathy, intensive cleaning) were assessed after 4 hours (first day), 24 and 48 hours. Temperature during the test was 25 °C; relative humidity was 60 - 77%. Bees were kept in darkness (except during observation).

Dates of work: May 21 to May 24, 2012

Results:

Validity Criteria:

Validity Criteria		Recommended	Obtained
Control Mortality		Contact Test	
	CO_2/water control	10%	0.0 %
		Oral Test	
	water/sugar syrup control	< 10%	0.0 %
LD_{50} of Reference Item (24 h)		Contact Test	
		0.10 - 0.30 µg a.s./bee	0.21 µg a.s./bee
		Oral Test	
		0.10 - 0.35 µg a.s./bee	0.14 µg a.s./bee

All validity criteria for the study were met.

Biological results:

Contact toxicity:
At the end of the contact toxicity test (48 hours after application), no mortality occurred at 100.0 µg a.s./bee. There was no mortality in the control group (water + 0.5 % Adhäsit). No test item induced behavioural effects were observed at any time in the contact toxicity test.

Oral toxicity:
In the oral toxicity test, the maximum nominal test level of iodosulfuron-methyl-sodium tech. (*i.e.* 100 µg a.s./bee) corresponded to an actual intake of 107.6 µg a.s./bee. This dose level led to 2.0 % mortality after 48 hours. In the control group (50 % sugar solution), no mortality occurred. In the oral test, during the 24 and 48 hrs assessment one bee was found apathetic, respectively.

Table CA 8.3.1.1-1: Toxicity of iodosulfuron-methyl-sodium tech. to honey bees; laboratory tests

Test Item	**Iodosulfuron-methyl-sodium tech.**	
Test Object	***Apis mellifera***	
Exposure	**contact (solution in Adhäsit (0.5 %)/water)**	**oral (sugar solution)**
Application rate µg a.s./bee	100.0	107.6
LD_{50} µg a.s./bee	> 100.0	> 107.6
ED_{20} µg a.s./bee	> 100.0	> 107.6
LD_{10} µg a.s./bee	> 100.0	> 107.6
NOED µg a.s./bee*	≥ 100.0	≥ 107.6

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

Conclusions:

The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 107.6 µg a.s./bee.

Report:	[redacted]; [redacted];2014;M-477331-01
Title:	Iodosulfuron-methyl sodium (tech.): Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No:	S13-01780
Document No:	M-477331-01-1
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 applicable**
GLP/GEP:	**yes**

Executive summary:

The contact toxicity of iodosulfuron-methyl sodium (tech.) to the bumble bee (*Bombus terrestris* L.) was determined in a limit test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001). In the test item treatment group, no mortality and no remarkable 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 iodosulfuron-methyl-sodium (tech.) was determined to be > 100 µg a.s./bumble bee.

Material and methods:

Test item: Name: Iodosulfuron-methyl-sodium (tech.)
TOX-No.: 09144-01
Origin Batch No.: ELIR003050
Purity: 93.0 % w/w (analysed)

The contact toxicity of iodosulfuron-methyl sodium (tech.) to the bumble bee (*Bombus terrestris* L.) was determined in a limit test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, bumble bees were exposed to 100 µg iodosulfuron-methyl sodium a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after treatment. The control groups were exposed for the same period of time under identical exposure conditions to tap water and acetone, respectively.

Dates of work: 24 September 2013 – 26 September 2013

Findings:

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

Table CA 8.3.1.1-2: LD_{50} values in the bumble bee contact toxicity test with iodosulfuron-methyl-sodium tech.

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

In the test item treatment group, no mortality and no remarkable 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 iodosulfuron-methyl-sodium (tech.) on bumble bees at the treatment level of 100 µg iodosulfuron-methyl-sodium a.s./bumble bee, caused no adverse effects regarding mortality, sub-lethal effects and behaviour.

Conclusion:

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

CA 8.3.1.1.1 - Acute oral toxicity

Study with technical iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1996;M-141821-01
Title:	Oral toxicity (LD50) to honey bees (Apis mellifera L.) Code: Hoe 115008 00 ZC89 0001
Report No:	A58108
Document No:	M-141821-01-1
Guidelines:	**EPPO: 170;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

LD_{50} >80 µg a.s./bee

CA 8.3.1.1.2 - Acute contact toxicity

Study with technical iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1996;M-141225-01
Title:	Contact toxicity (LD50) to honey bees (Apis mellifera L.) Code: Hoe 115008 00 ZC89 0001
Report No:	A57512
Document No:	M-141225-01-1
Guidelines:	**EPPO: 170; USEPA (=EPA): Subd.L,141-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

LD_{50} >150 µg a.s./bee

CA 8.3.1.2 - Chronic toxicity to bees

Study with technical iodosulfuron-methyl-sodium

Report:	[redacted];2014;M-479396-01
Title:	Iodosulfuron-methyl sodium (tech.) - Assessment of chronic effects to the honeybee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test
Report No:	S13-00142
Document No:	M-479396-01-1
Guidelines:	**no specific guideline available;not applicable**
GLP/GEP:	**yes**

Executive summary:

The chronic effects of the test item iodosulfuron-methyl sodium (tech.) on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding in the laboratory were assessed. The continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item iodosulfuron-methyl-sodium (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, sub-lethal effects and behaviour 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).The NOEC for mortality, sub-lethal effects and behaviour 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:	Iodosulfuron-methyl sodium (tech.)
	Tox No.:	09144-01
	Origin Batch No.:	ELIR003050AE F115008-01-03
	Purity:	93.0 % 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 iodosulfuron-methyl sodium (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 continuous exposure period. Furthermore, the daily food uptake was determined.

Dates of work (biology): 14 May 2013 – 24 May 2013

Findings:

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

The cumulative control mortality was 0.0 %, as determined at the final evaluation after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg iodosulfuron-methyl-sodium (tech.) was 2.0 % at the final evaluation.

At 120 mg a.s./kg iodosulfuron-methyl-sodium (tech.), no remarkable sub-lethal effects or behavioural abnormalities were observed throughout the entire observation period of 10 days. Only one single bee out of 100 was categorised as affected at evaluation E9.

After 10 days of continuous exposure, the accumulated nominal intake of the test item iodosulfuron-methyl-sodium (tech.) at the treatment level of 120 mg a.s./kg was 46.68 µg a.s./bee, the corresponding average daily dose was therefore 4.7 µg a.s./bee.

The overall mean daily consumption of the aqueous sucrose application 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 (38.9 mg/bee at 120 mg a.s./kg, compared to 38.3 mg/bee in the control group).

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

Table CA 8.3.1.2-1: Mean consumption of application solution, mean nominal intake of test item accumulated over all test days, average daily dose, cumulative mortality after ten days of continuous exposure (test end) as well as the LC_{50} and NOEC

Treatment Level[1]	Control	Test item at 120 mg a.s./kg (nominal)
Overall mean daily consumption of application (feeding) solution [mg/bee][2]	38.3	38.9
Mean nominal intake accumulated over ten test days [µg a.s./bee/10 d]	-	46.68
Average daily dose (nominal) throughout ten days of continuous exposure [µg a.s./bee/d]	-	4.7
Cumulative mortality after ten days of continuous exposure [%]	0.0	2.0
LC_{50}	> 120 mg a.s./kg (nominal)	
NOEC[3]	120 mg a.s./kg (nominal)	

1 The control group was fed with untreated 50 % (w/v) aqueous sucrose application solution containing 3 % acetone; the test item treatment group was fed with Iodosulfuron-methyl sodium (tech.)-treated 50 % (w/v) aqueous sucrose application solution containing 3 % acetone

2 The mean values per cage over the test period (non-rounded values) were used as basis for the calculation of the overall mean daily consumption of the aqueous sucrose application solution per treatment over the test period

3 Determined to be the NOEC based on mortality (not 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 iodosulfuron-methyl-sodium (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 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 except for the first day of exposure.

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

The NOEC for mortality, sub-lethal effects and behaviour 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). The NOEC for mortality, sub-lethal effects and behaviour 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];[redacted];2013;M-465335-01
Title:	Iodosulfuron-methyl-sodium WG 10 - A honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, Apis mellifera L. (Hymenoptera: Apidae)
Report No:	20110173
Document No:	M-465335-01-1
Guidelines:	**Oomen, P. A., de Ruijter, A. and van der Stehen, J. (1992). Method for honeybee brood feeding tests with insect growth-regulating insecticides. EPPO Bulletin, 22, 613-616 [1].; not specified**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to evaluate potential effects of iodosulfuron-methyl-sodium WG 10 administered together with the herbicide safener mefenpyr-diethyl WG 15 W on brood development and mortality of adult worker honey bees, *Apis mellifera* L.

To assess the potential effects of iodosulfuron-methyl-sodium WG 10 on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.243 g formulated test item/L (=0.025 g iodosulfuron-methyl-sodium/L) + 0.475 mL formulated herbicide safener/L (0.075 g mefenpyr-diethyl/L) per colony in summer 2012. Mortality of worker bees, larvae and pupae and behavior around the hive were observed for a period of 21 days after application. Condition of the colonies and brood development were also assessed. The method of investigating the development of the honey bee brood is based on the method of Oomen *et al.* (1992).

The administration of iodosulfuron-methyl-sodium WG 10 + the herbicide safener mefenpyr-diethyl WG 15 W to honey bee colonies caused no adverse effects on behaviour, colony strength, condition of the colonies, brood index and brood compensation index. In contrast, survival of adult bees, pupae and brood development (termination rate) in all brood stages (eggs, young and old larvae) was statistically significantly increased when compared to the control treatment.

Despite of the slightly elevated mortality and termination rates in the test item treatment group, overall colony performance was normal and not impaired.

Materials and Methods:

Test item: Iodosulfuron-methyl-sodium WG 10; Workorder: 12004631; Batch No.: AAIB00483; Sample description: TOX09722-00; Specification No.: 102000001346-01; Nominal content of a.s.: 100 g/kg; analysed content of a.s.: 103 g/kg.

Herbicide safener: Mefenpyr-diethyl WG 15 W; Sample code: 12006070; Batch No.: 2012-002193; Sample description: A.12000401; Specification No.: 102000027139; Nominal content of a.s.: 150 g/kg; analysed content of a.s.: 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.

Treatments:

Control: 50 % (w/v) aqueous sucrose solution, 1 L per colony.
Test item treatment: the test item iodosulfuron-methyl-sodium WG 10 and the herbicide safener mefenpyr-diethyl WG 15 W were both mixed together in 50 % (w/v) aqueous sucrose solution, at a final concentration of 0.025 g iodosulfuron-methyl-sodium/L and 0.075 g mefenpyr-diethyl/L, 1 L per colony.
Reference: 0.75 g fenoxycarb a.s./L, corresponding to 3.0 g (nominal) Insegar® 25 WG in 1 L 50% (w/v) aqueous sucrose solution, 1 L per colony.

Due to rainy weather and low flight activity of the honey bees, the treatment administration was conducted simultaneously to all hives in the afternoon 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 200 old larvae): one day before (= BFD0) and 5 (= BFD 6), 10 (= BFD 11), 14 (= BFD 15), 20 (= BFD 21) days after the application.

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

Results:

Validity:
The overall daily mean adult and pupae mortality of the reference item was significantly greater when compared to the control, indicating that sufficient exposure of the honeybees had taken place and thus the suitability of the test system to detect potential effects on the bee brood. The daily mean mortality of adult honeybees (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 Iodosulfuron-methyl-sodium WG 10 (+ Mefenpyr-diethyl WG 15 W) on honeybee mortality and honeybee brood development

Test item	Iodosulfuron-methyl-sodium WG 10 (+ 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	27.3 ± 6.3	31.0 ± 16.9
Post-application(DAT 1 to 21)	11.2 ± 0.9	21.6 ± 1.8 [a]	23.6 ± 7.4 [a]
	Mean mortality of pupae/colony		
Pre-application(DAT -3 to 0)	0.1 ± 0.1	0.8 ± 1.0	0.2 ± 0.3
Post-application(DAT 1 to 21)	0.5 ± 0.2	2.3 ± 0.7 [a]	34.8 ± 17.9 [a]
	Mean values of brood development (eggs)		
Brood termination rate (%) at BFD 21 (DAT 20)	41.1 ± 33.2	40.2 ±8.3 [b]	85.4± 10.9 [b]
Brood index at BFD 21 (DAT 20)	2.9 ± 1.7	3.0 ± 0.4	0.7 ± 0.5
Compensation index at BFD 21 (DAT 20)	3.7 ± 0.7	3.4 ± 0.2	1.0 ± 0.8
	Mean values of brood development (young larvae)		
Brood termination rate (%) at BFD 21 (DAT 20)	7.7 ± 4.5	35.5 ± 24.4 [b]	43.9 ± 35.6 [b]
Brood index at BFD 21 (DAT 20)	4.6 ± 0.2	3.2 ± 1.2	2.8± 1.7
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.1	4.2 ± 0.6	2.9 ± 1.8
	Mean values of brood development (old larvae)		
Brood termination rate (%) at BFD 21 (DAT 20)	5.0 ± 4.3	31.7 ± 22.9 [b]	51.8 ± 13.4 [b]
Brood index at BFD 21 (DAT 20)	4.7 ± 0.2	3.4 ± 1.2	2.4 ± 0.7 [c]
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.2	4.5 ± 0.4	2.8 ± 0.3 [c]

Values are mean ± SD

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

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

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

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

Mortality (adult and young worker bees)
The overall daily mean bee mortality observed on the days before application was similar in all treatments (22.8 to 31 bees per colony per day) indicating well adapted colonies.
The overall daily mean bee mortality after application of all treatments was 11.2, 21.6 and 23.6 in the control, test item and reference item treatment, respectively. Both, test item and the reference item treatment was statistically significantly greater when compared to the control.
Furthermore, the mean mortality was statistically significantly increased on DAT 4 and 5 (test item) and on DAT 5, 7 and 19 (reference item) when compared to the control treatment.

Mortality (pupae)
The overall daily mean pupae mortality observed on the days before application was low and similar in all treatments (0.1 to 0.8 pupae per colony per day).
The overall daily mean pupae mortality after application of all treatments was 0.5, 2.3 and 34.8 in the control, test item and reference item treatment, respectively. The test item and the reference item treatment were statistically significantly greater when compared to the control. Furthermore, statistically significant increased mean pupae mortality was observed in the test item at DAT 14, 16 and 18 (4.3 to 6 pupae per colony) and in the reference item treatment at DAT 10 to 21 (6.7 to 105 pupae per colony). This indicated that honey bee brood was well exposed during the test and that the test system was sensitive to detect potential brood effects of plant protection products.

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

Colony strength
The mean colony strength before treatment administration was 13600, 11317 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%, 21% and -27%, respectively and was at study termination 16617, 13750 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%, 4% 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
Selected eggs at BFD 0:
The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 41.1%, 40.2% and 85.4%, respectively.

Selected young larvae at BFD 0:
The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 7.7%, 35.5% and 43.9%, respectively.

Selected old larvae at BFD 0:
The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 5%, 31.7% and 51.8%, respectively.

Overall, the mean brood termination of the test item was at each brood assessment day statistically significantly greater for young and old larvae, whereas the selected eggs at BFD 0 were not statistically significantly different when compared to the control (Fisher's exact test, α=0.05, alternative one-sided smaller). In the reference item treatment, brood termination rate was statistically significantly higher at each brood assessment day in all selected brood stages (eggs, young and old larvae) when compared to the control (Fisher's exact test, α=0.05, alternative one-sided smaller). This indicated that the test system was sensitive to detect potential brood effects of plant protection products.

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

Selected eggs at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 2.9, 3 and 0.7, respectively.

Selected young larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.6, 3.2 and 2.8, respectively.

Selected old larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.7, 3.4 and 2.4, respectively.

Overall, the brood indices of the control and test item displayed a continuous and comparable increase, indicating a successful development of the brood. In contrast, the mean brood indices of the reference item were distinctly lower when compared to the control and was for the old larvae statistically significantly smaller at each brood assessment day when compared to the control (t-test, α=0.05, alternative one-sided greater).

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

Selected eggs at BFD 0:
The mean brood compensation index of the control, test item and reference item treatment at the last assessment (BFD 21) was 3.7, 3.4 and 1.0, respectively.

Selected young larvae at BFD 0:
The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.8, 4.2 and 2.9, respectively.

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

Overall, the brood compensation 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 for all brood staged when compared to the control. In the reference item treatment, at BFD 15 and 21 for the eggs, and at each brood assessment day for the old larvae, the brood compensation index was statistically significantly smaller when compared to the control (t-test, α=0.05, alternative one-sided greater).

Conclusions:

To assess the potential effects of iodosulfuron-methyl-sodium WG 10A on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.243 g formulated test item/L (=0.025 g iodosulfuron-methyl-sodium /L) + 0.475 g formulated herbicide safener/L (0.075 g mefenpyr-diethyl/L) per colony in summer 2012.
The administration of Iodosulfuron-methyl-sodium WG 10A + the herbicide safener mefenpyr-diethyl WG 15 W to honey colonies caused no adverse effects on behaviour, colony strength, condition of the colonies, brood index and brood compensation index. In contrast, survival of adult bees, pupae and brood development (termination rate) in all brood staged (eggs, young and old larvae) was statistically significantly increased when compared to the control treatment.
Despite of the slightly elevated mortality and termination rates in the test item treatment group, overall colony performance was normal and not impaired.

Report:	[redacted];[redacted];2014;M-477913-01
Title:	Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L): Effects on honey bee brood (Apis mellifera L.) under semi-field conditions - Tunnel test -
Report No:	79081033
Document No:	M-477913-01-1
Guidelines:	**OECD No. 75 (2007) and OEPP/EPPO No. 170 (4)(2010);The post-application exposure phase in the tunnel was reduced to 4 days due to the herbicide mode of action of the test item against the Phacelia-crop; at the end of the 4th day after application, the Phacelia-crop was no longer attractive to bees (faded) and did not longer support the confined colonies.**
GLP/GEP:	**yes**

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 maximum

rate (0.1 L) of Iodosulfuron-methyl sodium + mefenpyr-diethyl OD 400 (100+300 g/L) 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 iodosulfuron-methyl-sodium to honey bee colonies, including a very detailed assessment of brood development. Tunnels (20 m length x 5.5 m width x 2.5 m height) were set up on a ca. 75 m² plot of *Phacelia* (2 x 36 m²). Small bee colonies were introduced to the tunnels 3 days before the application. One honey bee colony was used per tunnel. The test item, water and a reference item was applied during honey bees actively foraging on the crop. The trial was carried out using four tunnels (i.e. replicates) for the test item treatment, the control and the reference item treatment (Insegar, 250 g/kg fenoxycarb), respectively. The confined exposure phase of the honey bees inside the treated crop was 4 days following the test item application. At the end of the 4th day after application, due to the herbicide mode of action of the test item, the *Phacelia*-crop was no longer attractive to bees (faded) and did not longer support the confined colonies. Thus, all bee colonies (i.e. the colonies from the test item, the water and the reference item group, respectively) were relocated after 4 complete days of confined exposure from their respective tunnels and placed in an area with no main flowering, bee attractive crops. The test item was applied under optimum foraging conditions. After foliar (spray) application of the water (control), test item (Iodosulfuron-methyl sodium + mefenpyr-diethyl OD 400 (100+300 g/L)) 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 a brood comb and taking a digital picture of the brood comb. After saving the file on a computer, 220 - 270 eggs per colony were marked at this first brood area fixing day BFD0 (BFD = Brood Area Fixing Day). For each subsequent brood assessment (BFDn), again, the respective comb was taken out of the hive and another digital photo was taken in order to investigate the progress of the brood development until day 21 following the application (BFD22 following BFD0). Statistical evaluation was done for mortality, foraging activity, colony strength and the brood termination rate using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student or Welch t- test (pairwise comparison.
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 Iodosulfuron-methyl sodium + mefenpyr-diethyl OD 400 (100+300 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate of 0.1 L product/ha (corresponding to 10 g iodosulfuron-methyl sodium a.s./ha), during honey bees actively foraging on a bee-attractive, flowering crop. The observed, characteristic brood effects of the reference item Insegar (a.s. fenoxycarb) in terms of typicality, time of 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:

Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L): iodosulfuron-methyl-sodium (AE F115008): 8.27 % w/w (93.84 g/L) (analysed), mefenpyr-diethyl (AE F107892): 26.6 % w/w (302.3 g/L) (analysed); Batch ID.: EFIT000452; Sample Description: TOX09939-00; Material No.: 06352286; Specification No.: 102000011563 - 06; density: 1.135 g/cm³ (20 °C).

Test Species:

Honey bees (*Apis mellifera carnica* L.); small bee colonies, maintained according to normal beekeeping practice, containing 11 combs with honey, pollen and brood. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply with nectar and pollen. The mean strength of the colonies per treatment group, one day before the application, was very similar and ranged between 4714 and 5018 adult bees per colony.

Test Design:

The test was conducted under forced/confined exposure conditions (tunnel), in order to assess potential effects of Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L) to honey bee colonies including brood development under semi-field conditions. Tunnels (20 m length x 5.5 m width x 2.5 m height) were set up on a ca. 75 m² plot of Phacelia tanacetifolia (2 x 36 m²). Small bee colonies were introduced to the tunnels 3 days before the application. One honey bee colony was used per tunnel.

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

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

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

Test Parameters:

- Mortality of adult bees and pupae: 2 days before to 27 days after application (= end of the trial);
- Behavioural abnormalities: 2 days before to 27 days after application (= end of the trial);
- Foraging activity of the bees: 2 days before to 4 days after application;
- Condition of the colonies (food stores, brood status and colony strength): 1 day before and 5, 9, 15, 21 and 27 days after application;
- Bee brood development (eggs): 1 day before (= BFD0) and 5 (= BFD 6), 9 (= BFD 10), 15 (= BFD 16), 21 (= BFD 22) days after the application.

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

Control: 400 L tap water/ha,
Test Item: 10 g iodosulfuron-methyl-sodium a.s./ha; 0.107 L (121 g) product in 400 L tap water/ha (corresponding to 0.303 g product/L),
Reference Item: 300 g fenoxycarb a.s. (1200 g product)/ha in 400 L spray solution/ha (corresponding to nominally 3.00 g product/L),
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 a very high honeybee foraging activity on the crop within the tunnels. Mean temperature during the whole experiment was between 12.9 and 29.1°C. First precipitation (28 mm) occurred in the night on day 2 (ca. 35 hours following the application). Thereafter, rain occurred on days 6 (13 mm), 8 (2 mm), 9 (7 mm), 10 (6 mm) and 14 (6 mm).

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

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

Results:

Mortality of the adult bees (worker bees)

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

Mortality of the pre-application phase in the control and the test item group was 24.8 and 25.8 dead bees/colony/day, respectively. The mortality in the reference item was 76.0 dead bees/colony/day. This was not statistically significantly different compared to the water control (Student t-test, pairwise comparison to the control, two-sided, $\alpha = 0.05$).

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

There was no sign of an acute effect on the mortality of the bees following the test item treatment. Average control mortality of adult bees during the exposition phase (day 0 to day 4 following the application) was 19.9 dead bees/colony/day. The average mortality in the test item group was slightly lower with 19.0 dead bees/colony/day and accordingly not statistically significant to the control values (Student t-test, pairwise comparison, one-sided greater, $\alpha = 0.05$). Reference Item mortality was 36.2 dead bees/colony/day (no statistical significant difference, Student t-test, pairwise comparison one-sided greater, $\alpha = 0.05$; Nota bene: The absence of acute effects of the reference item is in line to its mode of action).

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

An overall comparison of the mean number of dead bees found in the traps and on the gauze after the application from day 5 to day 27 did also not show a statistical significant difference between the control and the Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L) - treatment (Student t-test, pairwise comparison, one-sided greater, α = 0.05). A mean of 5.4 dead bees per day and tunnel was found for the period from day 5 to day 27 after treatment in the test item and control group, respectively.
There was no impact of the reference item to the adult bee mortality which is not to be expected due to mode of action of the reference item.

Mortality of pupae

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

Mortality of the pupae in the control, test item and reference item groups was 2.3, 0.3 and 3.4 dead pupae/colony/day, respectively. There was no statistically significant difference between the groups (Student t-test, pairwise comparison to the control, two-sided, α = 0.05).

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

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

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

Considering the period outside the tunnels, the test item treated group showed a slightly higher, but not statistically significant different pupae mortality rate compared to the control group. Pupae mortality in the reference item group was increased and statistically significant different to the control group.
Mean pupae mortality from day 5 to day 27 was 0.6 dead pupae/colony/day in the test item group and 0.4 dead pupae/colony/day in the control group. Reference item induced pupae mortality was 22.3 dead pupae/colony/day.

Foraging Activity

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

The mean foraging activity in the intended test item and reference item groups was comparable to the control group, resulting in overall daily mean values of 15.4, 18.3 and 19.6 bees/m²/day in the control, test item group and reference item groups, respectively. No statistically significant differences were found between the control, the test and reference item treatment groups at the overall daily mean comparison of this period.

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

There was a slight decrease in foraging activity after application in the test item group. Mean foraging activity on each occasion was lower compared to the control values on these days. Nevertheless, these lower flight activities were not statistically significant different (Student t-test, pair-wise comparison to the control, one-sided smaller, α = 0.05). The overall daily mean foraging activity from day 0 to day 4 in the test item group was 12.1 bees/m²/day compared to 15.7 bees/m²/day the control group.
The reference item (Insegar) resulted in no reduction of the foraging activity on the day of application and on all following days.

Behavioural abnormalities

After application of Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L) no behavioural abnormalities occurred at any time during the whole assessment period (up to day 27). No behavioural abnormalities were observed in the control group and in the reference item group.

Condition of the Colonies

At the beginning of the trial, all brood stages (eggs, larvae and closed brood), as well as a sufficient amount of nectar and pollen storage was found in all colonies as an indication of healthy colonies.
All queens and/or a sufficient presence of eggs were found in the test item treated colonies during all brood checks indicating that the queens were alive and healthy.
After application, no indication of a test item related effect on the condition of the colonies was observed. All test item treated colonies remained vital with increasing bee numbers and healthy brood. There was no indication of any hazard of the test item on the condition of the bee colonies.

Colony Strength

The mean number of honey bees per colony in all treatment groups was very similar one day before application and did not differ statistically (mean of 4714 to 5018 per colony). The subsequent development of the colony strength among the colonies in the control and test item treatment groups followed the same pattern. There was a continuous increase of colony strength observable, which was very similar in the test item group compared to the control group. 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	Day +5	Day +9	Day +15	Day +21	Day 27
Control	100%	123%	144%	159%	161%	148%
Test Item	100%	120%	130%	154%	151%	143%
Reference Item	100%	141%	152%	140%	137%	108%

Development of Bee Brood

Brood Termination Rate:

Following the assessment of single cells from the egg stage to the successfully hatched worker bee, the mean termination rate at BFD (Brood Fixing Day) 22 in the test item group was with a mean of 27.7 % lower compared to the control group (30.2 %). Accordingly, the Brood Termination Rate in the test item group was not statistically significantly different compared to the control group.

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

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 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 control. Differences in the Brood Compensation Index between test item and control were not statistically significant. The high 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 control.

Treatment Group	BFD +6	BFD +10	BFD +16	BFD +22
Control	2.7	3.5	3.5	4.4
Test Item	2.7 (n.s.)	3.1 (n.s.)	3.1 (n.s.)	4.0 (n.s.)
Reference Item	0.7 (*)	0.9 (*)	1.0 (*)	1.9 (*)

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 is an additional indicator for the bee brood development and facilitates a comparison between the different treatment groups. 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 test item and control were not statistically significant. After treatment with the reference item Insegar (a.s.: fenoxycarb), following the labelling of the eggs, the mean Brood Indices were statistically significant lower compared to the control indices.

Treatment Group	BFD +6	BFD +10	BFD +16	BFD +22
Control	2.6	3.3	3.3	4.1
Test Item	2.6 (n.s.)	3.0 (n.s.)	2.9 (n.s.)	3.6 (n.s.)
Reference Item	0.7 (*)	0.8 (*)	0.7 (*)	0.9 (*)

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- 2: Effects of Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L) on honey bee brood under semi-field conditions (Tunnel Test)

Parameter	Treatment group[1)]		
	Control	Test Item [0.1 L/ha]	Reference Item Insegar [0.3 kg a.i./ha]
Mean mortality of worker bees / colony / day [%] during			
pre-application phase [2)]	24.8 ± 11.6	25.8 ± 10.1 (n.s.)	76.0 ± 50.7 (n.s.)
exposure phase in the tunnels [2)]	19.9 ± 17.6	19.0 ± 10.8 (n.s.)	36.2 ± 16.9 (n.s.)
phase outside the tunnels [3)]	5.4 ± 4.9	5.4 ± 7.4 (n.s.)	6.8 ± 8.7 (n.s.)
overall after application	8.0 ± 9.9	7.9 ± 9.5 (n.s.)	12.0 ± 15.4 (n.s.)
Mean mortality of larvae and pupae [n] during			
pre-application phase [4)]	2.3 ± 2.7	0.3 ± 0.3 (n.s.)	3.4 ± 2.1 (n.s.)
exposure phase in the tunnels [4)]	0.8 ± 0.8	0.5 ± 0.7 (n.s.)	5.3 ± 3.7 (*)
phase outside the tunnels [5)]	0.4 ± 0.7	0.6 ± 1.2 (n.s.)	22.3 ± 28.6 (*)
overall after application	0.5 ± 0.7	0.6 ± 1.1 (n.s.)	19.3 ± 26.7 (*)
Mean foraging activity / m² / colony / day [n] during			
pre-application phase	15.4 ± 6.7	18.3 ± 5.2 (n.s.)	19.6 ± 7.2 (n.s.)
exposure phase in the tunnels	15.7 ± 5.3	12.1 ± 7.4 (n.s.)	16.3 ± 6.6 (n.s.)
Mean brood termination rate [%] [6)]	30.2	27.7 (n.s.)	82.3 (*)

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

Conclusions:

To assess the potential effects of Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L) on honey bee colonies including brood development, 0.107 L product in 400 L tap water/ha (corresponding to 10 g iodosulfuron-methyl-sodium a.s./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 Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate of 0.107 L product in 400 L tap water/ha (corresponding to 10 g iodosulfuron-methyl-sodium a.s./ha), during honey bees actively foraging on a bee-attractive, flowering crop. The observed, characteristic brood effects of the reference item Insegar (a.s. fenoxycarb) in terms of typicality, time of 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

For the representative formulation IMS + MPR OD 400 toxicity studies on the sensitive standard species *Typhlodromus pyri* and *Aphidius rhopalosiphi* were performed. Under laboratory conditions IMS + MPR OD 400 had only low effects on the mortality of *Typhlodromus pyri* and *Aphidius rhopalosiphi* (see section CA 8.3.2.1 and CA 8.3.2.2). The reproductive capacity of *Typhlodromus pyri* and *Aphidius rhopalosiphi* was not statistically significantly reduced up to the highest rate tested compared to the control. These studies showed that IMS + MPR OD 400 had no or only low effects on mortality and reproduction of these tested species. Details of the studies are provided in the table below.

Table CA 8.3.2-1: Toxicity data of iodosulfuron-methyl-sodium, formulated as OD 400, to non-target arthropods other than bees

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint		Reference Dossier-file-No.
Aphidius rhopalosiphi	IMS + MPR OD 400	LR_{50} > 300 mL prod./ha		[redacted] & [redacted], 2004
		Corr. Mortality [%]	Effect on Reproduction [%]	C039343
	Laboratory, glass plates			M-226797-01-1
	3.7 mL prod./ha	2.6	29.2	KCA 8.3.2.1 /03
	11.1 mL prod./ha	-2.6[A]	0.6	
	33.3 mL prod./ha	-2.6[A]	13.3	
	100.0 mL prod./ha	-2.6[A]	20.8	
	300.0 mL prod./ha	2.6	18.1	
Typhlodromus pyri	IMS + MPR OD 400	LR_{50} > 300 mL prod./ha		[redacted], 2004
		Corr. Mortality [%]	Effect on Reproduction [%]	C039089
	Laboratory, glass plates			M-226371-01-1
	3.7 mL prod./ha	5.0	-11.5[B]	KCA 8.3.2.2 /03
	11.1 mL prod./ha	11.7	5.1	
	33.3 mL prod./ha	10.0	15.4	
	100.0 mL prod./ha	10.0	26.9	
	300.0 mL prod./ha	13.3	11.5	

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

Report:	[redacted];[redacted];1997;M-142850-01
Title:	Toxicity to the ground dwelling predator (Poecilus cupreus L. Coleoptera, Carabidae) in the laboratory Code: AE F115008 02 WG20 B002
Report No:	A59158, CW97/018
Document No:	M-142850-01-1
Guidelines:	**BBA: VI 23 - 2.1.8;Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];1997;M-142891-01
Title:	Toxicity to the foliage dwelling predator (Chrysoperla carnea Steph. Neuroptera, Chrysopidae) in the laboratory Code: AE F115008 02 WG20 B002
Report No:	A59199, CW97/019
Document No:	M-142891-01-1
Guidelines:	**IOBC: (1984);Deviation not specified**
GLP/GEP:	**yes**

The endpoint of these studies, although listed in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final), is only relevant for the tested WG formulation.
No conclusion regarding ecotoxicological properties of the active substance itself or the representative formulation IMS + MPR OD 400 can be drawn from this study.

CA 8.3.2.1 - Effects on *Aphidius rhopalosiphi*

Report:	[redacted];[redacted];1997;M-142904-01
Title:	Acute toxicity to the aphid parasitoid (Aphidius rhopalosiphi Hymenoptera, Braconidae) in the laboratory Code : AE F115008 02 WG20 B002
Report No:	A59212, 97093/01-NLAp
Document No:	M-142904-01-1
Guidelines:	**IOBC: ;Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];1998;M-181805-01
Title:	Side-effects on the aphid parasitoid, Aphidius spp. (Hymenoptera, Aphidiidae) using and extended laboratory test Code: AE F115008 02 WG20 B002
Report No:	C001110, 97053/01-NEAp
Document No:	M-181805-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

The endpoint of these studies, although listed in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final), is only relevant for the tested WG formulation.
No conclusion regarding ecotoxicological properties of the active substance itself or the representative formulation IMS + MPR OD 400 can be drawn from this study.

Report:	[redacted];[redacted];[redacted];2004;M-226797-01
Title:	Effects of AE F115008 02 OD35 A202 on the parasitoid Aphidius rhopalosiphi in the laboratory - dose response test -
Report No:	C039343
Document No:	M-226797-01-1
Guidelines:	**IOBC: WPRS 2000;Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to produce a concentration-response curve for mortality effects seen over 48 h of exposure. Adult *Aphidius rhopalosiphi* (approximately 48 h old; 7 females and 3 males per replicate) were exposed on glass plates to application rates of 3.7, 11.1, 33.3, 100 and 300 ml product/ha (diluted in 200 L deionized water/ha) and were compared to those of deionized water treated controls (200 L/ha). Perfekthion (0.3 mL product/ha diluted in 200 L deionized water/ha) was

used as reference treatment. The duration of the mortality part was 48 hours. The reproductive performance of the survivors was examined for another 24 hour period using females from the control and from those test item concentrations where corrected mortality was < 50.0 %.
Under laboratory conditions the LR_{50} could not be calculated. It is estimated to be higher than 300 mL product/ha. The reproductive capacity of *A. rhopalosiphi* was not statistically significantly reduced up to 300 mL product/ha (the highest rate tested) compared to the control. All validity criteria according to the guideline were met.

Materials and Methods:

Test item. AE F115008 02 OD35 A202 (code for: AMS + MPR OD 400) active ingredients: AE F107892, content: 26.0 % w/w, AE F115008, content: 8.82 % w/w; Batch No.: AAIM01665; Density: 1.144 g/mL; Certificate of Analysis Ref. Code: AZ 11073.

Under laboratory conditions approximately 48 h old adult *Aphidius rhopalosiphi* (7 females and 3 males per replicate) were exposed to dried spray deposits of 3.7, 11.1, 33.3, 100 and 300 mL product/ha in 200 L deionised water/ha (corresponding to 0.0212, 0.0635, 0.190, 0.572 and 1.72 g product/L) on glass surfaces (4 replicates per treatment group). Deionised water was used as a control treatment and Perfekthion (0.3 ml product/ha diluted in 200 L deionised water/ha, containing nominally 400 g dimethoate/L) as a reference treatment. The duration of the mortality part was 48 hours. The reproductive performance of the survivors was examined for another 24 hour period using females from the control and from those test item concentrations where corrected mortality was < 50.0 %.

Toxic standard: Perfekthion (containing nominally (analysed) 400 g (401.2 g) dimethoate/L): 0.3 mL in 200 L deionised water/ha (corresponding to 1.5 µL Perfekthion/L); control: deionised water only (200 L/ha).

Dates of work: November 17, 2003 – December 15, 2003

Results:

Table CA 8.3.2.1-1: Validity criteria

Validity criteria	Recommended	Obtained
Control mortality	≤ 13 %	2.5 %
Control reproduction rate	≥ 5 mummies per female	36.6 mummies per female (mean value)
	≥ 2 parasitoids producing zero values	1 parasitoid producing zero values
Toxic standard mortality	≥ 50 %	100 %

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

Table CA 8.3.2.1-2: Effects on mortality and parasitisation efficiency of *Aphidius rhopalosiphi*, laboratory testing-dose response test

Test item	AE F115008 02 OD35 A202 (IMS + MPR OD 400)					
Test organism	*Aphidius rhopalosiphi*					
Exposure on	treated glass surfaces					
Treatment	**Mortality after 48 h[a] [%]**		**Corrected mortality after 48 h [%]**	**Mummies per female[b]**		**Reduction of parasitisation efficiency relative to the control [%]**
Control	2.5		-	36.6		-
3.7 mL product/ha	5.0	n.s.	2.6	25.9	n.s.	29.2
11.1 mL product/ha	0.0	n.s.	-2.6	36.3	n.s.	0.6
33.3 mL product/ha	0.0	n.s.	-2.6	31.7	n.s.	13.3
100 mL product/ha	0.0	n.s.	-2.6	28.9	n.s.	20.8
300 mL product/ha	5.0	n.s.	2.6	29.9	n.s.	18.1
0.3 mL Perfekthion/ha (Toxic reference)	100.0	*	100.0	n.a.		-

[a] n.s. = not significant, * = significant; Fisher Exact Test, α = 0.05
[b] n.s. = not significant; Dunnett-Test, α = 0.05
n.a. = not assessed

Conclusions:

Under laboratory conditions the LR_{50} could not be calculated due to the low effects of AE F115008 02 OD35 A202 (IMS + MPR OD 400). It is estimated to be higher than 300 mL product/ha.
The reproductive capacity of *A. rhopalosiphi* was not statistically significantly reduced up to 300 mL product/ha (the highest rate tested) compared to the control.

CA 8.3.2.2 - Effects on *Typhlodromus pyri*

Report:	[redacted];[redacted];1997;M-142912-01
Title:	Acute toxicity to the predatory mite (Typhlodromus pyri Scheuten Acari, Phytoseiidae) in the laboratory Code: AE F115008 02 WG20 B002
Report No:	A59222
Document No:	M-142912-01-1
Guidelines:	**IOBC:;Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];1998;M-180602-01
Title:	Acute toxicity to the predatory mite, Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory (addendum) Code: A EF115008 02 WG20 B002
Report No:	C000403
Document No:	M-180602-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoints of these studies, although listed in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final), is only relevant for the tested WG formulation.
No conclusion regarding ecotoxicological properties of the active substance itself or the representative formulation IMS + MPR OD 400 can be drawn from this study.

Report:	[redacted];[redacted];2004;M-226371-01
Title:	Effects of AE F115008 02 OD35 A202 on the predatory mite Typhlodromus pyri in the laboratory - dose response test
Report No:	C039079
Document No:	M-226371-01-1
Guidelines:	**EU (=EEC): Bluemel et al. (2000);Deviation not specified**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to produce a dose response curve for mortality effects seen after 7 days of exposure. Mites were exposed on glass plates to application rates of 3.7, 11.1, 33.3, 100 and 300 ml product/ha (diluted in 200 L deionized water/ha) and were compared to those of deionized water treated controls (200 L/ha). Perfekthion (10 mL product/ha diluted in 200 L deionized water/ha) was used as reference treatment. Assessment of the number of living, escaped and dead mites was conducted 2 and 7 days after application. For the reproduction assessment surviving mites from the control and from all test item groups displaying less than 50 % corrected mortality were sexed and the number of eggs per females was recorded at 4 assessment days within one week. Under worst case laboratory conditions the LR_{50} for *Typhlodromus pyri* was estimated to be > 300 mL product/ha in 200 L deionized water/ha. The reproduction of *Typhlodromus pyri* was not affected up to 300 ml product/ha in 200 L deionized water/ha. All validity criteria according to the guideline were met.

Materials and Methods:

Test item. AE F115008 02 OD35 A202 (code for IMS + MPR OD 400); active ingredients: AE F107892, content: 26.0 % w/w, AE F115008, content: 8.82 % w/w; Batch No.: AAIM01665; Density: 1.144 g/mL; Certificate of Analysis Ref. Code: AZ 11073.

Protonymphs (< 24 hours old) of *Typhlodromus pyri* (20 mites per replicate) were exposed to air dried spray deposits of 3.7, 11.1, 33.3, 100 and 300 mL product/ha in 200 L deionised water/ha (corresponding to 0.0212, 0.0635, 0.190, 0.572 and 1.72 g product/L) on glass plates (3 replicates per treatment group) under laboratory conditions. Deionised water (200 L/ha) was used as a control treatment and Perfekthion (10 mL product/ha diluted in 200 L deionised water/ha, containing nominally 400 g dimethoate/L) as a reference treatment. Initial evaluation of the test item was conducted in a range finding test. Based on these results a main test was designed. Assessment of the number of living, escaped and dead mites was conducted 2 and 7 days after application. For the reproduction assessment surviving mites from the control and from all test item groups displaying less than 50 % corrected mortality were sexed and the number of eggs per females was recorded at 4 assessment days within one week. The toxic standard treatment caused a 100 % corrected mortality.

Toxic standard: Perfekthion (containing nominally (analysed) 400 g (401.2 g) dimethoate/L): 10 mL in 200 L deionised water/ha (corresponding to 50 µL Perfekthionin/L); control: deionised water only (200 L/ha).

Dates of work: December 02, 2003 – December 16, 2003

Results:

Table CA 8.3.2.2-1: Validity criteria

Validity criteria	Recommended	Obtained
Control mortality	≤ 20 %	0 %
Control reproduction: Number of eggs per female for the second week	≥ 4 eggs	7.8 eggs
Toxic standard mortality (control corrected) at day 7 after test initiation	> 50 % (preferably < 100 %)	100 %

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

Mortality

There were no significant differences compared to the control up to 3.7 ml product/ha (Fisher Exact Test, a = 0.05). Significantly increased mortality to the control was observed at 11.1 mL up to and including 300 mL product/ha. The statistically significant differences occurring at these rates are not considered to be a test item effect, because mortality was below 20 % and is thus within the limit of the accepted range for the control mortality. The LR_{50} value could not be calculated due to the low effects of the product for the tested rates. Therefore, the LR_{50} was determined to be: LR_{50} > 300 mL AE F115008 02 OD35 A202/ha, the highest rate tested in the study. No abnormal behaviour or conditions were observed with the surviving mites.

Reproduction

There were no significant differences compared to the control in all rates tested (Bonferroni t-test (inhomog. Var.), a = 0.05).

Table CA 8.3.2.2-2: Effects on mortality and reproduction of *Typhlodromus pyri*, laboratory testing-dose response test

Test item	AE F115008 02 OD35 A202 (IMS + MPR OD 400)					
Test organism	*Typhlodromus pyri*					
Exposure on	Dried spray deposits on glass plates					
Treatment	**Mortality[a] [%]**		**Corrected mortality [%]**	**Reproduction[b] [eggs/female]**		**Effect on reproduction[c] [%]**
Control	0.0			7.8		
3.7 mL product/ha	5.0	n.s.	5.0	8.7	n.s.	-11.5
11.1 mL product/ha	11.7	*	11.7	7.4	n.s.	5.1
33.3 mL product/ha	10.0	*	10.0	6.6	n.s.	15.4
100 mL product/ha	10.0	*	10.0	5.7	n.s.	26.9
300 mL product/ha	13.3	*	13.3	6.9	n.s.	11.5
10 mL Perfekthion/ha (Toxic reference)	100	*	100	n.a.		n.a.
LR_{50}	**> 300 mL product/ha**					

[a] n.s. = not significant, * = significant; Fisher Exact Test, α = 0.05
[b] n.s. = not significant; Bonferroni-t-Test (inhomog. Var.), α = 0.05
[c] negative value indicates increased reproduction compared to the control
n.a. not applicable

Conclusions:

Under worst case laboratory conditions the LR_{50} of AE F115008 02 OD35 A202 (IMS + MPR OD 400) on artificial substrate (glass) on *Typhlodromus pyri* was determined to be LR_{50} > 300 ml

product/ha in 200 L deionised water/ha. The reproduction of *T. pyri* was not affected up to 300 mL AE F115008 02 OD35 A202/ha in 200 L deionised water/ha.

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. Three acute studies on the active substance and the metabolites AE F075736 (metsulfuron-methyl) and AE F059411 were submitted and reviewed for the first inclusion in Annex I. All these studies are added here.

Iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1998;M-143093-01
Title:	Acute toxicity to earthworms (Eisenia fetida) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59420, CE96/096
Document No:	M-143093-01-1
Guidelines:	**OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

$LC_{50} > 1000$ mg/kg

AE F075736

Report:	[redacted];[redacted];1998;M-181879-01
Title:	Acute toxicity to earthworms (Eisenia fetida) AE F075736 (metsulfuron-methyl) substance, technical metabolite of AE F115008 Code: AE F075736 00 1C92 0001
Report No:	C001153, CE98/091
Document No:	M-181879-01-1
Guidelines:	**EU (=EEC): 92/69/EWG; OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

$LC_{50} > 1000$ mg/kg

AE F059411

Report:	[redacted];[redacted];1998;M-181872-01
Title:	Acute toxicity to earthworms (Eisenia fetida) AE F059411 substance, technical metabolite of AE F115008 Code: AE F059411 00 1C99 0001
Report No:	C001150, CE98/086
Document No:	M-181872-01-1
Guidelines:	**EU (=EEC): 92/69/EWG; OECD: 207;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

$LC_{50} > 1000$ mg/kg

CA 8.4.1 - Earthworm, sub-lethal effects

For iodosulfuron-methyl-sodium and its metabolites AE F075736, AE F145741, AE F145740, AE 0002166, BCS-CW81253, AE F059411 and AE 0000119 reproductive toxicity studies on *Eisenia fetida* were performed. No-Observable-Effect levels ranged from 0.216 mg/kg dws for the metabolite AE F075736 to ≥ 100 mg/kg dws for the metabolites AE F145741, AE F145740, AE 002166, BCS-CW81253 and AE 0000119. No soil studies were performed for earthworms for the metabolite AE F161778, as data for earthworms from AE F145741 as preceding metabolite and data from BCS-CW81253 as succeeding metabolite are available and do not show any toxicity (NOEC ≥100 mg/kg dws).
Details of all studies are provided in the following table.

Table CA 8.4.1-1: Reproductive toxicity data of iodosulfuron-methyl-sodium and metabolites to *Eisenia fetida* presented in this chapter

Test substance	Test species, Test design	Endpoint	Reference
Iodosulfuron-methyl-sodium	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **9.3** **mg a.s./kg dws**[1)]	[redacted] & [redacted], 2010 10P29RR M-397577-01-1 KCA 8.4.1 /02
AE F075736	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item sprayed on soil surface	NOEC **≥50** **g/ha** **≥0.216** **mg/kg dws**[2)]	[redacted], 1998 GE98/092 M-182339-01-1 KCA 8.4.1 /01
AE F145741	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100** **mg/kg dws**	[redacted], 2013 82101022 M-457891-01-1 KCA 8.4.1 /03
AE F145740	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100** **mg/kg dws**	[redacted], 2013 82091022 M-457334-01-1 KCA 8.4.1 /04
AE 0002166	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100** **mg/kg dws**	[redacted], 2013 82111022 M-457338-01-1 KCA 8.4.1 /05
BCS-CW81253	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100** **mg/kg dws**	[redacted], 2013 13 10 48 091 S M-462824-01-1 KCA 8.4.1 /06
AE 0000119	*Eisenia fetida* reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC **≥100** **mg/kg dws**	[redacted], 2011 LRT-RG-R-104/11 M-404685-01-1 KCA 8.4.1 /07

Test substance	Test species, Test design	Endpoint	Reference
AE F059411	*Eisenia fetida* reproduction, 56 d, (5% peat in test soil)	NOEC **30** **mg/kg dws**	[redacted] (2011) LRT-RG-R-100/11 M-410930-01-1 KCA 8.4.1/08

[1] corrected to an analysed purity of 93.0%
[2] No observed effects at 10 g/ha and 50 g/ha; conversion from g/ha to mg as/kg dws with the following assumptions: calculated based on actual test rate, analysed purity of 92.2%, test vessel surface of 283.4 cm2 and test substrate of 850 g wet weight with moisture content of 28.8% per test vessel
dws = dry weight soil
Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];[redacted];2010;M-397577-01
Title:	Iodosulfuron-methyl-sodium: Reproduction toxicity to the earthworm *Eisenia fetida* in artificial soil test.
Report No:	10P29RR
Document No.:	M-397577-01-1
Guidelines:	**OECD Guideline 222 (2004); ISO 11268-2 Part 2 (1998); Deviations in the first and second test run**
GLP:	**Yes (certified laboratory)**

Executive summary:

The purpose of this study was to determine a NOEC/LOEC for the effects of the test item iodosulfuron-methyl-sodium on the reproduction (56 days after application) and the biomass development (28 days after application) of the earthworm *Eisenia fetida* (Lumbricidae) by dermal and alimentary uptake using a standardised artificial soil. Ten *Eisenia fetida* (clitellate adults) per replicate (8 for the control, 4 per test item concentration) were exposed to Iodosulfuron-methyl-sodium for 28 days at nominal conentrations of 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight (dw) (1st test run) and of 10, 18, 32, 56 and 100 mg test item/kg soil (dw) (2nd test run). As deviations from the guideline at the first test run, the soil moisture was at the end of the test at three treatments slightly higher than required by the guideline. At the end of the test of the second test run the soil moisture was not determined at the concentration of 18 mg test item/kg artificial soil (dw) and was slightly higher than required by the guideline at four treatments. At the end of the test the pH-value was in all treatment higher than required by the guideline. However, study results of the test have not been impacted. The duration of the exposure period (exposure of earthworms to the artificial soil containing the test item) was 56 days of each test run. The adult worms were removed from the substrate after 28 days.
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.

The following endpoints can be derived from the both test runs:
$NOEC_{Biomass} \geq 1000$ mg test item/kg soil (dw).
$LOEC_{Biomass} > 1000$ mg test item/kg soil (dw).
$NOEC_{Reproduction} = 10$ mg test item/kg soil (dw).
$LOEC_{Reproduction} = 18$ mg test item/kg soil (dw).

Material and Methods:

Test item. Iodosulfuron-methyl-sodium; CAS No.: 144550-36-7; Batch code: AE F115008-01-03; Origin Batch No.: ELIR003050; Sample description: TOX 08879-00; Specification No.: 102000000739, Purity: 93.0 % w/w; Certificate of Analysis No.: AZ 16393.

Ten *Eisenia fetida* (clitellate adults) per replicate (8 for the control, 4 per test item concentration) were exposed to Iodosulfuron-methyl-sodium for 28 days at nominal concentrations of 63, 125, 250, 500, and 1000 mg test item/kg soil (dw) (1st test run) and of 10, 18, 32, 56 and 100 mg test item/kg soil (dw) (2nd test run) artificial soil at 19.1 – 21.0 °C and 431 - 636 lx (1st test run) and at 1CA 8.9 – 20.8 °C and 517 - 710 lx (2nd test run). 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.

Dates of experimental work: July 19, 2010 – September 15, 2010 (1st test run)
September 22, 2010 – November 19, 2010 (2nd test run)

Results:

Validity criteria:

Table CA 8.4.1-2: Validity Criteria

First test run	Required	Obtained
Mortality of the adult test animals in the control	≤ 10 %	2.5 %
Number of juveniles per control replicate	≥ 30	337 - 431
Coefficient of variation for the number of juveniles in the control	≤ 30 %	CA 8.0 %

Second test run	Required	Obtained
Mortality of the adult test animals in the control	≤ 10 %	1.25 %
Number of juveniles per control replicate	≥ 30	224 - 310
Coefficient of variation for the number of juveniles in the control	≤ 30 %	13.1 %

The data provide evidence that the validity criteria have been fulfilled.

First test run:

All concentrations tested were based on the basis of the analysed content of the test item Iodosulfuron-methyl-sodium and were corrected for the purity of 93.0% (w/w).
At the control 2.5% mortality was observed. No mortality was observed at all concentrations of the test item tested except at the concentration of 63 mg test item/kg soil (dw) in which 5% mortality was determined.
Concerning the biomass of the adult worms after 28 days statistical analysis showed a statistically significant biomass increase (Williams t-test; 2-sided, $p \leq 0.05$) at the lowest concentration (i.e. 63 mg test item/kg soil (dw)) of the test item tested compared to the control.
Since the statistically significant biomass increase compared to the control was observed for the lowest concentration tested only (i.e. not related to the test item concentrations) the $NOEC_{Biomass}$ was

considered to be ≥ 1000 mg test item/kg soil (dw) and accordingly, the $LOEC_{Biomass}$ was regarded as > 1000 mg test item/kg soil (dw).
Concerning the number of juveniles statistical analysis (Williams t-test; 1-sided, $p \leq 0.05$) showed a significant difference between the control and all concentrations of the test item tested.
Therefore, the $NOEC_{Reproduction}$ could not be determined and was regarded as < 63 mg test item/kg soil (dw) and the $LOEC_{Reproduction}$ was considered to be ≤ 63 mg test item/kg soil (dw).

Table CA 8.4.1-3: Effects of Iodosulfuron-methyl-sodium on Mortality, Biomass and Reproduction of *Eisenia fetida* in the first test run

Concentration [mg test item/kg soil (dw)]	Adult mortality [%]	Biomass* [% of initial weight]	Number of Juveniles** [% of Control]
Control	2.5	176.0	100.0
63	5.0	189.9##	87.0#
125	0.0	170.6	6CA 8.6#
250	0.0	179.0	64.4#
500	0.0	176.1	57.8#
1000	0.0	175.1	41.4#
LC_{50}/EC_{50} [mg test item/kg soil (dw)] NOEC [mg test item/kg soil (dw)] LOEC [mg test item/kg soil (dw)]	- - -	- ≥ 1000 > 1000	- < 63 ≤ 63

not applicable;
* After 28 days of exposure
** After 56 days of exposure
Significantly different to control (Williams t-t-test; 2-sided, $p \leq 0.05$)
Significantly different to control (Williams t-test; 1-sided, $p \leq 0.05$)

Since the no observed effect concentration for reproduction could not be determined a second test run using a lower concentrations series was initiated.

Second test run:
All concentrations tested were based on the basis of the analysed content of the test item Iodosulfuron-methyl-sodium and were corrected for the purity of 93.0% (w/w).
At the control 1,25% mortality was observed. No mortality was observed at the concentrations of 10 and 100 mg test item/kg soil (dw) and 2,5% mortality were observed at the concentrations of 18, 32 and 56 mg test item/kg soil (dw).
Concerning the biomass of the adult worms after 28 days statistical analysis showed a statistically significant biomass increase (Williams t-test; 2-sided, $p \leq 0.05$) at the two lowest concentrations (i.e. 10 and 18 mg test item/kg soil (dw)) of the test item tested compared to the control.
Since the statistically significant biomass increase compared to the control was observed for the two lowest concentrations tested only (i.e. not related to the test item concentrations) the $NOEC_{Biomass}$ was considered to be ≥ 100 mg test item/kg soil (dw) and accordingly, the $LOEC_{Biomass}$ was regarded as > 100 mg test item/kg soil (dw).
Concerning the number of juveniles statistical analysis (Williams t-test; 1-sided, $p \leq 0.05$) showed a significant difference between the control and the concentrations of 18, 32, 56 and 100 mg test item/kg soil (dw).
Therefore, the $NOEC_{Reproduction}$ was determined as 10 mg test item/kg soil (dw) and accordingly, the $LOEC_{Reproduction}$ was determined as 18 mg test item/kg soil (dw).

Table CA 8.4.1-4: Effects of Iodosulfuron-methyl-sodium on Mortality, Biomass and Reproduction of *Eisenia fetida* in the second test run

Concentration [mg test item/kg soil (dw)]	Adult mortality [%]	Biomass* [% of initial weight]	Number of Juveniles** [% of Control]
Control	1.3	145.5	100.0
10	0.0	15CA 8.2##	90.9
18	2.5	160.5##	76.7#
32	2.5	154.6	67.1#
56	2.5	153.9	60.4#
100	0.0	149.7	50.1#
LC_{50}/EC_{50} [mg test item/kg soil (dw)]	-	-	-
NOEC [mg test item/kg soil (dw)]	-	≥ 100	10
LOEC [mg test item/kg soil (dw)]	-	> 100	18

not applicable;
* After 28 days of exposure
** After 56 days of exposure
Significantly different to control (Williams t-test; 2-sided, p ≤ 0.05).
Significantly different to control (Williams t-test; 1-sided, p ≤ 0.05).

The $LOEC_{Reproduction}$ value for Carbendazim tested as a reference item was 3.0 mg a.s./kg artificial soil (dw). The effects of Carbendazim confirm suitable sensitivity of the test system.

Conclusions:

Overall conclusions for both test runs:
$NOEC_{Biomass}$ ≥ 1000 mg test item/kg soil (dw).
$LOEC_{Biomass}$ > 1000 mg test item/kg soil (dw).
$NOEC_{Reproduction}$ = 10 mg test item/kg soil (dw).
$LOEC_{Reproduction}$ = 18 mg test item/kg soil (dw).

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F075736

Report:	[redacted];1998;M-182339-01
Title:	Effects on growth and reproduction of earthworms (Eisenia fetida) AE F075736 (metsulfuron-methyl) metabolite of AE F115008 substance technical Code: AE F075736 00 1C92 0001
Report No:	C001312, CE98/092
Document No:	M-182339-01-1
Guidelines:	**BBA: VI, 2-2 (1994);Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

NOEC 50 g/ha (corresponds to 0.216 mg/kg)

AE F145741

Report:	[redacted];[redacted];2013;M-457891-01
Title:	Iodosulfuron-methyl-sodium- AE F145741: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	82101022
Document No:	M-457891-01-1
Guidelines:	**OECD, Guideline for the testing of chemicals Nr. 222 "Earthworm, Reproduction Test" (adopted April 13, 2004); ISO-Guideline 11268-2, "Soil quality - Effects of pollutants on earthworm (Eisenia fetida) - Part 2: "Determination of effects on reproduction", International Organization for Standardization, 1998;none**
GLP/GEP:	**yes**

Executive summary:

The purpose of this study was to investigate the effects of AE F145740 (metabolite of Iodosulfuron-methyl-sodium) on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida.*

Adults of *Eisenia fetida* (with clitellum and weight range 304 to 598 mg, 11 to 12 months old) were exposed in artificial soil (with 10 % peat) to an untreated control and to the test concentration of 100 mg metabolite/kg soil dry weight (equivalent to the nominal concentration of 106 mg test item kg soil dry weight) in a 56-day test. The test item was incorporated into the soil. Eight replicates with ten worms each were used for the test item treatment and for the untreated control. For the control the same amount of quartz sand as in the test item treated groups was added and moistened with deionised water. After 28 days of exposure in treated artificial soil the adult worm mortality, behavioural effects and biomass development were assessed. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).

The No Observed Effect Concentration (NOEC) for mortality, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥100 mg test item/kg soil dry weight. The No Adverse Observed Effect Concentration (NOAEC) for growth was determined to be ≥100 mg metabolite/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) was determined to be >100 mg test item/kg soil dry weight.

Material and Methods:

Test item: AE F145741; BCS-Code: BCS-AU71532; Origin Batch No.: 25398-52; Batch code: AE F145741 00 1C94 0001; purity: 94.4% w/w; Certificate No.: AZ 16823.

Adult *Eisenia fetida* (with clitellum and weight range 302 to 589 mg, 11 to 12 months old, from an in-house culture; 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to an untreated control and to the nominal test concentration of 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days exposure of adult worms in treated artificial soil the mortality, behavioural effects and biomass development was carried out. After additional 28 days the reproduction rate (number of offspring) was assessed (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). Mortality, weight change, feeding activity and reproduction rate were determined as endpoints. The artificial soil contained 69.6 % fine quartz sand, 20 % kaolin clay, 10 % sphagnum peat, air dried and finely ground, and 0.4 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5 according to OECD 222; the pH was 5.8 to 5.9 at experimental start

and 5.9 to 6.3 at experimental end; the water content at experimental start was 30.6 % to 30.8 % (54.7 % to 55.0 % of the maximum water holding capacity) and at experimental end 32.7 % to 34.2 % (58.5 % to 61.1 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux.

Toxic standard (Luxan Carbendazim 500 FC): 0.57 – 0.87 – 1.30 – 1.96 – 2.91 mg a.s./kg soil dry weight (corresponds to 1.3 – 2.0 – 3.0 – 4.5 – 6.7 mg test item/ kg soil dry weight); control: same amount of quartz sand as in the test item treated groups moistened with deionised water, solvent control: none.

Dates of experimental work: March 21, 2013 to May 17, 2013

Results:

Validity criteria:

Table CA 8.4.1-5: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10%	0%
Reproduction of Control	≥ 30	215 - 301
Coefficient of variance of reproduction in the control	≤ 30%	11.0%

All study validity criteria were met.

Reference Item Test:
In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON P Number 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil dry weight and higher. The EC_{10}, EC_{20} and EC_{50} (reproduction) were calculated to be 1.2, 1.4 or 1.7 mg a.s./kg artificial soil dry weight.

Mortality:
No mortality was observed in any treatment group.

Weight change:
The body weight change of the earthworms after 4 weeks exposure to AE F145741 was statistically significantly different compared to the control at the single test item concentration of 100 mg metabolite/kg soil dry weight (Student t-test, $\alpha = 0.05$, two-sided). However, the body weight increase in the test item treated group was at a level, which is usual for weight changes in the control and is therefore not considered to be an adverse effect.

Reproduction:
The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg metabolite/kg soil dry weight (Student t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in the test item treated group was comparable to the control (see table below).

Table CA 8.4.1-6: AE F145741: Effects on earthworms (*Eisenia fetida*) in a 56-day reproduction study

AE F145741 [mg metabolite/kg soil dry weight]	Control	100
Mortality (day 28) [%]	0.0	0.0
Significance	-	-
Weight change (day 28) [%]	30.6	37.0
Significance [1)]	-	*
Mean No. of juveniles (day 56)	253.6	249.8
Significance [1)]	-	n.s.
Reproduction in [%] of control (day 56)	-	98.5
Food consumption [g]	25.0	25.0
	Endpoints [mg metabolite/kg soil dry weight]	
NOEC (day 28 mortality)	≥100	
NOAEC (day 28 weight)	≥100	
NOEC (day 56 reproduction)	≥100	
LOEC (day 56 reproduction)	>100	

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

Conclusions:

In an earthworm reproduction and growth study with AE F145741 the No Observed Effect Concentration (NOEC) for mortality, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥100 mg metabolite/kg soil dry weight.
The No Adverse Observed Effect Concentration (NOAEC) for growth was determined to be ≥100 mg metabolite/kg soil dry weight.
The Lowest Observed Effect Concentration (LOEC) or reproduction was determined to be >100 mg metabolite/kg soil dry weight.

AE F145740

Report:	[redacted];[redacted];2013;M-457334-01
Title:	Iodosulfuron-methyl-sodium- AE F145740: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	82091022
Document No:	M-457334-01-1
Guidelines:	**OECD, Guideline for the testing of chemicals Nr. 222 "Earthworm, Reproduction Test" (adopted April 13, 2004); ISO-Guideline 11268-2, "Soil quality - Effects of pollutants on earthworm (Eisenia fetida) - Part 2: "Determination of effects on reproduction", International Organization for Standardization, 1998;none**
GLP/GEP:	**yes**

Executive summary:

The purpose of this study was to investigate the effects of AE F145740 (metabolite of iodosulfuron-methyl-sodium) on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida.*
Adults of *Eisenia fetida* (with clitellum and weight range 302 to 589 mg, 11 to 12 months old) were exposed in artificial soil (with 10 % peat control) to the nominal test concentration of 100 mg test

item/kg soil dry weight in a 56-day test. The test item was incorporated into the soil. Eight replicates with ten worms each were used for the test item treatment and for the untreated control. For the control the same amount of quartz sand as in the test item treated groups was added and moistened with deionised water. After 28 days of exposure in treated artificial soil the adult worm mortality, behavioural effects and biomass development were assessed. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).
The No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* 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.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE F145740; Synonym: BCS-AU71533; customer Order No.: TOX-No.: 09988-00; Batch code: AE F145740-PU-02; Origin batch No.: GSE 61082-3-3; purity: 97.5% w/w; Certificate No.: AZ 18523.

Adult *Eisenia fetida* (with clitellum and weight range 302 to 589 mg, 11 to 12 months old, from an in-house culture; 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to an untreated control and to the nominal test concentration of 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days exposure of adult worms in treated artificial soil the mortality, behavioural effects and biomass development was carried out. After additional 28 days the reproduction rate (number of offspring) was assessed (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). Mortality, weight change, feeding activity and reproduction rate were determined as endpoints. The artificial soil contained 69.6 % fine quartz sand, 20 % kaolin clay, 10 % sphagnum peat, air dried and finely ground, and 0.4 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5 according to OECD 222; the pH was 5.8 to 5.9 at experimental start and 5.9 to 6.0 at experimental end; the water content at experimental start was 30.8 % to 31.5 % (55.0 % to 56.3 % of the maximum water holding capacity) and at experimental end 32.7 % to 33.0 % (58.5 % to 58.9 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux.

Toxic standard (Luxan Carbendazim 500 FC): 0.57 – 0.87 – 1.30 – 1.96 – 2.91 mg a.s./kg soil dry weight (corresponds to 1.3 – 2.0 – 3.0 – 4.5 – 6.7 mg test item/ kg soil dry weight); control: same amount of quartz sand as in the test item treated groups moistened with deionised water, solvent control: none.

Dates of experimental work: March 21, 2013 to May 17, 2013

Results:

Validity criteria:

Table CA 8.4.1-7: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10%	0%
Reproduction of Control	≥ 30	215 - 301
Coefficient of variance of reproduction in the control	≤ 30%	11.0%

All study validity criteria were met.

Reference Item Test:
In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher. The EC_{10}, EC_{20} and EC_{50} (reproduction) were calculated to be 1.2, 1.4 or 1.7 mg a.s./kg artificial soil dry weight.

Mortality:
No mortality was observed in any treatment group.

Weight change:
The body weight changes of the earthworms after 4 weeks exposure to AE F145740 was not statistically significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Student t-test, α = 0.05, two-sided).

Reproduction:
The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Student t-test, α = 0.05, one-sided smaller).

Behavioural abnormalities:
No behavioural abnormalities were observed in any of the treatment groups.

Feeding activity:
The feeding activity in the test item treated group was comparable to the control (see table below).

Table CA 8.4.1-8: AE F145740: Effects on earthworms (*Eisenia fetida*) in a 56-day reproduction study

AE F145740 [mg/kg soil dry weight]	Control	100
Mortality (day 28) [%]	0.0	0.0
Significance	-	-
Weight change (day 28) [%]	30.6	34.5
Significance [1)]	-	n.s.
Mean No. of juveniles (day 56)	254	278
Significance [1)]	-	n.s.
Reproduction in [%] of control (day 56)	-	109.8
Food consumption [g]	25.0	25.0
	Endpoints [mg/kg soil dry weight]	
NOEC (day 28 mortality and weight)	≥100	
NOEC (day 56 reproduction)	≥100	
LOEC (day 56 reproduction)	>100	

- = not applicable
n.s. = not significantly different compared to the control
1) Student t-test, α = 0.05, two-sided for weight changes and one-sided smaller for reproduction.

Conclusions:

In an earthworm reproduction and growth study with AE F145740 the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* 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.

AE 0002166

Report:	[redacted];[redacted];2013;M-457338-01
Title:	Iodosulfuron-methyl-sodium- AE 0002166: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	82111022
Document No:	M-457338-01-1
Guidelines:	**OECD, Guideline for the testing of chemicals Nr. 222 "Earthworm, Reproduction Test" (adopted April 13, 2004); ISO-Guideline 11268-2, "Soil quality - Effects of pollutants on earthworm (Eisenia fetida) - Part 2: "Determination of effects on reproduction", International Organization for Standardization, 1998;none**
GLP/GEP:	**yes**

Executive summary:

The purpose of this study was to investigate the effects of AE 0002166 (metabolite of iodosulfuron-methyl-sodium) on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida.*
Adults of *Eisenia fetida* (with clitellum and weight range 300 to 589 mg, 11 to 12 months old) were exposed in artificial soil (with 10 % peat control) to the nominal test concentration of 100 mg test item/kg soil dry weight in a 56-day test. The test item was incorporated into the soil. Eight replicates with ten worms each were used for the test item treatment and for the untreated control. For the control the same amount of quartz sand as in the test item treated groups was added and moistened with deionised water. After 28 days of exposure in treated artificial soil the adult worm mortality, behavioural effects and biomass development were assessed. Reproduction rate (number of offspring)

was assessed after additional 28 days (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).
The No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* 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.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE 0002166; CAS No.: 102394-28-5; Batch code: AE 0002166-PU-01; Origin batch No.: KATH4881-1-2; Purity: 95% w/w; Certificate No.: AZ 17846.

Adult *Eisenia fetida* (with clitellum and weight range 300 to 589 mg, 11 to 12 months old, from an in-house culture; 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to an untreated control and to the nominal test concentration of 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days exposure of adult worms in treated artificial soil the mortality, behavioural effects and biomass development was carried out. After additional 28 days the reproduction rate (number of offspring) was assessed (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). Mortality, weight change, feeding activity and reproduction rate were determined as endpoints. The artificial soil contained 69.6 % fine quartz sand, 20 % kaolin clay, 10 % sphagnum peat, air dried and finely ground, and 0.4 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5 according to OECD 222; the pH was 5.8 to 5.9 at experimental start and 5.9 to 6.0 at experimental end; the water content at experimental start was 30.4 % to 30.8 % (54.2 % to 55.0 % of the maximum water holding capacity) and at experimental end 32.3 % to 32.7 % (57.7 % to 58.5 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux.

Toxic standard (Luxan Carbendazim 500 FC): 0.57 – 0.87 – 1.30 – 1.96 – 2.91 mg a.s./kg soil dry weight (corresponds to 1.3 – 2.0 – 3.0 – 4.5 – 6.7 mg test item/ kg soil dry weight); control: same amount of quartz sand as in the test item treated groups moistened with deionised water, solvent control: none.

Dates of experimental work: March 21, 2013 to May 17, 2013

Results:

Validity criteria:

Table CA 8.4.1-9: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10%	0%
Reproduction of Control	≥ 30	215 - 301
Coefficient of variance of reproduction in the control	≤ 30%	11.0%

All study validity criteria were met.

Reference Item Test:
In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher. The EC_{10}, EC_{20} and EC_{50} (reproduction) were calculated to be 1.2, 1.4 or 1.7 mg a.s./kg artificial soil dry weight.

Mortality:
No mortality was observed in any treatment group.

Weight change:
The body weight changes of the earthworms after 4 weeks exposure to AE 0002166 was not statistically significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Mann-Whitney U-test, α = 0.05, two-sided).

Reproduction:
The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Student t-test, α = 0.05, one-sided smaller).

Behavioural abnormalities:
No behavioural abnormalities were observed in any of the treatment groups.

Feeding activity:
The feeding activity in the test item treated group was comparable to the control (see table below).

Table CA 8.4.1-10: AE 0002166: Effects on earthworms (*Eisenia fetida*) in a 56-day reproduction study

AE 0002166 [mg/kg soil dry weight]	Control	100
Mortality (day 28) [%]	0.0	0.0
Significance	-	-
Weight change (day 28) [%]	30.6	36.4
Significance [1]	-	n.s.
Mean No. of juveniles (day 56)	253.6	253.9
Significance [2]	-	n.s.
Reproduction in [%] of control (day 56)	-	100.1
Food consumption [g]	25.0	25.0
	Endpoints [mg/kg soil dry weight]	
NOEC (day 28 mortality and weight)	≥100	
NOEC (day 56 reproduction)	≥100	
LOEC (day 56 reproduction)	>100	

- = not applicable
n.s. = not significantly different compared to the control
[1] Mann-Whitney U-test, α = 0.05, two-sided for weight changes
[2] Student t-test, α = 0.05, one-sided smaller for reproduction

Conclusions:

In an earthworm reproduction and growth study with AE 0002166 the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm

Eisenia fetida was determined to be ≥100 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) was determined to be >100 mg test item/kg soil.

BCS-CW81253

Report:	[redacted];[redacted];2013;M-462824-01
Title:	Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine (BCS-CW81253): Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No:	13 10 48 091 S
Document No:	M-462824-01-1
Guidelines:	**OECD 222 (2004), ISO 11268-2 (1998);none**
GLP/GEP:	**yes**

Executive summary:

Adults of *Eisenia fetida andrei* (approximately 3 months old with clitellum) were exposed in artificial soil (with 10 % peat control) to concentration of 100 mg test item/kg soil dry weight in a 8-week test. The test item was incorporated into the soil. Eight replicates with ten worms each were used for the test item treatment and for the untreated control. For the control the same amount of quartz sand as in the test item treated groups was added. After 4 weeks of exposure in treated artificial soil the adult worm mortality, behaviour (including feeding activity) and biomass change were assessed. Reproduction rate (number of surviving juveniles) and behavioural and pathological symptoms were assessed after additional 4 weeks (assessed 8 weeks after application). The physic-chemical parameters of the artificial soil (water content, pH) were determined. The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004) as a limit test. 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.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine; BCS-code: BCS-CW81253; Batch code: BCS-CW81253-PU-01 ; Origin Batch No.: GSE 61145-5-3; LIMS No.: 1306024; Customer order No.: TOX 09918-00; Analysed purity: 99.0 % w/w; Certificate of analysis: AZ 18602.

Adult *Eisenia fetida andrei* (with clitellum and weight range 323 to 499 mg, about 3 months old, 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to an untreated control and to the test concentration of 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. The earthworms were fed with horse manure. After 28 days exposure of adult worms in treated artificial soil the mortality, behavioural effects (including feeding activity) and biomass development was carried out. After additional 4 weeks the number of surviving juveniles per replicate was assessed (assessed 8 weeks after application) and behavioural and pathological symptoms were observed. In addition, the physico-chemical parameters of the artificial soil (water content, pH) were determined. The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). The artificial soil contained 68.5 % fine quartz sand, 20 % kaolin clay, 10 % sphagnum peat, dried and finely ground, and 0.5 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5 according to OECD 222; the pH was 5.99 to 6.02 at experimental start and 5.76 to 5.73 at experimental end; the water content at experimental start was 35.0 % (54.1 % of the maximum water holding capacity) and at experimental end 34.5 % to

34.9 % (54.1 % of the maximum water holding capacity); temperature was within the range of 18.7°C to 21.8°C; the illumination was 16 h light : 8 h dark, light intensity was 520 lux.

Toxic standard Nutdazim 50 FLOW (Carbendazim SC 500): 5 and 10 mg product/kg soil dry weight; untreated control: same amount of quartz sand as in the test item treated groups, solvent control: none.

Dates of experimental work: April 05, 2013 to May 31, 2013

Results:

Validity criteria:

Table CA 8.4.1-11: Validity criteria (for the control group)

Validity criteria	Recommended	Obtained
Mortality of the adults	≤ 10%	0%
Number of juveniles per replicate	≥ 30	98, 119, 107, 116, 105, 84, 103 and 114
Coefficient of variance of reproduction	30%	10.4%

All study validity criteria were met.

Reference Item Test:
To verify the sensitivity of the test system, the reference item Nutdazim 50 FLOW (Carbendazim, SC 500) is routinely tested at concentrations of 5 and 10 mg product/kg soil dry weight.
In the most recent study with Nutdazim 50 FLOW (BioChem project No. R 12 10 48 004 S, dated October 29, 2012), the number of juveniles was reduced by 72.7 and 98.8 % at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 23 and 1) after 8 weeks of test duration when compared to control (mean number of juveniles = 84). Therefore, the observed effects assure a high sensitivity of the test system.

Mortality:
1.3 % mortality was found at 100 mg test item/kg soil d.w. No mortality (0 %) occurred in the control group.
No statistically significant effect (Fisher`s Exact Binomial Test, $p > 0.05$, one-sided greater) on mortality compared to the control group was recorded at 100 mg test item/kg soil d.w.

Behavioural effects:
No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.

Weight change:
The test item caused no statistically significant (Student-t-test, $p > 0.05$, one-sided smaller) change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group, i.e. a mean weight increase of 23.8 % was recorded in the control group and 22.7 % at 100 mg test item/kg soil d.w. (see table below).

Reproduction:
No statistically significant effect (Student-t-test, p > 0.05, one-sided smaller) on the number of juveniles compared to the control group was recorded at 100 mg test item/kg soil d.w. (see table below).

Table CA 8.4.1-12: Effects of BCS-CW81553 on growth (biomass change during 4 weks exposure), mortaliy and reproduction of adult earthworms

Endpoint	BCS-CW81253 [mg test item/kg d.w.]	
	Control	100
	Mortality of adult worms after 4 weeks	
Mortality (%)	0	1.3
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean (mg)	97.0	92.2
Mean (%)	23.8	22.7
	Number of juveniles per surviving adult worm after 8 weeks	
Mean	10.5	10.0
	Number of juveniles per replicate after 8 weeks	
Mean	105.4	99.3
	Reproduction compared to control (%)	
% to control	100	94

No statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test, p > 0.05, one-sided greater), biomass and reproduction (Student-t-test, p > 0.05, one-sided smaller)

Table CA 8.4.1-13: Effects of BCS-CW81253 on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	BCS-CW81253 *Eisenia fetida* Artificial soil		
	Mortality	Biomass change	Reproduction
	[mg test item/kg d.w.]		
LOEC	100	> 100	> 100
LC_{50}/EC_{50}	100	> 100	> 100
95 % confidence limit		-	-
NOEC	≥ 100	≥ 100	≥ 100

Conclusions:

BCS-CW81253 showed no statistically significantly adverse effects on mortality, growth and reproduction of the earthworm *Eisenia fetida* in artificial soil at 100 mg test item/kg soil dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/ kg soil d.w. and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil d.w.

AE 0000119

Report:	[redacted]; [redacted];2011; M-404685-01
Title:	BCS-AA10579-urea (AE 0000119): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artifical soil with 10% peat- limit test
Report No:	LRT-RG-R-104/11
Document No:	M-404685-01-1
Guidelines:	**ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; none**
GLP/GEP:	**yes**

Executive summary:

The purpose of this study was to assess the effect AE 0000119 (metabolite of iodosulfuron-methyl-sodium, further code: BCS-AA10579-urea), on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil at one test concentration (Limit test). The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004).
Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 100 mg test item/kg dry weight artificial soil, to an untreated control and to a toxic standard. The test item was mixed into the soil. After 27 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 29 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).
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.

Material and Methods:

Test Item: BCS-AA10579-urea (AE 0000119); Origin Batch No.: RDL 504-1-1; Batch Code.: AE 0000119-PU-01; LIMS No.: 0917101; content of a.s. (analysed): 97.8 % w/w; Certificate No.: AZ 15926.

Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals per treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 100 mg test item/kg dry weight artificial soil, to an untreated control and to a toxic standard. The test item was mixed into the soil. After 27 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil and the cocoons and juvenile earthworms remained in the test vessels. After further 29 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). Mortality, weight change and reproduction rate were determined as endpoints. The artificial soil contained 68.65 % industrial quartz sand, 20 % kaolin clay, 10 % sphagnum peat (shredded), 0.35 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5 according to OECD 222, and 1 % food (dried ground cow manure). Prior to the test, the mean pH value of the artificial soil was pH 6.09. At the end of the study the pH in the treatment group was 6.28 and in the control group 6.46. The mean soil moisture prior to the start of the test was 20.7 %. At Day 0 the mean soil moisture was 28.95 % and at Day 56 it was 27.75 %. The temperature was within the range of 18°C to 22°C. The illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux.

Toxic standard Carbendazim 360 g a.s./L (Derosal flüssig): 1.25 – 2.5 – 5.0 mg a.s./kg soil dry weight; untreated control: same amount of quartz sand as in the test item treated groups, solvent control: none.

Dates of experimental work: April 30, 2010 to July 02, 2010

Results:

Validity criteria:

Table CA 8.4.1-14: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10%	0%
Mean change in growth of the adult earthworms in the control during the exposure period of four weeks:	≥ -20%	+ 79.8%
Mean rate of reproduction of juveniles (Min – Max earthworms per control vessel)	≥ 30	271 (194 - 338)
Coefficient of variance of reproduction in the control	≤ 30%	15.7%

Validity criteria of the test according to the guideline were fulfilled.

Reference Item Test:
In the most recent test with the reference item Carbendazim 360 g a.s./L (Derosal flüssig) (Study No.: Rg 18/10; Report No.: LRG-Rg-R-Ref-13/10; NON-GLP; experimental work from January 2010 to March 2010), no mortality of the adult earthworms was observed 28 days after application.
The change of body weight of the adult earthworms of the test concentrations of 1.25 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (results of a Williams multiple sequential t-test, two-sided, α = 0.05).
No statistically significant different values for the biomass relative to the control were observed at the lowest test concentration of 2.5 mg a.s/kg dry weight artificial soil.
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the lowest test concentration of 1.25 mg a.s/kg dry weight artificial soil.
The number of juveniles per test vessel (determined after 56 days) of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significant reduced to the control (results of a Williams multiple sequential t-test, one-sided smaller, α = 0.05).
The results of the most recent toxic standard testing reference test item indicated that the test system was sensitive to the reference test item.

Mortality:
No mortality of adult earthworms was observed after 27 days of exposure at the control group. At the test concentration 100 mg test item/kg dry weight artificial soil just one worm died since 27 days. During the first 27 days of exposure, no reduced food consumption of the adults, could be observed.

Body weight:
The mean body weight of the adult earthworms in the control group had increased during the 4 weeks of exposure. The mean body weight was 0.53 g per worm.
The mean change in body weight of the test concentration of 100 mg test item/kg dry weight soil was + 78.7 %.
No statistically significant different value for the growth relative to the control was observed at the tested concentration 100 mg test item/kg dry weight artificial soil.

Reproduction:
In the control group, on average 270.6 juvenile earthworms per test vessel were found (corresponding to a mean reproduction rate of 27.1 juveniles per surviving adult).
At the test item treatment group exposed to 100 mg BCS-AA10579-urea /kg dry weight artificial soil, the mean reproduction rate was 100.6 % of the control value.
No statistically significant different values for the number of juveniles per test vessel relative to the control was observed at the tested concentrations 100 mg test item/kg dry weight artificial soil.

Table CA 8.4.1-15: Effects of AE 0000119 on mortality and changes in body weight of the adults after an exposure period of 27days and the number of offspring per test vessel after 56 days

Test object	*Eisenia fetida*	
Test item	Control	AE 0000119
Test concentration (mg test item/kg dws*)	---	100
Mortality of adult earthworms [%] after 27 days	0	1.25
Mean change of body weight of the adults from day 0 to day 27 [%]	+ 79.8	+ 78.7
Standard Deviation	± 7.9	± 10.8
Statistical comparison to the control **	---	n. s.
Mean number of offspring per test vessel after 56 days	271	272
Standard Deviation	± 42.4	± 42.6
Coefficient of variance	15.7	15.7
Statistical comparison to the control **	---	n. s.

* dws = dry weight artificial soil
** Result of a Student-t-test for homogenous Variances
n. s.: mean value not statistically significant different compared to the control ($p \geq 0.05$)

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE 0000119 related to growth is ≥ 100 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) related to growth is > 100 mg test item/ kg dry weight artificial soil. The No-Observed-Effect-Concentration (NOEC) related to reproduction is ≥ 100 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) related to reproduction is > 100 mg test item/ kg dry weight artificial soil.
Overall, it is concluded, that the NOEC for this study is greater than or equal 100 mg test item/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg test item/kg dry weight artificial soil.

AE F059411

Report:	[redacted];[redacted];2011;M-410930-01
Title:	Aminotriazine (AE F059411): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5 % peat
Report No:	LRT-RG-R-100/11
Document No:	M-410930-01-1
Guidelines:	**ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004;minor deviations, in both test runs the soil moisture at test end was higher than 60 % of the WHCmax**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of Aminotriazine (AE F059411, metabolite of iodosulfuron-methyl-sodium), on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil at 5 different test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004). As minor deviations from the guideline at both test runs, the soil moisture at test end was higher than 60 % of the WHC_{max}.
Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control and treatment group) were exposed in an artificial soil with 5 % peat content to the nominal test concentrations of 100 mg test item/kg dry weight artificial soil in the 1st test run. Ten months old *Eisenia fetida* (8 x 10 animals for control and 4 x 10 animals for each treatment group) were exposed to 9.5, 17, 30, 53, 95 mg test item/kg dry weight artificial soil in the 2nd test run. Endpoints were calculated based on the number of surviving animals and their weight alteration, as well as the number of offspring.
Based on the biological and statistical significance of the effects observed on reproduction, it is concluded, that the NOEC for this study is 30 mg test item/kg dry weight artificial soil. The overall LOEC is determined to be 53 mg test item/kg dry weight artificial soil.

Materials and Methods:

Test Item: Aminotriazine (AE F059411); Batch code: AE F059411 00 1B99 0002; Material: AE F059411, pure substance; Chemical name: 2-amino-4-methoxy-6-methyl-1,3,5-triazine; purity: 99.7% w/w.

Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control and treatment group) were exposed in an artificial soil with 5% peat content to the nominal test concentrations of 100 mg test item/kg dry weight artificial soil in the 1st test run. The artificial soil in the 1st test run was composed of 5% sphagnum peat, 20% kaolinite clay, 73.82% industrial quartz sand and 0.18% $CaCO_3$. Ten months old *Eisenia fetida* (8 x 10 animals for control and 4 x 10 animals for each treatment group) were exposed to 9.5, 17, 30, 53, 95 mg test item/kg dry weight artificial soil in the 2nd test run. The artificial soil in the 2nd test run was composed of 5 % peat, 20 % kaolinite clay, 74.8% industrial quartz sand and 0.2 % calcium carbonate $CaCO_3$. The test item was mixed into the soil for both test runs. 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.

Dates of experimental work: 1st test run: November 18, 2010 – January 20, 2011
2nd test run: Mach 03, 2011 – April 28, 2011

Results:

Validity criteria:

Table CA 8.4.1-16: Validity criteria of both test runs

Validity criteria (control values)	Recommended	Obtained 1st run	Obtained 2nd run
Mortality of the adults in the control	≤ 10 %	0 %	0 %
Mean rate of reproduction of juveniles (Min – Max juveniles per control vessel)	≥ 30	254.8 (231 - 296)	347 (254 - 395)
Coefficient of variance of reproduction in the control	≤ 30 %	7.9 %	15.0 %

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

Biological findings:
No mortality of adult earthworms was observed after 28 days of exposure in both test runs.
No statistically significant different values for the growth relative to the control were observed at test all concentrations of both test runs.
Statistically significant different values for the number of juveniles per test vessel relative to the control were observed in the 1st test run.
In the 2nd test run statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the two highest test concentrations (53 and 95 mg test item/kg dry weight artificial soil).

Table CA 8.4.1-17: Effects of AE F059411 on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days (values in this table are rounded values).

Test object	*Eisenia fetida*					
Test item	**Control**			**AE F059411**		
1st test run						
Test concentration (mg test item/kg dry weight artificial soil)	---			100		
Mortality of adult earthworms [%] after 28 days	0			0		
Mean change of body weight of the adults from day 0 to day 28 [%]	+ 72.8			+ 70.8		
Statistical comparison to the control*	---			n. s.		
Mean number of offspring per test vessel after 56 days	211.9			164.5		
Standard Deviation	30.2			34.2		
Statistical comparison to the control**	---			s.		
2nd test run						
Test concentration (mg test item/kg dry weight artificial soil)	---	9.5	17	30	53	95
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 [%]	45.5	46.1	42.5	39.5	39.0	34.5
Statistical comparison to the control*	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
Mean number of offspring per test vessel after 56 days	347	329	330	318	284	278
Standard Deviation	52	23	37	48	54	26
Statistical comparison to the control**	---	n. s.	n. s.	n. s.	s.	s.

* Result of a Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$
** Result of a Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$
n. s. mean value not statistically significant different compared to the control ($p > 0.05$)
s. mean value statistically significant different compared to the control ($p < 0.05$)

Conclusions:

No mortality of adult earthworms was observed after 28 days of exposure in both test runs. No statistically significant different values for the growth relative to the control were observed at test all concentrations of both test runs. Therefore:

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

Statistically significant different values for the number of juveniles per test vessel relative to the control were observed in the 1st test run.
In the 2nd test run statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the two highest test concentrations (53 and 95 mg test item/kg dry weight artificial soil). Therefore, based on statistical significance:

NOEC related to reproduction: 30 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 53 mg test item/kg dry weight artificial soil

Overall, based on the biological and statistical significance of the effects observed on reproduction, it is concluded, that the NOEC for this study is 30 mg test item/kg dry weight artificial soil.

The overall LOEC is determined to be 53 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 iodosulfuron-methyl-sodium and its metabolites AE F075736, AE F145741, AE F145740, AE 0002166, BCS-CW81253, AE F059411 and AE 0000119 reproductive toxicity studies on *Hypoaspis aculeifer* were performed. Reproductive studies on *Folsomia candida* were performed for iodosulfuron-methyl-sodium and its metabolites AE F075736, BCS-CW81253, AE F059411 and AE 0000119.
In the tests with the soil mite *Hyposapis aculeifer* the NOEC values ranged from ≥10 mg/kg dws for the metabolite AE F075736 to ≥1000 mg/kg dws for iodosulfuron. In the tests with the collembolan species *Folsomia candida* the NOEC values ranged from ≥10 mg/kg dws for the metabolite AE F075736 to 316 mg/kg dws for iodosulfuron. Details of all studies are provided in the following table.
No studies were performed for the metabolite AE F161778, as data for *Hypoaspis aculeifer* AE F145741 as preceding metabolite and data for *Hypoaspis aculeifer* and *Folsomia candida* from BCS-CW81253 as succeeding metabolite are available and do not show any toxicity (NOEC ≥100 mg/kg dws).

Table CA 8.4.2.1-1: Reproductive toxicity data of iodosulfuron-methyl-sodium and metabolites to other non-target macro-organisms presented in this chapter

Test substance	Test species	Endpoint		Reference
Iodosulfuron-methyl-sodium	*Hypoaspis aculeifer*	NOEC	**≥1000 mg a.s./kg dws**	[redacted], 2012 M-438590-01-1 KCA 8.4.2.1/01
	Folsomia candida	NOEC	**316 mg a.s./kg dws**	[redacted], 2012 M-438498-01-1 KCA 8.4.2.1/02
AE F075736	*Hypoaspis aculeifer*	NOEC	**≥ 10 mg/kg dws**	[redacted], 2013 M-456338-01-1 KCA 8.4.2.1/03
	Folsomia candida	NOEC	**≥ 10 mg/kg dws**	[redacted], 2013 M-464404-01-1 KCA 8.4.2.1/04
AE F145741	*Hypoaspis aculeifer*	NOEC	**≥ 100 mg/kg dws**	[redacted], 2013 M-462732-01-1 KCA 8.4.2.1/05
AE F145740	*Hypoaspis aculeifer*	NOEC	**≥ 100 mg/kg dws**	[redacted], 2013 M-459885-01-1 KCA 8.4.2.1/06
AE 0002166	*Hypoaspis aculeifer*	NOEC	**≥ 100 mg/kg dws**	[redacted], 2013 M-470489-01-1 KCA 8.4.2.1/07
BCS-CW81253	*Hypoaspis aculeifer*	NOEC	**≥ 100 mg/kg dws**	[redacted], 2013 M-453497-01-1 KCA 8.4.2.1/08
	Folsomia candida	NOEC	**≥ 100 mg/kg dws**	[redacted], 2013 M-462821-01-1 KCA 8.4.2.1/09
AE F059411	*Hypoaspis aculeifer*	NOEC	**100 mg/kg dws**	[redacted], 2010 M-452258-01-1 KCA 8.4.2.1/10
	Folsomia candida	NOEC	**≥ 100 mg/kg dws**	[redacted], 2011 M-400027-01-1 KCA 8.4.2.1/11
AE 0000119	*Hypoaspis aculeifer*	NOEC	**≥ 100 mg/kg dws**	[redacted], 2010 M-386844-01-1 KCA 8.4.2.1/12
	Folsomia candida	NOEC	**≥ 100 mg/kg dws**	[redacted], 2010 M-384229-01-1 KCA 8.4.2.1/13

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

Studies on iodosulfuron-methyl-sodium

Report:	[redacted]; [redacted] ;2012;M-438590-01
Title:	Iodosulfuron-methyl-sodium a.s. (BCS-BB66887): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	kra-HR-70/12
Document No:	M-438590-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil US EPA OCSPP: None;minor deviation**
GLP/GEP:	**yes**

Executive summary:

The purpose of the study was to assess the effects of Iodosulfuron-methyl-sodium a.s. on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 15 days in artificial soil comparing control and treatment.
10 adult, fertilized, female soil mites (females) per replicate (8 replicates for the control group and 4 replicates for each test item concentration) were exposed to control and to concentrations of 100, 178, 316, 562 and 1000 mg a.s./kg dry weight artificial soil corresponding to 107.6, 191.4, 339.8, 604.4, 1075.2 mg test item/kg dry weight artificial soil. After a period of 150 days, the number of juveniles and surviving parental mites was determined. The test was performed in accordance with the OECD Guideline 226 (2008). As deviation the duration of the test was 15 days instead of 14 days due to technical reason. This had no influence on the study.
The No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be ≥ 1000 mg a.s./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be > 1000 mg a.s./kg dry weight artificial soil. The validity criteria for the control group of the study were accomplished.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium a.s. (BCS-BB66887, AE F115008); Batch code: AE F115008-01-03; Origin Batch No.: ELIR003050; Customer order no.: TOX No.09144-00; Specification No.:102000000739; content: 93.0 %w/w.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg a.s./kg dry weight artificial soil were tested corresponding to 107.6, 191.4, 339.8, 604.4, 1075.2 mg test item/kg dry weight artificial soil. An amount of 20 g dry weight artificial soil was weighed into each test vessel. The *Hypoaspis aculeifer* were of uniform age not differing more than three days (34 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 15 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

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

Dates of experimental work: May 10, 2012 – June 04, 2012

Results:

Validity criteria:

Table CA 8.4.2.1-2: Validity criteria

Validity criteria (untreated control values)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	0 %
Mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	404.9
Coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	5.5 %

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

Reference test

The most recent non-GLP-test ([redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil. Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression. Confidence limits could not be determined due to mathematical reasons. The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were even after transformation not homogenous Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment procedure, $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 6.62 mg a. s./kg (95 % confidence limits from 6.02 mg a. s./kg to 2469.54 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression. The results are in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil and show, that the test organisms are sufficiently sensitive. 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 Test object Exposure	Iodosulfuron-methyl-sodium a.s. *Hypoaspis aculeifer* Artificial Soil		
mg a.s./kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**
Control	0.0	404.9 ± 22.4	-
100 (107.6)	0.0	407.5 ± 15.5	100.6 n.s.
178 (191.4)	0.0	436.0 ± 16.6	107.7 n.s.
316 (339.8)	3.3	431.3 ± 53.2	106.5 n.s.
562 (604.4)	7.5	384.8 ± 19.7	95.0 n.s.
1000 (1075.2)	5.0	419.5 ± 27.5	103.6 n.s.
			Reproduction
NOEC (mg a.s./kg dry weight artificial soil)			≥ 1000
LOEC (mg a.s./kg dry weight artificial soil)			> 1000

n.s. = statistically not significant (William's-t test one-sided-smaller, $\alpha = 0.05$)

Mortality:
In the control group 0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The LC_{50} could not be calculated and is considered to be >1000 mg a.s./kg dry weight artificial soil.

Reproduction:
Concerning the number of juveniles statistical analysis (Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment, 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 a.s./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 1000 mg a.s./kg dry weight artificial soil. The EC_{50}-values could not be calculated and is considered to be >1000 mg a.s./kg dry weight artificial soil.

Conclusions:

NOEC: ≥ 1000 mg a.s./kg dry weight artificial soil.
LOEC: > 1000 mg a.s./kg dry weight artificial soil.

Report:	[redacted];[redacted];2012;M-438498-01
Title:	Iodosulfuron-methyl-sodium a.s. (BCS-BB66887): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Report No:	FRM-Coll-140/12
Document No:	M-438498-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil;minor deviations**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of Iodosulfuron-methyl-sodium a.s. on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

10 collembolans (10-12 days old) per replicate (8 control replicates and 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg a.s./kg artificial soil dry weight corresponding to 107.6, 191.4, 339.8, 604.4, 1075.2 mg test item/kg artificial soil dry weight, at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.
The No-Observed-Effect-Concentration (NOEC) for reproduction is 316 mg a.s./kg artificial soil d.w.. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 562 mg a.s./kg artificial soil d.w.. An EC_{50} could not be calculated and is considered to be > 1000 mg a.s./kg artificial soil dry weight. All validity criteria (for the control group) according to the guideline were fulfilled.

Materials and Methods:

Test item. Iodosulfuron-methyl-sodium a.s. (BCS-BB66887); Batch code: AE F115008-01-03; Origin Batch No.: ELIR003050; CAS No.: 144550-36-7; analysed content of a.s.: 93.0 % w/w; certificate No.: AZ 16863.

10 collembolans (10-12 days old) per replicate (8 control replicates and 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg a.s./kg artificial soil dry weight corresponding to 107.6, 191.4, 339.8, 604.4, 1075.2 mg test item/kg artificial soil dry weight, at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

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

Dates of experimental work: May 10, 2012 – June 11, 2012

Results:

Validity criteria:

Table CA 8.4.2.1-4: Validity criteria

Validity criteria (for untreated control)	Recommended	Obtained
Mean adult mortality	≤ 20 %	1CA 8.8 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1054.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	1CA 8.1 %

All validity criteria for the study were met.

The most recent non-GLP-test (FRM-Coll-Ref-19/12, U. [redacted], May 25, 2012) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.
Boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95 % confidence limits from 98 mg to 137 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.
This shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1-5: Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	Iodosulfuron-methyl-sodium a.s. *Folsomia candida* Artificial soil				
mg test item (mg a.s.)/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD			Reproduction (% of control)
Control	1CA 8.8	1054.5	±	191.4	-
107.6 (100)	20.0	1171.5	±	90.3	111.1 $^{n.s.}$
191.4 (178)	20.0	88CA 8.3	±	91.6	84.2 $^{n.s.}$
339.8 (316)	15.0	945.8	±	124.5	89.7 $^{n.s.}$
604.4 (562)	35.0	849.5	±	89.0	80.6 *
1075.2 (1000)	17.5	860.0	±	59.2	81.6 *
$NOEC_{reproduction}$ (mg a.s./kg soil dry weight) $LOEC_{reproduction}$ (mg a.s./kg soil dry weight)					316 562

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

Mortality:
In the control group 1CA 8.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 1000 mg a.s./kg artificial soil dry weight.

Reproduction:
Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed statistically significant differences between control and the treatment groups with 562 and 1000 mg a.s./kg artificial soil dry weight.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 316 mg a.s./kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 562 mg a.s./kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 1000 mg a.s./kg artificial soil dry weight.

Conclusions:

$NOEC_{reproduction}$: 316 mg a.s. (339.8 mg test item)/kg artificial soil dry weight.
$LOEC_{reproduction}$: 562 mg a.s. (604.4 mg test item)/kg artificial soil dry weight.

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F075736

Report:	[redacted];[redacted];2013;M-465338-01
Title:	AE F075736 (BCS-AC12303): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	kra-HR-93/13
Document No:	M-465338-01-1
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 F075736 on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.
10 adult, fertilized, female soil mites per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and to 10 mg test item/kg soil dry weight. After a period of 14 days, the number of juveniles and surviving parental mites was determined. The test was performed as a limit test in accordance with the OECD Guideline 226 (2008).
The No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be ≥ 10 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be > 10 mg test item/kg soil dry weight. The validity criteria for the untreated control group of the study were accomplished.

Material and Methods:

Test item: AE F075736 (BCS-AC12303); common name: metsulfuron-methyl; analysed content of a.s.: 98.6 % w/w; Origin batch No.: 33074-238; Batch code: AE F075736 00 1B98 0002; Certificate No.: AZ 16744; LIMS No.: 1019427.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatment. A single concentration of 10 mg test item/kg artificial soil dry weight was tested. During the test, the *Hypoaspis aculeifer* were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a McFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Toxic standard (Dimethoate EC 400E G): 1.0 – 1.8 – 3.2 – 5.6 – 10.0 mg a.s./kg soil d.w.; control: quartz sand moistened with deionised water, solvent control: none.

Dates of experimental work: May 29, 2013 to June 20, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-6: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	1.3 %
Mean number of juveniles per replicate	≥ 50	295.4
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	9.4 %

All validity criteria for the study were met.

Reference test:
The most recent non-GLP-test ([redacted]-[redacted], kra/HR-O-12/13, April 08, 2013) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 4.32 mg a.s./kg (95 % confidence limits from 4.31 mg a. s./kg to 4.32 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 5.67 mg a.s./kg (95 % confidence limits from 5.58 mg a.s./kg to 5.79 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline for the EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a.s./kg dry weight artificial soil and shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1-7: Effects of AE F075736 on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE F075736 *Hypoaspis aculeifer* Artificial Soil					
mg test item/Kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.			Reproduction (% of control)	Significance (*)
Control	1.3	295.4	±	27.7	-	-
10	1.3	308.1	±	36.0	104.3	n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)					**≥ 10**	
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)					**> 10**	

(*)=Student-t-test one sided smaller; α=0.05
n.s.= non-significant

Mortality:
In the control group 1.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 10 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 10 mg test item/kg artificial soil dry weight.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE F075736 for reproduction was determined to be ≥ 10 mg test item/kg soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be > 10 mg test item/kg soil dry weight.

Report:	[redacted];[redacted];2013;M-464404-01
Title:	AE F075736 (BCS-AC12303): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-Coll-163/13
Document No:	M-464404-01-1
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 F075736 on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.
Adult collembolans were exposed to control (water treated) and 10 mg test item/kg artificial soil dry weight. The duration of the study was 28 days for exposition to the test item at 20 ± 2°C. After a period of 28 days, mortality and reproduction were determined.
The No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 10 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 10 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.

Material and Methods:

Test item: AE F075736 (BCS-AC12303); common name: metsulfuron-methyl; analysed content of a.s.: 98.6 % w/w; origin batch No.: 33074-238; Batch code: AE F075736 00 1B98 0002; Lims No.: 1019427; certificate no.: AZ 16744.

10 collembolans (10-12 days old) per replicate (8 replicates for the control group and for the treatment group) were exposed to control (water treated) and 10 mg test item/kg dry weight artificial soil containing 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C and a photoperiod: light : dark = 16 h : 8 h (400 - 800 lux). During the study, they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

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

Dates of experimental work: May 29, 2013 to July 5, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-8: Validity criteria

Validity criteria (untreated control)	Recommended	Obtained
Mean adult mortality	≤ 20 %	7.5 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1627.3
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	15.1 %

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

Toxic Reference test:
The most recent non-GLP-test (FRM-Coll-Ref-21/13, U. [redacted], March 26, 2013) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.
Boric acid showed an EC_{50} of 108 mg test item/kg artificial soil dry weight (95 % confidence limits from 98 mg to 120 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.
This shows that the test organisms are sufficiently sensitive.

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

Test item Test object Exposure	AE F075736 *Folsomia candida* Artificial soil					
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles per test vessel ± standard deviation			Reproduction (% of control)	Significance (*)
Control	7.5	1627.3	±	246.3	-	
10	6.3	1762.5	±	157.2	108.3	-
					Reproduction	
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)					≥10	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)					>10	

The calculations were performed with un-rounded values
(*) = (Student-t test-t test one-sided-smaller, $\alpha = 0.05$, + = significant, - = not significant)

Mortality:
In the control group 7.5 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥10 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >10 mg test item/kg artificial soil dry weight.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE F075736 for reproduction is ≥10 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >10 mg test item/ kg dry weight artificial soil.

AE F145741

Report:	[redacted];[redacted];2013;M-462732-01
Title:	Iodosulfuron-methyl-sodium-AE F145741 (BCS-AU71532): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	kra-HR-85/13
Document No:	M-462732-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals; Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; US EPA OCSPP: None;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F145741 (metabolite of iodosulfuron-methyl-sodium, further code: BCS-AU71532) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.
10 adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for the treated group) were exposed to control and treatment. A single concentration of 100 mg pure metabolite/kg artificial soil dry weight (corresponding to 106 mg test item/ kg artificial soil dry weight) was tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.
The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 100 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 100 mg pure metabolite/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE F145741 (BCS-AU71532); Batch code: AE F145741 00 1C94 0001; Origin batch No.: 25398-52; Certificate No.: AZ 16823; LIMS No.: 1023138; analysed content(s) of a.s.: 94.4 % w/w.

Ten adult, fertilized, female *Hypoaspis aculeife*r per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatment. A single concentration of 100 mg/kg pure metabolite/kg artificial soil dry weight (corresponding to 106 mg test item/kg artificial soil dry weight) was tested. During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a McFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

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

Dates of experimental work: February 01, 2013 – February 21, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-10: Validity criteria

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20 %	5.0 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	286.4
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	13.0 %

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

Reference test:
The most recent non-GLP-test with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 4.32 mg a.s./kg (95 % confidence limits from 4.31 mg a. s./kg to 4.32 mg a.s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 5.67 mg a.s./kg (95 % confidence limits from 5.58 mg a. s./kg to 5.79 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline for the EC_{50} based on the number of juveniles of 3.0–7.0 mg a.s./kg dry weight artificial soil and shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1-11: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE F145741 *Hypoaspis aculeifer* Artificial Soil			
mg pure metabolite /kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	5.0	286.4 ± 37.1	---	---
100	5.7	319.3 ± 26.9	111	n.s.
$NOEC_{reproduction}$ mg pure metabolite/kg dry weight artificial soil				**≥ 100**
$LOEC_{reproduction}$ mg pure metabolite /kg dry weight artificial soil				**> 100**

(*)=Student-t-test one sided smaller; α=0.05
n.s. = statistically not significant
(dw)= dry weight

Mortality:
In the control group 5.0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and treatment group.
Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg pure metabolite /kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg pure metabolite /kg artificial soil dry weight.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE F145741 for reproduction is ≥ 100 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg pure metabolite /kg artificial soil dry weight.

AE F145740

Report:	[redacted]; [redacted] 2013;M-459885-01
Title:	Iodosulfuron-methyl-sodium-AE F145740 (BCS-AU71533): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	kta-HR-84/13
Document No:	M-459885-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil US EPA OCSPP: None;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE F145740 (metabolite of iodosulfuron-methyl-sodium, further code: BCS-AU71533) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.
10 adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for the treated group) were exposed to control and treatment. A single concentration of 100

mg test item/kg dry weight (d.w.) of soil was tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.
The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 100 mg test item/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

Material and Methods:

Test item. Iodosulfuron-methyl-sodium-AE F145740 (BCS-AU71533); Batch code: AE F145740-PU-02; Origin batch No.: GSE 61082-3-3; Customer order No.: TOX-No.: 09988-00; LIMS No.: 1301958; analysed content(s) of a.s.: 97.5 % w/w iodosulfuron (AE F145740).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control and treatment. A single concentration of 100 mg test item/kg artificial soil dry weight was tested. During the test, the *Hypoaspis aculeifer* were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a McFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water, 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

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

Dates of experimental work: February 01, 2013 – February 21, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-12: Validity criteria

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20 %	5.0 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	286.4
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	13.0 %

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

Reference test:
The most recent non-GLP-test with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.32 mg a.s./kg (95 % confidence limits from 4.31 mg a. s./kg to 4.32 mg a.s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 5.67 mg a.s./kg (95 % confidence limits from 5.58 mg a.s./kg to 5.79 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline for the EC_{50} based on the number of juveniles of 3.0–7.0 mg a.s./kg dry weight artificial soil and shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1-13: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE F145740 *Hypoaspis aculeifer* Artificial Soil			
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (*)
Control	5.0	286.4 ± 37.1	---	---
100	1.3	315.3 ± 25.9	110.1	-
$NOEC_{reproduction}$ mg test item/kg dry weight artificial soil				**≥ 100**
$LOEC_{reproduction}$ mg test item/kg dry weight artificial soil				**> 100**

(*)=Student-t-test one sided smaller; α=0.05;
- : non-significant

Mortality:
In the control group 5.0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and treatment group.
Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE F145740 for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight.

AE 0002166

Report:	[redacted];[redacted];2013;M-470489-01
Title:	AE 0002166 (BCS-AW35544): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	LAR-HR-94/13
Document No:	M-470489-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: Not Applicable; OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil;none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE 0002166 (metabolite of iodosulfuron-methyl-sodium, further code: BCS-AW35544) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. Ten ault, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 8 replicates for each test item concentration) were exposed to control and treatment. A single concentration of 100 mg test item/kg artificial soil dry weight was tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.
The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 100 mg test item/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

Material and Methods:

Test item. AE 0002166; BCS code: BCS-AW35544; Batch code: AE 0002166-01-01; Customer order No.: TOX 10007-00; Origin batch No.: GSE 61266-1-3; LIMS No. 1319418; Analytical findings: 95.2 % w/w AE 0002166.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatment. A single concentration of 100 mg test item/kg artificial soil dry weight was tested. During the test, the *Hypoaspis aculeife*r were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied.
The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

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

Dates of experimental work: July 26, 2013 to August 14, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-14: Validity criteria

Validity criteria (control values)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	0 %
Mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	226.6
Coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	4.5 %

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

In the most recent non-GLP-test ([redacted]-[redacted], kra/HR-O-12/13, April 08, 2013), the LC_{50} (mortality) of the reference item, dimethoate, was calculated to be 4.32 mg a.s./kg dry weight artificial soil. The NOEC is calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the LOEC is 5.6 mg a.s./kg dry weight artificial soil. Dimethoate EC 400E G showed a EC_{50} (reproduction) of 5.67 mg a. s./kg dry weight artificial soil. The results of the reference test demonstrate the sensitivity of the test system.

Table CA 8.4.2.1-15: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE 0002166 *Hypoaspis aculeifer* Artificial Soil			
mg test item/Kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	0	226.6 ± 10.2		
100	1.3	243.4 ± 35.9	107.4	-
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			**≥ 100**	
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			**> 100**	

(*)=Welch-t-test one sided smaller; α=0.05 (-:non-significant; +: significant)

Mortality:
In the control group 0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Welch-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE 0002166 for reproduction was determined to be ≥ 100 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be > 100 mg test item/ kg dry weight artificial soil.

BCS-CW81253

Report:	[redacted];2013;M-453497-01
Title:	Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine (BCS-CW81253): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No:	13 10 48 090 S
Document No:	M-453497-01-1
Guidelines:	**OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproductiontest in soil;not applicable**
GLP/GEP:	**no**

Executive Summary:

The purpose of this study was to determine potential effects of BCS-CW81253 (metabolite of iodosulfuron-methyl-sodium) 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, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for the treated group) were exposed to control and treatment. A single concentration of 100 mg test item/kg artificial soil dry weight was tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted.
The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 100 mg test item/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

Material and Methods:

Test item. Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine (BCS-CW81253); Batch code: BCS-CW81253-PU-01; Origin batch No.: GSE 61145-5-3; Customer order No.: TOX-No.: 09918-00; Certificate No.: AZ 18602; LIMS-No.: 1306024; analysed purity: 99.0 % w/w.

10 adult soil mites (females) per replicate (8 replicates for the control group and 8 replicates for the treated group) were exposed to 100 mg test item/kg dry weight (d.w.) of soil containing 74.8 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.2 % $CaCO_3$, at 19.5 - 21.4°C and a photoperiod:light:dark = 16 h:8 h (593 lx). The Hypoaspis aculeifer adults were from a synchronised culture with an age difference of 3 days. They were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure. Therefore, surviving mites and juveniles of *Hypoaspis aculeifer* were extracted from each test replicate using a MacFadyen high-gradient extractor (heat/light extraction method). Following extraction, all juveniles and adults present in the fixing liquid were counted. Any adult mites not found after extraction were recorded as dead. From these data the mortality of the adult females and the reproductive output were calculated.

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

Dates of experimental work: March 14, 2013 – April 02, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-16: Validity criteria

Validity criteria (control values)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	1.3
Mean number of juveniles per replicate	≥ 50	289.4
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	13.8

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

Reference test:

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.i./ kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Mortality:

In the control group and in the test item treatment group a parental mortality of 1.3 % could be observed at the end of 14-day exposure period.

Reproduction:

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 289.4 in the control and 288.1 in the test item treatment group.

The test item caused no statistically significantly adverse effects on adult mortality (Fisher`s Exact Binomial 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.

Table CA 8.4.2.1-17: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	BCS-CW81253 *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 8.4.2.1-18: Observations on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	BCS-CW81253 (mg metabolite/kg soil d.w.)	
	control	100
Mortality of soil mites after 14 days (%)	1.3	1.3
Mean number of juveniles after 14 days	289.4	288.1
CV %	13.8	11.1
Reproduction (% to control)	100	100

No statistically significant differences compared to the control were calculated (Fisher`s Exact Binomial 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 unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group

Conclusions:

The test item BCS-CW81253 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 tobe > 100 mg test item/kg soil dry weight.

Report:	[redacted];[redacted];2013;M-462821-01
Title:	Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine (BCS-CW81253): Effects on the reproduction of the collembolan Folsomia candida
Report No:	13 10 48 089 S
Document No:	M-462821-01-1
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 BCS-CW81253 (metabolite of iodosulfuron-methyl-sodium) on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. 10 collembolans (9-12 days old) per replicate (8 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 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. 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.

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine (BCS-CW81253); Substance code: AE F153745; Batch code: BCS-CW81253-PU-01; Origin Batch No.: GSE 61145-5-3; Customer

order No.: TOX 09918-00; LIMS No.: 1306024; analysed purity: 99.0% w/w; certificate No.: AZ 18602.

10 *Collembola* (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to untreated control (quartz sand only) 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 % $CaCO_3$. The vessels were kept in a temperature-controlled room at 18.1 – 20.8 °C and a photoperiod: light : dark = 16 h : 8 h (710 lx). The collembolans 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 experimental work: March 14, 2013 – April 11, 2013

Results:

Validity criteria:

Table CA 8.4.2.1-19: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juveniles per replicate	≥ 100	686
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	15.6 %

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 3.4 %.

Reference test:
In a separate 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 results of the reference test demonstrate the sensitivity of the test system.

Table CA 8.4.2.1-20: Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	**BCS-CW81253** *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 8.4.2.1-21: Observations on mortality and reproduction of *Folsomia candida*

Endpoint	BCS-CW81253 (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	686	709
CV %	15.6	11.8
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: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated groups
R_c = mean number of juveniles observed in the control group

Mortality:
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, 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.

Reproduction:
The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 686 in the control and 709 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:

BCS-CW81253 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 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 F059411

Report:	[redacted];[redacted];2010;M-452258-01
Title:	IN-A4098: Effect on reproduction of the predatory mite Hypoaspis (Geolaelaps) aculeifer Canestrini (Acari: Laelapidae) in artificial soil
Report No:	S10-00288
Document No(s):	M-452258-01-1
Guidelines:	**OECD 226 (2008);not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was the assessment of the side effects of AE F059411 (metabolite of iodosulfuron-methyl-sodium, further code: IN-A4098) in soil on the reproductive output of the soil mite *Hypoaspis aculeifer* and the determination of the NOEC (No observed effect concentration) and if possible the EC_{50} (Effect concentration for 50 % effect) in a rate response test following 14-day exposure to artificial soil treated with the test item IN-A4098 under laboratory conditions. Based on the results of a GLP range-finding test, concentrations for the main test were chosen as follows: 9.53, 17.15, 30.86, 55.56 and 100.0 mg IN-A4098/kg soil dry weight. In this test each test item treatment group comprised 4 replicates, whereas 8 replicates were tested in both the water and the solvent (acetone) control group. A toxic reference item (Perfekthion) was tested in the testing facility as a separate study.

Mortality and reproduction were assessed after 14 days of exposure to treated substrate by counting surviving adult and juvenile mites.

The test item had no statistically significant effect on mortality and reproduction of *Hypoaspis* (Geolaelaps) aculeifer up to the highest test item concentration of 100.0 mg/kg soil dry weight. The 14-day NOEC (No observed effect concentration) was determined as 100.0 mg IN-A4098/kg soil dry weight

Materials and Methods:

Test item: IN-A4098 (=AE F059411); Test item code: 2010-000407; Batch/Lot number: 050942-015; CAS registry number: 1668-54-8; CAS name (uninverted): 4-Methoxy-6-methyl-1,3,5-triazin-2-amine; Purity: 98.7%.

A GLP range-finding test was performed including test item concentrations of 0.01, 0.1, 1.0, 10.0 and 100.0 mg test item/kg soil dry weight. Each treatment group in the range-finding test comprises two replicates with 10 adult individuals (32 days old females). A water and a solvent (acetone) control group were included in the test.

Based on the results of the range-finding test, 10 adult females (32 days old) per replicate were exposed in the main test to concentrations of 9.53, 17.15, 30.86, 55.56 and 100.0 mg test item/kg soil dry weight. Each test item treatment group comprised 4 replicates, whereas 8 replicates were tested in both the water and the solvent (acetone) control group. The artificial soil was composed of 5% sphagnum peat (air-dried and finely ground), 20% kaolin clay, 74% air-dried industrial sand and < 1% calcium carbonate ($CaCO_3$) in order to adjust pH to 6.0 ± 0.5.

Mortality and reproduction were assessed after 14 days of exposure to treated substrate by counting surviving adult and juvenile mites after 48-hour light/heat extraction using Tullgren-type extracting device. A toxic reference item (Perfekthion) to confirm sensitivity of the test organisms was tested at the testing facility in a separate study.

Dates of experimental work: March 16, 2010 – April 29, 2010

Results:

Validity criteria:

Table CA 8.4.2.1-22: Validity criteria

Validity criteria (control values of the main test)	Recommended	Obtained
Mean mortality of adult females in the solvent control	≤ 20%	3.8%
Mean number of juveniles per solvent control vessel	≥ 50	283.8
Coefficient of variation of reproduction in the solvent control	≤ 30%	11.3%

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

In a separate study (T. [redacted], S10-00085, May 2010) it was concluded that Perfekthion caused statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* starting with a concentration of 6.0 mg a.s./kg soil dry weight. The NOEC was determined as 4.0 mg a.s./kg soil dry weight, corresponding to 10.4 mg product/kg soil dry weight. The EC_{50} was determined as 5.8 mg a.s./kg soil dry weight (95 % confidence limits: 5.4 – 6.3 mg a.s./kg soil dry weight), corresponding to 15.1 mg product/kg soil dry weight (95 % confidence limits: 13.9 – 16.3 mg product/kg soil dry weight). The results of the reference test demonstrate the sensitivity of the test system.

Mortality

In the untreated control and the solvent control group a mortality of 6.3% and 3.8% was observed, respectively. The test item caused a mean mortality of 2.5%, 7.5%, 10.0%, 2.5% and 12.5% (mean corrected mortality: -1.4 %, 3.8%, 6.4%, -1.4% and 9.0%) at concentrations of 9.53, 17.15, 30.86, 55.56 and 100.0 mg/kg soil dry weight, respectively. No statistically significant effect on mortality was observed for any test item concentration (Fisher's Exact Test, Bonferroni-Holms corrected, one-tailed, $p \leq 0.05$).

Reproduction

Mean numbers of 262.4 and 283.8 juveniles per replicate were calculated for the untreated control and the solvent control group. The coefficient of variation in the untreated control group was calculated as 12.2 and for the solvent control 11.3 respectively. In the test item groups treated with 9.53, 17.15, 30.86, 55.56 and 100.0 mg test item/kg soil dry weight 281.0, 254.0, 266.3, 272.8 and 255.5 juveniles on average were produced within the exposure period of 14 days, respectively. The reduction in reproduction compared to the control group was calculated as 1.0%, 10.5%, 6.2%, 3.9% and 10.0%, respectively. No statistically significant differences in offspring numbers compared to the solvent control group were detected (Dunnett's t-Test, one-tailed, $p \leq 0.05$).

Table CA 8.4.2.1-23: Effects of AE F059411 on the mortality and reproduction of *Hypoaspis aculeifer*

	Test item concentration [mg/kg soil dry weight]						
	Untreated Control	**Solvent Control**	**9.53**	**17.15**	**30.86**	**55.56**	**100.0**
Mean mortality [%]	6.3	3.8	7.5	7.5	10.0	2.5	12.5
Corrected Mortality [1)] [%]	-	-	-1.4	3.8	6.4	-1.4	9.0
Mean no. of juveniles per replicate	262.4	238.8	281.0	254.0	266.3	272.8	255.5
Coefficient of variation [%]	12.2	11.3	7.7	9.9	4.4	5.7	9.7
Reduction in reproduction [%]	-	-	1.0	10.5	6.2	3.9	10.0
14-day NOEC	100						

[1)] : Corrected according to Schneider-Orelli (1947), referring to the solvent control

Conclusions:

AE F059411 had no statistically significant effect on mortality and reproduction of *Hypoaspis aculeifer* up to the highest test item concentration of 100 mg/kg soil dry weight.
The 14-day EC_{50} could not be calculated as the reduction of reproduction was below the trigger value of 50% for all treatment rates tested. It was estimated to be > 100.0 mg test item/kg soil dry weight.
The 14-day NOEC was determined as 100.0 mg/kg soil dry weight.

Report:	[redacted] [redacted];2011;M-400027-01
Title:	BCS-AA10579-aminotriazine (BCS-AA40997, AE F059411): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-Coll-110/11
Document No:	M-400027-01-1
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 effects of AE F059411 (aminotriazine, metabolite of iodosulfuron-methyl-sodium) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil. 10 collembolans (10 - 12 days old) per replicate were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight (limit test). After a period of 28 days, mortality and reproduction were determined.
The No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. All validity criteria for the untreated control of the study according to the OECD Guideline 232 have been fulfilled.

Materials and Methods:

Test item: Aminotriazine (metabolite of iodosulfuron-methyl-sodium), pure substance; synonyms: BCS-AA40997, AE F059411; batch code: AE F059411 00 1B99 0002; origin batch no: 001272; LIMS no.: 0723888;; purity: 99.7 % w/w.

10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight artificial soil containing 74.8% fine quartz sand, 20% kaolin clay, 5% sphagnum peat, air dried and finely ground, and 0.17% $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C and a photoperiod: light : dark = 16 h : 8 h (400 - 800 lux). Each test vessel 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.

Dates of experimental work: November 12, 2010 – December 15, 2010

Results:

Validity criteria:

Table CA 8.4.2.1-24: Validity criteria

Validity criteria (untreated control)	Recommended	Obtained
Mean adult mortality	≤ 20%	8.8%
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	955
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	24.4%

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

Reference test:
The most recent non-GLP-test (FRM-Coll-Ref-14/10, U. [redacted], March 03, 2010) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.
Boric acid showed an EC_{50} of 96 mg test item/kg artificial soil dry weight (95 % confidence limits from 87 mg to 105 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Biological findings:
Effects of the test item on growth and reproduction of *Folsomia candida* are presented in the table below:

Table CA 8.4.2.1-25: Effects on mortality and reproduction of *Folsomia candida*

Test item	AE F059411		
Test object	*Folsomia candida*		
Exposure	Artificial soil		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	8.8	955 ± 233	-
100	10	1126 ± 220	**118** ns
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			**≥100**
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			**>100**

The calculations were performed with un-rounded values
n.s. = statistically not significant (Student-t test one-sided-smaller, α = 0.05)

<u>Mortality</u>
In the control group 8.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

<u>Reproduction</u>
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant difference between the control group and the treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >100 mg test item/kg artificial soil dry weight.

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE F059411 for reproduction is ≥ 100 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/ kg dry weight artificial soil.

<u>AE 0000119</u>

Report:	[redacted];2010;M-386844-01
Title:	BCS-AA10579-urea (BCS-AB56501): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat
Report No:	KRA-HR-33/10
Document No:	M-386844-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; Minor deviations**
GLP/GEP:	**yes**

Executive Summary:

The purpose of the study was to assess the effects of AE 0000119 (metabolite of iodosulfuron-methyl-sodium, further codes: BCS-AA10579-urea, BCS-AB56501) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil with 5 % peat at 100 mg test item/kg dry weight artificial soil and control. Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 8 treatment replicates) were exposed to control (water treated) and 100 mg test item/kg dry weight artificial soil. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular. As minor deviations some

pH-values were marginal below the recommended range of the OECD guideline No. 226. This has no impact on the study.
The EC_{50} could not be calculated and is considered to be > 100 mg test item/kg dry weight artificial soil. The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 100 mg test item/kg dry weight artificial soil. The EC_{50}-value could not be calculated and was considered to be > 100 mg test item/kg dry weight artificial soil. All validity criteria (for the control replica) according to the guideline were met.

Material and Methods:

Test item: BCS-AA10579-urea (AE 0000119, BCS-AB56501); Batch Code: AE 0000119-PU-01; Material: AE 0000119, pure substance; Chemical name: (4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea; Origin Batch No.: RDL 504-1-1; LIMS No.: 0917101; analysed content: 97.8 % w/w; Certificate No.: AZ 15926.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 8 treatment replicates) were exposed to control (water treated) and 100 mg test item/kg dry weight artificial soil. The test item was applied by mixing a test item-quartz sand-mixture into the artificial soil. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (29 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2°C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and approximately 0.15 % 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 standard (Dimethoate EC 400): 1.0 – 1.8 – 3.2 – 5.6 – 10.0 mg a.s./kg dry weight artificial soil; control: quartz sand, solvent control: none.

Dates of experimental work: May 07, 2010 – May 27, 2010

Results:

Validity criteria:

Table CA 8.4.2.1-26: Validity criteria

Validity criteria (for control replicates)	Recommended	Obtained
Mean adult female mortality	≤ 20 %	2.5 %
mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	386.0
coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	5.7 %

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

In the most recent non-GLP-test (M.-A. [redacted], kra/HR-O-9/10, February 03, 2010) with the reference item dimethoate the LC_{50} was calculated to be 4.2 mg a. s./kg dry weight artificial soil for mortality of the adult mites according Probit analysis using maximum likelihood regression. The $NOEC_{reproduction}$ was calculated to be 3.2 mg a. s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.6 mg a. s./kg dry weight artificial soil according Williams-Test multiple t-test procedure, α = 0.05, one-sided. Dimethoate showed a EC_{50} of 5.7 mg a. s./kg dry weight artificial soil for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline of 3.0 – 7.0 mg a. s./kg dry weight artificial soil. This shows that the test organisms are sufficiently sensitive.

Mortality:
In the control group 2.5 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} cannot be calculated and is considered to be > 100 mg test item/kg dry artificial soil.

Reproduction:
Mean number of juveniles per control replicate (with 10 adult females introduced) was 386.0, which is above the recommended minimum of 50 juveniles.
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no significant differences between the control and treatment.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/ kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/ kg dry weight artificial soil. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg dry artificial soil.

Table CA 8.4.2.1-27: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE 0000119 *Hypoaspis aculeifer* Artificial Soil				
mg test item/kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.			Reproduction (% of control)
Control	2.5	386.0	±	22.1	---
100	2.5	391.5	±	24.6	101.4
					Reproduction
NOEC (mg test item/kg dry weight artificial soil)					≥ 100
LOEC (mg test item/kg dry weight artificial soil)					> 100

No statistical significance (Student-t test one-sided smaller, α = 0.05)

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE 0000119 for reproduction of *Hypoaspis aculeifer* is ≥ 100 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/ kg dry weight artificial soil.

Report:	[redacted];[redacted];2010;M-384229-01
Title:	BCS-AA10579-urea (BCS-AB56501): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Report No:	FRM-COLL-93/10
Document No:	M-384229-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil;minor deviations**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to assess the effect of AE 0000119 (metabolite of iodosulfuron-methyl-sodium, further codes: BCS-AA10579-urea, BCS-AB56501) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight. After a period of 28 days, mortality and reproduction were determined.

As Deviation from the guideline the pH-value for the control and the treatment group was marginal below the recommended value of 6 ± 0.5 at start of the test. At start of the study in one treatment replicate 11 collembolans instead of 10 collembolans were introduce by mistake. This has no impact on the study.

The No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Material and Methods:

Test item: BCS-AA10579-urea (AE 0000119, BCS-AB56501); analysed content 97.8 % w/w, batch code: AE 0000119-PU-01; origin batch no.: RDL 504-1-1; LIMS No.: 0917101; certificate no.: AZ 15926.

10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry containing 74.8 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and approximately 0.15 % $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C and a photoperiod: light : dark = 16 h : 8 h (400 - 800 lux). Each test vessel of the 8 control and the 4 treatment replicas plus the one for measurement purpose was filled up with 30 g wet weight artificial soil. During the test, the collembolans were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Dates of experimental work: May 07, 2010 to June 09, 2010

Results:

Validity criteria:

Table CA 8.4.2.1-28: Validity criteria

Validity criteria	Recommended	Obtained
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1472
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	6.7 %

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

Reference test:
The most recent non-GLP-test (FRM-Col-Ref-14/10, U. [redacted], March 03, 2010) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.
Boric acid showed an EC_{50} of 96 mg test item/kg artificial soil dry weight (95 % confidence limits from 87 mg to 105 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Mortality:
In the control group 3.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between the control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Table CA 8.4.2.1-29: Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE 0000119 *Folsomia candida* Artificial soil		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	3.8	1472 ± 99	-
100	2.5	1499 ± 50	102 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight) $LOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥ 100 > 100

The calculations were performed with un-rounded values
n.s. = statistically not significant (Student-t test one-sided-smaller, α = 0.05)

Conclusions:

The No-Observed-Effect-Concentration (NOEC) of AE 0000119 for reproduction of the collembolan species *Folsomia candida* is ≥ 100 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/ kg dry weight artificial soil.

CA 8.5 - Effects on soil nitrogen transformation

For iodosulfuron-methyl-sodium and its metabolites AE F145741, AE F145740, AE 0002166, AE F161778, BCS-CW81253, AE F059411 and AE 0000119 studies on the effect on soil nitrogen transformation were performed. For the Metabolite AE F075736 which is identical with the registered active substance metsulfuron-methyl data on effects on soil nitrogen transformation are available from the Review report for metsulfuron-methyl (SANCO 7593/VI/97-final from 14 Aug 2000). In none of the studies unacceptable effects were found at the highest tested dose level which ranged from 0.043 mg/kg dws to 0.4 mg/kg dws. Details of all studies are provided in the following table.

Table CA 8.5-1: Toxicity data of iodosulfuron-methyl-sodium and metabolites to soil non-target micro-organisms presented in this chapter

Test item	Test design	Ecotoxicological endpoint		Reference
N-transformation				
Iodosulfuron-methyl-sodium (tech.)	Study duration 28 d	**no unacceptable effects**	**≥0.0586 mg a.s./kg dws** [1)]	[redacted] (1996) M-141782-01-1 KCA 8.5/01
AE F075736	Study assumably duration 28 d	**no effect**	**0.2 mg/kg**	SANCO 7593/VI/97-final from 14 Aug 2000
AE F145741	Study duration 28 d	**no unacceptable effects**	**≥0.063mg/kg dws**	[redacted] (2013) M-457273-01-1 KCA 8.5/02
AE F145740	Study duration 28 d	**no unacceptable effects**	**≥0.063 mg/kg dws**	[redacted] (2013) M-457344-01-1 KCA 8.5/03
AE 0002166	Study duration 28 d	**no unacceptable effects**	**≥0.053 mg/kg dws**	[redacted] (2013) M-464391-01-1 KCA 8.5/04
AE F161778	Study duration 28 d	**no**	**≥0.049mg/kg dws**	[redacted] (2013)

		unacceptable effects	M-464817-01-1 KCA 8.5/05
BCS-CW81253	Study duration 28 d	**no unacceptable effects ≥0.043 mg/kg dws**	[redacted] (2013) M-459899-01-1 KCA 8.5/06
AE 0000119	Study duration 28 d	**no unacceptable effects ≥0.4 mg/kg dws**	[redacted] (2010) M-395864-01-1 KCA 8.5/07
AE F059411	Study duration 42 d	**no unacceptable effects ≥0.204 mg/kg dws**	[redacted] (2003) M-448838-01-1 KCA 8.5/08

dws = dry weight soil; a.s. = active substance; prod. = product
Bold values: endpoints used for risk assessment
1) Corrected to an analysed purity of 87.4%

Studies on iodosulfuron-methyl-sodium

Report:	[redacted];[redacted];1996;M-141782-01
Title:	Effects on soil microbial activity (nitrogen turnover) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A58058, CE96/094
Document No:	M-141782-01-1
Guidelines:	**BBA: VI,1-1;Deviation not specified**
GLP/GEP:	**yes**

Endpoint according to the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final):

No effects up to 50 g a.s./ha

(equivalent to ≥0.0586 mg a.s./kg dws; corrected to an analysed purity of 87.4%).

Studies on the metabolites of iodosulfuron-methyl-sodium

AE F145741

Report:	[redacted];[redacted];2013;M-457273-01
Title:	Iodosulfuron-methyl-sodium-AE F145741 (BCS-AU71532): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 024 N
Document No:	M-457273-01-1
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 F145741 (metabolite of iodosulfuron-methyl-sodium, further code: BCS-AU71532) 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 0.063 mg test item/kg soil dry weight. The control was prepared with quartz sand only. 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). No adverse effects of AE F145741 on nitrogen transformation in soil could be observed at

both test concentrations (0.012 mg/kg dry soil and 0.063 mg/kg dry soil) during the 28-day experiment. Differences from the control of -16.0 % (test concentration 0.012 mg/kg dry soil) and -13.2 % (test concentration 0.063 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE F145741; BCS-code: BCS-AU71532; Batch code: AE F145741 00 1C94 0001; Origin Batch No.: 25398-52; CAS No.: 887731-26-0; LIMS No.: 1023138; Analysed purity: 94.4 % w/w; Certificate No.: AZ 16823.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.012 and 0.063 mg test item/kg soil dry weight. Application rates were equivalent to 0.009 and 0.047 kg test item/ha. The control was prepared with quartz sand only. As toxic reference dinoterb was used in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)). A series of 3 replicates for each treatment was tested. 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). During the test period the samples were kept in a climatic room in darkness. The temperature range was 18.9 – 21.1°C. The water content of soil was 16.65 – 17.52 g/100 g soil dry weight (equivalent to 45.47 – 47.84 of maximum water-holding capacity (WHC)).
The coefficients of variation in the control (NO_3-N) were maximum 2.7 % and thus fulfilled the demanded range (≤15 %).

Dates of experimental work: March 21, 2013 - April 18, 2013

Results:

Validity criteria:
The coefficients of variation in the control (NO_3-N) were maximum 2.7 % and thus fulfilled the demanded range (≤15 %).

Reference test:
In the most recent test with the toxic standard (BioChem study code R 13 10 48 001 N, dated 04.01. - 01.02.2013), Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen turnover:
No adverse effects of AE F145741 on nitrogen transformation in soil could be observed at both test concentrations (0.012 mg/kg dry soil and 0.063 mg/kg dry soil) during the 28-day experiment. Differences from the control of -16.0 % (test concentration 0.012 mg/kg dry soil) and -13.2 % (test concentration 0.063 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5-2: Effects on nitrogen transformation in soil after treatment with AE F145741

Time Interval (days)	Control			0.012 mg test item/kg soil dry weight equivalent to 0.009 kg test item/ha				0.063 mg test item/kg soil dry weight equivalent to 0.047 kg test item/ha			
	Nitrate-N 1)			Nitrate-N 1)			% difference to control	Nitrate-N 1)			% difference to control
0-7	3.80	±	0.03	3.84	±	0.22	**+0.9** n.w.	3.70	±	0.15	**-2.8** n.s.
7-14	1.20	±	0.15	1.19	±	0.40	**-1.2** n.s.	1.31	±	0.18	**+8.7** n.s.
14-28	1.11	±	0.06	0.94	±	0.22	**-16.0** n.s.	0.97	±	0.19	**-13.2** 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.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE F145741 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) during the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.063 mg test item/kg soil dry weight.

AE F145740

Report:	[redacted];[redacted];2013;M-457344-01
Title:	Iodosulfuron-methyl-sodium-AE F145740 (BCS-AU71533): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 025 N
Document No:	M-457344-01-1
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 F145740 (metabolite of Iodosulfuron-methyl-sodium, further code: BCS-AU71533) 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 0.063 mg test item/kg soil dry weight. The control was prepared with quartz sand only. 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). The AE F145740 (BCS-AU71533) a temporary inhibition of the daily nitrate rate at the tested concentrations of 0.012 mg/kg and 0.063 mg/kg dry soil at time interval 7-14 days after application.
However, no adverse effects of AE F145740 on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +7.1 % (test concentration 0.012 mg/kg dry soil) and -14.2 % (test concentration 0.063 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE F145740; Substance code: AE F145740; BCS-code: BCS-AU71533; Batch code: AE F145740-PU-02; Customer order no.: TOX09988-00; Origin Batch No.: GSE 61082-3-3; CAS No.: 185119-76-0; LIMS No.: 1301958; analysed purity: 97.5 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.012 and 0.063 mg test item/kg soil dry weight. Application rates were equivalent to 0.009 and 0.047 kg test item/ha. The control was prepared with quartz sand only. As toxic reference dinoterb was used in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)). A series of 3 replicates for each treatment was tested. 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). During the test period the samples were kept in a climatic room in darkness. The temperature range was 18.9 – 21.1°C. The water content of the soil was 16.71 – 18.00 g/100 g soil dry weight (equivalent to 45.64 – 49.17 of maximum water-holding capacity (WHC)).
The coefficients of variation in the control (NO_3-N) were maximum 2.9 % and thus fulfilled the demanded range (≤15 %).

Dates of experimental work: March 21, 2013 – April 18, 2013

Results:

Validity criteria:
The coefficients of variation in the control for NO_3-N were maximum 2.9 % and thus fulfilled the demanded range (≤15 %).

Reference test:
In the most recent test (BioChem study code R 13 10 48 001 N, dated 04.01. - 01.02.2013), the toxic standard Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen turnover:
The test item AE F145740 caused a temporary inhibition of the daily nitrate rate at the tested concentrations of 0.012 mg/kg and 0.063 mg/kg dry soil at time interval 7-14 days after application. However, no adverse effects of AE F145740 on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +7.1 % (test concentration 0.012 mg/kg dry soil) and -14.2 % (test concentration 0.063 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5-3: Effects on nitrogen transformation in soil after treatment with AE F145740

Time Interval (days)	Control			0.012 mg test item/kg soil dry weight equivalent to 0.009 kg test item/ha				0.063 mg test item/kg soil dry weight equivalent to 0.047 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.14	±	0.03	3.27	±	0.26	**+4.1** n.w.	3.59	±	0.08	**+14.3** *s.
7-14	2.20	±	0.04	1.50	±	0.28	**-31.7** *w.	1.53	±	0.27	**-30.7** *w.
14-28	0.97	±	0.12	1.04	±	0.19	**+7.1** n.s.	0.83	±	0.13	**-14.2** 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.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

*w. = statistically significantly different to control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE F145740 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.063 mg test item/kg soil dry weight.

<u>AE 0002166</u>

Report:	[redacted];2013;M-464391-01
Title:	Iodosulfuron-methyl-sodium-AE 0002166 (BCS-AW35544): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	13 10 48 026 N
Document No:	M-464391-01-1
Guidelines:	**OECD 216 (2000);not specified**
GLP/GEP:	**yes**

Executive summary:

The purpose of this study was to determine the effects of AE 0002166 (metabolite of iodosulfuron-methyl-sodium, further code: BCS-AW35544) 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 0.011 and 0.053 mg test item/kg soil dry weight. The control was prepared with quartz sand only. 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). No adverse effects of AE 0002166 on nitrogen transformation in soil could be observed at both test concentrations (0.011 mg/kg dry soil and 0.053 mg/kg dry soil) during the 28 day experiment. Differences from the control of -17.3 % (test concentration 0.011 mg/kg dry soil) and +9.2 % (test concentration 0.053 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE 0002166; BCS-code: BCS-AW35544; Batch code: AE 0002166-01-01; Origin Batch No.: GSE 61266-1-3; LIMS No.: 1319418; Certificate No.: AZ 18786; Customer order No.: TOX-No: 10007-00; CAS No.: 102394-28-5; analysed purity: 95.2 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.011 and 0.053 mg test item/kg soil dry weight. Application rates were equivalent to 0.008 and 0.040 kg test item/ha. The control was prepared with quartz sand only. As toxic reference dinoterb was used in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)). A series of 3 replicates for each treatment was tested. 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). During the test period the samples were kept in a climatic room in darkness. The temperature range was 18.4 – 20.7°C. The water content of the soil was 16.44 – 17.19 g/100 g soil dry weight (equivalent to 46.74 – 48.87 of maximum water-holding capacity (WHC)).
The coefficients of variation in the control (NO_3-N) were maximum 1.7 % and thus fulfilled the demanded range (≤15 %).

Dates of experimental work: July 11, 2013 - August 08, 2013

Results:

Validity criteria:
The coefficients of variation in the control for NO_3-N were maximum 1.7 % and thus fulfilled the demanded range (≤15 %).

Reference toxic:
In the most recent test (BioChem study code R 13 10 48 001 N, dated 04.01. - 01.02.2013), the toxic standard Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen turnover:
No adverse effects of AE 0002166 on nitrogen transformation in soil could be observed at both test concentrations (0.011 mg/kg dry soil and 0.053 mg/kg dry soil) after 28 days. Differences from the control of -17.3 % (test concentration 0.011 mg/kg dry soil) and +9.2 % (test concentration 0.053 mg/kg dry soil) were measured at the end of the 28-day incubation period.

Table CA 8.5-4: Effects on nitrogen transformation in soil after treatment with AE 0002166

Time Interval (days)	Control			0.011 mg test item/kg soil dry weight equivalent to 0.008 kg test item/ha				0.053 mg test item/kg soil dry weight equivalent to 0.040 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.30	±	0.10	3.51	±	0.16	**+6.3** n.s.	3.87	±	0.24	**+17.3** *s.
7-14	1.07	±	0.09	1.23	±	0.24	**+14.7** n.s.	0.96	±	0.22	**-10.7** n.s.
14-28	1.00	±	0.05	0.83	±	0.23	**-17.3** n.s.	1.10	±	0.25	**+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, $p \leq 0.05$)

*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE 0002166 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) during the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.053 mg test item/kg soil dry weight.

<u>AE F161778</u>

Report:	[redacted]; [redacted];2013;M-464817-01
Title:	Iodosulfuron-methyl-sodium-AE F161778 (BCS-AU85549): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 027 N
Document No:	M-464817-01-1
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 F161778 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 0.010 and 0.049 mg test item/kg soil dry weight. The control was prepared with quartz sand only. 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). The test item AE F161778 caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.049 mg/kg dry soil at time interval 7-14 days after application.

However, no adverse effects of AE F161778 on nitrogen transformation in soil could be observed at both tested concentrations (0.010 mg and 0.049 mg test item/kg dry soil) at the end of the test, 28 days after application (time interval 14-28). Differences to the control of +16.3 % (test concentration 0.010 mg/kg dry soil) and +4.6 % (test concentration 0.049 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-AE F161778; BCS-code: BCS-AU85549; Origin Batch No.: 1540-1; Batch code: AE F161778 00 1C94 0001; LIMS No.: 1300203; CAS No.: 126312-31-0; Certificate No.: AZ 10492 of March 11, 2003 and AZ 18502 of February 08, 2013; analysed purity: 94.7 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.010 and 0.049 mg test item/kg soil dry weight. Application rates were equivalent to 0.007 and 0.037 kg test item/ha. The control was prepared with quartz sand only. As toxic reference dinoterb was used in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)). A series of 3 replicates for each treatment was tested. 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). During the test period the samples were kept in a climatic room in darkness. The temperature range was 18.4 – 20.5°C. The water content of the soil was 16.37 – 17.11 g/100 g soil dry weight (equivalent to 46.54 – 48.64 of maximum water-holding capacity (WHC)).
The coefficients of variation in the control (NO_3-N) were maximum 3.4 % and thus fulfilled the demanded range (≤15 %).

Dates of experimental work: July 18, 2013 - August 15, 2013

Results:

Validity criteria:
The coefficients of variation in the control (NO_3-N) were maximum 3.4 % and thus fulfilled the demanded range (≤15 %).

Reference toxic:
In the most recent test (BioChem study code R 13 10 48 001 N, dated 04.01. - 01.02.2013), the toxic standard Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen turnover:
The test item AE F161778 caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.049 mg/kg dry soil at time interval 7-14 days after application.
However, no adverse effects of AE F161778 on nitrogen transformation in soil could be observed at both tested concentrations (0.010 mg and 0.049 mg test item/kg dry soil) at the end of the test, 28 days after application (time interval 14-28). Differences to the control of +16.3 % (test concentration 0.010 mg/kg dry soil) and +4.6 % (test concentration 0.049 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5-5: Effects on nitrogen transformation in soil after treatment with AE F161778

Time Interval (days)	Control			0.010 mg test item/kg soil dry weight equivalent to 0.007 kg test item/ha				0.049 mg test item/kg soil dry weight equivalent to 0.037 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	4.02	±	0.22	3.73	±	0.06	**-7.1** n.s.	3.67	±	0.40	**-8.8** n.s.
7-14	0.95	±	0.15	1.18	±	0.17	**+24.0** n.s.	1.30	±	0.07	**+37.0** *s.
14-28	0.88	±	0.08	1.02	±	0.11	**+16.3** n.s.	0.92	±	0.05	**+4.6** 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, $p \leq 0.05$)

*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE F161778 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) during the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.049 mg test item/kg soil dry weight.

BCS-CW81253

Report:	[redacted];[redacted];2013;M-459899-01
Title:	Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine (BCS-CW81253): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 028 N
Document No:	M-459899-01-1
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 BCS-CW81253 (also called iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine) 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 0.008 and 0.043 mg test item/kg soil dry weight. The control was prepared with quartz sand only. 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). No adverse effects of BCS-CW81253 on nitrogen transformation in soil could be observed at both test concentrations (0.008 mg/kg dry soil and 0.043 mg/kg dry soil) during the 28 day experiment. Differences from the control of -17.1 % (test concentration 0.008 mg/kg dry soil) and -8.3 % (test concentration 0.043 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Material and Methods:

Test item: Iodosulfuron-methyl-sodium-des-iodo-carbamoyl-guanidine; BCS-code: BCS-CW81253; Batch code: BCS-CW81253-PU-01; Origin Batch No.: GSE 61145-5-3; LIMS No.: 1306024; Customer order No.: TOX-No. 09918-00; analysed purity: 99.0 % w/w; Certificate of analysis: AZ 18602.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.008 and 0.043 mg test item/kg soil dry weight. Application rates were equivalent to 0.006 and 0.032 kg test item/ha. The control was prepared with quartz sand only. As toxic reference dinoterb was used in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)). A series of 3 replicates for each treatment was tested. 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). During the test period the samples were kept in a climatic room in darkness. The temperature range was 19.8 – 21.1°C. The water content of soil was 16.62 – 17.69 g/100 g soil dry weight (equivalent to 45.40 – 48.31 of maximum water-holding capacity (WHC)).
The coefficients of variation in the control (NO_3-N) were maximum 11.8 % and thus fulfilled the demanded range (≤15 %).

Dates of experimental work: April 09, 2013 – May 14, 2013

Results:

Validity criteria:
The coefficients of variation in the control for NO_3-N were maximum 11.8 % and thus fulfilled the demanded range (≤15 %).

Reference toxic:
In the most recent test with the toxic standard (BioChem study code R 13 10 48 001 N, dated 04.01. - 01.02.2013), Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen turnover:
No adverse effects of BCS-CW81253 on nitrogen transformation in soil could be observed at both test concentrations (0.008 mg/kg dry soil and 0.043 mg/kg dry soil) during the 28 day experiment. Differences from the control of -17.1 % (test concentration 0.008 mg/kg dry soil) and -8.3 % (test concentration 0.043 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5-6: Effects on nitrogen transformation in soil after treatment with BCS-CW81253

Time Interval (days)	Control			0.008 mg test item/kg soil dry weight equivalent to 0.006 kg test item/ha				0.043 mg test item/kg soil dry weight equivalent to 0.032 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.99	±	0.20	3.86	±	0.19	**-3.2** n.s.	3.70	±	0.45	**-7.2** n.s.
7-14	1.26	±	0.79	1.29	±	0.07	**+1.9** n.w.	1.12	±	0.63	**-10.9** n.s.
14-28	1.03	±	0.38	0.85	±	0.20	**-17.1** n.s.	0.95	±	0.14	**-8.3** n.s.

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

BCS-CW81253 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) during the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.043 mg test item/kg soil dry weight.

AE 0000119

Report:	[redacted];[redacted];2010;M-395864-01
Title:	BCS-AA10579-urea (BCS-AB56501): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	10 10 48 048 N
Document No:	M-395864-01-1
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 0000119 (metabolite of iodosulfuron-methyl-sodium, further codes: BCS-AA10579-urea, BCS-AB56501) 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 0.4 mg test item/kg soil dry weight. The application rate was equivalent to 0.3 kg test item/ha. The control was left untreated, i.e. is prepared with quartz meal only. The nitrogen transformation (NO3-nitrogen production) was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH4-nitrogen, NO3- and NO2-nitrogen were determined by an Autoanalyzer II (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment). At time interval 7-14 days after application, BCS-AA10579-urea (BCS-AB56501) caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.4 mg/kg dry soil. However, no adverse effects of AE 0000119 on nitrogen transformation in soil could be observed at the test concentration of 0.4 mg/kg dry soil, 28 days after application. Only a negligible difference to control of -2.9 % (test concentration 0.4 mg/kg dry soil) was measured at the end of the 28-day incubation period (time interval 14-28).

Material and Methods:

Test item: BCS-AA10579-urea (BCS-AB56501); Material: AE 0000119, pure substance; Batch code: AE 0000119-PU-01; Origin Batch No.: RDL 504-1-1; Analysed purity: 97.8 % w/w; Certificate of analysis: AZ 15926.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.4 mg test item/kg soil dry weight. The application rate was equivalent to 0.3 kg test item/ha. The control was left untreated, i.e. is prepared with quartz meal only. As toxic reference dinoterb was used in a separate study to verify the sensitivity of the test system (6.80, 16.00 and 27.00 mg dinoterb/kg soil dry weight (28 days)). A series of 3 replicates for each treatment was tested. The nitrogen transformation (NO_3-nitrogen production) was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyzer II (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment). During the test period the samples were kept in a climatic room in darkness. The temperature range was 19.0 – 20.9°C. The water content of the soil was 16.69 – 17.68 g/100 g soil dry weight (equivalent to 46.11 – 48.85 of maximum water-holding capacity (WHC)).
The coefficients of variation in the control (NO_3-N) were maximum 3.3 % and thus fulfilled the demanded range (≤15 %).

Dates of experimental work: October 07, 2010 – November 09, 2010

Results:

Validity criteria:
The coefficients of variation in the control (NO_3-N) were maximum 3.3 % and thus fulfilled the demanded range (≤15 %).

Reference test:
In the separate test (BioChem study code R 10 10 48 002 N, dated 07.01. - 18.02.2010), the toxic standard Dinoterb caused an effect of +37.6 %, +51.4 % and +27.1 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg dinoterb/kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen turnover:
At time interval 7-14 days after application, AE 0000119 caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.4 mg/kg dry soil. However, no adverse effects of AE 0000119 on nitrogen transformation in soil could be observed at the test concentration of 0.4 mg/kg dry soil, 28 days after application. Only a negligible difference to control of -2.9 % (test concentration 0.4 mg/kg dry soil) was measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5-7: Effects on nitrogen transformation in soil after treatment with AE 0000119

Time Interval (days)	Application rate						
	Control			AE 0000119			
				0.4 mg/kg dry weight soil			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control
0-7	1.66	±	0.05	1.58	±	0.47	**-4.6** n.s.
7-14	0.38	±	0.03	0.27	±	0.43	**-27.8** n.s.
14-28	0.73	±	0.09	0.71	±	0.15	**-2.9** n.s.

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$; Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE 0000119 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 (time interval 14-28). The study was performed in a field soil at a concentration of 0.4 mg test item/kg soil, which is equivalent to an application rate of 0.3 kg test item/ha.

AE F059411

Report:	[redacted];[redacted];2003;M-448838-01
Title:	IN-A4098: Assessment of the effects on soil microflora
Report No:	Dupont-12117
Document No:	M-448838-01-1
Guidelines:	**OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Guideline 216, dated 21 January 2000 OECD-Guideline for the Testing of Chemicals, Soil Microorganisms: Carbon Transformation Test, Guideline 217, dated 21 January 2000;not specified**
GLP/GEP:	**yes**

Executive Summary:

Aim of this study was to examine the potential effect of AE F059411 (metabolite of iodosulfuron-methyl-sodium, further code: IN-A4098) on short-term, substrate-induced respiration and nitrogen turnover (ammonification and nitrification) in soil. One experiment determined the carbon mineralisation of the microbiological biomass. A second experiment determined the nitrogen mineralisation. The test was performed in accordance with OECD guideline 216 (2000) and OECD guideline 217 (2000).
AE F059411 was incubated in a single loamy sand soil over a period of 28 days (short-term respiration) and 42 days (nitrogen turnover) at rates of 30 g and 150 g test item/ha, equivalent to 0.041g and 0.204 mg test item/kg soil dry weight. The control consisted of soil treated with acetone treated quartz sand. For the nitrogen turnover the soil was thoroughly mixed with the ground Lucerne meal before application. For the short-term respiration a concentration of 3 g glucose/kg soil wet weight was used in the test. The nitrate content was statistically significantly different from control at the end of the study for both test concentrations, even the difference from control was clearly below the 25 % trigger value given by the OECD 216 guideline. The rate of nitrate turnover in soil, calculated as the nitrate formation rate per day, was also below the 25 % trigger value according to the OECD guideline 216. AE F059411 at rate equivalent to 30 g and 150 g test item/ha had no significant effect on the short-term, substrate-induced respiration. At the end of the 28 day study, the deviations in

respiration rates compared to control soil were below the 25% trigger value according to the OECD guideline 217. According to the OECD guidelines 216/217, no long term effects were observed.

Materials and Methods:

Test item: IN-A4098 (AE F059411); Batch No.: IN-A4098-005; CAS registry number: 1668-54-8; CAS name: 4-Methoxy-6-methyl-1,3,5-triazin-2-amine; Purity: 98.7%.

For each experiment (i.e. the short-time respiration and the soil nitrogen turnover) a soil treated with acetone treated quartz sand (control), a soil treated with 0.041 mg test item/kg soil dry weight (equivalent to 30 g a.s./ha) and a soil treated with 0.204 mg test item/kg soil dry weight (equivalent to 150 g a.s./ha) with 3 replicates each, were tested.
For the nitrogen turnover the soil was thoroughly mixed with the ground Lucerne meal before application. The final concentration of the dried lucerne meal was 0.5 % of the soil dry weight. For the short-term respiration the amount of glucose needed to obtain maximum short-term rates of respiration in the test soil was determined prior to the beginning of the test. A concentration of 3 g glucose/kg soil wet weight was found to be the optimum and was used in the test. AE F059411 containing quartz sand and acetone treated quartz sand (control) were applied to the soil, thoroughly mixed and then incubated at 20 ± 2 °C in the dark. Samples were taken after 0, 7, 14 and 28 days after applying the test material. The nitrogen turnover test was prolonged up to 42 days. At each sampling date the mineral nitrogen content, the rate of nitrate formation, the short-term respiration, the dry weight and pH were determined. Changes in the mineral nitrogen level of the soil and rate of nitrate formation were used to assess the potential effects of AE F059411 on nitrogen turnover. The short-term respiration was measured in soil samples taken on Day 0, 7, 14 and 28 after adding 3 g glucose/kg soil wet weight to the soil sample. The rate of oxygen uptake was measured for up to 24 hours following the addition of glucose.

Dates of experimental work: April 08, 2003 – May 23, 2003

Results:

Validity Criteria:
The results of the study can be regarded to be valid according to criteria established in OECD 216/217, since the variation between the replicate control samples was less than 15% for both nitrate levels and the rate of soil respiration.

Reference test:
In separate studies (IBACON study codes 12453080 and 15751080, October to December 2002) the toxic standards Dinoterb and Dinoterb Acetate at concentrations of 85 mg/kg and 75 mg/kg soil dry weight, respectively, were tested. At day 28, the soil respiration rates of the toxic standards differed by -39.8% and -46.6% from control. The nitrate-N content of the toxic standards treated soils differed from control by 57.9% and 158 %, respectively. The variation of replicate control samples was clearly below the 15% value given by the OECD test guidelines 216/217 (exception: soil nitrogen turnover test day 7: 17.3%).

Nitrogen turnover:
The nitrate content was statistically significantly different from control at the end of the study for both test concentrations, even the difference from control was clearly below the 25 % trigger value given by the OECD 216 guideline. The rate of nitrate turnover in soil, calculated as the nitrate formation rate

per day, was also below the 25 % trigger value according to the OECD guideline 216.

Short-term respiration:
AE F059411 at a rate equivalent to 30 g and 150 g test item/ha (0.041g and 0.204 mg test item/kg soil dry weight) had no significant effect on the short-term, substrate-induced respiration. At the end of the 28 day study, the deviations in respiration rates compared to control soil were below the 25 % trigger value according to the OECD guideline 217.

Table CA 8.5-8: Effect of AE F059411 on soil respiration and nitrogen turnover in a loamy sand soil after 28 and 42 days of exposure

Application rate	IN-A4098 concentration [mg/kg soil dry weight]	Nitrogen turnover[1] (deviation from control after 42 days) [%]	Rate of nitrate formation[2] (deviation from control after 42 days) [%]	Respiration (deviation from control after 28 days) [%]
30 g test item/ha	0.041	-15.1	-22.9	4.97
150 g test item/ha	0.204	-14.1	-21.7	-6.25

[1] based on the sum of NH_4 -N and NO_3^- -N
[2] based on the rate of nitrate formation

Conclusions:

The impact of AE F059411 on soil microbial processes is negligible up to concentrations of 0.204 mg/kg soil dry weight. According to the OECD guidelines 216/217, no long term effects were observed.

Information from peer reviewed open literature

Report:	[redacted];[redacted];[redacted];[redacted];[redacted];[redacted];2005;M-467357-01
Title:	Effects of iodosulfuron-methyl-sodium on several biological indicators in soil
Report No:	M-467357-01-1
Document No:	M-467357-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**n.a.**

Executive summary

This article reports on the impact of iodosulfuron-methyl sodium (0, 0.5, 1 and 5 mg/kg soil) on urease and catalase activity in the soil, soil respiration and soil microbial biomass.
Effects on urease and catalase activity, soil respiration and soil microbial biomass were detected during the first days after substance application compared to the control but at the end of the test (latest 30 days after application), all parameters reached the control level.

Material and methods

A. Material

1. Test material

Test item:	Iodosulfuron-methyl sodium
Active substance(s):	sodium salt of methyl-4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) sulfonylurea] benzoate ($C_{14}H_{13}IN_5NaO_6S$)
Chemical state and description:	n/a
Source of test item:	[redacted]
Batch number:	n/a
Purity:	>98%
Storage conditions:	n/a
Water solubility:	n/a
Molecular mass:	529.3

B. Study design and methods

1. Soil sampling:

Name / Classification	Sandy loam
Source:	Samples were selected from quaternary red clay developed in plots of parent material to which iodosulfuron-methyl sodium had never been applied. Source: see Table 1
Sampling technique:	The soil column method was used to collect the surface layer (0 - 20 cm) of soil.
Pre-treatment and storage conditions:	A part of the sampled soil underwent air-drying, weed removal, grinding and filtration through a 20-mesh sieve. Another part was preserved at -5°C after direct filtration through a 10-mesh sieve. For physical and chemical properties see Table 1

2. Biological measurement methods

Urease activity:	Indophenol blue colorimetric method
Catalase activity:	Potassium permanganate titration method
Microbial biomass carbon levels	Fumigation-extraction method
Respiration strength	Sealed stand-alone CO_2 method

3. Impact on soil urease activity, catalase activity and microbial biomass carbon levels

Test soil quantity :	No numerical value stated
Test concentrations/Treatments:	Iodosulfuron-methyl sodium standard solution was added to make the concentration in soil 1 mg/kg, mixed thoroughly and distilled water added to adjust the water level in soil to about 50% of the saturated water level
Replicates:	3
Control:	A blank test was run.
Test conditions:	Cultured at 25 ±1°C
Duration:	35 days
Sampling time:	1, 4, 7, 14, 21, 24, 28 and 35 days
Measurements:	The air-dried soil was used to measure enzyme activity and the fresh soil was used to measure microbial biomass carbon levels

4. Impact of iodosulfuron-methyl sodium on soil respiration

Soil pre-treatment:	100 g of air-dried soil was weighed. 2 g glucose was added and mixed thoroughly. Distilled water was added to adjust the water level in the soil to about 50% of its saturated water level. Soil placed into a Ø 18 cm × 21 cm sealed specimen bottle and pre-cultured for 7 days in a 25 ±1°C incubator
Test soil quantity :	3 samples a 20 g
Test concentrations/Treatments:	A fixed quantity of iodosulfuron-methyl sodium standard solution was added to each sample, so that quantities in the soil were at 0.5, 1 and 5 mg/kg; the soil was mixed thoroughly, added to the sealed specimen bottle and at the same time a small beaker loaded with

	30 mL of 0.1 mol/L sodium hydroxide was also placed into the sealed specimen bottle.
Replicates:	3
Control:	At the same time a blank test was run
Test conditions:	Cultured in an incubator
Duration:	15 days
Sampling time:	At 2, 5, 7, 10, 12 and 15 days respectively after pesticide treatment, the small beaker loaded with sodium hydroxide was extracted; 0.2 mol/L hydrochloric acid was used to titrate the remaining sodium hydroxide; at the same time it was replaced with a new small beaker loaded with the same sodium hydroxide and the culturing continued.
Measurements:	The results for each test were calculated according to the formula below for the quantity of CO_2 released in 100 g soil and expressed using W(mg) (in the formula 50 is the acid-base titration constant): W = (blank value – titration value) × hydrochloric acid molar concentration × CO_2 relative molecular weight × 50/dry soil quality

5. Chemical analysis

Not mentioned.

Table CA 8.5-9: Physical and chemical properties of the soil studied

Soil	Sampling sites	Organic matter (g/kg)	CEC (c mol/kg)	pH (H_2O)	Texture
Red soil	[redacted] Agricultural University	32.0	7.3	5.14	Sandy loam

Results

Effects of iodosulfuron-methyl sodium on soil urease

Compared to the blank (CK) control, in the first 14 days after culturing the soil urease activity of iodosulfuron-methyl-sodium-treated soil was significantly inhibited (its inhibition rate was 25.21 - 41.21%; Fig. CA 8.5-1). After 21 days the soil urease activity started to return to control levels, which were reached after 30 days.

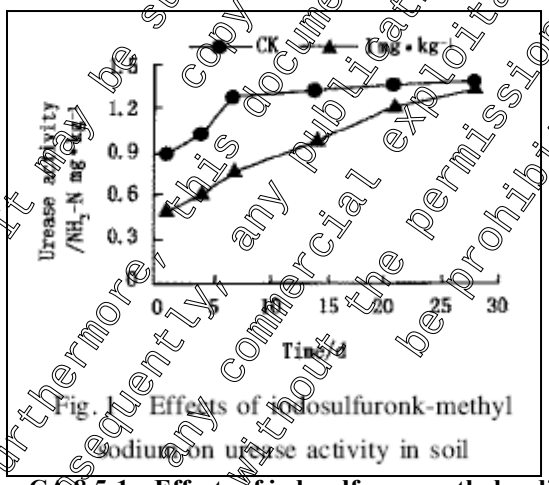


Fig. 1 Effects of iodosulfuronk-methyl sodium on urease activity in soil

Figure CA 8.5-1: Effects of iodosulfuron-methyl sodium on soil urease

Effects of iodosulfuron-methyl sodium on soil catalase

After the application of iodosulfuron-methyl sodium, in the first 9 days there was a slight inhibition action on soil catalase activity, while after 9 days the catalase activity of the pesticide-applied soil exceeded that of the control group, reaching the maximum difference relative to the control group at 21 days. After 30 days catalase activity was basically consistent in the pesticide-applied soil and the control group soil (Fig. CA 8.5-2).

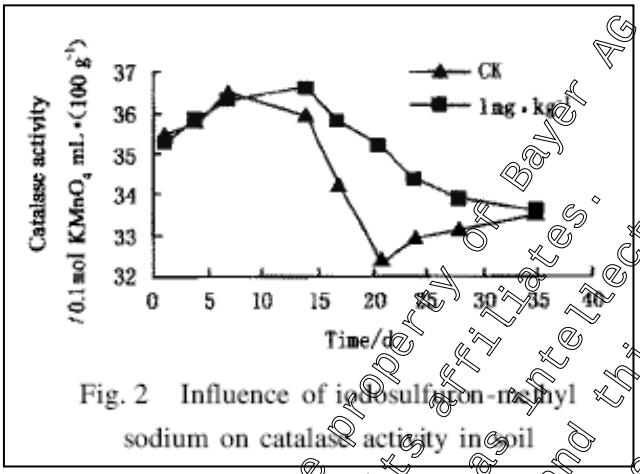


Fig. 2 Influence of iodosulfuron-methyl sodium on catalase activity in soil

Figure CA 8.5-2: Effects of iodosulfuron-methyl sodium on soil catalase

Effects of iodosulfuron-methyl sodium on soil respiration

Two days after pesticide application all of the treatments showed definite inhibition effects on soil respiration. The higher the pesticide concentration, the greater the inhibition effect on the intensity of soil respiration. The inhibition effects were most pronounced in the group treated with 5 mg/kg, with a reduction of 55.06 % compared to the control group. Over time the 0.5 mg/kg treatment at 5 days after pesticide application and the 1 and 5 mg/kg treatments at 7 days after pesticide application showed an increase in soil respiration compared to the control. At 12 days the pesticide-applied soil and the control group soil were basically identical in terms of respiration.

Table CA 8.5-10: Influence of iodosulfuron-methyl sodium on soil respiration (rate of release CO_2, mg/d)

Table 2 Influence of iodosulfuron-methyl sodium on soil respiration (The rate of release CO_2, mg · d^{-1})

Concentration of pesticide, mg · kg^{-1}	Time/d					
	0 ~ 2	2 ~ 5	5 ~ 7	7 ~ 10	10 ~ 12	12 ~ 15
CK	11.37 ± 1.03	11.44 ± 0.64	11.26 ± 2.16	10.05 ± 1.57	6.80 ± 1.39	5.31 ± 0.16
0.5	9.03 ± 0.29	10.08 ± 1.93	11.34 ± 1.68	12.84 ± 0.46	7.79 ± 2.38	5.11 ± 1.12
1	8.48 ± 1.68	9.61 ± 2.00	10.22 ± 1.52	14.07 ± 1.68	8.09 ± 0.65	5.52 ± 0.49
5	5.11 ± 0.79	8.16 ± 0.71	9.71 ± 0.71	13.76 ± 1.73	7.91 ± 1.97	5.01 ± 0.76

Effects of iodosulfuron-methyl sodium on microbial biomass carbon in the soil

See Figure CA 8.5-3 for the trend in the impact of iodosulfuron-methyl sodium on microbial biomass carbon levels in the soil over time. The results indicated that after iodosulfuron-methyl sodium was applied, for the first 7 days the magnitude of the reduction was relatively significant (its inhibition rate was 31.42 - 35.14%); later microbial biomass carbon levels in the soil recovered; after 14 days the change was not significant and basically tended to be consistent with the control.

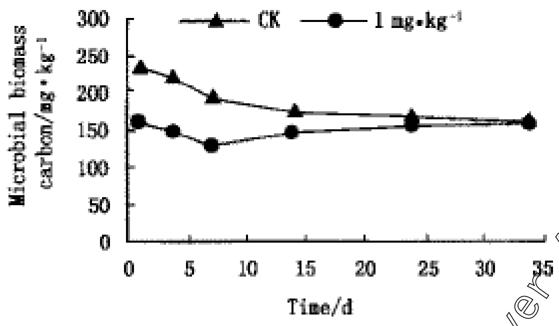


Fig. 3 Influence of iodosulfuron-methyl sodium on microbial biomass carbon

Figure CA 8.5-3: Impact of iodosulfuron-methyl sodium on microbial biomass carbon levels in the soil

Results summary

<u>Soil urease activity</u> of iodosulfuron-methyl sodium-treated soil was significantly reduced in the first 14 days after treatment but returned to control levels 30 days after treatment.
<u>Catalase activity</u> in the treatment group showed first a slight inhibition followed by a stimulation. After 30 days catalase activity was at the same level in the pesticide-applied soil and the control group soil.
Inhibition of <u>soil respiration</u> was observed in all treatments after pesticide application but after 12 days all treatment groups had returned to control levels.
For the first 7 days after application <u>microbial biomass carbon</u> in the soil was significantly reduced, but recovered over time. After 14 days it was consistent with the control.

Comments by the notifier

The information contained in the article is considered supplementary information since all reported effects on microbial communities are ≤ 25% after 30 days, confirming the conclusions of the risk assessment.

Report:	[redacted];[redacted];[redacted];[redacted];[redacted];2012;M-460898-01
Title:	Effects of environmental conditions and microbes on degradation of iodosulfuron-methyl-sodium in soil.
Report No:	M-460898-01-2
Document No:	M-460898-01-2
Guidelines:	**not applicable;not applicable**
GLP/GEP:	**no**

Executive summary

This article mainly studied the impact of iodosulfuron-methyl sodium on catalase activity and respiration in the soil. The study demonstrated that the quantity of iodosulfuron-methyl sodium used did not have a major impact on soil catalase activity; iodosulfuron-methyl sodium impact on soil catalase activity was definitely correlated to soil properties and culture duration. Iodosulfuron-methyl sodium showed significant inhibition on soil respiration intensity at two days after pesticide

application; the larger the concentration the stronger the inhibition. 12 days after pesticide application respiration intensity in the soil had basically recovered and was consistent with the control group.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Iodosulfuron-methyl sodium
Active substance(s):	((IUPAC name: sodium salt of methyl-4-iodo-2-(4-methoxy-6-methyl-1,3,5-diazine-2-yl) sulfonylurea benzoate, molecular formula: $C_{14}H_{13}N_5NaO_6S$)
Chemical state and description:	Not given
Source of test item:	[redacted]
Batch number:	Not given
Purity:	≥ 980 g/kg
Storage conditions:	Not given
Water solubility:	Not given
Molecular weight:	529.3

2. Soil:

Name / Classification	Sandy loam, loam and clay loam
Source, sampling date and storage conditions	Samples were collected from fields to which iodosulfuron-methyl sodium had never been applied. For sampling site, see Table 1. The soil column method was used to collect topsoil (0-20 cm), which was air dried, debris was removed, it was pulverised and passed through a 20-mesh sieve.
Soil type:	see Table 1
pH:	see Table 1
Organic carbon content:	see Table 1

Table CA 8.5-11: Physical and Chemical Properties of Soil Samples

Soil	Collection site	Water levels in soil (g/kg)	Organic matter (g/kg)	CEC (cmol/kg)	pH (H_2O)	Soil property
Quaternary red earth	[redacted] Agricultural University field	4.3	3.20	7.3	5.14	Sandy loam soil
Hechao earth	[redacted] Changsha County	3.8	2.93	9.1	6.84	Loam
Purple earth	[redacted] Hengshan County	3.9	3.45	15.8	4.41	Clay loam

B. Study design and methods

1. Study design

Study type:	Laboratory
Study duration:	35 days

2. Methods and Measurements

Physico-chemical measurements:	The basic physical and chemical properties of the soil were tested in accordance with the methods by Lao, 1988[6].

[6] Lao Jiacheng. Handbook for Soil Agrochemical Analysis. Beijing: Agriculture Press, 1988.

Methods/protocols:	Catalase activity in soil was measured using the method of [redacted] and [redacted], 1986[7]. The millilitre count of 1 kg dry soil to consume 0.1 mol/L potassium permanganate was used to express catalase activity. For soil respiration intensity, the sealed static CO_2 test method was used ([redacted] and [redacted], 1986).
Treatments/test concentrations:	Test concentrations were mixed thoroughly into test soils: Control, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/kg for catalase activity at different Iodosulfuron concentrations 1 mg/kg for catalase activity of different culture times and different soil types Control, 0.5, 1.0 and 5.0 mg/kg for testing soil respiration
Replicates:	3
Test conditions:	
- impact of different pesticide usage quantities on **catalase activity**	Six parts quaternary red earth were weighed, air dried and filtered; 10 g each were placed into a 250 ml stoppered triangular flask.; Different usage quantities of the iodosulfuron-methyl sodium were added, mixed thoroughly, set aside for 30 minutes, then catalase activity was measured.
- impact of different culture times on soil **catalase activity**	Four parts quaternary red earth were weighed, air dried and filtered; 80 g each were placed into a 250 ml stoppered triangular flask and numbered A, B, C and D. A fixed amount of iodosulfuron-methyl sodium standard solution was added; after waiting for the solvent to volatilise, distilled water was added to wet the soil (so that the water level in the soil was about 50% of saturation level). The sample was cultured in a constant-temperature incubator at (25 ±1)°C, and at days 1, 4, 7, 14 and 21 after culturing the samples were tested for their catalase activity
- impact of different types of soil on **catalase activity**	Three soil samples (Hechao earth, purple earth, quaternary red earth) in a total of 12 parts were weighed, air dried and filtered, four parts per soil type; 10 g each were placed into a 250 ml stoppered triangular flask; a fixed quantity of iodosulfuron-methyl sodium standard solution was added, set aside for 30 minutes and catalase activity was then measured.
Soil respiration intensity test conduct:	100 g of quaternary red earth and 2 g glucose were weighed, air dried and filtered in a 150 ml beaker, and a small amount of water was added to wet the soil. The beaker was placed into an 18 cm × 21 cm sealed specimen bottle and pre-cultured for seven days in a (25 ±1)°C incubator. Three parts weighed and pre-cultured soil samples, 20 g each, were placed into a stoppered triangular flask; iodosulfuron-methyl sodium was mixed thoroughly at 3 concentration levels (see above). After waiting for the solvent to volatilise, distilled water was added to wet the soil (to make the water level in the soil about 50% of saturation level). Each was placed into an 18 cm × 21 cm sealed specimen bottle; at the same time a small beaker containing 30 ml 0.1 mol/L NaOH solution was placed inside. Then it was placed into a (25 ±1)°C incubator and cultured. At days 2, 5, 7, 10, 12 and 15 after pesticide treatment, the small beaker containing NaOH solution was removed; 0.2 mol/L HCl was used to titrate the remaining NaOH solution and at the same time replaced with a new small beaker containing NaOH, and culturing was continued.

[7] [redacted] Guanghui. Handbook for Soil Microbe Analysis Methods. Beijing: Agriculture Press, 1986

Data analysis:	The formula below was used to calculate the quantity of CO_2 released in 100 g soil[8]. *W = (blank value – titration value) × molar concentration of hydrochloric acid ×CO_2 molecular weight × 50/dry soil mass* (W is the quantity of CO_2 released in 100 g dry soil (mg)).

Results

<u>Impact of different pesticide usage quantities on catalase activity:</u>

At the recommended iodosulfuron-methyl sodium application quantity in fields (effective ingredient 0.625-1.25 mg/kg), there was no major impact on soil catalase activity and its inhibition rate was only 0.8-2.45% (Table 2). Even when the pesticide usage quantity was higher than seven times the quantity applied in fields, the inhibition rate for soil catalase activity was still only 10.11%. From the regression equation it can be seen that the quantity of iodosulfuron-methyl sodium used presented a significant correlation to soil catalase activity.

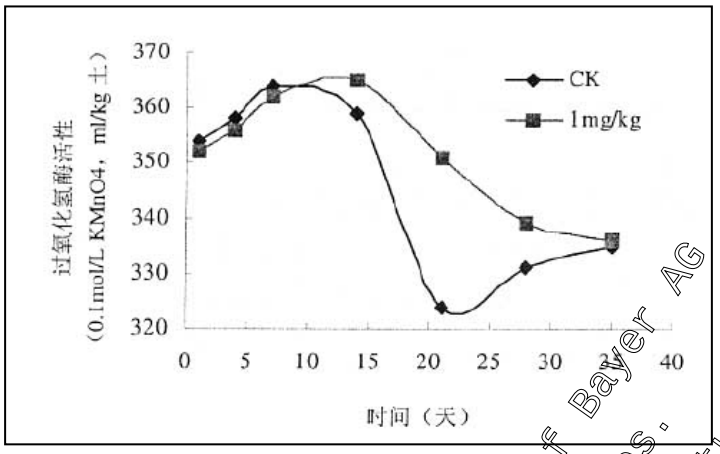
Table CA 8.5-11: Impact of Different Pesticide Usage Quantities on Catalase Activity in the Soil (0.1 mol/L $KMnO_4$, ml/kg)

Pesticide usage quantity (mg/kg)	**0.0**	**0.5**	**1.0**	**2.5**	**5.0**	**10.0**
Enzyme activity	356	353	348	343	340	320
Inhibition rates (%)		0.84	2.45	3.65	4.49	10.11
After regression analysis was performed on the results, the mathematical equation used to obtain pesticide usage quantity and catalase activity was: $y = 0.3336 + 35.39$ ($R^2 = 0.9685$, $n = 6$, $p \leq 0.01$) In the formula: x represents the pesticide usage quantity; y represents catalase activity.						

<u>The impact of different culture times on soil catalase activity:</u>

The first nine days after pesticide application iodosulfuron-methyl sodium presented a mild inhibitory action on soil catalase activity (Figure CA 8.5-4). As time went on it was seen that starting on day 9 catalase activity in soil to which pesticide had been applied exceeded that of the control group and enzyme activity was stimulated. At day 21 stimulation reached its peak, and after 28 days the catalase activity of the soil to which pesticide had been applied was basically consistent with the soil catalase activity in the control group.

[8] Zhu Lusheng, Wang Jun. Impact of acetochlor and atrazine on soil microbes and safety assessment. Soil and Environmental Sciences, 2000, 9(1): 71-72.

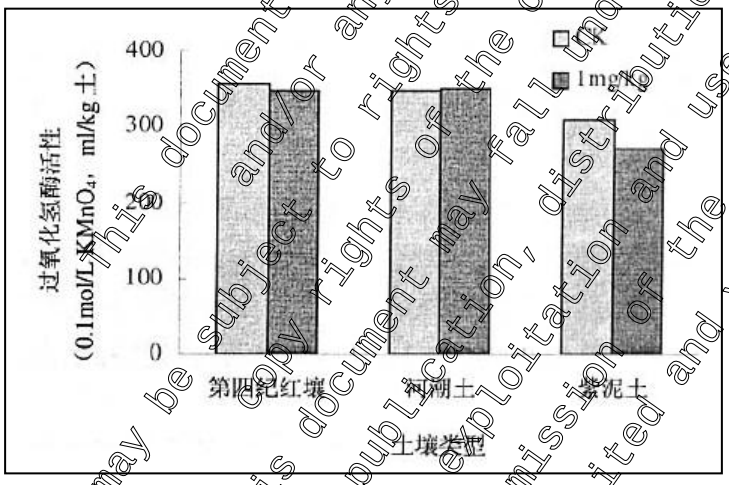


[x-axis:] Time (days)
[y-axis:] Catalase activity

Figure CA 8.5-4. Impact of Iodosulfuron-Methyl Sodium on Soil Catalase Activity at Different Culture Times

Impact of different types of soil on soil catalase activity:

In neutral Hechao earth with low organic matter and relatively high pH, iodosulfuron-methyl sodium presented a slight stimulation action on catalase activity; whereas, in purple earth with relatively high organic matter and acidic pH, catalase activity presented an inhibitory action and its enzyme activity inhibition rate reached 12.9% (Figure CA 8.5-5). From this it can be seen that iodosulfuron-methyl sodium inhibition on soil catalase is intensified as soil organic matter increases and pH decreases.

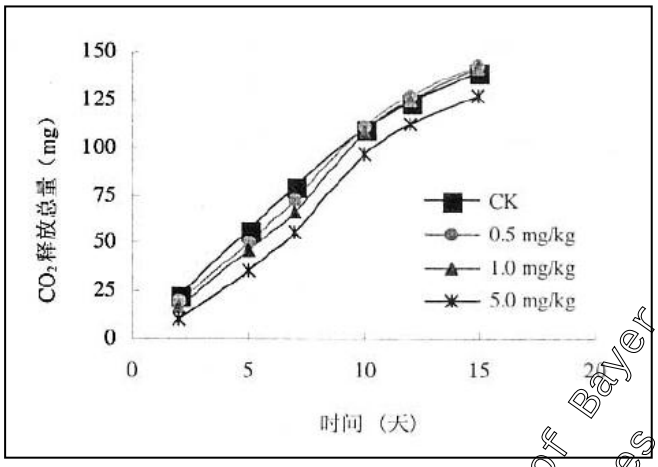


[column 1:] Quaternary red earth
[column 2:] Hechao earth
[column 3:] Purple earth
[x-axis:] Soil type
[y-axis:] Catalase activity

Figure CA 8.5-5: Impact of Different Soil Types on Soil Catalase Activity

Impact of iodosulfuron-methyl sodium on soil respiration:

In the early stages of pesticide application, iodosulfuron-methyl sodium presented an inhibition effect on soil respiration, the higher its concentration the greater its inhibition on soil respiration intensity (Figure CA 8.5-6). At 0.5 mg/kg and 1.0 mg/kg, the quantities of CO_2 released in the soil were basically restored at 10 and 12 days respectively after pesticide application, slightly exceeding the quantities of CO_2 released in the control group. Soil treated with 5.0 mg/kg still showed an inhibitory effect on soil respiration of 8.63% 15 days after treatment (Table CA 8.5-12).



[x-axis:] Time (days)
[y-axis:] Total CO_2 released

Figure CA 8.5-6: Impact of Iodosulfuron-Methyl-Sodium on Soil Respiration

Assessment of the impact of iodosulfuron-methyl-sodium on soil respiration:

Impact of pesticides on microbial respiration was assessed according to Cai et al, 1986[9]. The tested iodosulfuron-methyl sodium pesticide had no significant impact on soil respiration (Table CA 8.5-12). For none of the iodosulfuron-methyl sodium treatment concentrations did the risk coefficients exceed 20, demonstrating that iodosulfuron-methyl sodium was a pesticide with low toxicity or no actual toxicity to microbes in the soil.

Table CA 8.5-12: Risk Coefficients for Each Iodosulfuron-Methyl-Sodium Treatment to Soil Microbes

Pesticide mass fraction (mg/kg)	CO_2 cumulative release quantity (mg)	Inhibition intensity (%)	Inhibition time (days)	Risk coefficient
0	139.35			
0.5	142.29	2.11	15	0.633
1.0	141.16	1.30	15	0.195
5.0	127.33	8.63	15	0.259

Results summary

(1) The study demonstrated that the quantity of iodosulfuron-methyl-sodium used did not have a major impact on soil catalase activity; iodosulfuron-methyl-sodium impact on soil catalase activity was definitely correlated to soil properties and culture duration.
(2) Iodosulfuron-methyl-sodium showed significant inhibition of soil respiration intensity at two days after pesticide application; the larger the concentration, the stronger the inhibition. But as time went on, it was gradually converted from an inhibition action to a stimulation action and after 12 days of pesticide application respiration intensity in the soil to which pesticides had been applied had basically recovered and was consistent with the control group.
(3) The observed reduction of microbial indicators in the soil is consistent with effects reported for this class of herbicides. The short degradation time of iodosulfuron-methyl-sodium could explain that initially observed effects on catalase activity and soil respiration were receding 12 to 28 days after treatment.

[9] Cai Daoji, Jiang Xiliu, Cai Yuqi. Assessing the safety of chemical pesticides on ecology and environment. Study I: Impact and assessment of chemical pesticides on microbes in soil. Rural Eco-Environment, 1986, (2): 9-13

Comments by the notifier

The information contained in the article is considered supplementary information since all reported effects on microbial communities are ≤ 25% after 30 days, confirming the conclusions of the risk assessment.

CA 8.6 - Effects on terrestrial non-target higher plants

CA 8.6.1 - Summary of screening data

For iodosulfuron-methyl-sodium, a screening study on higher plant species was performed. As expected for a sulfonyl urea herbicide the compound showed significant herbicidal activity to several plants. Details of the studies submitted in the original EU dossier are provided in the following table.

Table CA 8.6-1: Effect data of a straight iodosulfuron-methyl-sodium to higher terrestrial plants

Test design	Test species	Ecotoxicological endpoint	Reference
Iodosulfuron-methyl-sodium			
Greenhouse, seedling emergence and growth, 28 d	Crop plants (8 species) Broadleaf plants (28 species) Grass plants (13 species)	Iodosulfuron-methyl-sodium is a broad spectrum herbicide acting pre-emergence through soil as well as post-emergence through foliar uptake. It controls broad leaves and grass weeds at very low rates. None of the 8 crops tested is fully tolerant to the active substance.	[redacted], 1998 M-182753-01-1 KCA 8.6.1 /01

Report:	[redacted];1998;M-182753-01
Title:	Efficacy of the herbicide iodosulfuron-methyl-sodium (AE F115008) on higher plant species as applied under greenhouse conditions
Report No:	C001486
Document No:	M-182753-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted];[redacted];1998;M-182688-01
Title:	Effectivity of the herbicide AE F115008 on entomology screening species
Report No:	C00146[illegible]
Document No:	M-182688-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

CA 8.6.2 - Testing on non-target plants

Test results of studies on non-target plants are, by nature, related to the tested formulation. In the original dossier for Annex I inclusion, a test with the formulation iodosulfuron-methyl-sodium + mefenpyr-diethyl WG 20 (5+15) was submitted, which was the respresentative formulation in that process. This study is no longer considered relevant as a different representative formulation have been chosen for the Renewal of the Approval of iodosulfuron-methyl-sodium.
In preparation of the submission for the Renewal of the Approval of iodosulfuron-methyl-sodium, new tier 2-tests have been performed with the new representative formulation: iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400 (100+300). For herbicides and plant growth regulators, it is considered unprofitable to conduct tier 1 studies as it is inevitable that these will lead to tier 2 or dose response studies in order to generate data suitable for deterministic or probabilistic risk assessments, i.e. ER_{50} values for 6-10 species, representing a broad range of plant species. From both tier 2- studies a most sensitive species and a respective lowest EC_{50} could be derived (see Table CA 8.6.2-1). These studies are presented and discussed in Section 10 of MCP document. Also the risk assessment based on these endpoints is presented in MCP.
Nevertheless, study results for both formulations are repeated in the table below for sake of completeness.

Table CA 8.6.2-1: Survey of non-target plant tests performed with formulated iodosulfuron-methyl-sodium

Terrestrial Non-Target Plants			
Number of species tested (species)	**Test method** **Test substance** **Application rate**	**Effects**	**Reference**
Iodosulfuron-methyl-sodium + mefenpyr-diethyl WG 20			
Dicotyledoneae: 3 (mustard, tomato, pea) Monocotyledoneae: 3 (maize, ryegrass, onion)	Tier 2 vegetative vigour IMS + MPR WG 20 0 (control), 0.032, 0.100, 0.320, 1.0, 3.2 and 10.0 mL a.s./ha (in terms of iodosulfuron-methyl-sodium) for mustard, tomato, pea, maize, ryegrass and onion with visually observations on Days 7, 14 and 21, dry weight measurements on Day 21	most sensitive species: mustard; lowest EC50: 0.042 g a.s./ha [1]	[redacted], 1999; C006692 M-194440-01-1 KCA 8.6.2/01
Iodosulfuron-methyl-sodium + mefenpyr-diethyl OD 400			
Dicotyledoneae: 7 (sugar beet, oilseed rape, radish, cucumber, sunflower, soybean, tomato) Monocotyledoneae: 3 (onion, oat, corn)	Tier 2 vegetative vigour IMS + MPR OD 400 0 (control), 1.56, 3.13, 6.25, 12.5, 25 and 50 mL prod./ha for corn and oats 0 (control), 0.39, 0.78, 1.56, 3.13, 6.25 and 12.5 mL prod./ha for cucumber and onion 0 (control), 0.1, 0.2, 0.39, 0.78, 1.56 and 3.13 mL prod./ha for sugar beet, oilseed rape, radish, sunflower, soybean and tomato with visually observations on Days 7, 14 and 21, dry weight measurements on Day 21	most sensitive species: sunflower; **lowest EC_{50}:** **2.43 mL prod./ha**	[redacted] & [redacted], 2004; C042604 M-232956-01-1 **KCP 10.6.2/01**
Dicotyledoneae: 7 (sugar beet, oilseed rape, radish, cucumber, sunflower, soybean, tomato) Monocotyledoneae: 2 (oat, corn)	Tier 2 seedling emergence IMS + MPR OD 400 0 (control), 1.56, 3.13, 6.25, 12.5, 25 and 50 mL prod./ha with daily assessments of germination until 65% emergence of control seedlings, and assessments of number of plants and mortality on Days 7 and 14 after this time; measurement of dry weight on Day 14	most sensitive species: sugar beet; **lowest EC_{50}:** **5.62 mL prod./ha**	[redacted] & [redacted], 2004; C042664 M-233058-01-1 **KCP 10.6.2/02**

[1] In the study endpoints are given in g iodosulfuron-methyl-sodium /ha.

Studies on iodosulfuron-methyl-sodium (formulated)

Report:	[redacted];[redacted];1999;M-194440-01
Title:	Acute phytotoxicity to non-target terrestrial plants following the OECD Guideline 208 (proposal 1998) and US-EPA OPPTS 850.4250 vegetative vigor, Tier II (public draft 1996) Code: AE F115008 02 WG20 B002
Report No:	C006692
Document No:	M-194440-01-1
Guidelines:	**OECD: 208; USEPA (=EPA): OPPTS 850.4250;Deviation not specified**
GLP/GEP:	**yes**

The endpoint of these studies, although listed in the Review Report for iodosulfuron-methyl-sodium (SANCO/10166/2003-Final), is only relevant for the tested WG formulation.

No conclusion regarding ecotoxicological properties of the active substance itself or the representative formulation IMS + MPR OD 400 can be drawn from this study.

Information from peer reviewed open literature

Report:	[redacted];[redacted];[redacted];[redacted];2005;M-458576-01
Title:	Response of Arabidopsis thaliana to 22 ALS inhibitors: Baseline toxicity and cross-resistance of csr1-1 and csr1-2 resistant mutants.
Report No:	M-458576-01-1
Document No:	M-458576-01-1
Guidelines:	**not applicable; not applicable**
GLP/GEP:	**no**

Executive Summary:

Acetolactate synthase (ALS) is the target site of the herbicide family known as ALS inhibitors. The intensive use of the ALS inhibitors, together with an apparently high weed mutation rate and/or a wide range of resistance, have resulted in an increased occurrence of weed population resistance.
The aim was to study the relationships among 22 ALS-inhibiting herbicides using two *Arabidopsis thaliana* susceptible lines and to assess the cross-resistance pattern of chlorsulfuron- and imazapyr-resistant lines to these 22 ALS-inhibiting herbicides.
Two susceptible (S) and two resistant (R) lines of A. thaliana: Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. ED_{50} values for the Col and Ler susceptible lines of *A. thaliana* were 333 mg/ha and 506 mg/ha, respectively.

Material and methods:

A. Material

1. Test material

Test item:	Iodosulfuron-methyl-sodium was obtained directly from the marketing company who provided a formulation containing the ALS inhibitor as the single herbicide active ingredient.
Active substance(s):	Iodosulfuron-methyl-sodium
Adjuvant / Surfactant:	Not given
Source of test item:	Bayer CropSciences ([redacted], [redacted])
Lot/Batch number:	Not given
Purity:	10% a.i. (wt/wt)
Stability of test item:	Not given
Water solubility:	Not given

2. Test organism(s)

Species:	Two susceptible (S) and two resistant (R) lines of *A. thaliana*: Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. The *A. thaliana* chlorsulfuron-resistant (csr1-1 or GH50) and imazapyr-resistant (csr1-2 or GH90) mutants isolated by Haughn and Somerville (1986, 1990)[10] from ethylmethane- sulfonate (EMS) mutagenized populations of the wild-type susceptible Col line were used.

[10] Haughn GW & Somerville CR (1986) Sulfonylurea-resistant mutants of Arabidopsis thaliana. Molecular and General Genetics 204, 430–434.
Haughn GW & Somerville CR (1990) A mutation causing imidazolinone resistance maps to the csr1 locus of Arabidopsis thaliana. Plant Physiology 92, 1081–1085.

	The csr1-1 mutant is resistant due to a point mutation resulting in a Pro to Ser substitution at the 197th amino acid, while the csr1-2 mutant is resistant due to a point mutation resulting in a Ser to Asn substitution at the 653rd amino acid (Haughn et al., 1988; Sathasivan et al., 1990, 1991)[11].
Cultivar:	Not given
Source of test species:	[redacted], UK).
Crop growth stage at treatment:	Post-emergence

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory assays
Guideline/method:	Not specified
Duration of study:	From seedlings to 20 days after 4 to 5 leaf stage
Conduction:	Seeds of *A. thaliana* were sown in 1-L plastic pots filled with a commercial soil (Terreau Semis Bouturage Repiquage; Composana, Roche-les-Beaupré, France). They were grown in the greenhouse at 20/25°C (night/ day) under natural light supplemented by artificial sodium light to provide a 16-h photoperiod. The pots were regularly rotated during the growing period. The plants were watered twice a week with a standard nutrient solution.
Application rates:	Applied post-emergence at rates: 0.034, 0.103, 0.309, 0.926, 2.778, 8.33 and 25 g a.i./ha
Number of replicates:	3 (randomized)
Plot size:	Before spraying, plants were thinned to 40 per pot.
Application device / nozzles:	Laboratory track sprayer delivering 1 spray solution with a 110-04 nozzle operated at 400 kPa
Water volume:	300 L ha
Verification of dispersion:	Not speicifed

2. Test conditions

Soil type at study site:	Commercial soil (Terreau Semis Bouturage Repiquage; Composana, [redacted]´, France)
pH:	Not specified
Organic matter (C_{org}):	Not specified
Others:	Not specified

2. Observations and measurements:

Treatment at end of test:	Two weeks after treatment plants were cut off at soil level and shoots were oven-dried at 70°C for 48 h.
Biological parameters measured:	An observation corresponded to the dry shoot biomass of 40 plants per pot.
Statistical analyses:	Data were expressed as percentages of their untreated respective controls to standardise comparisons between Col

[11] Haughn GW, [redacted] J, Mazur B & Somerville C (1988) Transformation with a mutant Arabidopsis acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. Molecular & General Genetics 211, 266-271.
Sathasivan K, Haughn GW & Murai N (1990) Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant Arabidopsis thaliana var. Columbia. Nucleic Acids Research 18, 2188.
Sathasivan K, Haughn GW & Murai N (1991) Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var. Columbia. Plant Physiology 97, 1044–1050.

and Ler lines. For each line a non-linear regression was used to describe the response of lines to ALS inhibiting herbicides. Following Kudsk and Streibig (1993)[12], we used the equation given below and fitted the dose–response curve using SYSTAT[13]. An F-test ($P = 0.05$) was used to test significant differences of the regression parameters. Bonferroni's correction was applied to adjust the observed significance level for the fact that multiple comparisons were made (Scherrer, 1984)[14]. Comparisons of ED_{50} values among herbicides were carried out by examining the overlap between the 95% Wald's confidence limits. Wilcoxon's signed-rank test was then performed to test the effect of the Col or Ler genetic background of the S line on the ED_{50} (Scherrer, 1984).

Results:

1. Biological findings:

Baseline toxicity: For each susceptible line the herbicide application rates were sufficient to establish the dose–response curve. ED_{50} was used to characterize the baseline toxicity of the ALS-inhibiting herbicides studied for *A. thaliana*. Results for iodosulfuron-methyl-sodium are shown in Table CA 8.6.2-2.

Table CA 8.6.2-2: ED_{50} for the Col and Ler susceptible lines and resistance ratios (R:S) for the chlorsulfuron-resistant csr1-1 and imazapyr-resistant csr1-2 lines of *Arabidopsis thaliana* treated with 22 ALS-inhibiting herbicides – results for iodosulfuron-methyl-sodium

Herbicide	*Arabidopsis thaliana*				csr1-1 R:S	csr1-2 R:S
	Col		Ler			
	ED_{50} [mg/ha]	CL* [mg/ha]	ED_{50} [mg/ha]	CL* [mg/ha]		
Iodosulfuron-methyl-sodium	132	84-179	287	75-499	11	1

*CL: 95% Wald confidence limits
R = resistant; S = susceptible

Data from 14 species were considered to be suitable for the study of the relationships between ED_{50} for *A. thaliana* and other weed species. Iodosulfuron-methyl-sodium was not included in the comparison.

Cross-resistance: A cross-resistance pattern could be directly assessed by the inhibition of ALS enzyme activity. Here, the cross-resistance pattern on the 22 ALS-inhibiting herbicides, including iodosulfuron-methyl-sodium , used in the study was assessed for the homozygous chlorsulfuron- and imazapyr-resistant lines by recording plant dry matter. The resistance ratios for the csr1-1 and csr1-2

[12] Kudsk P & Streibig JC (1993) Formulations and adjuvants. In: Herbicide Bioassays (eds JC Streibig & P Kudsk), 99–116. CRC Press, Boca Raton, FL, USA.
[13] SYSTAT 10 (2000) SYSTAT, Release 10 for Windows. SPSS, Chicago, IL, USA.
[14] Scherrer B (1984) Biostatistiques (ed. B Scherrer), 593–596. Chicoutimi: Gae¨tan Morin Editeur, Quebec, Canada.

lines are indicated in Table CA 8.6.2-2. The csr1-2 imazapyr-resistant line conferred little or no resistance to some sulfonylurea herbicides, including iodosulfuron-methyl-sodium (R:S ratio < 5). The csr1-1 chlorsulfuron-resistant line exhibited low resistance to two sulfonylurea herbicides, including iodosulfuron-methyl-sodium (R:S ratio ≥ 5).

Results summary:

ED_{50} values (dry shoot biomass) for the Col and Ler susceptible lines of *Arabidopsis thaliana* were 132 mg/ha and 287 mg/ha, respectively.

Comments by the notifier

Although the paper as a whole can be regarded as reliable, the endpoints presented in this paper are not considered in the risk assessment for iodosulfuron-methyl-sodium for the following reasons:

1. The test was conducted with strains which were susceptible to ALS-inhibitors and not to naturally occurring phenotypes of *A. thaliana*.
2. As far as described in the paper the test method used does not fully apply to OECD 2017. Especially the plant density (40 plants in a 1 L pot) was exceptionally high.

Therefore, this paper is considered as supplementary information only.

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

For iodosulfuron-methyl-sodium 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 iodosulfuron-methyl-sodiumWG 20 to entomology screening species presented in this chapter

Test design	Test species	Ecotoxicological endpoint	Reference
Iodosulfuron-methyl-sodium, formulated as WG 20			
Root systemicity test, different treated stages (eggs, larvae, all stages), 7 d	*Spodoptera littoralis*, *Heliothis virescens*, *Aphis fabae*, *Metaparsia lugens*, *Diabrotica undecimpunctata*, *Meloidogyne incognita*, *Tetranychus urticae*, *Blattella germanica*, *Vicia faba*, *Aphis fabae* (root systemic activity)	The test item is not effective on any tested species. most sensitive species: *Meoidogyne incognita* (larvae)	[redacted], 1998 M-182688-01-1 KCA 8.6.1 /02

CA 8.8 - Effects on biological methods for sewage treatment

For iodosulfuron-methyl-sodium, studies with soil microflora, activated sludge and *Pseudomonas putida* have been conducted and presented in the original EU dossier. Details of all studies are provided in the following table. No additional study is deemed necessary for the Annex I renewal of iodosulfuron.

Table CA 8.8-1: Effect data of iodosulfuron-methyl-sodium to activated sludge presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Iodosulfuron-methyl-sodium			
Soil microbial activity	Short-term respiration, 0-91 days	loamy sand and loamy silt soil 13.33 & 66.65 µg a.s./kg soil (equivalent to 10 & 50 g a.s./ha) At both application rates negligible effect on soil microbial respiration in **loamy sand** after 28 days (<± 15 % deviation of the control treatment) At the application rate of 10 g/ha negligible effect on soil microbial respiration in **loamy silt soil** after 28 days (<± 15 % deviation of the control treatment) At the application rate of 50 g/ha tolerable effect on soil microbial respiration in **loamy silt soil** after 28 days (<± 21.9 % deviation of the control treatment)	[redacted], 1997 M-143028-01-1 KCA 8.8. /01
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-141820-01-1 KCA 8.8. /02
Pseudomonas putida	Cell multiplication inhibition test, 16 ± 1 h (DIN 38412 part 8 (1991))	*Pseudomonas putida*, inhibitory effect of water-soluble test substances: EC_{10} 10 mg/L (Range: 1-10 mg/L) EC_{50} 26 mg/L (Range: 10-100 mg/L) harmful effect of the test substance to bacteria	[redacted], 1996 M-141031-01-1 KCA 8.8. /03

Report:	[redacted];1997;M-143028-01
Title:	Effects on soil microbial activity (short-term respiration) AE F115008 substance, technical Code: AE F115008 00 1C89 0001
Report No:	A59351, CE96/095
Document No:	M-143028-01-1
Guidelines:	**BBA: VI, 1-1, March 1990;Deviation not specified**
GLP/GEP:	**yes**

The results from this study were not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted];[redacted];1996;M-141820-01
Title:	Respiration inhibition to activated sludge of AE F115008 substance, technical
Report No:	A58107
Document No:	M-141820-01-1
Guidelines:	**OECD: 209;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

Report:	[redacted]; [redacted];1996;M-141031-01
Title:	Inhibitory effect of water constituents on bacteria (Pseudomonas cell multiplication inhibition test) Hoe 115008 substance, technical
Report No:	A57292
Document No:	M-141031-01-1
Guidelines:	**DIN: 38412 part 8; ISO: 10 712;Deviation not specified**
GLP/GEP:	**yes**

The endpoint from this study was not mentioned in the Review Report for iodosulfuron-methyl-sodium (SANCO/ 10166/2003-Final).

CA 8.9 - Monitoring data

Monitoring data concerning adverse effects of the active substance to non-target organisms are not available.