Propoxycarbazone-sodium

Herbicide

Dossier for Renewal of Approval according to Commission Regulation 844/2012

Document M-CA, Section 8

Ecotoxicological studies on the active substance

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Introduction

Propoxycarbazone-sodium is an herbicidal active substance.
Ecotoxicological data of propoxycarbazone-sodium and its major metabolites were submitted within the EU Dossier (Baseline Dossier), which resulted in the Annex inclusion under Directive 91/414/EEC. In this Supplemental Dossier P-010245-02 for Renewal of Approval of propoxycarbazone-sodium only those ecotoxicological studies are described in Section 8, which were not submitted within the Baseline Dossier. However, for a better understanding, all endpoints are presented in summary tables for each Point CA 8.1 to 8.9. To differentiate between studies already evaluated during the last Annex I listing and new studies submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval, studies already evaluated were shaded in grey in the endpoint tables.

Experimental details of ecotoxicological studies done with the formulated product ATTRIBUT SG70 that also satisfy data requirements specified in Commission Regulation (EU) No 283/2013 were included in this Document M-CA. ATTRIBUT SG70 is considered to be ecotoxicologically equivalent to MKH 6561 WG 70, the representative product of the Baseline Dossier. For further details please refer to CONFIDENTIAL information provided separately in Document J of this Supplemental Dossier P-010245-02.

The codes and structures of propoxycarbazone-sodium and its metabolites addressed in this section are presented in Document N3 of the dossier. For convenience, a short summary of codes used in ecotoxicological studies of the Baseline dossier and this Supplemental Dossier is given in the table below:

Table 8.1-1 Codes of propoxycarbazone-sodium and metabolites used in ecotoxicological studies

Name	Alternative code(s)
Propoxycarbazone-sodium	MKH 6561
Attribut SG70	Propoxycarbazone-sodium SG 70, MKH 6561 WG 70; MKH 6561 70 WG
M04	MKH 6561-carboxylic acid; MKH 7018
M05	MKH 6561-sulfonamide methyl ester; STJ 4934)
M06	MKH 6561-sulfonamide Acid; MKH 7283
M07	MKH 6561-saccharin; MKH 7284
M08	MKH 6561-4-hydroxy-saccharin, KTS 9357
M09	MKH 6561-propoxytriazolinonamide; KTS 9304
M10	MKH6561-N-methyl propoxytriazolinone; MKH 7017
M11	MKH6561-methoxy-saccharin

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on birds

A summary of all available relevant and compliant data for propoxycarbazone-sodium on acute and long-term toxicity to birds is presented in the tables below.

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Table 8.1-1 Acute toxicity of propoxycarbazone-sodium to birds

Test item	Species	LD_{50} [mg a.s./kg bw]	NOEL [mg a.s./kg bw]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	Bobwhite quail	> 2000	2000	[redacted] (1999) 108741 M-007896-01-1 KCA 8.1.1.1 /01	Yes

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

Table 8.1-2 Short-term toxicity of propoxycarbazone-sodium to birds

Test item	Species	LC_{50} [mg a.s./kg feed]	NOEL/ NOAEL [mg a.s./kg feed]	LD_{50} [mg a.s./kg bw/day]	NOEL/ NOAEL [mg a.s./kg bw/day]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	Bobwhite quail	> 10000	10000	> 2000	2000	[redacted] (1999) 108802 M-015907-01-1 KCA 8.1.1.2 /01	Yes
	Mallard duck	> 10000	10000	> 2203	2203	[redacted] & [redacted] (1999) 109144 M-018780-01-1 KCA 8.1.1.2 /02	Evaluated during the first EU review

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

Table 8.1-3 Long-term toxicity to birds of propoxycarbazone-sodium to birds

Test item	Species	Test design	NOEL/ NOAEL [mg a.s./kg feed]	NOEL/ NOAEL [mg a.s./kg bw/day]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	Bobwhite quail	Reproduction, one generation, 22 weeks	1250	94.1 [a]	[redacted] (1999) 108910 M-018752-01-1 KCA 8.1.1.3 /01	Yes
	Mallard duck	Reproduction one generation, 20 weeks	1250	165 [a]	[redacted] et al. (1999) 109381 M-023611-01-1 KCA 8.1.1.3 /02	Yes
Propoxy-carbazone-sodium	Bobwhite quail	Reproduction one generation, 25 weeks	324	45	[redacted] & [redacted] (2013) EBMIL003 M-449836-01-1 KCA 8.1.1.3 /03	New study

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[a] For re-calculation of the endpoint, please refer to Table 8.1-4 below.
Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

Conversion of avian reproduction study results from feed concentration to daily dose

The TER values for long-term exposure of birds are calculated on the basis of a dietary dose or level. Thus, in case the endpoint in the study is only given in ppm, conversion of endpoints from ppm to mg a.s./kg bw/d is necessary as recommended by the "Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC" (SANCO/4145/2000-final). For this purpose generally the mean body weight and the mean food consumption over the exposure period have to be calculated. In the table below the re-calculation of the endpoints is shown for the two studies submitted for the first EU review. In the newly conducted study ([redacted] & [redacted] (2013), LBMIL003, M-449836-01-1) endpoints are already given in ppm and mg a.s./kg bw/d.

Table 8.1-4 Daily dose conversion from propoxycarbazone-sodium avian reproduction studies

Nominal Dose (mg a.s./kg feed)	Daily mean food consumption (g feed/bird/day)	Mean body weight (g)	Daily dose (mg a.s./kg bw/day)
Bobwhite quail ([redacted] (1999), 108910, M-018732-01-1)			
Control	17.0	219	0
50	16.4	217	3.8
250	16.9	223	19.0
1250	16.9	225	94.1
Mallard duck ([redacted] et al. (1999), 109381, M-023611-01-1)			
Control	145	1108	0
50	160	1096	7.3
250	145	1085	33.0
1250	**148**	**1122**	**165**

CA 8.1.1.1 Acute oral toxicity to birds

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph from the first Annex I inclusion.

CA 8.1.1.2 Short-term dietary toxicity to birds

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

One additional study on reproductive toxicity to birds was conducted for re-registration in the USA which was not submitted during the first Annex I inclusion process. The study is submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal as the study resulted in a lower endpoint for propoxycarbazone-sodium that needs to be considered for the risk assessment presented in M-CP, Section 10, Point CP 10.1.1. The study is summarised below.

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Report:	[redacted];[redacted];[redacted];2013;M-449836-01
Title:	Toxicity of MKH6561 (propoxycarbazone-sodium) on reproduction to the northern bobwhite quail (Colinus virginianus)
Report No:	EBMIL003
Document No:	M-449836-01-1
Guidelines:	**OECD 206** **USEPA OPPTS 850.2300**
Deviations:	The age of adult birds deviated from the OECD guideline specification of 9 months old at experimental start. The ages of birds for the study were at least 16 weeks old at experimental start. The cage size varied from the suggested specifications in the guidelines. The deviations were considered minor and did not have a significant impact on the quality of the study.
GLP/GEP:	**yes**

Executive Summary
The objective of the study was to evaluate the effects upon the adult quail of dietary exposure to propoxycarbazone-sodium technical over a period of approximately 25 weeks. Nominal dietary feeding levels for the study were 0 (control), 111, 333, and 1000 mg a.s./kg feed. Effects on adult health, body weight, and feed consumption were evaluated. In addition, the effects of adult exposure to propoxycarbazone-sodium technical on the number of eggs laid, fertility, embryo viability, hatchability, offspring survival, and eggshell quality (strength and thickness) were evaluated.
The No Observed Effect Level (NOEL) for both parental toxicity and reproduction endpoints of bobwhite quail exposed to propoxycarbazone-sodium technical was 333 ppm (nominal test level). This value corresponds to a daily dietary dose levels of 45 mg a.s./kg bw/d. The Lowest Observed Effect Level (LOEL) was 1000 mg ppm (nominal test level) corresponding to a dose level of 140 mg a.s./kg bw/day.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561 (propoxycarbazone-sodium); technical
Description:	White powder
Lot/Batch #:	Batch Code: AE 0298618-01-07; Origin Batch No.: K782016
Purity:	97.7% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Approximately 14 weeks of age
Sex:	Seventy-two bird pairs were utilized for the study. One reproductive pair of birds (i.e. one male & one female) was housed per cage.
Weight:	Adult body weights were measured the day prior to experimental start, on Week's 3, 5, 7 and 9, and at termination the adult phase. No adult body weights were taken during the egg production phase.

Source:	Farm raised quails obtained from [redacted], [redacted], Ohio
Loading:	One reproductive pair of birds (i.e. one male & one female) was housed per cage
Diet/Food:	Teklad Bayer Gamebird Ration and local tap water *ad libitum*
Acclimatisation:	Acclimated to the laboratory environment for approximately four weeks prior to experimental start

4. Environmental conditions:

Temperature:	20.9°C (average value, adult birds)
Relative humidity:	60.1% (average value, adult birds)
Photoperiod:	7 h light/17 h dark during acclimation of the birds and 8 weeks short day length phase, afterwards the photoperiod was increased to 17 h light/7 h dark

B. STUDY DESIGN

1. Experimental treatments

Adult bobwhite quail (*Colinus virginianus*) were exposed to propoxycarbazone-sodium technical for approximately 25 weeks to nominal dietary levels of control, 111, 333, and 1000 mg a.s./kg feed. Bobwhite quail were approximately 18 weeks old at experimental start with 18 pairs of birds at each treatment level. Birds were observed for mortality, abnormal behaviour and signs of toxicity; adult body weight and feed consumption were measured; gross pathology was conducted; reproductive parameters, as well as hatchling health, growth and survival, were examined.

2. Observations

The test birds were acclimated to the test facility and study cages for approximately four weeks prior to experimental start. During the acclimation, all birds were observed daily. Birds exhibiting abnormal behaviour or debilitating physical injuries were not used for the test. During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed daily from hatching until 14 days of age. A record was maintained for all clinical observations and mortalities.

Adult body weights were measured the day prior to experimental start, on weeks 3, 5, 7 and 9, and at termination the adult phase. No adult body weights were taken during the egg production phase.

Adult feed consumption was measured weekly by cage throughout the study. A measured quantity of feed was added to feed pans on Thursday of each study week. The amount of any additional feed added during the study was recorded. At the end of each study week, the quantity of remaining feed was weighed to determine feed consumption.

Adult birds that died or were euthanized during the course of the study were subjected to gross necropsy. At the conclusion of the exposure period, all surviving birds were necropsied.

3. Statistical calculations

The No Observed Effect Level (NOEL) and Lowest Observed Effect Level (LOEL) were identified for each parameter using hypothesis testing methodology. All hypotheses testing were performed with a specialized statistical program designed to analyse avian reproduction data. All data was analysed independently according to each end-point. Data from treatment groups were compared to controls using the Shapiro-Wilk's test for normality and Levene's test of equal variance to determine if dose groups had unequal variances. If assumption of normality ($p \leq 0.01$) and homogeneity of variance ($p \leq 0.05$) were met, then parametric analyses were conducted using analysis of variance (ANOVA)

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followed by Dunnett's test or William's test. If variances were unequal, then the non-parametric analyses were conducted using the Jonckheere or Mann-Whitney procedures. Statistical analyses were performed using SAS® statistical software for personal computers with conclusions of statistical significance at the $\alpha = 0.05$ (95% confidence level).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.1-5 Effects of propoxycarbazone-sodium on reproductive performance of bobwhite quail over 25 weeks

Propoxycarbazone-sodium [mg a.s./kg feed]	Control	111	333	1000
Reproductive performance				
Mean number of eggs laid per hen	55.9	49.6	51.8	41.5*
Mean number of eggs cracked per hen	1.5	0.6	0.4	0.2
Eggs non-cracked/eggs laid per hen [%]	97.4	98.3	99.4	99.6
No. of eggs set per hen	49.3	44.1	46.6	37.1
Eggs set of eggs laid per hen [%]	87.5	87.4	87.7	87.6
No. of viable embryos per hen	48.1	42.1	43.1	36.2
Viable embryos of eggs set [%]	97.4	95.1	90.7	96.5
No. of 3-week live embryos per hen	47.5	41.7	42.7	36.1
Live embryos of viable embryos per hen [%]	98.6	98.7	98.9	99.7
Mean number of hatchlings per female	45.7	40.8	39.8	33.9**
Hatched of eggs laid per hen [%]	80.9	80.4	72.2	80.0
Hatched of eggs set per hen [%]	92.5	91.7	82.0	91.2
Hatched of live embryos [%]	96.2	97.4	91.1	94.8
No. of 14 day survived hatchlings per hen	44.8	40.4	39.0	33.4**
14-day-old survivors/egg set [%]	90.4	90.8	80.1	89.9
14-day-old survivors of no. hatched [%]	97.7	99.0	97.9	98.5
Eggshell thickness				
Mean shell thickness [mm]	0.22	0.22	0.22	0.22
Eggshell strength				
Eggshell strength [kg]	0.78	0.79	0.77	0.81
Body weight of hatchling				
Mean body weight [g]	6.3	6.3	6.2	6.2
Body weight of 14-day old survivors				
Mean body weight [g]	36.6	36.5	34.8	35.7

* Statistically significant from control (Dunnett's test, $p \leq 0.05$)
**Statistically significant from control (Williams test, $p \leq 0.05$)

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The overall NOEL and LOEL are given below:

Endpoints	
NOEL reproduction	333 mg a.s./kg feed (nominal test level), corresponding to 45 mg a.s./kg bw/day
LOEL reproduction	1000 mg a.s./kg feed (nominal test level), corresponding to 140 mg a.s./kg bw/day

B. OBSERVATIONS

Dietary Concentration

The nominal amounts of propoxycarbazone-sodium technical in the dietary feed were administered at levels of 0 (control), 111, 333, and 1000 mg a.s./kg feed. The average measured amounts of propoxycarbazone-sodium technical for weeks 1, 5, 10, 15, 20, and 25 were determined as 0, 110, 324, and 999 mg a.s./kg feed representing percent nominal values of 99%, 97%, and 100%, respectively. These values correspond to daily dietary dose levels of 0, 16, 45, and 140 mg a.s./kg bw/day, respectively.

The dietary concentrations is summarised in the table below:

Nominal Dietary Level [mg a.s./kg feed]	Measured Dietary Level [mg a.s./kg feed]	Percent of Nominal	Measured Daily Dietary Dose [mg a.s./kg bw/day]
0 (control)	0	-	-
111	110	99%	16
333	324	97%	45
1000	999	100%	140

Adult Bird Observations

Clinical observations of adult birds exhibited no treatment related signs of toxicity. Incidental clinical observations noted during the study included those that are normally associated with injuries and penwear. Such signs included feather loss on tail, neck, back, and head, foot injuries, and abrasions/lacerations on head, back, feet, and neck. Except for the incidental findings, all birds were normal in appearance and behaviour throughout the study.

Adult Bird Mortality

Nine incidental adult mortalities occurred during the test, with one bird in the control group, two birds in the 111 mg a.s./kg level, two birds in the 333 mg a.s./kg level, and four birds in the 1000 mg a.s./kg level. In summary, all bird mortality was attributed to injuries sustained in the cage and was not a condition of treatment related effects to the test substance.

Adult Bird Necropsy

Necropsy observations of adult birds exhibited feather loss in all treatment levels including the control. Minor skin lesions/abrasions were observed in all levels. These observations were due to normal cage wear for laboratory reared bobwhite quail in the reproductive phase. Reproductive organs appeared normal at all treatment levels with the exception of eight female birds with regressed ovaries in the following: control (2 birds), 111 ppm (3 birds), 333 ppm (3 birds), and 1000 ppm (3 birds). All male reproductive organs appeared normal for all treatment levels with the exception of two males with regressed testes: one in the control group, one in the 333 ppm treatment level. One female in the 111 ppm treatment level and one female in the 1000 ppm treatment level had lesions/growths on the liver.

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Adult Bird Body Weight
The adult body weights in the quail reproduction study were measured prior to dosing and every other week up to the egg production phase (i.e. weeks 3, 5, 7, and 9) and prior to adult sacrifice. There were no statistically significant effects for either adult male or female body weight gain at any test level. The NOEL for the adult weight gain endpoint was determined to be 1000 ppm for this study.

Adult Bird Feed Consumption
Adult bird food consumption was measured weekly over a 25-week period in the quail reproduction study. There were no statistically significant differences at any treatment level as compared to the control for adult bird food consumption and the NOEL was determined to be 1000 ppm for this study.

Reproductive Effects
Data for the egg production endpoints: eggs laid, eggs cracked, percent eggs not cracked of laid, eggs set and percent eggs set of eggs laid, eggshell strength and thickness were evaluated. The embryo endpoints included the number of viable embryos, the number of live embryos, the percent viable embryos of eggs set, and the percent live embryos of viable embryos. In summary, there were statistically significant differences at the 1000 ppm treatment level as compared to the control for the number of eggs laid. The NOEL for the egg endpoints was determined to be 333 ppm for the quail reproduction study.

Hatchling Effects

The endpoints analysed included the number hatched and hatchling survival, percent number hatched of eggs set, percent number hatched of eggs laid, percent number hatched of live embryos, percent 14-day survivors of eggs set, and percent 14-day survivors of number hatched. The mean number of hatchlings and 14-day hatchling survivors were statistically significant for the 1000 ppm treatment level as compared to the control. The NOEL was determined to be 333 ppm for the hatchling endpoints.

Validity criteria
The following validity criteria for the quail reproduction study were fulfilled as stated in the OPPTS 850.2300 and OECD 206 guidelines.

- Adult Control Mortality: One control mortality occurred for the 18 pair of adult birds during the study. Total control mortality for the study was ≤10%.
- Analytical Verification: Analysis of propoxycarbazone-sodium in the feed resulted in mean measured concentrations ≥80% recovery of the nominal concentrations.
- 14-Day Old Survivors: The 14-day old survivor's per hen in the control group was 45, exceeding the requirement of 12 per hen for bobwhite quail in this study design.
- Eggshell Thickness: The eggshell thickness for the control group was 0.22 mm which was in excess of the stated validity criteria value of 0.19 mm for bobwhite quail.

III. CONCLUSIONS

The No Observed Effect Level (NOEL) for both parental toxicity and reproduction endpoints of bobwhite quail exposed to propoxycarbazone-sodium technical was 333 ppm (nominal test level). This value corresponds to a daily dietary dose levels of 45 mg a.s./kg bw/d. The Lowest Observed Effect Level (LOEL) was 1000 mg ppm (nominal test level) corresponding to a dose level of 140 mg a.s./kg bw/day.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

A summary of the ecotoxicological relevant data for propoxycarbazone-sodium on acute and long-term toxicity to mammals is presented in the table below. For details please refer to Document M-CA Section 5.

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Table 8.1-6 Acute and long-term toxicity of propoxycarbazone-sodium to mammals; endpoints relevant for the ecotoxicological risk assessment

Test item	Test design	Species	Endpoint	Reference	EU agreed endpoint (SANCO/4067/2001-final)
Propoxy-carbazone-sodium	acute, oral	Rat	LD_{50} > 5000 mg a.s./kg bw	[redacted] (1994) 23480 M-001552-01-1 KCA 5.2.1 /01	Yes
Propoxy-carbazone-sodium	2-generation	Rat	NOAEL 16000 ppm corresponding to 1231 mg/kg bw/d	[redacted] (1999) 109096 M-012427-03-1 KCA 5.6.1/02	Yes

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

CA 8.1.2.1 Acute oral toxicity to mammals

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph. For details please refer to Document M-CA Section 5.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph. For details please refer to Document M-CA Section 5.

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

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

As the log P_{OW} of the active substance propoxycarbazone-sodium is below the trigger (< 3), no evaluation of secondary poisoning is needed. Please refer to M-CP, Section 10, Point CP 10.1.1.2 for details.

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

Since propoxycarbazone-sodium is of low toxicity in birds and laboratory rodents, no risk for reptiles and amphibians is to be expected.

CA 8.1.5 Endocrine disrupting properties

The following definitions were used as the basis for evaluating the potential impact of propoxycarbazone-sodium to wildlife:

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*

[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.

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adverse effects in an intact organism, or its progeny, or (sub)populations." An adverse effect has been defined 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."*

Wild mammals

The existing toxicological data package is sufficient to exclude relevant endocrine disrupting (ED)-like potential of propoxycarbazone-sodium. This is based on the absence of effects on the weight of hormone-sensitive tissues like reproductive organs, thyroids and pituitary. In addition, the available fertility studies showed no effects on male or female fertility, which may be considered sensitive targets of ED-like activity.

Birds

The population relevant effects of propoxycarbazone-sodium on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. For both species there were no effects on adult birds, offspring or reproductive parameters up to and including 333 ppm. Reproduction was not affected in two studies with bobwhite quail ([redacted] (1999), 108910, M-018752-01-1) and mallard duck ([redacted] et al. (1999), 109381, M-023611-01-1) up to and including 1250 ppm. In the new reproduction study with bobwhite quail ([redacted] & [redacted] (2013), EBMIL003, M-449836-01-1) statistically significant differences at the 1000 ppm treatment level were found for the number of laid eggs, the mean number of hatchlings and 14-day hatchling survivors. However, as no endocrine disrupting potential was found in mammals there is no indication that these effects were caused by an endocrine mode of action but rather the result of a general toxicity. Therefore, no further testing for endocrine disrupting properties is warranted.

CA 8.2 Effects on aquatic organisms

In order to complete the aquatic risk assessment and to address data requirements according to Commission Regulation (EU) No 283/2013, additional studies were performed. For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

To address data requirements, an additional study with propoxycarbazone-sodium on the dicotyledonous aquatic macrophytes *Myriophyllum spicatum* was conducted.

To complete the aquatic data package, several new studies were conducted with the major aquatic metabolite M06 (acute fish, acute *Daphnia*, algae and *Lemna*) and as well with soil metabolites M07 (acute fish, acute *Daphnia*, algae and *Lemna*) and M08 (acute *Daphnia*, algae and *Lemna*) which can be transported to surface water bodies via run-off and drainage. For further details please refer to Doc M-CA, Section 7.

For soil metabolites M09 and M11, no studies on aquatic organisms were conducted. In the soil degradation pathway propoxycarbazone-sodium is degraded in first steps via cleavage of the ester bond yielding carboxylic acid (M04) and/or cleavage of the triazolinone amide bond resulting in sulfonamide methyl ester (M05) or N-methyl propoxy triazolinone amide (M09) and N-methyl propoxy triazolinone (M10). A full data package with aquatic organisms is available for metabolite M10 showing no significant effects on any of the tested organisms. As M09 has a similar molecular structure to M10 (M09 is the amide of M10) and M09 is further degraded to M10, it is not expected that M09 poses a risk to aquatic organisms and no studies were conducted for this metabolite. In addition, M09 showed no herbicidal activity in a screening test for herbicidal activity (see also CA 8.6.1 below) as can be expected since the toxophore (i.e. the sulfonylurea group) is no longer present in the molecule. The newly detected metabolite M11 was formed via aerobic and anaerobic transformation of metabolite M08 (4-hydroxy-

[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.

saccharin). As structurally similar precursor metabolites of M11 (i.e. M07 and M08) do not give indication to any toxicity and herbicidal activity (the toxophor sulfonylurea is no longer present in any of the metabolites), testing of aquatic organism with M11 was not considered necessary.

CA 8.2.1 Acute toxicity to fish

A summary of all available relevant and compliant data for propoxycarbazone-sodium on acute toxicity to fish is presented in the table below.

Table 8.2-1 Acute toxicity of propoxycarbazone-sodium and metabolites to fish

Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/2001-final)
Propoxy-carbazone-sodium	*Oncorhynchus mykiss*	96 h, static	LC_{50} > 77.2 (nm)	[redacted] (1998) 108066 M-004219-01-1 KCA 8.2.1 /01	Yes
	Lepomis macrochirus	96 h, static	LC_{50} > 110 (nom)	[redacted] (1997) 107748 M-001668-01-1 KCA 8.2.1 /02	Evaluated during the first EU review
M04	*Lepomis macrochirus*	96 h, static	LC_{50} > 100 (nom)	[redacted] (1998) DOM 98054 M-005115-01-1 KCA 8.2.1 /03	Evaluated during the first EU review
M05	*Brachydanio rerio*	96 h, semi-static	LC_{50} > 79 (mm)	[redacted] (1999) 742072 M-017346-01-1 KCA 8.2.1 /05	Evaluated during the first EU review
M06	*Oncorhynchus mykiss*	96 h, static	LC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30183230 M-278097-01-1 KCA 8.2.1 /06	New study
M07	*Oncorhynchus mykiss*	96 h, static	LC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30193230 M-278099-01-1 KCA 8.2.1 /07	New study
M10	*Lepomis macrochirus*	96 h, static	LC_{50} > 100 (nom)	[redacted] (1998) DOM 98052 M-005052-01-1 KCA 8.2.1 /04	Evaluated during the first EU review

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For detailed information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

To complete the aquatic data package on fish, additional acute studies with metabolites M06 and M07 were performed, which were not submitted for the first Annex I inclusion and are submitted within this

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Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval. These studies are summarised below.

Metabolite M06

Report:	[redacted];[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**
Deviations:	None
GLP/GEP:	**yes**

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Executive Summary

The purpose of this study was to evaluate the acute toxicity of MKH 6561-Sulfonamide Acid to fish. For this purpose, juvenile Rainbow trout were exposed in a static test to aqueous test media containing the test item at a concentration of 100 mg test item/L under defined conditions (limit test). The recorded effects were the mortality and visible abnormalities of the fish.
In the control and in the test medium of 100 mg test item/L all fish survived until the end of the test and no visible abnormalities were observed. The 96-hour NOEC of MKH 6561-Sulfonamide Acid to Rainbow trout was determined to be 100 mg test item/L. The 96-hour LC_{50} can be determined to be greater than 100 mg test item/L. The endpoints were expressed in terms of nominal concentrations.

1. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-Sulfonamide Acid
Description:	Solid, white powder
Lot/Batch #:	AE 1234964-PU-01; Origin Batch No: M00102
Purity:	99% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juveniles
Size and weight:	The mean body length of the fish* in the test was 5.12 ± 0.21 cm (Mean ± SD), the mean body wet weight 1.16 ± 0.26 g (Mean ± SD). * 10 fish from the test fish batch used for the test were measured during the course of the test
Source:	The test fish were obtained from [redacted], Germany.

Holding conditions:	In accordance with the test guidelines the fish were held in test water in the laboratories of IBACON for at least 12 days prior to the start of the test without any medication. During this period until one day before test start, the fish were fed regularly with a commercial fish diet for Rainbow trout. During the last 12 days prior to the start of the test no fish (0%) died in the test fish batch used and all fish were healthy.
Diet/Food:	None during the test
Acclimatisation:	For at least 12 days before the start of the test the fish were acclimated to the test water and test temperature.

4. **Test conditions:**

Test concentration:	100 mg test item/L (limit test), and a control (test water only)
Number of replicates:	1
Fish per replicate	10
Maximum loading rate	1 g fish/L test water

5. **Environmental conditions:**

Temperature:	17°C
Photoperiod:	16 h light : 8 h dark; 380 – 720 lux
pH:	pH 7.8 to 7.9
Dissolved oxygen:	At least 60 % of the air saturation value for the duration of the study
Hardness:	2.5 mmol/L (= 250.0 mg/L) as $CaCO_3$
Aeration of the test water:	The test media were slightly aerated during the test.

B. STUDY DESIGN

1. Experimental treatments

Ten fish were exposed to nominal concentrations of 100 mg MKH 6561-Sulfonamide Acid/L. For the determination of the test concentrations a non GLP range-finding test was performed. A negative control (test water without addition of the test item) was tested in parallel.

2. Observations

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 water temperature, pH-values and the dissolved oxygen concentrations were determined daily in the test media of the only test concentration of 100 mg/L and the control.
The behaviour of the test item in the only test concentration of 100 mg/L was observed once every day during the test.
Duplicate samples from the freshly prepared test media of the only test concentration and the control were taken at the start of the test and at the end of exposure (after 96 hours of exposure). The concentration were analysed via HPLC.

3. Statistical calculations

No statistical analysis was performed. The LC_{50} was determined directly from the raw data.

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II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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.

The 96 hour LC_{50} and NOEC values are presented below.

Endpoints	MKH 6561-Sulfonamide Acid [mg/L]
LC_{50} (96 h)	> 100
NOEC (96 h)	100

B. OBSERVATIONS

The biological observations recorded during the test are presented in the table below.

Table 8.2-2 Effects of MKH 6561-Sulfonamide Acid to rainbow trout

Nominal concentration of MKH 6561-Sulfonamide Acid [mg/L]	Number of dead fish / number of fish with intoxication symptoms and observed symptoms				
	0 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
100	0	0	0	0	0

All validity criteria according to OECD 203 were fulfilled, as no mortality occurred in the control group, dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

The toxic effect of the test item, MKH 6561-Sulfonamide Acid to Rainbow Trout (*Oncorhynchus mykiss*) was assessed in a static, limit test. The 96-hour NOEC value was determined to be 100 mg test item/L, the LC_{50} was > 100 mg test item/L.

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Metabolite M07

Report:	[redacted];[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**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary
The purpose of this study was to evaluate the acute toxicity of MKH 6561-Saccharin to fish. For this purpose, juvenile Rainbow trout were exposed in a static test to aqueous test media containing the test item only at the concentration of 100 mg test item/L under defined conditions (limit test). The recorded effects were the mortality and visible abnormalities of the fish.
In the control and in the test medium of 100 mg test item/L all fish survived until the end of the test and no visible abnormalities were observed. The 96-hour NOEC of MKH 6561-Saccharin to Rainbow trout was determined to be 100 mg test item/L. The 96-hour LC_{50} can be determined to be greater than 100 mg test item/L. The endpoints were expressed in terms of nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: MKH 6561-Saccharin
Description: Solid, white powder
Lot/Batch #: Product code: AE F159737 00 1B99 0002; Batch No: M00402
Purity: 99.9% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species: Rainbow trout (*Oncorhynchus mykiss*)
Age: Juveniles
Size and weight: The mean body length of the fish* in the test was 5.12 ± 0.21 cm (Mean ± SD), the mean body wet weight 1.16 ± 0.26 g (Mean ± SD).
* 10 fish from the test fish batch used for the test were measured during the course of the test
Source: The test fish were obtained from [redacted], Germany.

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Holding conditions:	In accordance with the test guidelines the fish were held in test water in the laboratories of IBACON for at least 12 days prior to the start of the test without any medication. During this period until one day before test start, the fish were fed regularly with a commercial fish diet for Rainbow trout. During the last 12 days prior to the start of the test no fish (0%) died in the test fish batch used and all fish were healthy.
Diet/Food:	None during the test
Acclimatisation:	For at least 12 days before the start of the test the fish were acclimated to the test water and test temperature.

4. Test conditions:

Test concentration:	100 mg test item/L (limit test), and a control (test water only)
Number of replicates:	1
Fish per replicate	10
Maximum loading rate	1 g fish/L test water

5. Environmental conditions:

Temperature:	17°C
Photoperiod:	16 h light : 8 h dark; 380 – 720 lux
pH:	pH 7.8 to 7.9
Dissolved oxygen:	At least 60 % of the air saturation value for the duration of the study
Hardness:	2.5 mmol/L (= 250.0 mg/L) as $CaCO_3$
Aeration of the test water:	The test media were slightly aerated during the test.

B. STUDY DESIGN

1. Experimental treatments

Ten fish were exposed to nominal concentrations of 100 mg MKH 6561-Saccharin/L. For the determination of the test concentrations a non GLP range-finding test was performed. A negative control (test water without addition of the test item) was tested in parallel.

2. Observations

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 water temperature, pH-values and the dissolved oxygen concentrations were determined daily in the test media of the only test concentration of 100 mg/L and the control.
The behaviour of the test item in the only test concentration of 100 mg/L was observed once every day during the test.
Duplicate samples from the freshly prepared test media of the only test concentration and the control were taken at the start of the test and at the end of exposure (after 96 hours of exposure). The concentration were analysed via HPLC.

3. Statistical calculations

No statistical analysis was performed. The LC_{50} was determined directly from the raw data.

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II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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.

The 96 hour LC_{50} and NOEC values are presented below.

Endpoints	MKH 6561-Saccharin [mg/L]
LC_{50} (96 h)	> 100
NOEC (96 h)	100

B. OBSERVATIONS

The biological observations recorded during the test are presented in the table below.

Table 8.2-3 Effects of MKH 6561-Saccharin to rainbow trout

Nominal concentration of MKH 6561-Saccharin [mg/L]	Number of dead fish / number of fish with intoxication symptoms and observed symptoms				
	0 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
100	0	0	0	0	0

All validity criteria according to OECD 203 were fulfilled, as no mortality occurred in the control group, dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

The toxic effect of the test item, MKH 6561-Saccharin to Rainbow Trout (*Oncorhynchus mykiss*) was assessed in a static, limit test. The 96-hour NOEC value was determined to be 100 mg test item/L, the LC_{50} was > 100 mg test item/L.

CA 8.2.2 Long-term and chronic toxicity to fish

A summary of all available relevant and compliant data for propoxycarbazone-sodium on long-term toxicity to fish is presented in the table below.

Table 8.2-4 Long-term toxicity of propoxycarbazone-sodium to fish

Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	*Pimephales promelas*	ELS, flow-through 35 d	NOEC 105 (mm)	[redacted] & [redacted] (1999) 108453 M-015904-01-1	Yes

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Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
				KCA 8.2.2.1 /01	

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

CA 8.2.2.1 Fish early life stage toxicity test

See Point CA 8.2.2. No additional studies were performed.

CA 8.2.2.2 Fish full life cycle test

See Point CA 8.2.2. No additional studies were performed.

CA 8.2.2.3 Bioconcentration in fish

As propoxycarbazone has a log P_{OW} of -1.55 at pH 7 (for details please refer to Document M-CA, Section 2, Point CA 2.7) a study on bioconcentration in fish is not required.

CA 8.2.3 Endocrine disrupting properties

Population relevant effects of propoxycarbazone-sodium on fish were studied in an early life-stage test (ELS) under flow-through conditions with fathead minnow (*Pimephales promelas*). As no effects on any parameter in fish were observed in the ELS test, the overall NOEC was 100 mg/L (nominal concentration), the highest concentration tested.
Based on the absence of relevant effects it can be concluded that propoxycarbazone-sodium is not a (potential) endocrine disrupter. No further testing is indicated to evaluate the endocrine disrupter potential of propoxycarbazone-sodium to fish.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

A summary of all available relevant and compliant data for propoxycarbazone-sodium on acute toxicity to *Daphnia magna* is presented in the table below.

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Table 8.2-5 Acute toxicity of propoxycarbazone-sodium and metabolites to *Daphnia magna*

Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/2001-final)
Propoxy-carbazone-sodium	*Daphnia magna*	48 h, static	EC_{50} > 110 (nom)	[redacted] (1998) 107841 M-002122-01-1 KCA 8.2.4.1 /01	Yes
M04	*Daphnia magna*	48 h, static	EC_{50} > 100 (nom)	[redacted] (1998) HBF/Dm 199 M-005032-01-1 KCA 8.2.4.1 /03	Yes
M05	*Daphnia magna*	48 h, semi-static	EC_{50} > 63 (mm)	[redacted] (1999) 742050 M-017326-01-1 KCA 8.2.4.1 /04	Yes
M06	*Daphnia magna*	48 h, static	EC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30183220 M-278971-01-1 KCA 8.2.4.1 /05	New study
M07	*Daphnia magna*	48 h, static	EC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30192220 M-278973-01-1 KCA 8.2.4.1 /06	New study
M08	*Daphnia magna*	48 h, static	EC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30202220 M-278974-01-1 KCA 8.2.4.1 /07	New study
M10	*Daphnia magna*	48 h, static	EC_{50} > 100 (nom)	[redacted] (1998) HBF/Dm 198 M-005036-01-1 KCA 8.2.4.1 /02	Yes

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

To complete the aquatic data package on *Daphnia magna*, additional acute studies with metabolites M06, M07 and M08 were performed, which were not submitted for the first Annex I inclusion and are submitted within this Supplemental Dossier for the propoxycarbazone-sodium Renewal of Approval. The studies are summarised below.

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Metabolite M06

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.**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary
The purpose of this study was to evaluate the influence of the test item, MKH 6561-Sulfonamide Acid, on the immobilisation (survival) of *Daphnia magna*. Young *Daphnia* (< 24 hours old) were exposed for 48 hours under static test conditions in a limit test to a nominal concentration of 100 mg test item/L and a control.
No immobilisation was observed at 100 mg test item/L after 48 hours test duration. Therefore, the 48 h NOEC was determined to be 100 mg test item/L and the 48 h EC_{50} > 100 m test item /L.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-Sulfonamide Acid
Description:	Solid, white powder
Lot/Batch #:	AE 1284964-PU-01; Origin Batch No: M00102
Purity:	99% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Daphnia magna* (Straus), clone 5
Age:	< 24 h (16.5 to 23 hours old)
Source:	[redacted], Germany
Loading:	5 individuals per test vessel (Glass beakers of 100 mL volume containing 80 mL test medium).
Diet/Food:	Once a week the Daphnia of the stock culture were fed with a Tetra Min-extract and at least all other working days with green algae (*Desmodesmus subspicatus*); not fed during the study.
Acclimatisation:	For 16.5 hours under test conditions

4. Environmental conditions:

Temperature:	20°C
Photoperiod:	16 h light : 8 h dark
Light intensity:	207 – 376 lux
pH:	Control: 7.8 (test start) – 7.1 (test end)
	Test item: 6.6 (test start) – 7.1 (test end)
Dissolved oxygen:	Control: 8.6 mg/L (test start) – 8.5 mg/L (test end)
	Test item: 8.6 mg/L (test start) – 8.3 mg/L (test end)
Conductivity:	< 5 μScm^{-1}
Hardness	2.5 mmol/L (= 250 mg/L) as $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-Sulfonamide Acid on *Daphnia magna* were evaluated in a 48-hour static toxicity limit test. 30 *Daphnia* (6 replicates of 5 animals per test beaker) per control and test item concentration were exposed to 100 mg test item/L and an untreated control. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations

Daphnids were observed for immobilisation or mortality at 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. At the same times the water temperature was determined in the test medium of one control beaker. The behaviour of the test item in test water was observed at the start of the test and after 24 and 48 hours test duration in the only test concentration of 100 mg/L. Samples for the determination of the concentrations of MKH 6561-Sulfonamide Acid in the test medium were taken from the control and from the test media of 100 mg test item/L at the start and at the end of the test.

3. Statistical calculations

No statistical analysis was performed. The NOEC (i.e. 0% immobility) was directly determined from the raw data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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 concentration of the test item.

The EC_{50} and NOEC values for MKH 6561-Sulfonamide Acid are given below based on nominal concentrations.

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Endpoints (48 h)	MKH 6561-Sulfonamide Acid [mg/L]
EC_{50}	> 100
NOEC	100

B. OBSERVATIONS

Influence of MKH 6561-Sulfonamide Acid on the mobility of *Daphnia magna* is summarised in the table below.

Table 8.2-6 Effects of MKH 6561-Sulfonamide Acid to *Daphnia magna*

Nominal concentration of MKH 6561-Sulfonamide Acid [mg/L]	No. of *Daphnia* tested	No. of immobilized *Daphnia* after		% of immobilized *Daphnia* after	
	-	24 h	48 h	24 h	48 h
Control	30	0	0*	0	0
100	30	0+	0+*	0	0

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

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

The 48-h EC_{50} for *Daphnia magna* exposed to MKH 6561-Sulfonamide Acid was determined to be > 100 mg/L based on nominal concentration. No immobilisation was observed at 100 mg test item/L after 48 hours test duration.

<u>Metabolite M07</u>

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.**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

The purpose of this study was to evaluate the influence of the test item, MKH 6561-Saccharin, on the immobilisation (survival) of *Daphnia magna*. Young *Daphnia* (<24 hours old) were exposed for 48 hours under static test conditions in a limit test to a nominal concentration of 100 mg test item/L and a control.

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3 *Daphnia* (10%) were immobile after 48 hours test duration. Therefore, the 48 h EC_{50} was determined to be > 100 mg test item/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-Saccharin
Description:	Solid, white powder
Lot/Batch #:	Product code: AE F159737 00 1B99 0002; Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Daphnia magna* (Straus), clone 5
Age:	< 24 h (16.5 to 23 hours old)
Source:	[redacted], Germany
Loading:	5 individuals per test vessel (Glass beakers of 100 mL volume containing 80 mL test medium).
Diet/Food:	Once a week the Daphnia of the stock culture were fed with a Tetra Min-extract and at least all other working days with green algae (*Desmodesmus subspicatus*), not fed during the study.
Acclimatisation:	For 16.5 hours under test conditions

4. Environmental conditions:

Temperature:	20°C
Photoperiod:	16 h light : 8 h dark
Light intensity:	207 – 376 lux
pH:	Control: 7.8 (test start) – 7.1 (test end) Test item: 6.5 (test start) – 7.1 (test end)
Dissolved oxygen:	Control: 8.6 mg/L (test start) – 8.5 mg/L (test end) Test item: 8.7 mg/L (test start) – 8.5 mg/L (test end)
Conductivity:	< 5 µScm^{-1}
Hardness	2.5 mmol/L (= 250 mg/L) as $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-Saccharin on *Daphnia magna* were evaluated in a 48-hour static toxicity limit test. 30 *Daphnia* (6 replicates of 5 animals per test beaker) per control and test item concentration

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were exposed to 100 mg test item/L and an untreated control. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations

Daphnids were observed for immobilisation or mortality at 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile. The pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. At the same times the water temperature was determined in the test medium of one control beaker. The behaviour of the test item in test water was observed at the start of the test and after 24 and 48 hours test duration in the only test concentration of 100 mg/L. Samples for the determination of the concentrations of MKH 6561-Saccharin in the test medium were taken from the control and from the test media of 100 mg test item/L at the start and at the end of the test.

3. Statistical calculations

The 24- and 48-hour EC_{50} values were not calculated, since the immobilisation was less than 50% (10% after 48 hours of exposure) and only a limit-test was performed. The 0% immobility, 100% immobility, NOEC and EC_{50} were determined directly from the raw data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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 concentration of the test item.

The EC_{50} and NOEC values for MKH 6561-Saccharin are given below based on nominal concentrations.

Endpoints (48 h)	MKH 6561-Saccharin [mg/L]
EC_{50}	> 100
NOEC	100

B. OBSERVATIONS

Influence of MKH 6561-Saccharin on the mobility of *Daphnia magna* is summarised in the table below.

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Table 8.2-7 Effects of MKH 6561-Saccharin to *Daphnia magna*

Nominal concentration of MKH 6561-Saccharin [mg/L]	No. of *Daphnia* tested	No. of immobilized *Daphnia* after		% of immobilized *Daphnia* after	
	-	24 h	48 h	24 h	48 h
Control	30	0	0*	0	0
100	30	1	3	3	10

*:1 *Daphnia* in the control showed unusual behaviour (trapping at surface of water)

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was > 3 mg/L in all test vessels.

III. CONCLUSIONS

The 48-h EC_{50} for *Daphnia magna* exposed to MKH 6561-Saccharin was determined to be > 100 mg/L based on nominal concentration.

Metabolite M08

Report:	[redacted]; [redacted]; [redacted]; 2006; M-278974-01
Title:	Acute toxicity of MKH 6561-4-hydroxy-saccharine to Daphnia magna in a 48-hour immobilization test
Report No:	30202220
Document No:	M-278974-01-1
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.**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

The purpose of this study was to evaluate the influence of the test item, MKH 6561-4-Hydroxy-Saccharin, on the immobilisation (survival) of *Daphnia magna*. Young *Daphnia* (<24 hours old) were exposed for 48 hours under static test conditions in a limit test to a nominal concentration of 100 mg test item/L and a control.

No immobilisation was observed at 100 mg test item/L after 48 hours test duration. Therefore, the 48 h NOEC was determined to be 100 mg test item/L and the 48 h EC_{50} > 100 m test item/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-4-Hydroxy-Saccharin
Description:	Solid, beige powder
Lot/Batch #:	Batch Code: AE 1364277-PU-01; Origin Batch No: M00832
Purity:	99.0% w/w

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2. **Vehicle and/or positive control:** n/a

3. **Test organisms:**

Species:	*Daphnia magna* (Straus), clone 5
Age:	< 24 h (6.5 to 23.5 hours old)
Source:	[redacted], Germany
Loading:	5 individuals per test vessel (Glass beakers of 100 mL volume containing 80 mL test medium).
Diet/Food:	Once a week the Daphnia of the stock culture were fed with a Tetra Min-extract and at least all other working days with green algae (*Desmodesmus subspicatus*); not fed during the study.
Acclimatisation:	For 6.5 hours under test conditions

4. **Environmental conditions:**

Temperature:	20 - 21°C
Photoperiod:	16 h light : 8 h dark
Light intensity:	160 – 320 lux
pH:	Control: 7.9 (test start) – 7.7 (test end) Test item: 6.9 (test start) – 7.2 (test end)
Dissolved oxygen:	Control: 8.4 mg/L (test start) – 8.6 mg/L (test end) Test item: 9.0 mg/L (test start) – 8.8 mg/L (test end)
Conductivity:	< 5 µScm^{-1}
Hardness	2.5 mmol/L (≈ 250 mg/L) as $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-4-Hydroxy-Saccharin on *Daphnia magna* were evaluated in a 48-hour static toxicity limit test. 30 *Daphnia* (6 replicates of 5 animals per test beaker) per control and test item concentration were exposed to 100 mg test item/L and an untreated control. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations

Daphnids were observed for immobilisation or mortality at 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile. The pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. At the same times the water temperature was determined in the test medium of one control beaker. The behaviour of the test item in test water was observed at the start of the test and after 24 and 48 hours test duration in the only test concentration of 100 mg/L. Samples for the determination of the concentrations of MKH 6561-4-Hydroxy-Saccharin in the test medium were taken from the control and from the test media of 100 mg test item/L at the start and at the end of the test.

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3. Statistical calculations

No statistical analysis was performed. The NOEC (i.e. 0% immobility) was directly determined from the raw data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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 concentration of the test item.

The EC_{50} and NOEC values for MKH 6561-4-Hydroxy-Saccharin are given below based on nominal concentrations.

Endpoints (48 h)	MKH 6561-4- Hydroxy-Saccharin [mg/L]
EC_{50}	> 100
NOEC	100

B. OBSERVATIONS

Influence of MKH 6561-4-Hydroxy-Saccharin on the mobility of *Daphnia magna* is summarised in the table below.

Table 8.2-8 Effects of MKH 6561-4-Hydroxy-Saccharin to *Daphnia magna*

Nominal concentration of MKH 6561-4-Hydroxy-Saccharin [mg/L]	No. of *Daphnia* tested	No. of immobilized *Daphnia* after		% of immobilized *Daphnia* after	
		24 h	48 h	24 h	48 h
Control	30	0	0	0	0
100	30	0	0	0	0

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

The 48-h EC_{50} for *Daphnia magna* exposed to MKH 6561-4-Hydroxy-Saccharin was determined to be > 100 mg/L based on nominal concentration. No immobilisation was observed at 100 mg test item/L after 48 hours test duration.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

As propoxycarbazone-sodium is an herbicide, studies on additional aquatic invertebrate species are not required.

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CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

A summary of all available relevant and compliant data for propoxycarbazone-sodium on long-term toxicity to *Daphnia magna* is presented in the table below.

Table 8.2-9 Long-term toxicity of propoxycarbazone-sodium and metabolites to *Daphnia magna*

Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/2001-final)
Propoxy-carbazone-sodium	*Daphnia magna*	static renewal, 21 d, limit test	NOEC 110 (nom)	[redacted] & [redacted] (1998) 108140 M-005116-01-1 KCA 8.2.5.1 /01	Evaluated during the first EU review
		static renewal, 21 d, dose-response test	NOEC 110 (nom)	[redacted] & [redacted] (1999) 108845 M-016508-01-1 KCA 8.2.5.1/02	Yes

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

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

No reproductive and development toxicity studies on additional aquatic invertebrate species are required since propoxycarbazone-sodium is not an insecticide and does not show an insecticidal mode of action.

CA 8.2.5.3 Development and emergence in *Chironomus* species

No studies on development and emergence in *Chironomus* species are required since propoxycarbazone-sodium is not an insecticide and does not show an insecticidal mode of action.

CA 8.2.5.4 Sediment dwelling organisms

No studies on sediment dwelling organisms are required since propoxycarbazone-sodium is not an insecticide and does not show an insecticidal mode of action and accumulation in the sediment is not indicated.

CA 8.2.6 Effects on algal growth

A summary of all available relevant and compliant data for propoxycarbazone-sodium on effects on algae is presented in the table below.

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Table 8.2-10 Effects of propoxycarbazone-sodium and metabolites on algal growth

Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	*Pseudokirch-neriella subcapitata* [a]	96 h	E_rC_{50} 7.36 (mm) E_bC_{50} 1.57 (mm)	[redacted] & [redacted] (1999) 108820 M-012242-01-1 KCA 8.2.6.1 /01	Yes
	Anabaena flos-aquae	96 h	E_dC_{50} 11.3 (mm) [b]	[redacted] (1997) 107718 M-001647-01-1 KCA 8.2.6.1 /02	Yes
M04	*Pseudokirch-neriella subcapitata* [a]	72 h	E_rC_{50} > 100 (nom) E_bC_{50} > 100 (nom)	[redacted] (1999) DOM 98051 M-007702-01-1 KCA 8.2.6.1 /04	Yes
M05	*Scenedesmus subspicatus*	72 h	E_rC_{50} > 62 (mm) E_bC_{50} > 62 (mm)	[redacted] (1999) 742094 M-017343-01-1 KCA 8.2.6.1 /05	Yes
M06	*Pseudokirch-neriella subcapitata*	72 h	E_rC_{50} > 100 (nom) E_bC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30181210 M-293396-01-1 KCA 8.2.6.1 /06	New study
M07	*Pseudokirch-neriella subcapitata*	72 h	E_rC_{50} > 100 (nom) E_bC_{50} > 100 (nom)	[redacted] & [redacted] (2006) 30191210 M-281243-01-1 KCA 8.2.6.1 /07	New study
M08	*Pseudokirch-neriella subcapitata*	72 h	E_rC_{50} 30.8 (gmm [c]) E_bC_{50} 23.9 (nom)	[redacted] & [redacted] (2006) 30201210 M-281220-01-1 KCA 8.2.6.1 /08	New study
M10	*Pseudokirch-neriella subcapitata* [a]	96 h	E_rC_{50} > 100 (nom) E_bC_{50} > 100 (nom)	[redacted] (1998) DOM 98049 M-006193-01-1 KCA 8.2.6.1 /03	Yes

[a] formerly *Selenastrum capricornutum*
[b] Endpoint is reported based on density only; E_dC_{50} corresponds to a biomass endpoint
[c] geometric mean of the measured test concentration
Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). .

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

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CA 8.2.6.1 Effects on growth of green algae

To complete the data package for algae, additional studies with metabolites M06, M07 and M08 on green algae *Pseudokirchneriella subcapitata* were performed, which were not submitted during the first Annex I inclusion and are submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval. The studies are summarised below.

Metabolite M06

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.**
Deviations:	None
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item 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 100, 32, 10, 3.2 and 1.0 mg test item/L and a control. Cell density and the inhibition of growth in relation to control cultures were determined over a test period of 72 hours, and thus over several algal generations.

The 72 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to MKH 6561-Sulfonamide Acid was determined to be > 100 mg/L, based on nominal concentration of the test item, the no observed effect concentration (NOEC) based on growth rate was 100 mg/L.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-Sulfonamide Acid
Description:	Solid, white powder
Lot/Batch #:	AE 1234964-PU-01; Origin Batch No: M00102
Purity:	99% w/w

2. Vehicle and/or positive control: n/a

3. **Test organisms:**

Species:	*Pseudokirchneriella subcapitata*
Source:	The algae were supplied by the [redacted] Germany. The algae were cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines.
Initial cell concentration:	5000 algal cells per mL test medium
Acclimatisation:	These cells were taken from an exponentially growing pre-culture, which was set up 3 days prior to the test start at the same conditions as in the test.

4. **Environmental conditions:**

Temperature:	23 - 24°C
Photoperiod:	Continuous illumination
Light intensity:	7088 Lux (mean value), range: 6590 to 7600 Lux
pH:	8.0 to 8.3 (test start) 9.2 to 9.6 (test end)
Hardness:	0.24 mmol/L (= 24 mg/L) as $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-Sulfonamide Acid on *Pseudokirchneriella subcapitata* were evaluated in a 72-hour static toxicity test performed at nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L, and an untreated control. Test vessels (3 replicates per test concentration and 6 replicates in the control) were 50 mL Erlenmeyer flasks containing 30 mL algal suspension per replicate. The test was started by inoculation of a biomass of 5000 algal cells per mL test medium. The test vessels were incubated for 72 hours in a water bath and the algae suspensions were continuously stirred by magnetic stirrers. Additionally, one replicate per test concentration was prepared without algae to provide as ″blank″ for the spectrophotometrical measurements and incubated under the same conditions.

2. Observations

Defined volumes of the algae suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The cell densities in the samples were determined by spectrophotometrical measurement. For the determination of algal cell densities, the absorption of the blanks was subtracted from the absorption of the samples with algae.

The cell density in one control was counted by microscope after 72 hours test duration. Based on the counted cell densities and based on the determined absorption of the control and five dilutions of the control a linear regression was performed for the calculation of the cell densities in all other samples measured spectrophotometrically during the test.

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 taken after the test period of 72 hours. The shape of the treated algal cells compared to the control was microscopically examined.

The pH-values were determined in the test media at the beginning and at the end of the test. During the test duration the test media temperatures were measured daily in an Erlenmeyer flask filled with water

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and incubated under the same conditions as the test flasks. The behaviour of the test item in test water was visually determined daily in all test concentrations. Samples for the determination of the concentrations of MKH 6561-Sulfonamide Acid in the test medium were taken from all test concentrations and the control at the start and at the end of the test.

3. Statistical calculations

The EC_{50} values (the concentrations of the test item corresponding to 50% inhibition of dry weight (biomass) or growth rate for frond number and compared to the control), and their 95 %-confidence limits were calculated by Probit analysis.

For the determination of the LOEC and NOEC, the calculated growth rates and mean biomass at the test concentrations were tested on significant differences to the control values by the Williams Multiple Sequential t-test procedure (ToxRat Version 2.09 2001-2005).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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.

The 72 h-E_rC_{50} and NOEC values for MKH 6561-Sulfonamide Acid are given below based on nominal concentrations.

Parameter (0 - 72 h)	**Growth rate µ MKH 6561-Sulfonamide Acid [mg/L]**
E_rC_{50}	> 100
E_rC_{10}	> 100
NOEC	100

B. OBSERVATIONS

At the microscopic examination of the shape of the algal cells after 72 hours test period no morphological difference was observed between the algae growing in the test concentration of nominal 100 mg test item/L and the algal cells in the control.

Mean cell densities and inhibition of growth rate over the test period are summarised in the table below.

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Table 8.2-11 Mean cell densities and percentage of inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to MKH 6561-Sulfonamide Acid

Test parameters	Control	MKH 6561-Sulfonamide Acid [mg/L]				
	-	1.0	3.2	10	32	100
Mean cell densities (0-72 h) (x 10000 cells/mL)	195	191	195	189	203	196
Inhibition of growth rate μ (0-72 h) [% of control]	-	0.4	0.0	0.5	- 0.7	0.1

- % inhibition: increase in growth relative to that of control

The growth rate in the control cultures increased by a factor of > 391 within 72 hours, the coefficient of variance for section specific growth rates must not exceed ≤ 35% (was 20.8%), for the whole test period it must not exceed ≤ 7% (was 5.5%). The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSIONS

The 72 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to MKH 6561-Sulfonamide Acid was determined to be > 100 mg/L, based on nominal concentration of the test item, the no observed effect concentration (NOEC) based on growth rate was 100 mg/L.

Metabolite M07

Report:	[redacted]; [redacted]; [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.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item MKH 6561-Saccharin 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 100, 32, 10, 3.2 and 1.0 mg test item/L and a control. Cell density and the inhibition of growth in relation to control cultures were determined over a test period of 72 hours, and thus over several algal generations.

The 72 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to MKH 6561-Saccharin was determined to be > 100 mg/L, based on nominal concentration of the test item, the no observed effect concentration (NOEC) based on growth rate was 100 mg/L.

I. MATERIALS AND METHODS

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A. MATERIALS

1. Test material:

Test item:	MKH 6561-Saccharin
Description:	Solid, white powder
Lot/Batch #:	Product code: AE F159737 00 1B99 0002; Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Pseudokirchneriella subcapitata*
Source:	The algae were supplied by the [redacted] Germany.
	The algae were cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines.
Initial cell concentration:	5000 algal cells per mL test medium
Acclimatisation:	These cells were taken from an exponentially growing pre-culture, which was set up 3 days prior to the test start at the same conditions as in the test.

4. Environmental conditions:

Temperature:	23 - 24°C
Photoperiod:	Continuous illumination
Light intensity:	7088 Lux (mean value), range: 6590 to 7600 Lux
pH:	7.9 to 8.2 (test start) 8.7 to 9.2 (test end)
Hardness:	0.24 mmol/L (= 24 mg/L) as $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-Saccharin on *Pseudokirchneriella subcapitata* were evaluated in a 72-hour static toxicity test performed at nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L, and an untreated control. Test vessels (3 replicates per test concentration and 6 replicates in the control) were 50 mL Erlenmeyer flasks containing 30 mL algal suspension per replicate. The test was started by inoculation of a biomass of 5000 algal cells per mL test medium. The test vessels were incubated for 72 hours in a water bath and the algae suspensions were continuously stirred by magnetic stirrers. Additionally, one replicate per test concentration was prepared without algae to provide as "blank" for the spectrophotometrical measurements and incubated under the same conditions.

2. Observations

Defined volumes of the algae suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The cell densities in the samples were

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determined by spectrophotometrical measurement. For the determination of algal cell densities, the absorption of the blanks was subtracted from the absorption of the samples with algae.

The cell density in one control was counted by microscope after 72 hours test duration. Based on the counted cell densities and based on the determined absorption of the control and five dilutions of the control a linear regression was performed for the calculation of the cell densities in all other samples measured spectrophotometrically during the test.

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 taken after the test period of 72 hours. The shape of the treated algal cells compared to the control was microscopically examined.

The pH-values were determined in the test media at the beginning and at the end of the test. During the test duration the test media temperatures were measured daily in an Erlenmeyer flask filled with water and incubated under the same conditions as the test flasks. The behaviour of the test item in test water was visually determined daily in all test concentrations.

Samples for the determination of the concentrations of MKH 6561-Saccharin in the test medium were taken from all test concentrations and the control at the start and at the end of the test.

3. Statistical calculations

Based on the calculated cell densities the E_bC_{50} and E_rC_{50} and their 95%-confidence limits were calculated by Probit Analysis.

For the determination of the LOEC and NOEC, the calculated growth rates and mean biomass at the test concentrations were tested on significant differences to the control values by the Williams Multiple Sequential t-test procedure (mean biomass) and Bonferroni t-test (growth rates) (ToxRat Version 2.09, 2001-2005).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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.

The 72 h-E_rC_{50} and NOEC values for MKH 6561-Saccharin are given below based on nominal concentrations.

Parameter (0 - 72 h)	Growth rate µ MKH 6561-Saccharin [mg/L]
E_rC_{50}	> 100
E_rC_{10}	> 100
NOEC	100

B. OBSERVATIONS

At the microscopic examination of the shape of the algal cells after 72 hours test period no morphological difference was observed between the algae growing in the test concentration of nominal 100 mg test item/L and the algal cells in the control.

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Mean cell densities and inhibition of growth rate over the test period are summarised in the table below.

Table 8.2-12 Mean cell densities and percentage of inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to MKH 6561-Saccharin

Test parameters	Control	MKH 6561-Saccharin [mg/L]				
	-	1.0	3.2	10	32	100
Mean cell densities (0-72 h) (x 10000 cells/mL)	195	204	181	167	202	188
Inhibition of growth rate μ (0-72 h) [% of control]	-	- 0.8	1.2	2.6*	0.6	0.6

- % inhibition: increase in growth relative to that of control
* mean value significantly different from the control (Bonferroni t-test, $p \leq 0.05$), however, as there is no dose-response relation, the significance is considered to be coincidently and not test item related

The growth rate in the control cultures increased by a factor of > 391 within 72 hours, the coefficient of variance for section specific growth rates must not exceed ≤ 35% (was 20.8%), for the whole test period it must not exceed ≤ 7% (was 5.5%). The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSIONS

The 72 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to MKH 6561-Saccharin was determined to be > 100 mg/L, based on nominal concentration of the test item, the no observed effect concentration (NOEC) based on growth rate was 100 mg/L.

Metabolite M08

Report:	[redacted], 2006, M-281220-01
Title:	Toxicity of MKH 6561-4-Hydroxy-Saccharine to Pseudokirchneriella subcapitata in an algal growth inhibition test
Report No:	30201210
Document No:	M-281220-01-1
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.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item MKH 6561-4-Hydroxy-Saccharin 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 100, 32, 10, 3.2 and 1.0 mg test item/L and a control. Cell density and the inhibition of growth in relation to control cultures were determined over a test period of 72 hours, and thus over several algal generations. Nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L correspond to the geometric mean of measured concentrations of 93.4, 26.6, 7.3, 2.2 and 0.72 mg test item/L.

The 72 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to MKH 6561-4-Hydroxy-Saccharin was determined to be 30.8 mg test item/L (based on geometric mean of the measured test concentrations), the no observed effect concentration (NOEC) based on growth rate was 7.3 mg/L (based on geometric mean of the measured test concentrations).

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-4-Hydroxy-Saccharin
Description:	Solid, beige powder
Lot/Batch #:	Batch Code: AE 1364277-PU-01; Origin Batch No: M00832
Purity:	99.0% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Pseudokirchneriella subcapitata*
Source:	The algae were supplied by the [redacted], Germany. The algae were cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines.
Initial cell concentration:	5000 algal cells per mL test medium
Acclimatisation:	These cells were taken from an exponentially growing pre-culture, which was set up 3 days prior to the test start at the same conditions as in the test.

4. Environmental conditions:

Temperature:	23 - 24°C
Photoperiod:	Continuous illumination
Light intensity:	7090 Lux (mean value), range: 6830 to 7440 Lux
pH:	8.0 (test start) 8.0 to 8.9 (test end)
Hardness:	0.24 mmol/L (= 24 mg/L) as $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-4-Hydroxy-Saccharin on *Pseudokirchneriella subcapitata* were evaluated in a 72-hour static toxicity test performed at nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L, and an untreated control. Test vessels (3 replicates per test concentration and 6 replicates in the control) were 50 mL Erlenmeyer flasks containing 30 mL algal suspension per replicate. The test was started by inoculation of a biomass of 5000 algal cells per mL test medium. The test vessels were incubated for 72 hours in a water bath and the algae suspensions were continuously stirred by magnetic stirrers. Additionally, one replicate per test concentration was prepared without algae to provide as ″blank″ for the spectrophotometrical measurements and incubated under the same conditions.

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2. Observations

Defined volumes of the algae suspensions from all replicates and from the blanks were sampled after 24, 48 and 72 hours of exposure, and were not replaced. The cell densities in the samples were determined by spectrophotometrical measurement. For the determination of algal cell densities, the absorption of the blanks was subtracted from the absorption of the samples with algae.

The cell density in one control was counted by microscope after 72 hours test duration. Based on the counted cell densities and based on the determined absorption of the control and five dilutions of the control a linear regression was performed for the calculation of the cell densities in all other samples measured spectrophotometrically during the test.

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 taken after the test period of 72 hours. The shape of the treated algal cells compared to the control was microscopically examined.

The pH-values were determined in the test media at the beginning and at the end of the test. During the test duration the test media temperatures were measured daily in an Erlenmeyer flask filled with water and incubated under the same conditions as the test flasks. The behaviour of the test item in test water was visually determined daily in all test concentrations. Samples for the determination of the concentrations of MKH 6561-4-Hydroxy-Saccharin in the test medium were taken from all test concentrations and the control at the start and at the end of the test.

3. Statistical calculations

Based on the calculated cell densities the E_bC_{50} and E_rC_{50} and their 95%-confidence limits were calculated by Probit Analysis.

For the determination of the LOEC and NOEC, the calculated growth rates and mean biomass at the test concentrations were tested on significant differences to the control values by the Williams Multiple Sequential t-test procedure (growth rate) and Bonferroni t-test (mean biomass) (ToxRat Version 2.09, 2001-2005).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: At the start of the test 101% of the nominal test concentrations were found. After 72 hours test duration 62% of the nominal values were determined. Therefore, all reported results are related to nominal and the geometric mean of the measured concentrations of the test item. Nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L correspond to the geometric mean of measured concentrations of 93.4, 26.6, 7.3, 2.2 and 0.72 mg test item/L.

The concentration of the test item in the aged test media of nominal 1 mg/L were actually below the Limit of Quantification. However, these values were reported, since they were considered reasonable. This was not considered to influence the integrity of the study, since this test concentration was below the NOEC determined in this test.

The 72 h-E_rC_{50}, E_rC_{10}, NOEC and LOEC values for MKH 6561-4-Hydroxy-Saccharin are given below based on nominal and the geometric mean of the measured concentrations.

Parameter **(0 - 72 h)**	**Growth rate µ** **MKH 6561-4-Hydroxy--Saccharin (nominal) [mg/L]**	**Growth rate µ** **MKH 6561-4-Hydroxy--Saccharin (geometric mean of measured) [mg/L]**
E_rC_{50} (95% conf. limits)	36.6 (27.7 – 48.5)	30.8 (22.8 – 41.9)

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E_rC_{10} (95% conf. limits)	12.3 (5.0 – 18.2)	9.2 (3.4 – 14.2)
NOEC	10	7.3
LOEC	32	26.6

B. OBSERVATIONS

At the microscopic examination of the shape of the algal cells after 72 hours test period no morphological difference was observed between the algae growing in the test concentration of nominal 100 mg test item/L and the algal cells in the control.

Mean cell densities and inhibition of growth rate over the test period are summarised in the table below.

Table 8.2-13 Mean cell densities and percentage of inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to MKH 6561-4-Hydroxy-Saccharin

Test parameters	Control	MKH 6561-4-Hydroxy-Saccharin [mg/L]				
	-	1.0	3.2	10	32	100
Mean cell densities (0-72 h) (x 10000 cells/mL)	86	81	95	82	13	2.6
Inhibition of growth rate μ (0-72 h) [% of control]		1.1	- 1.6	1.0	47.2*	84.6*

- % inhibition: increase in growth relative to that of control
* mean value significantly different from the control (Williams t-test, $p \leq 0.05$)

The growth rate in the control cultures increased by a factor of > 172 within 72 hours, the coefficient of variance for section specific growth rates must not exceed ≤ 35% (was 32.5%), for the whole test period it must not exceed ≤ 7% (was 2.2%). The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSIONS

The 72 h E_rC_{50} for *Pseudokirchneriella subcapitata* exposed to MKH 6561-4-Hydroxy-Saccharin was determined to be 30.8 mg test item/L (based on geometric mean of the measured test concentrations), the no observed effect concentration (NOEC) based on growth rate was 7.3 mg/L (based on geometric mean of the measured test concentrations).

CA 8.2.6.2 Effects on growth of an additional algal species

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.
Further studies are not required.

CA 8.2.7 Effects on aquatic macrophytes

A summary of all available relevant and compliant data for propoxycarbazone-sodium on effects on aquatic macrophytes is presented in the table below.

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Table 8.2-14 Toxicity of propoxycarbazone-sodium and metabolites to aquatic plants

Test item	Species	Test design	Endpoint [mg/L]	Reference	EU agreed endpoint (SANCO/4067/2001-final)
Propoxy-carbazone-sodium	*Lemna gibba*	14-day, static-renewal	$EC_{50\ (biomass)}$ 0.0064 (mm)	[redacted] & [redacted] (1999) 108338 M-009972-01-1 KCA 8.2.7 /01	Yes
Propoxy-carbazone-sodium	*Lemna gibba*	7 day, static	$E_rC_{50\ (frond\ no)}$ 0.00664 (nom) $E_rC_{50\ (frond\ area)}$ 0.00453 (nom)	[redacted] (2004) DOM 23101 M-001604-01-1 KCA 8.2.7 /05	New study
Propoxy-carbazone-sodium	*Myrio-phyllum spicatum*	14 d, static water-sediment system	$E_rC_{50\ (wet\ weight)}$ 0.063 (nom) $E_rC_{50\ (wet\ weight)}$ 0.0292 (nom)	[redacted] (2013) 70401245 M-466605-02-1 KCA 8.2.7 /06	New study
M04	*Lemna gibba*	7 day, static	EC_{50} 14.2 (mm)	[redacted] (1999) DOM 98094 M-009770-02-1 KCA 8.2.7 /03	Yes
M05	*Lemna gibba*	7 day, static	EC_{50} > 89.4 (mm)	[redacted] (1999) DOM 99081 M-018594-01-1 KCA 8.2.7 /04	Yes
M06	*Lemna gibba*	7 day, pilot study	EC_{50} > 5	- [a]	Yes [a]
M06	*Lemna gibba*	7 day, static	EC_{50} > 100 (nom)	[redacted] (2006) 30184240 M-281240-01-1 KCA 8.2.7 /07	New study
M07	*Lemna gibba*	7 day, static	EC_{50} > 100 (nom)	[redacted] (2006) 30194240 M-281250-01-1 KCA 8.2.7 /08	New study
M08	*Lemna gibba*	7 day, static	EC_{50} > 100 (nom)	[redacted] (2006) 30203240 M-281362-01-1 KCA 8.2.7 /09	New study
M10	*Lemna gibba*	7 day, static	EC_{50} > 100 (nom)	[redacted] (1999) DOM 98114 M-009757-01-1 KCA 8.2.7 /02	Yes

[a] The result for M06 presented in the final list of endpoints (SANCO/4067/2001-final) was obtained from a non-GLP pilot study on *Lemna* and was submitted to RMS Germany on request during the first Annex inclusion (SANCO/4089/2001 rev.0-2 (05.12.02)). However, as a new study with M06 on *Lemna* is available, the endpoint can be replaced by the result of the newly conducted GLP study (EC_{50} > 100 mg/L, [redacted], 2006; M-281240-01-1).

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Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

An additional study on the most sensitive species *Lemna* with propoxycarbazone-sodium was conducted in 2004 ([redacted], DOM 23101, M-001604-01-1) to demonstrate technical equivalence of the active substance after the change of specification of propoxycarbazone-sodium technical. The E_rC_{50} values for average growth rate derived from the new study were 6.64 µg a.s./L for frond numbers and 4.53 µg a.s./L for total frond area. As the new endpoint is slightly lower than the current EU endpoint, the risk assessment provided in M-CP, Section 10, Point CP 10.2 should be based on the lower endpoint as worst case approach.

To address data requirements according to Commission Regulation (EU) No 283/2013, an additional study with propoxycarbazone-sodium on the dicotyledonous aquatic macrophytes *Myriophyllum spicatum* was conducted. The study ([redacted] (2013), 70401245, M-466605-02-1) revealed a slightly higher but overall comparable sensitivity of dicotyledonous macrophytes to the compound. As the most sensitive endpoint results from the studies with *Lemna*, it is considered appropriate to focus on *Lemna gibba* in the risk assessment; no further testing with *M. spicatum* (metabolites, formulation) is necessary.

Furthermore, to complete the data package for *Lemna*, additional studies with metabolites M06, M07 and M08 were performed.

All studies that were not submitted during the first Annex I inclusion process and that are submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval are summarised below.

<u>Propoxycarbazone-sodium</u>

Report:	[redacted];[redacted];2004;M-001604-01
Title:	MKH 6561 EU - Influence on the growth of Lemna gibba G3 in a static test
Report No:	DOM 23101
Document No:	M-001604-01-1
Guidelines:	**OECD No. 221, Lemna Growth Inhibition Test (Draft October 2000), under consideration of the new draft guideline July 2002**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The aim of the study was to determine the toxicity of MKH 6561 (propoxycarbazone-sodium) to *Lemna gibba* G3.

3 x12 fronds per test concentration were exposed in a chronic multi-generation test for 7 days under static test conditions to nominal concentrations of 1.00, 3.20, 10.0, 32.0, 100, and 320 µg a.s./L against a control. The response of the plants is quantified by measurements of frond numbers, dry weights of plants and frond area.

The pH values ranged from 7.6 to 8.7 in all test levels and the incubation temperature ranged from 23.3°C to 23.4°C measured in an additional incubated glass vessel over the whole period of testing (mean 23.3).

Recoveries of MKH 6561 were measured in all freshly prepared test levels on day 0 and in all aged test levels on day 7.

After 7 days, the EC_{50} for average growth rate were 6.64 µg/L for frond numbers, 4.53 µg/L for total frond area and >320 µg a.s./L for dry weight of plants. All values based on nominal concentrations.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561 (propoxycarbazone-sodium); technical
Description:	White powder
Lot/Batch #:	Batch number: 05649/0054
Purity:	96.3%

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Lemna gibba* G3
Source:	In-house laboratory stock cultures; The original culture was obtained from [redacted] U.S.A.
Acclimatisation:	Stock cultures are maintained under sterile conditions in glass dishes filled with 20X AAP medium under constant illumination of 6500 - 10000 lux and temperature of 24 ± 2°C for a minimum of three weeks. Transfers are made regularly into fresh medium to provide 7-10 days old colonies as test inoculum.

4. Environmental conditions:

Temperature:	23.3 – 23.4°C
Photoperiod:	Continuous illumination
Light intensity:	7335 lux (mean of total of 5 measurements on day 0)
pH:	7.6 – 8.3 (test start); 8.4 – 8.7 (test end)

B. STUDY DESIGN

1. Experimental treatments

A 7 day static toxicity test on *Lemna gibba* was performed with test concentrations of 1.00, 3.20, 10.0, 32.0, 100, and 320 µg propoxycarbazone-sodium/L and an untreated control. 3 replicates per test concentration and control were tested under the same conditions. Test vessels were filled with 20X AAP medium and 3 plants, preferably consisting of 4 fronds each (for a total of 12 fronds) were aseptically added to each test vessel. Test vessels were kept in an incubator at a temperature of 24 ± 2°C during the 7-day study.

2. Observations

Fronds were counted and total frond area was determined on study days 0, 3, 5 and 7. In addition, visible changes in plant development were observed and pH was measured. On day 7 also dry weight was determined.

Samples were analysed for the actual concentration of propoxycarbazone-sodium present in the test medium at each treatment level and in the control on day 0 and day 7. Aliquots for the day 0 analyses were sampled from the prepared volume of each test treatment level. At exposure termination, the fronds were removed from the test vessels, the contents of all three replicate vessels were combined, and the pH was measured. The combined test solutions were then submitted for the day 7 analyses.

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3. Statistical calculations

Growth data, based on (a) average-growth rates of frond numbers, (b) average growth rates of frond area, and (c) average-growth rates of dry weights of plants were used to conduct the statistical analyses. Calculations were carried out using Microsoft Excel spreadsheets.

Statistical analyses, LOEC determinations, and EC_{50} calculations were conducted using a commercial computer program ToxRat Professional (2003) with conclusions of statistical significance based on a 95 percent confidence level ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The quantities of MKH 6561, measured as free acid of MKH 6561, and recalculated found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 91 and 107% (average 100%). In 7 d-aged test levels analytical findings were between 99 and 103% (average 101%) of nominal. All results based on nominal concentrations of MKH 6561.

The EC_{50}, NOEC and LOEC values for MKH 6561 (propoxycarbazone-sodium) are given below based on nominal concentrations.

Endpoint	Average growth rate for frond no. MKH 6561 [µg a.s./L]	Average growth rate for total frond area MKH 6561 [µg a.s./L]	Average growth rate for final dry weight of plants MKH 6561 [µg a.s./L]
EC_{50} (95% conf. limits)	6.64 (3.25 – 13.1)	4.53 (2.01 – 9.46)	320 (84.0 – n.d.)
NOEC	1.0	1.0	1.0
LOEC	3.2	10	3.2

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

B. OBSERVATIONS

Smaller and curled fronds were observed on days 5 and 7 for test concentrations of 3.2 and 10.0 mg/L. In the highest test concentration, one necrotic frond was observed on day 3 and two necrotic fronds were observed on days 5 and 7.

Frond number, total frond area and dry weights of plants and their percent inhibition based on average growth rate are presented in the table below.

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Table 8.2-15 Frond number, total frond area and dry weights of plants and their percent inhibition based on average growth rate after 7 days of exposure to propoxycarbazone-sodium

Test item concentration [µg a.s./L]	Final frond no. (replicate means, day 7)	Total frond area (replicate means) [mm²]	Final dry weight of plants (replicate means, day 7) [mg]	% inhibition		
				average growth rate for frond no.	average growth rate for total frond area	average growth rate for final dry weight of plants
Control	102	861	14.1	-	-	-
1.0	98	747	12.4	2.0	7.3*	4.9
3.2	43	314	8.0	40.5*	47.5*	22.3*
10	25	191	6.2	65.1*	72.9*	32.4*
32	20	146	5.6	77.1*	85.0*	36.3*
100	14	125	4.7	92.9*	89.0*	43.4*
320	15	127	5.4	91.2*	91.4*	37.8*

* Results significantly different from control (based on Dunnett's Multiple t-test; α = 0.05)

The validity criterion for the study was fulfilled:

- the doubling time (T_d) of frond number in the control must be less than 2.5 days (was 2.3 days).

III. CONCLUSIONS

After 7 days, the EC_{50} of propoxycarbazone-sodium for average growth rate were 6.64 µg/L for frond numbers, 4.53 µg/L for total frond area and >320 µg a.s./L for dry weight of plants. All values based on nominal concentrations.

Report:	[redacted];2013;M-466605-02
Title:	Toxicity of propoxycarbazone-sodium to the aquatic plant Myriophyllum spicatum in a static growth inhibition test with a prior rooting phase
Report No:	70401245
Document No:	M-466605-02-1
Guidelines:	**GLP compliant study based on the ring test protocol for a proposed test method for the rooted aquatic macrophyte, myriophyllum spec., 2009 and ring test protocol: standardized method for investigating test substance impact on rooted aquatic macrophytes, 2011**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of propoxycarbazone-sodium on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum* in a static water-sediment system. Following a 7-day pre-rooting phase, plants of *Myriophyllum spicatum* were exposed to test concentrations of 0.95, 3.05, 9.77, 31.3 and 100 µg propoxycarbazone-sodium/L and an untreated control under defined conditions. The inhibition of growth in relation to control cultures was determined over an exposure period of 14 days where the plants were incubated under controlled environmental conditions (20 ± 2°C, 16/8 photoperiod).

Recoveries of propoxycarbazone-sodium were measured in all freshly prepared test levels on day 0 and in all aged test levels on day 14.

The E_rC_{50} values for growth rate were > 100 µg/L for total shoot length, 63 µg/L for wet weight and >100 µg/L for dry weight of plants of *Myriophyllum spicatum* following 14 day exposure to propoxycarbazone-sodium. The 14-day E_yC_{50} values for yield were calculated to be 57.0, 29.2 and

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64.2 µg a.s./L for total shoot length, wet weight and dry weight, respectively. All values based on nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Propoxycarbazone-sodium; technical
Description:	White solid
Lot/Batch #:	Batch code: AE 0298618-01-09; Origin Batch No: 2012-000352
Purity:	95.1% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Myriophyllum spicatum*
Source:	Sterile plants were obtained from the [redacted], Germany.
Acclimatisation:	The plants of the stock culture are maintained in modified Andrews' medium containing 3% sucrose under sterile conditions. They are cultured under continuous illumination at 6500 to 10000 lux and a temperature range of 20 to 25 °C.

4. Environmental conditions:

Temperature:	19 - 21°C for the 14-days exposure period
Photoperiod:	16 h light : 8 h dark
Light intensity:	9447 lux (mean value) with a range of 8870 - 10000 lux
Concentration of Dissolved Oxygen	6.5 - 7.8 mg/L at test start 1.2 - 7.6 mg/L on day 4 9.6 - 13.6 mg/L on day 10 8.6 - 13.0 mg/L at test end Low oxygen concentrations were measured on day 4 and appeared in all concentrations and the control. Therefore, it is not considered as a test item effect. No adverse effects on the plants due to the low oxygen concentration were detected.
pH:	7.5 at test start; 7.2 - 7.3 on day 4 8.5 - 9.5 on day 10 8.4 - 9.5 at test end

B. STUDY DESIGN

1. Experimental treatments

A 14 day static toxicity test on *Myriophyllum spicatum* was performed with test concentrations of 0.95, 3.05, 9.77, 31.3 and 100 µg propoxycarbazone-sodium/L and an untreated control. Five shoot apices from healthy culture plants (without any flowers) were carefully planted into small plant pots filled with sediment (prepared according to OECD test guideline 219 with added N and P nutrients at a concentration of 200 mg nutrient/kg dry sediment, pH 6.9). The pots were placed into 2 L test beakers and the test medium (Smart & Barko Medium, pH 7.5) was added very carefully in order to avoid any

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disturbance of the sediment. To induce root development, the plants were incubated under test conditions (20 ± 2°C; 16/8 photoperiod) for 7 days. After the pre-rooting phase, two of the five plants in each test beaker were removed leaving three in size and appearance homogeneous plants per test beaker (= replicate). 3 replicates per test concentration and 6 replicates per control were prepared. Before application of the test item, the test medium was changed in all replicates to reduce growth of micro-organisms. Defined volumes of the test media per test concentration were removed in each replicate and carefully spiked with the respective volume of stock solution to obtain the respective test concentrations. The day of application of the test item was designated as Day 0 (= start of the test). The plants were then incubated for further 14 days.

2. Observations

At test start (Day 0), Day 4, Day 10 and at test end (Day 14) the shoot length and the length of any side shoot above the sediment was measured for all plants. Fresh and dry weight of every test plant was measured at the test start and at test end. Any sublethal symptoms e.g. chlorosis or necrosis were recorded twice during the test (Day 4 and 10) and at the end of the test. At test end the existence of roots and their appearance were also recorded.
Samples were analysed for the actual concentration of propoxycarbazone-sodium present in the test medium at each treatment level and in the control at the start and at the end of the test.

3. Statistical calculations

The $EC_{10/20/50}$ for growth rate and yield and their 95%-confidence limits were calculated by Probit analysis.
For the determination of the 14-days LOE$_y$C and NOE$_y$C values significant differences at the test concentrations compared to the control values were tested by the Welch t-test (total shoot length) and Williams t-test (wet and dry weight), respectively.
For the determination of the 14-days LOE$_r$C and NOE$_r$C values significant differences at the test concentrations compared to the control values for total shoot length, wet weight and dry weight were tested by the Williams t-test.
The statistical evaluation based on the mean values per replicate.
The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat® Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: At the start of the test 93% of the nominal test concentration was found (average of all test concentrations). So correct dosing could be demonstrated. After 14 days test duration, 85% of the nominal values were determined (average of all test concentrations). Hence, the test item was sufficiently stable under the conditions of the test. Therefore, all reported results refer to nominal concentrations.

The $EC_{50/20/10}$, NOEC and LOEC values for yield and growth rate are given below based on nominal concentrations.

Endpoint	Yield (total shoot length) [µg a.s./L]	Growth (total shoot length) [µg a.s./L]	Yield (wet weight) [µg a.s./L]	Growth (wet weight) [µg a.s./L]	Yield (dry weight) [µg a.s./L]	Growth (dry weight) [µg a.s./L]
14-day EC_{50} (95% conf. limits)	57.0 (43.8 – 78.2)	> 100 (> 100)	29.2 (12.8 – 69.8)	63.0 (35.0 - > 100)	64.2 (26.7 - > 100)	> 100 (83.4 - > 100)
14-day EC_{20} (95% conf. limits)	17.8 (10.1 – 24.8)	51.3 (38.5 - 60.9)	10.6 (0.674 - 20.1)	15.2 (1.94 - 28.5)	5.01 (0.226 - 12.9)	13.6 (2.01 - 30.6)
14-day EC_{10}	9.69	32.8	6.26	7.22	1.32	3.06

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Endpoint	Yield (total shoot length) [µg a.s./L]	Growth (total shoot length) [µg a.s./L]	Yield (wet weight) [µg a.s./L]	Growth (wet weight) [µg a.s./L]	Yield (dry weight) [µg a.s./L]	Growth (dry weight) [µg a.s./L]
(95% conf. limits)	(4.21 – 15.2)	(20.3 - 42.5)	(0.110 - 13.8)	(0.267 - 16.6)	(< 0.95 - 4.92)	(< 0.95 - 9.25)
14-day NOEC	9.77	9.77	9.77	9.77	9.77	9.77
14-day LOEC	31.3	31.3	31.3	31.3	31.3	31.3

B. OBSERVATIONS

Over the whole test period no sublethal effects were recorded. All plants developed healthy roots and no difference could be detected between the roots of the control and the exposed plants. Side shoots frequently occurred in the control and the lower concentration ranges. With increasing test item concentrations, the number of side shoots decreased. The side shoot lengths were considered in the total shoot length evaluation.
The test media were clear and colourless.

Yields and growth rates based on total shoot length, wet weight and dry weight after 14 days of exposure and their percentage inhibition are presented in the tables below.

Table 8.2-16 Yields and percentage inhibition of *Myriophyllum spicatum* after 14 days of exposure to propoxycarbazone-sodium

Test item concentration [µg a.s./L]	Yield based on			% inhibition		
	total shoot length (replicate means) [cm]	final wet weight (replicate means) [mg]	final dry weight (replicate means) [mg]	average yield for shoot length	average yield for final wet weight	average yield for final dry weight
Control	36.5	1047	81.7	-	-	-
0.95	34.0	793	86.7	6.8	24.3	-6.2
3.05	37.3	987	77.8	-2.2	5.7	4.7
9.77	34.1	1092	61.6	6.3	-4.3	24.6
31.3	25.5	364	41.5	30.1 **	65.3 *	49.2 *
100	11.9	257	39.7	67.4 **	75.4 *	51.4 *

- % inhibition: increase in growth relative to that of control
* Results significantly different from control (based on Williams t-test, α = 0.05, one-sided)
** Results significantly different from control (based on Welch t-test, α = 0.05, one-sided)

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Table 8.2-17 Growth rates and percentage inhibition of *Myriophyllum spicatum* after 14 days of exposure to propoxycarbazone-sodium

Test item concentration [µg a.s./L]	Growth rate based on			% inhibition		
	total shoot length (replicate means) [1/day]	final wet weight (replicate means) [1/day]	final dry weight (replicate means) [1/day]	average growth rate for shoot length	average growth rate for final wet weight	average growth rate for final dry weight
Control	0.130	0.114	0.088	-	-	-
0.95	0.127	0.104	0.089	2.8	9.3	-0.9
3.05	0.128	0.114	0.087	1.8	0.0	1.7
9.77	0.128	0.117	0.072	1.8	-2.3	18.0
31.3	0.119	0.060	0.056	8.7 *	47.2 *	36.5 *
100	0.076	0.050	0.055	41.9 *	56.0 *	37.2 *

- % inhibition: increase in growth relative to that of control
* Results significantly different from control (based on Williams t-test, α < 0.05, one-sided)

Validity criteria were not defined at performance of the test:

- The validity criterion under discussion during performance of the test and mentioned in the study plan (at least 50% growth compared to the initial length / biomass in the controls) was met: Compared to initial values (100%) control values after 14 days were 613%, 542% and 355% for total shoot length, wet weight and dry weight, respectively.

III. CONCLUSIONS

The E_rC_{50} values for growth rate were > 100 µg/L for total shoot length, 63 µg/L for wet weight and >100 µg/L for dry weight of plants of *Myriophyllum spicatum* following 14 day exposure to propoxycarbazone-sodium. The 14-day E_yC_{50} values for yield were calculated to be 57.0, 29.2 and 64.2 µg a.s./L for total shoot length, wet weight and dry weight, respectively. All values based on nominal concentrations.

Metabolite M06

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.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item MKH 6561-Sulfonamide Acid on the growth of the freshwater aquatic plant *Lemna gibba* in a static test design after 7 days of exposure. Cultures of *Lemna gibba* were exposed to nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L against a control. The response of the plants is quantified by measurements of frond numbers and dry weights of plants to determine the inhibition of growth for growth rate of frond number and for growth rate of dry weight of plants in relation to control cultures. The test item MKH 6561-Sulfonamide Acid was analysed after 0 and 7 days.

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The 7-days EC_{50} values for *Lemna gibba* exposed to MKH 6561-Sulfonamide Acid were > 100 mg/L for growth rate of frond number and for growth rate of dry weight, based on nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-Sulfonamide Acid
Description:	Solid, white powder
Lot/Batch #:	AE 1234964-PU-01; Origin Batch No: M00102
Purity:	99% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Lemna gibba* G3
Source:	The plants were supplied by [redacted], Germany
Acclimatisation:	The plants are cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines. Plants are pre-cultured for 14 days under test conditions.

4. Environmental conditions:

Temperature:	23 – 24°C
Photoperiod:	Continuous illumination
Light intensity:	7933 Lux (mean value), range: 6990 to 8440 Lux
pH:	7.4 – 7.6 (test start); 8.6 – 8.9 (test end)

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-Sulfonamide Acid on *Lemna gibba* were evaluated in a 7 day static toxicity test performed at nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L, and an untreated control. Test vessels (3 replicates per test concentration and 6 replicates in the control) were 250 mL glass flasks with about 170 mL 20X AAP-Growth Medium per replicate. Colonies consisting of 3 fronds were transferred in a randomised order from the inoculum culture. Each test vessel contained a total of 12 fronds. Test vessels were incubated for 7 day under controlled conditions.

2. Observations

Fronds were counted and total frond area was determined on study days 0, 3, 5 and 7. In addition, visible changes in plant development were observed and pH was measured. On day 7 also dry weight was determined.

The pH-values were determined in the test media at the start and at each observation day. During the test duration the test media temperatures were measured daily in a test vessel filled with test medium and incubated under the same conditions as the test flasks. The behaviour of the test item in test water

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was visually determined at each observation day in all test concentrations. Light intensity was measured once during the test.

Samples for the determination of the concentrations of MKH 6561- Sulfonamide Acid in the test medium were taken from the test concentrations of nominal 100, 32, 10, 3.2, 1.0 and 0.32 mg test item/L and the control at the start and at the end of the test. The lowest test concentration of nominal 0.1 mg test item/L was not analysed, since it was below the 7 day NOEC, determined in this test.

3. Statistical calculations

The EC_{50} values (the concentrations of the test item corresponding to 50% inhibition of dry weight (biomass) or growth rate for frond number and compared to the control), and their 95%-confidence limits were calculated by Probit analysis.
For the determination of the LOEC and NOEC values, the calculated growth rates at the test concentrations were tested on significant differences to the control values by the Williams Multiple Sequential t-test Procedure (growth rate of frond number and growth rate of dry weight) (ToxRat Version 2.09, 2001-2005).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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* were exposed to a mean of 95% of nominal. Therefore, all reported results are related to nominal concentrations of the test item.

The EC_{50}, NOEC and LOEC values for MKH 6561- Sulfonamide Acid are given below based on nominal concentrations.

Endpoint	**Growth rate for frond number MKH 6561- Sulfonamide Acid [mg/L]**	**Growth rate for dry weight MKH 6561- Sulfonamide Acid [mg/L]**
EC_{50}	> 100	> 100
7-day NOEC	0.32	0.32
7-day LOEC	1.0	1.0

B. OBSERVATIONS

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.

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Table 8.2-18 Frond number and dry weights of plants and their percent inhibition based on average growth rate after 7 days of exposure to MKH 6561-Sulfonamide Acid

Test item concentration [mg/L]	Final frond no. (replicate means, day 7)	Final dry weight of plants (replicate means, day 7) [mg]	% inhibition	
			growth rate for frond no. (0-7 days)	growth rate for dry weight (after 7 days)
Control	173	21.4	-	
0.1	194	23.5	-4.2	-3.3
0.32	186	22.6	-2.8	-1.9
1.0	145	15.5	6.7*	11.1*
3.2	102	10.6	19.8*	24.2*
10	90	9.3	24.3*	28.9*
32	78	8.4	29.8*	33.5*
100	71	7.9	33.6*	34.9*

- % inhibition: increase in growth relative to that of control
* Results significantly different from control (based on Williams Multiple Sequential t-test; $\alpha = 0.05$, one-sided)

The validity criterion for the study was fulfilled:

- the doubling time (T_d) of frond number in the control must be less than 2.5 days (was 1.82 days, corresponding to an approximately 14.4-fold increase in 7 days).

III. CONCLUSIONS

The 7-days EC_{50} values for *Lemna gibba* exposed to MKH 6561-Sulfonamide Acid were > 100 mg/L for growth rate of frond number and for growth rate of dry weight, based on nominal concentrations.

Metabolite M07

Report:	[redacted]; [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.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item MKH 6561-Saccharin on the growth of the freshwater aquatic plant *Lemna gibba* in a static test design after 7 days of exposure. Cultures of *Lemna gibba* were exposed to nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L against a control. The response of the plants is quantified by measurements of frond numbers and dry weights of plants to determine the inhibition of growth for growth rate of frond number and for growth rate of dry weight of plants in relation to control cultures. The test item MKH 6561-Saccharin was analysed after 0 and 7 days.

The 7-days EC_{50} values for *Lemna gibba* exposed to MKH 6561-Saccharin were > 100 mg/L for growth rate of frond number and for growth rate of dry weight, based on nominal concentrations.

I. MATERIALS AND METHODS

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A. MATERIALS

1. Test material:

Test item:	MKH 6561-Saccharin
Description:	Solid, white powder
Lot/Batch #:	Product code: AE F159737 00 1B99 0002; Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Lemna gibba* G3
Source:	The plants were supplied by [redacted], Germany
Acclimatisation:	The plants are cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines. Plants are pre-cultured for 8 days under test conditions.

4. Environmental conditions:

Temperature:	23 – 24°C
Photoperiod:	Continuous illumination
Light intensity:	7307 Lux (mean value), range: 7100 to 7600 Lux
pH:	7.4 – 7.5 (test start); 8.7 – 8.9 (test end)

B. STUDY DESIGN

1. Experimental treatments

The effects of MKH 6561-Saccharin on *Lemna gibba* were evaluated in a 7 day static toxicity test performed at nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L, and an untreated control. Test vessels (3 replicates per test concentration and 6 replicates in the control) were 250 mL glass flasks with about 170 mL 20X AAP-Growth Medium per replicate. Colonies consisting of 3 fronds were transferred in a randomised order from the inoculum culture. Each test vessel contained a total of 12 fronds. Test vessels were incubated for 7 day under controlled conditions.

2. Observations

Fronds were counted and total frond area was determined on study days 0, 3, 5 and 7. In addition, visible changes in plant development were observed and pH was measured. On day 7 also dry weight was determined.

The pH-values were determined in the test media at the start and at each observation day. During the test duration the test media temperatures were measured daily in a test vessel filled with test medium and incubated under the same conditions as the test flasks. The behaviour of the test item in test water was visually determined at each observation day in all test concentrations. Light intensity was measured once during the test.

Samples for the determination of the concentrations of MKH 6561-Saccharin in the test medium were taken from the test concentrations of nominal 100, 32, 10, 3.2, 1.0, 0.32 and 0.1 mg test item/L and the control at the start and at the end of the test.

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3. Statistical calculations

The EC_{50} values (the concentrations of the test item corresponding to 50% inhibition of dry weight (biomass) or growth rate for frond number and compared to the control), and their 95%-confidence limits were calculated by Probit analysis.

For the determination of the LOEC and NOEC values, the calculated growth rates of frond number and growth rates for dry weight were tested on significant differences to the control values by the Dunnett´s Multiple t-test Procedure (ToxRat Version 2.09, 2001-2005).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: 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.

The EC_{50}, NOEC and LOEC values for MKH 6561-Saccharin are given below based on nominal concentrations.

Endpoint	Growth rate for frond number MKH 6561-Saccharin [mg/L]	Growth rate for dry weight MKH 6561-Saccharin [mg/L]
EC_{50}	> 100	> 100
7-day NOEC	0.32	10
7-day LOEC	1.0	32

B. OBSERVATIONS

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 10 mg test item/L. At 32 and 100 mg test item/L necrosis was observed after 5 and 7 days of exposure.

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Table 8.2-19 Frond number and dry weights of plants and their percent inhibition based on average growth rate after 7 days of exposure to MKH 6561-Saccharin

Test item concentration [mg/L]	Final frond no. (replicate means, day 7)	Final dry weight of plants (replicate means, day 7) [mg]	% inhibition	
			growth rate for frond no. (0-7 days)	growth rate for dry weight (after 7 days)
Control	174	23.8	-	-
0.1	134	19.1	9.1	7.5
0.32	139	20.2	7.9	5.8
1.0	105	18.0	18.3*	9.8
3.2	82	18.2	27.8*	9.5
10	80	18.2	28.9*	9.8
32	67	15.0	35.6*	17.2**
100	65	12.5	36.7*	22.6**

- % inhibition: increase in growth relative to that of control
* Results significantly different from control (based on Dunnett's test; $p \leq 0.05$)
** Results significantly different from control (based on Dunnett's Multiple t- test; $\alpha = 0.05$, one-sided)

The validity criterion for the study was fulfilled:

- the doubling time (T_d) of frond number in the control must be less than 2.5 days (was 1.8 days, corresponding to an approximately 14.5-fold increase in 7 days).

III. CONCLUSIONS

The 7-days EC_{50} values for *Lemna gibba* exposed to MKH 6561-Saccharin were > 100 mg/L for growth rate of frond number and for growth rate of dry weight, based on nominal concentrations.

<u>Metabolite M08</u>

Report:	[redacted];[redacted];2006;M-281362-01
Title:	Toxicity of MKH 6561-4-Hydroxy-Saccharine to the aquatic plant Lemna gibba in a growth inhibition test
Report No:	30203240
Document No:	M-281362-01-1
Guidelines:	**Revised Proposal for a new OECD Guideline 221: "Lemna sp. Growth Inhibition Test", October 22, 2004.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The purpose of this test was to determine the inhibitory effect of the test item MKH 6561-4-Hydroxy-Saccharin on the growth of the freshwater aquatic plant *Lemna gibba* in a static test design after 7 days of exposure. Cultures of *Lemna gibba* were exposed to nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L against a control. The response of the plants is quantified by measurements of frond numbers and dry weights of plants to determine the inhibition of growth for growth rate of frond number and for growth rate of dry weight of plants in relation to control cultures. The test item MKH 6561-4-Hydroxy-Saccharin was analysed after 0 and 7 days.

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The 7-days EC_{50} values for *Lemna gibba* exposed to MKH 6561-4-Hydroxy-Saccharin were > 100 mg/L for growth rate of frond number and for growth rate of dry weight, based on nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-4-Hydroxy-Saccharin
Description:	Solid, beige powder
Lot/Batch #:	Batch Code: AE 1364277-PU-01, Origin Batch No: M00832
Purity:	99.0% w/w

2. Vehicle and/or positive control:
n/a

3. Test organisms:

Species:	*Lemna gibba* G3
Source:	The plants were supplied by [redacted], Germany
Acclimatisation:	The plants are cultivated in the laboratories of IBACON under standardised conditions according to the test guidelines. Plants are pre-cultured for 8 days under test conditions.

4. Environmental conditions:

Temperature:	23 – 24°C
Photoperiod:	Continuous illumination
Light intensity:	7120 Lux (mean value), range: 6750 to 7400 Lux
pH:	7.5 (test start); 8.8 – 8.9 (test end)

B. STUDY DESIGN

1. Experimental treatments
The effects of MKH 6561-4-Hydroxy-Saccharin on *Lemna gibba* were evaluated in a 7 day static toxicity test performed at nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg test item/L, and an untreated control. Test vessels (3 replicates per test concentration and 6 replicates in the control) were 250 mL glass flasks with about 170 mL 20X AAP-Growth Medium per replicate. Colonies consisting of 3 fronds were transferred in a randomised order from the inoculum culture. Each test vessel contained a total of 12 fronds. Test vessels were incubated for 7 day under controlled conditions.

2. Observations
Fronds were counted and total frond area was determined on study days 0, 3, 5 and 7. In addition, visible changes in plant development were observed and pH was measured. On day 7 also dry weight was determined.

The pH-values were determined in the test media at the start and at each observation day. During the test duration the test media temperatures were measured daily in a test vessel filled with test medium and incubated under the same conditions as the test flasks. The behaviour of the test item in test water

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was visually determined at each observation day in all test concentrations. Light intensity was measured once during the test.

Samples for the determination of the concentrations of MKH 6561-4-Hydroxy-Saccharin in the test medium were taken from the test concentrations of nominal 100, 32, 10, 3.2 and 1.0 mg test item/L and the control at the start and at the end of the test. The two lowest test concentrations of nominal 0.32 and 0.1 mg test item/L were not analysed.

3. Statistical calculations

The EC_{50} values (the concentrations of the test item corresponding to 50% inhibition of dry weight (biomass) or growth rate for frond number and compared to the control), and their 95 %-confidence limits were calculated by Probit analysis.
For the determination of the LOEC and NOEC values, the calculated growth rates at the test concentrations were tested on significant differences to the control values by the Williams Multiple Sequential t-test Procedure (growth rate of frond number and growth rate of dry weight) (ToxRat Version 2.09, 2001-2005).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: At the start of the test 100% of the nominal test concentration was found (average test concentrations of nominal 1 to 100 mg test item/L). After 7 days test duration 60% of the nominal value was determined (average test concentrations of nominal 10 to 100 mg test item/L). In the aged test media with a concentration of 100 mg/L a mean value of 80% of nominal was found. Since the NOEC of this test was 100 mg test item/L (nominal), all reported results are related to nominal concentrations of the test item.
The test media with a nominal concentration of 0.1 and 0.32 mg test item/L were not analysed. This was not considered to influence the integrity of the study (NOEC = 100 mg test item/L).

The EC_{50}, NOEC and LOEC values for MKH 6561-4-Hydroxy-Saccharin are given below based on nominal concentrations.

Endpoint	Growth rate of frond number MKH 6561-4-Hydroxy-Saccharin [mg/L]	Growth rate of dry weight MKH 6561-4-Hydroxy-Saccharin [mg/L]
EC_{50}	> 100	> 100
7-day NOEC	100	100
7-day LOEC	100	100

B. OBSERVATIONS

The shape of fronds and colonies after the test period of 7 days was not different to those in the control.

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Table 8.2-20 Frond number and dry weights of plants and their percent inhibition based on average growth rate after 7 days of exposure to MKH 6561-4-Hydroxy-Saccharin

Test item concentration [mg/L]	Final frond no. (replicate means, day 7)	Final dry weight of plants (replicate means, day 7) [mg]	% inhibition	
			growth rate for frond no. (0-7 days)	growth rate for dry weight (after 7 days)
Control	174	23.8	-	-
0.1	155	21.7	4.1	3.6
0.32	176	23.9	-0.9	0.1
1.0	186	24.5	-3.2	-1.3
3.2	148	21.5	5.4	3.5
10	153	23.5	4.5	0.4
32	164	23.7	1.5	0.2
100	144	24.0	6.7	-0.3

- % inhibition: increase in growth relative to that of control

The validity criterion for the study was fulfilled:

- the doubling time (T_d) of frond number in the control must be less than 2.5 days (was 1.8 days, corresponding to an approximately 14.4-fold increase in 7 days).

III. CONCLUSIONS

The 7-days EC_{50} values for *Lemna gibba* exposed to MKH 6561-4-Hydroxy-Saccharin were > 100 mg/L for growth rate of frond number and for growth rate of dry weight, based on nominal concentrations.

CA 8.2.8 Further testing on aquatic organisms

Further testing on aquatic organisms is not considered necessary.

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

A summary of all available relevant and compliant data for propoxycarbazone-sodium on effects on bees is presented in the table below.

Table 8.3-1 Effects of propoxycarbazone-sodium on bees

Test substance	Test species / test design	Endpoint	Reference	EU agreed endpoint
Propoxycarbazone-sodium, tech.	Honeybee, 48 h oral and contact toxicity	oral LD_{50} > 319 µg a.s./bee contact LD_{50} > 200 µg a.s./bee	[redacted] (1998) 4150036 M-006195-01-1 KCA 8.3.1.1.1 /01 KCA 8.3.1.1.2 /01	Yes

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Test substance	Test species / test design	Endpoint	Reference	EU agreed endpoint
Propoxycarbazone-sodium	Honeybee, 10 d chronic adult feeding study	NOEC ≥ 1600 mg a.s./kg feeding solution NOED ≥ 47.6 µg a.s./bee/d	[redacted] (2014) 70407136 M-484627-01-1 KCA 8.3.1.2 /01	New study
ATTRIBUT SG70	Honeybee brood feeding (Oomen et al., 1992)	No adverse effects on bee brood development (eggs, young larvae, old larvae) and mortality of adult bees and pupae by feeding honey bee colonies sugar syrup at a concentration of 0.175 g a.s./L	[redacted] (2013) 70473031 M-466734-01-1 KCA 8.3.1.3 /01	New study

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

To address data requirements according to Commission Regulation (EU) No 283/2013, a chronic 10-day adult feeding limit test was conducted with propoxycarbazone-sodium. Furthermore, in order to investigate the intrinsic properties of propoxycarbazone-sodium on immature honey bee live stages, a honey bee brood feeding study has been performed with the product ATTRIBUT SG70.

These additional studies were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval. The studies are summarised under Point CA 8.3.1.2 and CA 8.3.1.3.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

CA 8.3.1.1.2 Acute contact toxicity

Please refer to Point CA 8.3.1.1.1.

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CA 8.3.1.2 Chronic toxicity to bees

Report:	[redacted];[redacted];[redacted];2014;M-484627-01
Title:	Chronic oral toxicity test of propoxycarbazone-sodium technical on the honey bee (Apis mellifera L.) in the laboratory
Report No:	70407136
Document No:	M-484627-01-1
Guidelines:	**OECD 213: OECD Guideline for the Testing of Chemicals on Honeybee, Acute Oral Toxicity Test, (adopted 21st September 1998)** **CEB No.: 230: Method used to assess the Effects of Crop Protection Products on Honeybees, Apis mellifera L., 1st Edition, November 2003).**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary
The effects of propoxycarbazone-sodium on honey bees *(Apis mellifera)* were tested in a chronic oral exposure laboratory test with regards to mortality and behavioural abnormalities. Bees were fed with test item treated sugar solution *ad libitum* for 10 consecutive days. Nominal concentration of 1600, 800, 400, 200 and 100 mg a.s./kg feeding solution were tested, corresponding to doses of 47.6, 29.5, 13.7, 6.20 and 3.70 µg a.s./bee per day (based on actual mean intake). An untreated control (50% aqueous sugar syrup solution) and a reference item (dimethoate) were run in parallel.
After 10 days of oral exposure, 3.3% mortality occurred in the untreated control group. Mortality of 3.3% was found in the 3.70 µg a.s./bee/day test item group. No mortality was observed in the 47.6, 29.5, 13.7 and 6.20 µg a.s./bee/day test item treated groups. The reference item caused 100% mortality after 7 days at a dose of 0.029 µg dimethoate/bee/day. No test item related behavioural abnormalities occurred at any time of the test.

The LC_{50} value (10 days) was > 1600 mg a.s./kg feeding solution, corresponding to an LD_{50} value (10 days) of > 47.6 µg a.s./bee/day based on actual food intake. The NOEC and NOED values (10 days) were ≥ 1600 mg a.s./kg feeding solution and ≥ 47.6 µg a.s./bee per day, respectively.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Propoxycarbazone-sodium
Description:	White solid
Lot/Batch #:	Batch code: AE 0298618-01-09; Origin Batch No: 2012-000352
Purity:	93.1% w/w

2. Vehicle and/or positive control:

Positive control: Perfekthion EC (analytical content: 411.7 g dimethoate/L)

3. Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Freshly emerged adult worker bees
Source:	Honey bee colonies, disease-free and queen-right, bred by [redacted]
Diet/Food:	50% aqueous solution of commercial ready-to-use syrup (Apiinvert; 30% sucrose, 31% glucose, 39% fructose) *ad libitum*

4. Environmental conditions:

Temperature:	32 - 34°C
Relative humidity:	39 - 79%; mean relative humidity: 71%
Light:	24 h darkness (except during observation)
Ventilation:	Ventilation to avoid possible accumulation of pesticide vapour

B. STUDY DESIGN

1. Experimental treatments

Freshly emerged female worker bees were exposed to nominal concentrations of 1600, 800, 400, 200 and 100 mg a.s./kg feeding solution, an untreated control and a reference item (1 mg dimethoate/kg feeding solution).

One day before the start of the test two brood combs were selected from one hive with sealed brood in which bees were visibly starting to emerge. The combs contained pollen which was used as a first feeding source for the freshly hatched bees. The combs were taken from the hive and adult bees were swept out.

Afterwards the combs were placed in an excluder box and brought back to the hive for a further day. The freshly hatched bees remained in the excluder box.

The following day (start of the test), freshly emerged worker bees were taken out from the excluder box with forceps and were transferred to the ready-prepared test units (cages) without the use of smoke and without anaesthetics in order to start the test.

Three replicates per treatment group were tested, each consisting of 10 bees per test cage. The bees were fed with a 50% aqueous solution of commercial ready-to-use syrup (Apiinvert; sugar content: 30% sucrose, 31% glucose, 39% fructose) containing either a respective concentration of the test item or the reference item (test and reference item treatment group). The control group were fed with untreated 50% aqueous sugar syrup solution only. The treated and untreated food was offered for 10 consecutive days *ad libitum* to each cage in syringes. The syringes were replaced and weighed daily to calculate the food uptake per bee per day.

The final test solutions were prepared once for the entire time of the experiment (10 days), directly before start of the experiment and were kept cool in a refrigerator (4°C ± 4°C) in the dark.

2. Observations

Number of dead bees was assessed daily during the exposure period of 10 days. Dead bees were removed from the test units on each assessment day. Behavioural abnormalities were assessed daily (day 1 to day 10). Food uptake was recorded daily.

3. Statistical calculations

The LC_{50} was determined directly from the raw data without statistical analysis. The NOEC/NOED was estimated using Fisher's Exact test (pairwise comparison, one-sided greater, $\alpha = 0.05$). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

The test item was daily administered to the bees in sugar solution at the following concentrations: 1600, 800, 400, 200 and 100 mg a.s./kg feeding solution. Mean consumption of feeding solution in the test item treatment groups ranged from 29.8 to 37.0 mg/bee/day. Based on actual daily food consumption, the concentrations correspond to mean doses of 47.6, 29.5, 13.7, 6.20 and 3.70 µg a.s./bee/day, respectively.

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Mean consumption of feeding solution in the control group was 32.2 mg/bee/day and in the reference item group 24.9 mg/bee/day.

After 10 days of oral exposure, 3.3% mortality occurred in the untreated control group. Mortality of 3.3% was found in the 3.70 µg a.s./bee/day test item group. No mortality was observed in the 47.6, 29.5, 13.7 and 6.20 µg a.s./bee/day test item treated groups. The reference item (dimethoate) at a dose of 0.029 µg dimethoate/bee/day caused 100% mortality until day 7.

No test item related behavioural abnormalities occurred at any time of the test.

Table 8.3-2 10 days chronic oral toxicity of propoxycarbazone-sodium technical to honey bees

Test Item	**Propoxycarbazone-sodium, technical**	
Test Organism	***Apis mellifera* L.**	
Exposure	**Oral** **10 days chronic exposure *via* 50% aqueous sugar solution**	
Application Rate	Concentration [mg a.s./kg feeding solution]	Dose [µg a.s./bee per day]
	1600, 800, 400, 200 and 100	47.6, 29.5, 13.7, 6.20 and 3.70
Endpoints *	LC_{50}: > 1600	LD_{50}: > 47.6
	NOEC: 1600	NOED: 47.6

* The NOEC/NOED was estimated using Fisher's Exact test (pairwise comparison, one-sided greater, $\alpha = 0.05$)

Validity criteria of the test (control mortality ≤ 15%; mortality of the reference item (dimethoate) > 50%) are fulfilled.

III. CONCLUSIONS

In a 10 day oral exposure study with propoxycarbazone-sodium on bees, the LC_{50} value was determined to be > 1600 mg a.s./kg feeding solution, corresponding to an LD_{50} value of > 47.6 µg a.s./bee/day. The NOEC and NOED values were 1600 mg a.s./kg feeding solution and 47.6 µg a.s./bee per day, respectively.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	[redacted];[redacted];2013;M-466734-01
Title:	Study on the effects of propoxycarbazone-sodium SG 70 W on honey bees brood (apis mellifera l.) - Bbrood feeding test
Report No:	70473031
Document No:	M-466734-01-1
Guidelines:	**according to Oomen et al. (1992)**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

A bee brood test was conducted in order to assess the effect of Propoxycarbazone-sodium SG 70 to the honey bee brood. 0.25 g test item in 1 L commercial ready-to-use syrup per colony, equivalent to an active substance concentration of 0.175 g propoxycarbazone-sodium a.s./L was tested. An untreated control and a toxic reference were included in the study. Three bee colonies were used per treatment group. The test item and reference item solutions were mixed with ready-to-use syrup (Apiinvert) and applied to the bee

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colonies via a feeding trough. Pure syrup was used for the controls. Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a period of 22 days following the application for each treatment group and colony. Assessment was conducted one day before (= BFD0; Brood Area Fixing Day) and 4 (= BFD 5), 8 (= BFD 9), 15 (= BFD 16), 22 (= BFD 23) days after the application by taking digital photos of selected brood combs. Mortality of adult bees and pupae was assessed daily.

Honey bee colonies or bee brood development was not adversely affected by Propoxycarbazone-sodium SG 70 at a concentration of 0.175 g propoxycarbazone-sodium a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Propoxycarbazone-sodium SG 70 (ATTRIBUT SG70)
Description:	Light-beige solid
Lot/Batch #:	Batch ID: EFKE002041; Specification No.: 102000011542 - 04
Purity:	71.0% w/w (analytical) propoxycarbazone-sodium (MKH 6561)

2. Vehicle and/or positive control: Positive control: Fenoxycarb 250 g/kg (Insect growth regulator)

3. Test organisms:

Species:	Honey bee (*Apis mellifera carnica* L.)
Age:	All ages and all stages.
Source:	Honey bee colonies, maintained according to normal beekeeping practice, by [redacted]. No varroacide has been used in the colonies for at least 2 months prior to the experimental start date.
Diet/Food:	Natural food and water sources As a deviation to that, on day 12 following the application, 1 L commercial ready-to-use syrup (Apiinvert; 30% sucrose, 31% glucose, 39% fructose) was supplied to each of the colonies. During the assessments on BFD+9, it was observed that some of the colonies had an insufficient amount of nectar/honey stores. Therefore, it was decided that an additional, exact feeding of all colonies was needed in order to avoid a suboptimal supply of the colonies. This situation was caused by the very limited natural food resources available to the colonies at this location during the assessment period.
Preparation of the Honey Bees:	Colonies were well fed and queen-right, each colony occupied two magazines ("Deutsch Normalmaß, DN") with 11 frames each. At the start of the experiment, each colony had 10-14 brood combs containing eggs, larvae and capped cells and a sufficient amount of honey and pollen. The colonies were assembled at the same time with healthy queens in order to guarantee uniform bee material in all treatments. 1-2 years old queens were used. The colonies contained about 16.600 - 19.500 adult honey bees. All colonies were equipped with a dead bee trap at the entrance.

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4. Environmental conditions:

Test site:	All colonies were set up at the same location. Test side was characterized by uncultivated fields; the surrounding area underlies agricultural use mainly with arable crops and meadows.
Conditions:	Natural conditions; temperature, relative humidity and precipitation was recorded for the entire experimental time.

B. STUDY DESIGN

1. Experimental treatments

1 L commercial ready-to-use syrup (Apiinvert) was offered in a feeding trough, which was put directly into each colony on top of the second magazine. The sugar solution was either untreated for the control treatment or mixed with 0.25 g test item (equivalent to a concentration of 0.175 g propoxycarbazone-sodium/L). As toxic reference fenoxycarb was administered with the sugar solution at a nominal concentration of 0.75 g/L. Three bee colonies per treatment group were tested. After 24 h, the uptake of the food by the colonies was complete (with the exception of one colony in the reference item group that needed 26 hours).

2. Observations

To evaluate bee mortality, dead bees were collected from dead-bee traps. The collected dead bees were separated during counting into adult worker bees, larvae and pupae. Mortality and behavioural abnormalities were assessed once per day from 3 days before application to day 21 after application.

Honey bee brood was assessed at different expected stages during the development, covering one complete development period of the honey bee. The development of the bee brood in individual marked cells was observed by photographing one or several combs per individual colony. One day prior to application, 120 - 150 cells containing eggs, 120 - 150 cells with young larvae and 150 cells with old larvae were selected, automatically numbered and marked using an image analysis program (ImageJ), in order to follow up the progress over a complete honey bee brood cycle, which lasts normally around 21 days. In most of the cases 150 cells were marked. Bee brood development (eggs, young- and old larvae) was assessed one day before (= BFD0; Brood Area Fixing Day) and 4 (= BFD 5), 8 (= BFD 9), 15 (= BFD 16), 22 (= BFD 23) days after the application.

The climatic conditions (temperature, relative humidity and precipitation) were recorded throughout the experimental period. The days following the application frequently rain occurred, meaning that the bees were not excessively foraging on other crops and reverting to the offered contaminated food in their colonies. In general, the early summer must be characterised as unusual cold and wet. The mean daily temperature following application, from day 0 to day 21, was between 11.0 and 20.8°C. The weather over the entire time of the experiment was unsettled and frequently some rain occurred.

3. Statistical calculations

The data were tested for normal distribution using Shapiro-Wilk`s test and homogeneity of variance using Levene`s test.

<u>Mortality:</u>

A pairwise comparison ($\alpha = 0.05$) was conducted for the mortality data (two-sided before application and one-sided greater, after application) using Student t-test for homogeneous variances.

<u>Brood Development:</u>

A pairwise comparison (one-sided greater, $\alpha = 0.05$) was conducted for the comparison of the brood data (egg and larvae termination rates), using Student t-test for homogenous variances.

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The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

Adult bee mortality in the test item treatment group was lower and thus not statistically significantly different when compared to the control group.

Pupae mortality was higher (4.7 dead pupae/day/colony) when compared to the control (3.4 dead pupae/day/colony), but not statistically significantly different.

No behavioural impairments were noted at any time in any of the test or reference item treatment groups until test end. Also no behavioural abnormalities were observed in the control group.

The termination rate of eggs was higher in the test item treatment group (25.2%) when compared to the values from the control group (16.0%). This is due to the fact, that one of the colonies has an unusually high termination rate of 42.0%, compared to the other two colonies (20.8% and 12.7%, respectively). Nevertheless, this slightly increased termination rate was not statistically significant compared to the control group.

No effect on the development of young larvae was observed after consumption of the test item. The mean termination rates of young larvae in the test item treatment group were lower, with a mean of 2.1% compared to 18.9% in the control group.

There was also no effect on the development of old larvae after consumption of the test item. The termination rates of old larvae in the test item treatment group were 1.1% compared to 2.7% in the control group.

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

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Table 8.3-3 Effects of Propoxycarbazone-sodium SG 70 on honey bee brood

Test item	**Propoxycarbazone-sodium SG 70**		
Test species	**Honey bees (*Apis mellifera* L.) (complete colonies)**		
Exposure	**via treated sugar solution**		
Treatment	**Untreated control**	**Propoxycarbazone-sodium SG 70**	**Reference Item (Insegar, a.i. = fenoxycarb)**
Rate per L sugar solution [product] [a]	-	0.25 g/L	3.0 g/L
Rate per L sugar solution [a.s.] [a]	-	0.175 g a.s./L	0.75 g a.s./L
Termination rate of the eggs [%] [b]	16.0%	25.2% (n.s.)	97.4% (*)
Termination rate of the young larvae [%] [b]	18.9%	2.1% (n.s.)	83.1% (*)
Termination rate of the old larvae [%] [b]	2.7%	1.1% (n.s.)	16.2% (*)
Mean brood termination rate over all stages	12.5%	9.5% (n.s.)	65.6% (*)
Mean mortality of worker bees/colony/day during pre-application phase [c] during the entire post-application phase [c]	 15.3 22.5	 9.6 (n.s.) 19.1 (n.s.)	 12.7 (n.s.) 54.7 (*)
Mean mortality of pupae/colony/day during pre-application phase [d] during the entire post-application phase [d]	 0.1 3.4	 0.4 (n.s.) 4.7 (n.s.)	 0.6 (n.s.) 11.9 (*)
Mean Number of Bees before Application	18165	16590	19485

[a] test and reference item was mixed in sugar solution
[b] mean termination rate of 3 colonies per treatment group
[c] mean number of dead honeybees per day and colony found in dead bee traps
[d] mean number of dead pupae/larvae per day and colony found in dead bee traps
Statistics: n.s. = not statistically significantly different compared to the control; * = statistically significantly different compared to the control; Student t-test, α = 0.05, pairwise comparison, two-sided (before application), one-sided greater (after application)

Validity criteria of the test (control mortality not considerable, high number of impacted brood due to the reference item fenoxycarb) are fulfilled.

III. CONCLUSIONS

Honey bee colonies or bee brood development was not adversely affected by Propoxycarbazone-sodium SG 70 at a concentration of 0.175 g propoxycarbazone-sodium a.s./L.

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 all studies conducted with propoxycarbazone-sodium, sub-lethal effects, if occurring, are described and reported.

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CA 8.3.2 Effects on non-target arthropods other than bees

Studies on non-target arthropods have been performed with the representative formulation of ATTRIBUT SG70 (tested as MKH 6561 WG 70). A summary of all available relevant and compliant data is presented in the table below.

Table 8.3-4 Effects of ATTRIBUT SG70 on non-target arthropods other than bees

Species	Test substance / Test design	Ecotoxicological endpoint		Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Aphidius rhopalosiphi	MKH6561 WG 70 lab., glass plates [g product/ha] 5 100	LR_{50} > 100 g/ha corr. Mortality [%] 3.0 0.0	Effect on Parasitation Efficiency [%] 0.0 5.0	[redacted] & [redacted] (1999) BAY-98-2 M-006190-01-1 KCA 8.3.2.1 /01	Yes
Typhlodromus pyri	MKH6561 WG 70 lab., glass plates [g product/ha] Control 100 200	LR_{50} > 200 g/ha corr. Mortality [%] 12.0 19.0	Effect on Reproduction [%] 1.0 22.0	[redacted] (1999) B041TPL M-016607-01-1 KCA 8.3.2.2 /01	Yes
Coccinella septempunctata	MKH6561 WG 70 lab., glass plates [g product/ha] Control 100	LR_{50} > 100 g/ha corr. Mortality [%] - 0.0	No of larvae/female 46 224	[redacted] (1999) SXR/Cs016 M-011866-01-1 KCA 8.3.2 /02	Yes
Pardosa ssp	MKH6561 WG 70 lab., quartz sand [g product/ha] 100	LR_{50} > 100 g/ha corr. Mortality [%] 0.0	Effect on Food Uptake [%] -3 [a]	[redacted] (1999) 98 10 48 082 M-006613-01-1 KCA 8.3.2 /01	Yes

[a] A negative value indicates a higher feeding activity in the treatment than in the control.
Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

No additional studies were conducted. Please refer to Point CA 8.3.2.

CA 8.3.2.2 Effects on *Typhlodromus pyri*

No additional studies were conducted. Please refer to Point CA 8.3.2.

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

Although no longer a data requirement according to Commission Regulation (EU) No 283/2013, a summary of all available relevant and compliant data for propoxycarbazone-sodium on acute effects on earthworms is presented in the table below for completeness.

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Table 8.4-1 Acute toxicity of propoxycarbazone-sodium and metabolites to earthworms

Test item	Species	Test design	Endpoint [mg/kg soil]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	*Eisenia fetida*	acute, 14 d	LC_{50} > 1000	[redacted] 1998 HBF/Rg 277 M-004250-01-1 KCA 8.4 /01	Yes
M05	*Eisenia fetida*	acute, 14 d	LC_{50} > 1000	[redacted] (1999) 736661 M-009647-01-1 KCA 8.4 /02	Yes
M07	*Eisenia fetida*	acute, 14 d	LC_{50} > 1000	[redacted] (1999) 736672 M-009308-01-1 KCA 8.4 /03	Yes
M10	*Eisenia fetida*	acute, 14 d	LC_{50} > 1000	[redacted] 1999 HBF/Rg 313 M-024206-01-1 KCA 8.4 /04	Yes

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

CA 8.4.1 Earthworms – sub-lethal effects

A summary of all available relevant and compliant data for propoxycarbazone-sodium on long-term effects on earthworms is presented in the table below.

Table 8.4-2 Long-term toxicity of propoxycarbazone-sodium and metabolites to earthworms

Test item	Species	Test design	NOEC [mg/kg soil]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	*Eisenia fetida*	reproduction, 56 d	5.0	[redacted] (2012) 70403022 M-466608-01-1 KCA 8.4.1 /03	New study
M05	*Eisenia fetida*	reproduction, 56 d	10	[redacted] (2012) 70415022 M-466675-01-1 KCA 8.4.1 /04	New study
M07	*Eisenia fetida*	reproduction, 56 d (limit test)	< 10	[redacted] (2012) 70424022 M-466689-01-1 KCA 8.4.1 /05	New study
		reproduction, 56 d (dose response)	5.0	[redacted] (2013) 70425022 M-466699-01-1 KCA 8.4.1 /06	New study
M08	*Eisenia fetida*	reproduction, 56 d	5.0	[redacted] (2014) 71792022 M-485902-01-1 KCA 8.4.1 /07	New study

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Test item	Species	Test design	NOEC [mg/kg soil]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
M09	*Eisenia fetida*	reproduction, 56 d	316	[redacted] (1999) HBF/Rg 315 M-024207-01-1 KCA 8.4.1 /02	Yes
M10	*Eisenia fetida*	reproduction, 56 d	5.0	[redacted] (2014) 71822022 M-484633-01-1 CA 8.4.1 /08	New study
M11	*Eisenia fetida*	reproduction, 56 d	5.0	[redacted] (2014) 71812022 M-485903-01-1 CA 8.4.1 /09	New study

[a] NOEC given in study report is 0.350 kg a.s./ha; endpoint was re-calculated during first Annex I review, considering a vessel surface area of 198 cm² and 500 g dws per vessel, NOEC was highest tested concentration.
Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

In order to address data requirements according to Commission Regulation (EU) No 283/2013, several additional studies on chronic exposure to earthworm have been performed with propoxycarbazone-sodium and the soil metabolites M05, M07, M08, M10 and M11 and are submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval. These studies are summarised below.

Propoxycarbazone-sodium

Report:	[redacted];[redacted];2012;M-466608-01
Title:	Effects of propoxycarbazone-sodium on reproduction and growth of earthworms eisenia fetida in artificial soil with 5 percent peat
Report No:	70403022
Document No:	M-466608-01-1
Guidelines:	**OECD 222, 2004 and ISO 11268-1, 1998**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of propoxycarbazone-sodium on *Eisenia fetida* were tested in a 56 day sublethal laboratory test with regards to mortality, behavioural effects, weight change, feeding activity and reproduction rate in artificial soil prepared according to OECD 222 with 5% peat. The test was conducted with five nominal test concentrations of 1.25, 2.5, 5, 10 and 20 mg propoxycarbazone-sodium/kg dry soil. Defined amounts of the test item were first mixed with fine quartz sand and then thoroughly mixed with artificial soil. The soil was moistened with deionised water. In addition a control group was exposed to soil mixed with the same amount of fine quartz sand as in the test item groups and moistened with deionised water.

After 28 days, the test item caused no mortality at any tested concentration. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass when compared to the control group. Reproduction rates (assessed after 56 days) were significantly reduced compared to the control at 2.5, 10 and 20 mg test item/kg dry soil, but the significance at 2.5 mg test item/kg dry soil was not considered to be treatment

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related since there was no dose-response relation and no statistical significance at the higher concentration of 5 mg test item/kg dry soil. All validity criteria according to OECD guideline 222 were fulfilled.

The NOEC for mortality and weight was determined to be 20 mg test item/kg dry soil. The NOEC for reproduction was determined to be 5 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Propoxycarbazone-sodium
Description:	White solid
Lot/Batch #:	Batch code: AE 0298618-01-09; Origin Batch No: 2012-000352
Purity:	95.1% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Eisenia fetida*
Age:	Adults, approx. 12 months old with clitellum
Weight:	330 – 600 mg
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light, 8 h dark, 400 – 800 lux
Soil pH:	Test start: 6.4 – 6.5
	Test end: 6.4 – 6.5
Soil moisture content:	Test start: 20.9% to 23.0% (48.7 – 53.6% of the maximum WHC)
	Test end: 22.7% to 25.9% (52.7 – 60.3% of the maximum WHC)

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to five concentrations of the test substance in an artificial soil substrate (5% Sphagnum-peat, 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate; according to OECD 222 and EPPO (2003), 5% of peat was used in the artificial soil considering the potential influence of the properties of the test item on bioavailability). Propoxycarbazone-sodium was mixed with fine quartz sand and added to artificial soil, resulting in the following nominal concentrations: 1.25, 2.5, 5, 10 and 20 mg propoxycarbazone-sodium/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. While thoroughly mixing the artificial soil, the soil of each treatment group was moistened with deionised water.

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Four replicate test containers (test item) and 8 replicate test containers (control) with 616.8 g soil wet weight (corresponding to 500 g dry weight soil, 111.8 g deionised water and 5 g food) and 4 - 5 cm soil depth were prepared for each treatment group. 10 adult earthworms per replicate (80 individuals per control, 40 individuals per test item treated group) were exposed.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations
At test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56 days after application), the number of surviving juveniles per replicate were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded. Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

3. Statistical calculations
Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and Kolmogorov-Smirnov test for weight changes and the Levene's test. Dunnett's t-test was used to compare treatment and control values (multiple comparison, $\alpha = 0.05$ two-sided for weight changes and one-sided smaller for reproduction). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group. The body weight of the earthworms at test end was not significantly different compared to the control group up to and including the highest tested concentration. The reproduction rate was significantly different compared to the control at concentrations of 2.5, 10 and 20 mg test item/kg dry soil. However, the statistical significance at 2.5 mg test item/kg dry soil was not considered to be treatment related, since there was no dose-response relation and no statistical significance at the higher concentration of 5 mg test item/kg dry soil.

Table 8.4-3 Lethal and sublethal effects of propoxycarbazone-sodium on earthworm

Propoxycarbazone-sodium [mg test item/kg dry soil]	**Control**	**1.25**	**2.5**	**5**	**10**	**20**
Mortality of adult worms after 28 days (%)	0	0	0	0	0	0
Mean weight change after 28 days (%)	37.0	42.7	48.0	33.2	33.9	42.9
Mean number of juveniles after 56 days	368	332	279*	315	294*	286*
Change of reproduction compared to control (%)	-	90.1	75.7	85.4	79.8	77.6
Food consumption	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg test item/kg soil]					
NOEC (day 28 mortality and weight)	20					
NOEC (day 56 reproduction)	5					

* Significantly different compared to the control (Dunnett´s t-test, $\alpha = 0.05$, one-sided smaller); however significance is not considered treatment related since there was no dose-response relation and no statistical significance at the higher concentration of 5 mg test item/kg dry soil.

The validity criteria according to guideline OECD 222 are fulfilled:

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- mortality in the control group was 0% (should be ≤ 10%)
- the number of juvenile worms per replicate was 245 to 463 and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)
- the coefficient of variation of reproduction in the control was 17.4% (should be ≤ 30).

In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46644022 from September 2011 to November 2011), there were statistically significant effects on reproduction at a concentration of 0.67 mg carbendazim/kg soil and higher; the EC_{50} for reproduction was calculated as 1.11 mg carbendazim/kg soil. These results show the sensitivity of the test system.

III. CONCLUSIONS

In an earthworm reproduction and growth study with propoxycarbazone-sodium the no observed effect concentration (NOEC) for mortality and weight was determined to be 20 mg test item/kg dry soil. The NOEC for reproduction was determined to be 5 mg test item/kg dry soil.

Metabolite M05

Report:	[redacted]; [redacted]; 2012; M-466675-01
Title:	Effects of MKH 6561-sulfonamide on reproduction and growth of earthworms eisenia fetida in artificial soil with 5 percent peat
Report No:	70415022
Document No:	M-466675-01-1
Guidelines:	**OECD 222, 2004 and ISO 11268-2, 1998**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-sulfonamide on *Eisenia fetida* were tested in a 56 day sublethal laboratory test with regards to mortality, behavioural effects, biomass development and reproduction rate in artificial soil prepared according to OECD 222 with 5% peat. The test was conducted with a single concentration of 10 mg MKH 6561-sulfonamide/kg dry soil. The test item was first mixed with fine quartz sand and then thoroughly mixed with the artificial soil. The soil was moistened with deionised water. In addition a control group was exposed to soil mixed with the same amount of fine quartz sand as in the test item group and moistened with deionised water.

After 28 days, the test item caused no mortality in any treatment group. Body weight increased significantly in the test item group compared to the control. However, a stronger weight increase is not considered to be an adverse effect. No effects on behaviour (including feeding activity) of the worms were observed during the test. Reproduction rates (assessed after 56 days) were not significantly different compared to the control. All validity criteria according to the OECD guideline 222 were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil. The NOAEC for body weight changes was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

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A. MATERIALS

1. Test material:

Test item:	MKH 6561-sulfonamide
Description:	White solid
Lot/Batch #:	Batch code: AE F073550-01-01; Origin Batch No: BCOO 5771-1-1
Purity:	99.4% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Eisenia fetida*
Age:	Adults, approx. 11 months old with clitellum
Weight:	300 – 600 mg
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light/ 8 h dark, 400 – 800 lux
Soil pH:	Test start: 6.3 – 6.4 Test end: 6.2
Soil moisture content:	Test start: 23.5% (54.5% of the maximum WHC) Test end: 24.3% to 24.7% (56.5% to 57.4% of the maximum WHC)

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate; according to OECD 222 and EPPO (2003), 5% of peat was used in the artificial soil considering the potential influence of the properties of the test item on bioavailability). MKH 6561-sulfonamide was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-sulfonamide/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. While thoroughly mixing the artificial soil, the soil of each treatment group was moistened with deionised water. Eight replicate test containers (for the test item and the control) with 617.5g soil wet weight (corresponding to 500 g dry weight soil, 112.5 g deionised water and 5 g food) and 4 - 5 cm soil depth were prepared for each treatment group. 10 adult earthworms per replicate (a total of 80 worms per test item treated and control group) were exposed for 56 days.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations

At test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56

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days after application), the number of surviving juveniles per replicate were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded. Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

3. Statistical calculations

Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and the Levene's test. Student t-test was used to compare treatment and control values (pair-wise comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group. The body weight of the earthworm at test end was significantly higher compared to the control group which is not considered to be an adverse effect. The reproduction rate, behaviour and feeding activity was not significantly different compared to the control.

Table 8.4-4 Lethal and sublethal effects of MKH 6561-sulfonamide on earthworm

MKH 6561-sulfonamide [mg test item/kg dry soil]	Control	10
Mortality of adult worms after 28 days (%)	0	0
Mean biomass change after 28 days (%)	55.7	67.8 *
Mean number of juveniles after 56 days	130	125
Change of reproduction compared to control (%)	-	96.2
Food consumption [g]	22.0	22.0
Endpoints [mg test item/kg soil]		
NOEC (day 28 mortality)	10	
NOAEC (weight)	10	
NOEC (day 56 reproduction)	10	

* Significantly different compared to the control (but not considered to be an adverse effect)

The validity criteria according to guideline OECD 222 are fulfilled:

- mortality in the control group was 0% (should be ≤ 10%)
- the number of juvenile worms per replicate was 94 to 188 and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)
- the coefficient of variation of reproduction in the control was 23.1% (should be ≤ 30).

In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46644022 from September 2011 to November 2011), there were statistically significant effects on reproduction at a concentration of 0.67 mg carbendazim/kg soil and higher; the EC_{50} for

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reproduction was calculated as 1.11 mg carbendazim/kg soil. These results show the sensitivity of the test system.

III. CONCLUSIONS

In an earthworm reproduction and growth study with MKH 6561-sulfonamide the 28 d NOAEC based on mortality and weight was determined to be 10 mg test item/kg dry soil. The NOEC based on reproduction after 56 days was determined to be 10 mg test item/kg dry soil.

Metabolite M07

Report:	[redacted]; [redacted];2012;M-466689-01
Title:	Effects of MKH 6561-saccharine on reproduction and growth of earthworms eisenia fetida in artificial soil with 5 percent peat
Report No:	70424022
Document No:	M-466689-01-1
Guidelines:	**OECD 222, 2004 and ISO 11268-2, 1998**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-saccharin on *Eisenia fetida* were tested in a 56 day sublethal laboratory test with regards to mortality, behavioural effects, biomass development and reproduction rate in artificial soil prepared according to OECD 222 with 5% peat. The test was conducted with a single concentration of 10 mg MKH 6561-saccharin/kg dry soil. The test item was first mixed with fine quartz sand and then thoroughly mixed with the artificial soil. The soil was moistened with deionised water. In addition a control group was exposed to soil mixed with the same amount of fine quartz sand as in the test item group and moistened with deionised water.

After 28 days, the test item caused no mortality in any treatment group. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass when compared to the control group. Reproduction rates (assessed after 56 days) were significantly reduced compared to the control. All validity criteria according to the OECD guideline 222 were fulfilled.

The no observed effect concentration (NOEC) for on mortality and weight was determined to be 10 mg test item/kg dry soil. The NOEC for reproduction was determined to be < 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-saccharin
Description:	Off-White solid
Lot/Batch #:	Batch code: AE F159737 00 1 B99 0002; Origin Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control: n/a

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3. Test organisms:

Species:	*Eisenia fetida*
Age:	Adults, approx. 11 months old with clitellum
Weight:	300 – 600 mg
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light, 8 h dark, 400 – 800 lux
Soil pH:	Test start: 6.3 – 6.4 Test end: 6.2
Soil moisture content:	Test start: 23.5% to 24.2% (54.5% to 56.3% of the max. WHC) Test end: 24.3% to 24.6% (56.5% to 57.2% of the max. WHC)

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz sand and 0.2% calcium carbonate; according to OECD 222 and EPPO (2003), 5% of peat was used in the artificial soil considering the potential influence of the properties of the test item on bioavailability). MKH 6561-saccharin was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-saccharin/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. While thoroughly mixing the artificial soil, the soil of each treatment group was moistened with deionised water. Eight replicate test containers (for the test item and the control) with 617.5g soil wet weight (corresponding to 500 g dry weight soil, 112.5 g deionised water and 5 g food) and 4 - 5 cm soil depth were prepared for each treatment group. 10 adult earthworms per replicate (a total of 80 worms per test item treated and control group) were exposed for 56 days.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations

At test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56 days after application), the number of surviving juveniles per replicate, were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded. Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

3. Statistical calculations

Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and the Levene's test. Student t-test was used to compare treatment and control values (pair-wise comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

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II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group. The body weight changes in the group treated with MKH 6561-saccharin were not statistically significantly different compared to the control (Student t-test, $\alpha = 0.05$, two-sided). The reproduction rate of the earthworms after 4 weeks exposure to the test concentration of 10 mg MKH 6561-saccharin/kg soil was statistically significantly reduced compared to the control (Student t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Table 8.4-5 Lethal and sublethal effects of MKH 6561-saccharin on earthworm

MKH 6561-saccharin [mg test item/kg dry soil]	**Control**	**10**
Mortality of adult worms after 28 days (%)	0	0
Mean biomass change after 28 days (%)	55.7	59.4
Mean number of juveniles after 56 days	130	95*
Change of reproduction compared to control (%)		73.3
Food consumption [g]	22.0	22.0
	Endpoints [mg test item/kg soil]	
NOEC (day 28 mortality and weight)	10	
NOEC (day 56 reproduction)	< 10	

* Significantly different compared to the control (Student t-test, $\alpha = 0.05$, one-sided smaller)

The validity criteria according to guideline OECD 222 are fulfilled:

- mortality in the control group was 0% (should be ≤ 10%)
- the number of juvenile worms per replicate was 94 to 188 and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)
- the coefficient of variation of reproduction in the control was 23.1% (should be ≤ 30).

III. CONCLUSIONS

In an earthworm reproduction and growth study with MKH 6561-saccharin the no observed effect concentration (NOEC) for mortality and weight was determined to be 10 mg test item/kg dry soil. The NOEC for reproduction was determined to be < 10 mg test item/kg dry soil.

As a $NOEC_{reproduction}$ could not be determined in the limit test, an additional dose response test was conducted with metabolite M07. In contrast to the limit test, peat content in soil was 10%.

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Report:	[redacted];[redacted];2013;M-466699-01
Title:	Effects of MKH 6561-saccharin on reproduction and growth of earthworms eisenia fetida in artificial soil
Report No:	70425022
Document No:	M-466699-01-1
Guidelines:	**OECD 222, 2004 and ISO 11268-2, 1998**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-saccharin on *Eisenia fetida* were tested in a 56 day sublethal laboratory test with regards to mortality, behavioural effects, biomass development and reproduction rate in artificial soil prepared according to OECD 222 with 10% peat. The test was conducted with five nominal test concentrations of 0.50, 0.89, 1.58, 2.81 and 5.0 mg MKH 6561-saccharin/kg dry soil. Defined amounts of the test item were solved in acetone, mixed with fine quartz sand and after evaporation of the solvent thoroughly mixed with artificial soil. The soil was moistened with deionised water. In addition a control group was exposed to soil mixed with the same amount of acetone treated quartz sand as in the test item groups and moistened with deionised water.

After 28 days, the test item caused no mortality at any tested concentration. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant body weight changes when compared to the control group. Reproduction rates (assessed after 56 days) were not significantly different compared to the control in any of the test item groups. All validity criteria according to the OECD guideline 222 were fulfilled.

The no observed effect concentration (NOEC) for mortality, body weight and reproduction was determined to be 5.0 mg test item/kg dry soil, i.e. the highest concentration tested.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-saccharin
Description:	Off-White solid
Lot/Batch #:	Batch code: AE F159737 00 1 B99 0002; Origin Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control:

Acetone

3. Test organisms:

Species:	*Eisenia fetida* (Savigny 1826)
Age:	Adults, approx. 8 months old with well-developed clitellum
Weight:	303 – 598 mg
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light/ 8 h dark, 400 – 800 lux
Soil pH:	Test start: 6.0 – 6.1
	Test end: 6.1 – 6.3
Soil moisture content:	Test start: 24.3% - 26.7% (48.6% to 53.4% of the maximum WHC)
	Test end: 27.1% - 29.5% (54.2% to 58.9% of the maximum WHC)

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to five concentrations of the test substance in an artificial soil substrate (10% Sphagnum-peat; 20% kaolin clay, 69.5% fine quartz-sand and 0.5% calcium carbonate). A defined amount of MKH 6561-saccharin was dissolved in acetone and a sequential dilution series was prepared. The dilutions were added to fine quartz sand and the mixture was left for approximately two hours in a fume hood until the solvent had evaporated. The sand was mixed and added to artificial soil resulting in the following nominal concentrations: 0.50, 0.89, 1.58, 2.81 and 5.0 mg MKH 6561-saccharin/kg dry soil. The control was treated with the same amount of acetone treated quartz sand as the test item groups. While thoroughly mixing the artificial soil, the soil of each treatment group was moistened with deionised water.

Four replicate test containers (test item) and 8 replicate test containers (control) with 634.9 g soil wet weight (corresponding to 500 g dry weight soil, 129.9 g deionised water and 5 g food) and 4 - 5 cm soil depth were prepared for each treatment group. 10 adult earthworms per replicate (a total of 80 individuals for the control and 40 individuals per test item treatment group) were exposed for 56 days.

In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations

At test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56 days after application), the number of surviving juveniles per replicate were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded. Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

3. Statistical calculations

Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and Cochron's test. Further statistical evaluation was performed using Williams t-test (multiple comparison, two-sided, $\alpha = 0.05$) for body weight data and Bonferroni-Welch t-test (multiple comparison, one-sided smaller, $\alpha = 0.05$) for reproduction data. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group except for one dead worm in the control. The body weight changes in the group treated with MKH 6561-saccharin were not statistically significantly different

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compared to the control (Williams t-test, α = 0.05, two-sided). The reproduction rates of the earthworms after 4 weeks exposure to the test concentrations up to and including 5.0 mg MKH 6561-saccharin/kg soil were not statistically significantly reduced compared to the control (Bonferroni-Welch t test, α = 0.05, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Table 8.4-6 Lethal and sublethal effects of MKH 6561-saccharin on earthworms

MKH 6561-saccharin [mg test item/kg dry soil]	Control	0.50	0.89	1.58	2.81	5.0
Mortality of adult worms after 28 days (%)	1.3	0.0	0.0	0.0	0.0	0.0
Mean weight change after 28 days (%)	26.9	21.4	21.4	25.6	22.8	27.0
Mean number of juveniles after 56 days	270	237	230	253	253	282
Change of reproduction compared to control (%)	-	87.9	85.3	93.8	93.8	104.3
Food consumption	24.8	24.0	24.8	25.0	25.0	25.0
Endpoints [mg test item/kg soil]						
NOEC (day 28 mortality and weight)	5.0					
NOEC (day 56 reproduction)	5.0					

The validity criteria according to guideline OECD 222 are fulfilled:

- mortality in the control group was 1.3% (should be ≤ 10%)
- the number of juvenile worms per replicate was 225 to 358 and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)

the coefficient of variation of reproduction in the control was 15.9% (should be ≤ 30).

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_{50} for reproduction was calculated as 1.7 mg carbendazim/kg soil. These results show the sensitivity of the test system.

III. CONCLUSIONS

In an earthworm reproduction and growth study with MKH 6561-saccharin the No Observed Effect Concentration (NOEC) for mortality, body weight changes and reproduction was determined to be 5.0 mg test item/kg soil, i.e. the highest concentration tested.

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Metabolite M08

Report:	[redacted];[redacted];2014;M-485902-01
Title:	Effects of MKH6561-4-hydroxy-saccharin on reproduction and growth of earthworms eisenia fetida in artificial soil
Report No:	71792022
Document No:	M-485902-01-1
Guidelines:	**OECD, Guideline for the testing of chemicals No. 222, Earthworm, Reproduction Test (adopted April 13, 2004)** **ISO-Guideline 11268-2, Soil quality - Effects of pollutants on earthworms - Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei, International Organization for Standardization, 2012**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-4-hydroxy-saccharin on *Eisenia fetida* were tested in a 56 day sublethal laboratory study design with regards to mortality, behavioural effects, biomass development and reproduction rate in artificial soil prepared according to OECD 222 with 10% peat. A 1st experiment was conducted with a single test concentration of 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil. As a NOEC for reproduction could not be determined, a 2nd experiment was performed with five nominal test concentrations of 0.50, 0.89, 1.58, 2.81 and 5.0 mg test item/kg dry soil. In both experiments, the test item was added to deionised water to prepare a stock solution and defined amounts of the solution were thoroughly mixed with artificial soil. The soil was moistened with deionised water. A control group, moistened with deionised water only, was run in parallel.

After 28 days, the test item caused no mortality at any treatment group except for one dead worm at the concentration of 0.89 mg test item/kg soil, which was not statistically significantly different compared to the control. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass when compared to the control group. Reproduction rates (assessed after 56 days) were not statistically significantly different compared to the control up to and including the test concentration of 5.0 mg test item/kg soil. All validity criteria according to the OECD guideline 222 were fulfilled.

The no observed effect concentration (NOEC) for mortality, weight changes and feeding activity and reproduction was determined to be 10.0 mg test item/kg dry soil. The no observed effect concentration (NOEC) for reproduction was determined to be 5.0 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561-4-hydroxy-saccharin
Description:	White solid
Lot/Batch #:	Batch code: AE 1364277-01-01; Origin Batch No: BCOO 6427-19-15
Purity:	99.5% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Eisenia fetida* (Savigny 1826)
Age:	Adults, approx. 7 months (1st experiment) and 12 month (2nd experiment) old with well-developed clitellum
Weight:	300 mg to 600 mg (1st experiment) 310 mg to 597 mg (2nd experiment)
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions (both experiments)

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light/ 8 h dark, 400 – 800 lux
Soil pH:	1st experiment: start: 5.5 to 5.6; end: 6.0 2nd experiment: start: 5.8 to 5.9; end: 5.9
Soil moisture content:	1st experiment: start: 29.9% to 30.6%; end: 30.8% to 30.9% 2nd experiment: start: 29.4% to 30.3%; end: 30.5% to 32.7%

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (10% Sphagnum-peat; 20% kaolin clay; 69.5% fine quartz-sand and 0.5% calcium carbonate). A stock solution was prepared in both experiments by mixing deionised water with MKH6561-4-hydroxy-saccharin. Defined amounts of the solution were thoroughly mixed with artificial soil and moistened with deionised water to achieve the following nominal concentrations: 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil (1st experiment) and 0.50, 0.89, 1.58, 2.81 and 5.0 mg MKH6561-4-hydroxy-saccharin/kg dry soil (2nd experiment). The control groups were moistened with deionised water only.
In the 1st experiment eight replicate test containers (test item and control), in the 2nd experiment, four replicate test containers (test item) and 8 replicate test containers (control) were prepared, both with 642.9 g soil wet weight (corresponding to 500 g dry weight plus 142.9 g deionised water). The height of the soil layer in the containers was approximately 4 - 5 cm. 5 g food/container was scattered on the soil surface after application (1st experiment) and at day 1 after application (2nd experiment) and was moistened with 5 g deionised water. 10 adult earthworms per replicate (a total of 80 worms per test item treated and control group in the 1st experiment and a total of 80 individuals for the control and 40 individuals per test item treatment group in the 2nd experiment) were exposed.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations

In both tests, at test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56 days after application), the number of surviving juveniles per replicate were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded.
Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

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3. Statistical calculations

Mortality data were analysed for significance by using the Fisher's Exact test (one-sided greater, $\alpha = 0.05$). Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and the Levene's test. Further statistical evaluation was performed using Student t-test (pairwise comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$) in the 1st experiment. In the 2nd experiment the Williams t-test (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group except for one dead worm at the concentration of 0.89 mg test item/kg soil, which was not statistically significantly different compared to the control (Fisher's Exact test, one-sided greater, $\alpha = 0.05$).
The body weight changes of the earthworms after 4 weeks exposure to MKH6561-4-hydroxy-saccharin were not statistically significantly different compared to the control up to and including the highest test concentration of 10.0 mg test item/kg soil (Student t-test for the 1st experiment and Williams t-test for the 2nd experiment, $\alpha = 0.05$, two-sided).
The reproduction rate was not statistically significantly different compared to the control up to and including the test concentration of 5.00 mg test item/kg soil (2nd experiment, Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test item concentration of 10.0 mg test item/kg soil reproduction was statistically significantly reduced compared to the control (1st experiment, Student t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Table 8.4-7 Lethal and sublethal effects of MKH6561-4-hydroxy-saccharin on earthworms (1st experiment: limit test)

1st experiment (limit test)		
MKH6561-4-hydroxy-saccharin [mg test item/kg dry soil]	**Control**	**10**
Mortality of adult worms after 28 days (%)	0	0
Mean biomass change after 28 days (%)	18.9	23.6
Mean number of juveniles after 56 days	229	204 *
Change of reproduction compared to control (%)	-	89.0
Food consumption [g]	25.0	25.0
	Endpoints [mg test item/kg soil]	
NOEC (day 28 mortality and weight)	10	
NOEC (day 56 reproduction)	< 10	

* Significantly different compared to the control (Student t-test, $\alpha = 0.05$, one-sided smaller)

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Table 8.4-8 Lethal and sublethal effects of MKH6561-4-hydroxy-saccharin on earthworms (2nd experiment: dose response test)

2nd experiment (dose response test)						
MKH6561-4-hydroxy-saccharin [mg test item/kg dry soil]	**Control**	**0.50**	**0.89**	**1.58**	**2.81**	**5.0**
Mortality of adult worms after 28 days (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean weight change after 28 days (%)	28.7	27.2	28.6	24.2	27.4	24.0
Mean number of juveniles after 56 days	215	214	223	210	210	188
Change of reproduction compared to control (%)	-	99.6	103.7	97.7	97.7	87.5
Food consumption	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg test item/kg soil]					
NOEC (day 28 mortality and weight)	10.0					
NOEC (day 56 reproduction)	5.0					

The validity criteria according to guideline OECD 222 are fulfilled:

- mortality in the control group was 0% in both experiments (should be ≤ 10%)
- the number of juvenile worms per replicate was 202 to 274 (1st experiment) and 168 to 250 (2nd experiment) and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)
- the coefficient of variation of reproduction in the control was 11.8% (1st experiment) and 12.6% (2nd experiment) (should be ≤ 30).

In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 466A6022 from August 2013 to October 2013), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg carbendazim/kg soil). The EC_{50} for reproduction was calculated as 1.32 mg carbendazim/kg soil, which is in the range of the 5 most recent studies, where EC_{50} values between 1.11 and 1.59 mg carbendazim/kg soil were determined. These results show the sensitivity of the test system.

III. CONCLUSIONS

In an earthworm reproduction and growth study with MKH6561-4-hydroxy-saccharin the no observed effect concentration (NOEC) for mortality, weight changes and feeding activity and reproduction was determined to be 10.0 mg test item/kg dry soil. The no observed effect concentration (NOEC) for reproduction was determined to be 5.0 mg test item/kg dry soil.

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Metabolite M10

Report:	[redacted];[redacted];2014;M-484633-01
Title:	Effects of MKH6561-N-methyl propoxytriazolinone on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	71822022
Document No:	M-484633-01-1
Guidelines:	**OECD, Guideline for the testing of chemicals No. 222, Earthworm, Reproduction Test (adopted April 13, 2004)** **ISO-Guideline 11268-2, Soil quality - Effects of pollutants on earthworms - Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei, International Organization for Standardization, 2012**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-N-methyl propoxytriazolinone on *Eisenia fetida* were tested in a 56 day sublethal laboratory study design with regards to mortality, behavioural effects, biomass development and reproduction rate in artificial soil prepared according to OECD 222 with 10% peat. A 1st experiment was conducted with a single test concentration of 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil. As a NOEC for reproduction could not be determined, a 2nd experiment was performed with five nominal test concentrations of 0.50, 0.89, 1.58, 2.81 and 5.0 mg test item/kg dry soil. In both experiments, the test item was added to deionised water to prepare a stock solution and defined amounts of the solution were thoroughly mixed with artificial soil. The soil was moistened with deionised water. A control group, moistened with deionised water only, was run in parallel.

After 28 days, the test item caused no mortality at any tested concentration. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass when compared to the control group. Reproduction rates (assessed after 56 days) were not statistically significantly different compared to the control up to and including the test concentration of 5.0 mg test item/kg soil. All validity criteria according to the OECD guideline 222 were fulfilled.

The no observed effect concentration (NOEC) for mortality, weight changes and feeding activity and reproduction was determined to be 10.0 mg test item/kg dry soil. The no observed effect concentration (NOEC) for reproduction was determined to be 5.0 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Test item:	MKH6561-N-methyl propoxytriazolinone
Description:	White solid
Lot/Batch #:	Batch code: AE 1364263-01-01; Origin Batch No: NLL 5797-6-5
Purity:	99.0% w/w

2. **Vehicle and/or positive control:** n/a

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3. Test organisms:

Species:	*Eisenia fetida* (Savigny 1826)
Age:	Adults, approx. 9 months (1st experiment) and 6 month (2nd experiment) old with well-developed clitellum
Weight:	310 mg to 596 mg (1st experiment) 300 mg to 600 mg (2nd experiment)
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions (both experiments)

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light/ 8 h dark, 400 – 800 lux
Soil pH:	1st experiment: start: 6.0 to 6.2; end: 6.1 to 6.2 2nd experiment: start: 5.5; end: 5.8 to 5.9
Soil moisture content:	1st experiment: start: 30.5% to 30.6%; end: 31.0% to 31.4% 2nd experiment: start: 29.9% to 30.8%; end: 31.0% to 33.7%

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (10% Sphagnum-peat; 20% kaolin clay, 69.6% (1st experiment) and 69.5% (2nd experiment) fine quartz-sand and 0.4% (1st experiment) and 0.5% (2nd experiment) calcium carbonate). A stock solution was prepared in both experiments by mixing deionised water with MKH6561-N-methyl propoxytriazolinone. Defined amounts of the solution were thoroughly mixed with artificial soil and moistened with deionised water to achieve the following nominal concentrations: 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil (1st experiment) and 0.50, 0.89, 1.58, 2.81 and 5.0 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil (2nd experiment). The control groups were moistened with deionised water only.
In the 1st experiment eight replicate test containers (test item and control) with 644.0 g soil wet weight (corresponding to 500 g dry weight plus 144.0 g deionised water) were prepared. In the 2nd experiment, four replicate test containers (test item) and 8 replicate test containers (control) with 643.8 g soil wet weight (corresponding to 500 g dry weight plus 143.8 g deionised water) were prepared. The height of the soil layer in the containers was approximately 4 - 5 cm. 5 g food/container was scattered on the soil surface at day 1 after application (1st experiment) and after application (2nd experiment) and was moistened with 5 g deionised water. 10 adult earthworms per replicate (a total of 80 worms per test item treated and control group in the 1st experiment and a total of 80 individuals for the control and 40 individuals per test item treatment group in the 2nd experiment) were exposed.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations

In both tests, at test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56 days after application), the number of surviving juveniles per replicate were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded.

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Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

3. Statistical calculations

Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and the Levene's test. Further statistical evaluation was performed using Student t-test (pairwise comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$) in the 1st experiment. In the 2nd experiment the Williams t-test (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group.
The body weight changes of the earthworms after 4 weeks exposure to MKH6561-N-methyl propoxytriazolinone were not statistically significantly different compared to the control up to and including the highest test concentration of 10.0 mg test item/kg soil (Student t-test for the 1st experiment and Williams t-test for the 2nd experiment, $\alpha = 0.05$, two-sided). The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 5.0 mg test item/kg soil (2nd experiment, Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test item concentration of 10.0 mg test item/kg soil reproduction was statistically significantly reduced compared to the control group (1st experiment, Student t-test, $\alpha = 0.05$, one-sided smaller). The feeding activity in all the treated groups was comparable to the control.

Table 8.4-9 Lethal and sublethal effects of MKH6561-N-methyl propoxytriazolinone on earthworms (1st experiment: limit test)

1st experiment (limit test)		
MKH6561-N-methyl propoxytriazolinone [mg test item/kg dry soil]	**Control**	**10**
Mortality of adult worms after 28 days (%)	0	0
Mean biomass change after 28 days (%)	35.2	38.8
Mean number of juveniles after 56 days	280	242*
Change of reproduction compared to control (%)	-	86.3
Food consumption [g]	25.0	25.0
	Endpoints [mg test item/kg soil]	
NOEC (day 28 mortality and weight)	10	
NOEC (day 56 reproduction)	< 10	

* Significantly different compared to the control (Student t-test, $\alpha = 0.05$, one-sided smaller)

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Table 8.4-10 Lethal and sublethal effects of MKH6561-N-methyl propoxytriazolinone on earthworms (2nd experiment: dose response test)

2nd experiment (dose response test)						
MKH6561-N-methyl propoxytriazolinone [mg test item/kg dry soil]	**Control**	**0.50**	**0.89**	**1.58**	**2.81**	**5.0**
Mortality of adult worms after 28 days (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean weight change after 28 days (%)	14.9	18.3	20.9	19.1	18.0	22.4
Mean number of juveniles after 56 days	174	167	156	163	154	166
Change of reproduction compared to control (%)	-	96.1	89.3	93.5	88.4	95.5
Food consumption	24.8	24.8	25.0	24.8	25.0	25.0
	Endpoints [mg test item/kg soil]					
NOEC (day 28 mortality and weight)	10.0					
NOEC (day 56 reproduction)	5.0					

The validity criteria according to guideline OECD 222 are fulfilled:

- mortality in the control group was 0% in both experiments (should be ≤ 10%)
- the number of juvenile worms per replicate was 240 to 316 (1st experiment) and 146 to 210 (2nd experiment) and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)
- the coefficient of variation of reproduction in the control was 9.3% (1st experiment) and 12.6% (2nd experiment) (should be ≤ 30).

In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 4664/6022 from August 2013 to October 2013), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg carbendazim/kg soil). The EC_{50} for reproduction was calculated as 1.32 mg carbendazim/kg soil, which is in the range of the 5 most recent studies, where EC_{50} values between 1.11 and 1.59 mg carbendazim/kg soil were determined. These results show the sensitivity of the test system.

III. CONCLUSIONS

In an earthworm reproduction and growth study with MKH6561-N-methyl propoxytriazolinone the no observed effect concentration (NOEC) for mortality, weight changes and feeding activity and reproduction was determined to be 10.0 mg test item/kg dry soil. The no observed effect concentration (NOEC) for reproduction was determined to be 5.0 mg test item/kg dry soil.

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Metabolite M11

Report:	[redacted];[redacted];2014;M-485903-01
Title:	Effects of MKH6561-methoxy-saccharin on reproduction and growth of earthworms eisenia fetida in artificial soil
Report No:	71812022
Document No:	M-485903-01-1
Guidelines:	**OECD, Guideline for the testing of chemicals No. 222, Earthworm, Reproduction Test (adopted April 13, 2004)** **ISO-Guideline 11268-2, Soil quality - Effects of pollutants on earthworms - Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei, International Organization for Standardization, 2012**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-methoxy-saccharin on *Eisenia fetida* were tested in a 56 day sublethal laboratory study design with regards to mortality, behavioural effects, biomass development and reproduction rate in artificial soil prepared according to OECD 222 with 10% peat. A 1st experiment was conducted with a single test concentration of 10 mg MKH6561-methoxy-saccharin/kg dry soil. As a NOEC for reproduction could not be determined, a 2nd experiment was performed with five nominal test concentrations of 0.50, 0.89, 1.58, 2.81 and 5.0 mg test item/kg dry soil. In both experiments, the test item was added to deionised water to prepare a stock solution and defined amounts of the solution were thoroughly mixed with artificial soil. The soil was moistened with deionised water. A control group, moistened with deionised water only, was run in parallel.

After 28 days, the test item caused no mortality at treatment group except for one dead worm at the concentration of 0.50 mg test item/kg soil, which was not statistically significantly different compared to the control. No effects on behaviour (including feeding activity) of the worms were observed during the test. The body weight changes were statistically significantly reduced at test concentration of 0.50 mg test item/kg soil. However, this reduction is not considered treatment related as at all higher concentrations no significant difference compared to the control was found. Reproduction rates (assessed after 56 days) were not statistically significantly different compared to the control up to and including the test concentration of 5.0 mg test item/kg soil. All validity criteria according to the OECD guideline 222 were fulfilled.

The no observed effect concentration (NOEC) for mortality, weight changes and feeding activity and reproduction was determined to be 10.0 mg test item/kg dry soil. The no observed effect concentration (NOEC) for reproduction was determined to be 5.0 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Test item:	MKH6561-methoxy-saccharin
Description:	Light yellow
Lot/Batch #:	Batch code: BCS-AG71018-01-01; Origin Batch No: BCOO 6413-13-5
Purity:	99.7% w/w

2. **Vehicle and/or positive control:** n/a

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3. Test organisms:

Species:	*Eisenia fetida* (Savigny 1826)
Age:	Adults, approx. 7 months (1st experiment) and 10 month (2nd experiment) old with well-developed clitellum
Weight:	300 mg to 600 mg (1st experiment) 300 mg to 600 mg (2nd experiment)
Source:	In-house culture
Diet/Food:	Finely ground cattle manure
Acclimatisation:	1 day in artificial soil under test conditions (both experiments)

4. Environmental conditions:

Temperature:	18 – 22 °C
Photoperiod:	16 h light/ 8 h dark, 400 – 800 lux
Soil pH:	1st experiment: start: 5.5 to 5.6; end: 6.0 2nd experiment: start: 5.8 to 6.1; end: 6.0 to 6.2
Soil moisture content:	1st experiment: start: 29.9% to 32.3%; end: 30.9% to 33.9% 2nd experiment: start: 30.5% to 33.3%; end: 33.1% to 35.2%

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (10% Sphagnum-peat; 20% kaolin clay; 69.5% fine quartz-sand and 0.5% calcium carbonate). A stock solution was prepared in both experiments by mixing deionised water with MKH6561-methoxy-saccharin. Defined amounts of the solution were thoroughly mixed with artificial soil and moistened with deionised water to achieve the following nominal concentrations: 10 mg MKH6561-methoxy-saccharin/kg dry soil (1st experiment) and 0.50, 0.89, 1.58, 2.81 and 5.0 mg MKH6561-methoxy-saccharin/kg dry soil (2nd experiment). The control groups were moistened with deionised water only.

In the 1st experiment eight replicate test containers (test item and control) with 642.9 g soil wet weight (corresponding to 500 g dry weight plus 142.9 g deionised water) were prepared. In the 2nd experiment, four replicate test containers (test item) and 8 replicate test containers (control) with 648.6 g soil wet weight (corresponding to 500 g dry weight plus 148.6 g deionised water) were prepared. The height of the soil layer in the containers was approximately 4 - 5 cm. 5 g food/container was scattered on the soil surface at after application (1st experiment) and 1 day after application (2nd experiment) and was moistened with 5 g deionised water. 10 adult earthworms per replicate (a total of 80 worms per test item treated and control group in the 1st experiment and a total of 80 individuals for the control and 40 individuals per test item treatment group in the 2nd experiment) were exposed.

In a separate study, earthworms were exposed to the toxic reference substance carbendazim.

2. Observations

In both tests, at test initiation and after 28 days, the mean body weight of adult earthworms was recorded. Mortality, behavioural and morphological abnormalities were recorded after 28 days. After additional 28 days (56 days after application), the number of surviving juveniles per replicate were counted. The amount of food added to each test container (which approximately reflects the amount of food eaten) was recorded.

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Temperature and light intensity were monitored continuously. Water content and pH measurements were performed at test initiation and at test termination.

3. Statistical calculations

Mortality data were analysed for significance by using the Fisher's Exact test (one-sided greater, $\alpha = 0.05$). Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using Shapiro-Wilk's test and and the Levene's test. Further statistical evaluation was performed using Student t-test (pairwise comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$) in the 1st experiment. In the 2nd experiment the Williams t-test (multiple comparison, two-sided for weight and one-sided smaller for reproduction, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

No mortality was observed in any treatment group except for one dead worm at the test concentration of 0.50 mg test item/kg soil, which was not statistically significantly different compared to the control (Fisher's Exact test, one-sided greater, $\alpha = 0.05$).
The body weight changes of the earthworms after 4 weeks exposure to MKH6561-methoxy-saccharin were statistically significantly different compared to the control at the test concentration of 0.50 mg test item/kg soil. However, this reduction was not considered to be a treatment related effect since at all higher test concentrations up to and including the highest test concentration of 10.0 mg test item/kg soil no statistical difference compared to the control could be observed (Student t-test for the 1st experiment and Williams t-test for the 2nd experiment, $\alpha = 0.05$, two-sided).
The reproduction rate was not statistically significantly different compared to the control up to and including the test concentration of 5.00 mg test item/kg soil (2nd experiment, Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 10.0 mg test item/kg soil reproduction was statistically significantly reduced compared to the control (1st experiment, Student t-test, $\alpha = 0.05$, one-sided smaller).
No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Table 8.4-11 Lethal and sublethal effects of MKH6561-methoxy-saccharin on earthworms (1st experiment: limit test)

1st experiment (limit test)		
MKH6561-methoxy-saccharin [mg test item/kg dry soil]	**Control**	**10**
Mortality of adult worms after 28 days (%)	0	0
Mean biomass change after 28 days (%)	18.9	16.5
Mean number of juveniles after 56 days	229	193*
Change of reproduction compared to control (%)	-	84.1
Food consumption [g]	25.0	25.0
	Endpoints [mg test item/kg soil]	
NOEC (day 28 mortality and weight)	10	
NOEC (day 56 reproduction)	< 10	

* Significantly different compared to the control (Student t-test, $\alpha = 0.05$, one-sided smaller)

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Table 8.4-12 Lethal and sublethal effects of MKH6561-methoxy-saccharin on earthworms (2nd experiment: dose response test)

2nd experiment (dose response test)						
MKH6561-methoxy-saccharin [mg test item/kg dry soil]	**Control**	**0.50**	**0.89**	**1.58**	**2.81**	**5.0**
Mortality of adult worms after 28 days (%)	0.0	2.5	0.0	0.0	0.0	0.0
Mean weight change after 28 days (%)	22.5	14.9*	24.3	19.9	19.8	20.7
Mean number of juveniles after 56 days	249	223	247	238	234	233
Change of reproduction compared to control (%)	-	89.7	99.3	95.5	94.2	93.5
Food consumption	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg test item/kg soil]					
NOEC (day 28 mortality and weight)	10.0					
NOEC (day 56 reproduction)	5.0					

* Significantly different compared to the control (Student t-test, α = 0.05, two-sided smaller), however reduction not considered treatment related as all higher concentrations are not significantly different compared to the control

The validity criteria according to guideline OECD 222 are fulfilled:

- mortality in the control group was 0% in both experiments (should be ≤ 10%)
- the number of juvenile worms per replicate was 202 to 274 (1st experiment) and 225 to 290 (2nd experiment) and so this validity criterion was met (should be ≥ 30 juveniles by the end of the test)
- the coefficient of variation of reproduction in the control was 11.8% (1st experiment) and 7.6% (2nd experiment) (should be ≤ 30).

In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46646022 from August 2013 to October 2013), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg carbendazim/kg soil). The EC_{50} for reproduction was calculated as 1.32 mg carbendazim/kg soil, which is in the range of the 5 most recent studies, where EC_{50} values between 1.11 and 1.59 mg carbendazim/kg soil were determined. These results show the sensitivity of the test system.

III. CONCLUSIONS

In an earthworm reproduction and growth study with MKH6561-methoxy-saccharin the no observed effect concentration (NOEC) for mortality, weight changes and feeding activity and reproduction was determined to be 10.0 mg test item/kg dry soil. The no observed effect concentration (NOEC) for reproduction was determined to be 5.0 mg test item/kg dry soil.

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

A summary of all available relevant and compliant data for propoxycarbazone-sodium on effects on non-target soil meso- and macrofauna (other than earthworms) is presented in the table below.

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Table 8.4-13 Long-term toxicity of propoxycarbazone-sodium and its metabolites to other non-target soil macro-organisms

Test item	Species	Test design	NOEC [mg/kg soil]	Reference	EU agreed endpoint (SANCO/4067-2001-final)
Propoxy-carbazone-sodium	*Folsomia candida*	reproduction, 28 d	500	[redacted] (2012) 70404016 M-466609-01-1 KCA 8.4.2 /01	New study
	Hypoaspis aculeifer	reproduction, 14 d	1000	[redacted] (2012) 70405089 M-466611-01-1 KCA 8.4.2 /02	New study
M05	*Folsomia candida*	reproduction, 28 d, limit test	10	[redacted] (2012) 70412016 M-466656-01-1 KCA 8.4.2 /03	New study
	Hypoaspis aculeifer	reproduction, 14 d, limit test	10	[redacted] (2012) 70411089 M-466654-01-1 KCA 8.4.2 /04	New study
M07	*Folsomia candida*	reproduction, 28 d	9.0	[redacted] (2012) 70422016 M-466684-01-1 KCA 8.4.2 /05	New study
	Hypoaspis aculeifer	reproduction, 14 d, limit test	10	[redacted] (2012) 70422016 M-466684-01-1 KCA 8.4.2 /06	New study
M08	*Folsomia candida*	reproduction, 28 d, limit test	10	[redacted] (2014) 71793016 M-484422-01-1 KCA 8.4.2 /07	New study
	Hypoaspis aculeifer	reproduction, 14 d, limit test	10	[redacted] (2014) 71794089 M-484430-01-1 KCA 8.4.2 /08	New study
M09	*Folsomia candida*	reproduction, 28 d, limit test	10	[redacted] (2012) 70445016 M-466718-01-1 KCA 8.4.2 /09	New study
	Hypoaspis aculeifer	reproduction, 14 d, limit test	10	[redacted] (2012) 70444089 M-466715-01-1 KCA 8.4.2 /10	New study
M10	*Folsomia candida*	reproduction, 28 d, limit test	10	[redacted] (2014) 71823016 M-484425-01-1	New study

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Test item	Species	Test design	NOEC [mg/kg soil]	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
				KCA 8.4.2 /11	
	Hypoaspis aculeifer	reproduction, 14 d, limit test	10	[redacted] (2014) 71824089 M-484437-01-1 KCA 8.4.2 /12	New study
M11	*Folsomia candida*	reproduction, 28 d, limit test	10	[redacted] (2014) 71813016 M-484423-01-1 KCA 8.4.2 /13	New study
	Hypoaspis aculeifer	reproduction, 14 d, limit test	10	[redacted] (2014) 71814089 M-484433-01-1 KCA 8.4.2 /14	New study

In order to address data requirements according to Commission Regulation (EU) No 283/2013, several additional studies on chronic exposure to other non-target soil macro-organisms, represented by Collembola *Folsomia candida* and soil mite *Hypoaspis aculeifer*, have been performed with propoxycarbazone-sodium and the soil metabolites M05, M07, M08, M09, M10 and M11 and are submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval. These studies are summarised below.

Propoxycarbazone-sodium

Report:	[redacted];[redacted];2012;M-466609-01
Title:	Effects of propoxycarbazone-sodium on reproduction of the collembola folsomia condida in artificial soil with 5 percent peat
Report No:	70404016
Document No:	M-466609-01-1
Guidelines:	**GLP compliant study based on OECD 232, 2009 and ISO 11267, 1999**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of propoxycarbazone-sodium on Collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. The test was conducted with five test concentrations, encompassing 62.5, 125, 250, 500 and 1000 mg propoxycarbazone-sodium/kg dry soil. In addition a control group was exposed to soil mixed without test item.

After 28 days, the test item caused statistically significantly increased mortality and decreased reproduction at 1000 mg propoxycarbazone-sodium/kg dry soil when compared to the control group. No effects on behaviour of the springtails were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 28 days was determined to be 500 mg test item/kg dry soil. The overall lowest observed effect concentration (LOEC) was determined to be 1000 mg test item/kg soil. The EC_{50} for reproduction after 28 days was calculated to be 922 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Propoxycarbazone-sodium
Description:	White solid
Lot/Batch #:	Batch code: AE 0298618-01-09; Origin Batch No: 2012-000352
Purity:	95.1% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast
Acclimatisation:	Individuals were kept under breeding conditions until test start

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 at test start and 6.1-6.4 at test end
Water content:	54.3% to 56.3% of the maximum WHC at test start 52.1% to 53.8% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to five concentrations of the test substance in an artificial soil substrate (5% Sphagnum peat; 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. Propoxycarbazone-sodium was mixed with fine quartz sand and added to artificial soil, resulting in the following nominal concentrations: 62.5, 125, 250, 500 and 1000 mg propoxycarbazone-sodium/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Four replicate test containers (test item) and 8 replicate test containers (control) each with 30 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, Collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 2, 8, 11, 14, 18, 21 and 25 by opening the lids for a short period.

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2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The ECx values for reproduction were calculated by Probit Analysis (Finney 1971). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A slight mortality of up to 15% was observed in the test item treated groups up to and including 500 mg/kg soil, which was not statistically significantly different compared to the control, where 9% of the collembola died. A statistically significant mortality of 38% was observed in the test item group of 1000 mg/kg soil compared to the control (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the collembolans exposed to propoxycarbazone-sodium was not statistically significantly different compared to the control up to and including the test concentration of 500 mg/kg soil whereas at the test item concentration of 1000 mg/kg soil reproduction was statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-14 Lethal and sublethal effects of propoxycarbazone-sodium on Collembola (*Folsomia candida*)

Propoxycarbazone-sodium [mg test item/kg dry soil]	Control	62.5	125	250	500	1000
Mortality after 28 days (%)	9	10	8	15	15	38 *
Mean number of juveniles after 28 days	304	329	365	315	290	125 **
Change of reproduction compared to control (%)	-	108	120	104	95	41
	Endpoints [mg test item/kg soil]					
NOEC (mortality)	500					
LC_{50} (mortality)	> 1000					
NOEC (reproduction)	500					
EC_x (reproduction)	EC_{10}		EC_{20}		EC_{50}	
	577.8		678.4		922.0	

* Significantly different compared to the control (Fisher's Exact test, $\alpha = 0.05$, one-sided greater)

** Significantly different compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller)

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 9% (should be ≤ 20%)

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- number of juvenile per replicate was 243 to 346 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 13.2% (should be ≤ 30).

In the most recent test with the reference item Boric acid (performed under IBACON study code 61402016 from August to October 2011), there were statistically significant effects on reproduction at concentrations of ≥ 53.7 mg/kg soil; the EC_{50} for reproduction was calculated to be 75.7 mg/kg soil. Mortality was statistically significantly higher compared to the control at 137.5 mg/kg soil and above.

III. CONCLUSIONS

In a reproduction study with *Folsomia candida* exposed to propoxycarbazone-sodium the no observed effect concentration (NOEC) for mortality and reproduction was determined to be 500 mg test item/kg dry soil. The LOEC was determined to be 1000 mg test item/kg dry soil for mortality and reproduction. The EC_{50} for reproduction was calculated to be 922.0 mg test item/kg soil.

Report:	[redacted]; [redacted]; 2012-M-466611-01
Title:	Effects of propoxycarbazone-sodium on reproduction of the predatory mite hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	70405089
Document No:	M-466611-01-1
Guidelines:	**OECD 226, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of propoxycarbazone-sodium on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with five test concentrations, encompassing 62.5, 125, 250, 500 and 1000 mg propoxycarbazone-sodium/kg dry soil homogenously mixed into the soil. In addition a control group was exposed to soil mixed without test item.

After 14 days, the test item caused no statistically significant effects on mortality and reproduction up to and including 1000 mg propoxycarbazone-sodium/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria according to the guideline were fulfilled.

The no observed effect concentration (NOEC) based on mortality and reproduction after 14 days was determined to be 1000 mg test item/kg dry soil. The lowest observed effect concentration (LOEC) based on mortality and reproduction after 14 days was determined to be greater than 1000 mg test item/kg dry soil. The LC_{50} and EC_{50} for mortality and reproduction were determined to be greater than 1000 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Propoxycarbazone-sodium
Description:	White solid
Lot/Batch #:	Batch code: AE 0298618-01-09; Origin Batch No: 2012-000352
Purity:	95.1% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 10 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 2, 4, 8 and 11

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 at test start and 6.3 - 6.4 at test end
Water content:	54.3% to 56.3% of the maximum WHC at test start 51.2% to 53.9% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to five concentrations of the test substance in an artificial soil substrate (5% Sphagnum peat; 20% kaolin clay; 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. Propoxycarbazone-sodium was mixed with fine quartz sand and added to artificial soil, resulting in the following nominal concentrations: 62.5, 125, 250, 500 and 1000 mg propoxycarbazone-sodium/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Four replicate test containers (test item) and 8 replicate test containers (control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on days 2, 4, 8, and 11 by opening the lids for a short period.

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2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 8 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A slight mortality of up to 18% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the adult mites died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the predatory mites exposed to propoxycarbazone-sodium was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).
No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 8.4-15 Lethal and sublethal effects of propoxycarbazone-sodium on the Predatory Mite *Hypoaspis aculeifer*

Propoxycarbazone-sodium [mg test item/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality after 14 days (%)	8	8	8	10	18	15
Mean number of juveniles after 14 days	242	223	243	239	213	200
Change of reproduction compared to control (%)	-	92	100	99	88	83
	Endpoints [mg test item/kg soil]					
NOEC (mortality)	1000					
LC_{50} (mortality)	> 1000					
NOEC (reproduction)	1000					
EC_{50} (reproduction)	> 1000					

The validity criteria according to guideline OECD 226 are fulfilled:

- mortality in the control group was 8% (should be ≤ 20%)
- number of juvenile per replicate was 213 to 266 and so this validity criterion was met (should be ≥ 50 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 8.3% (should be ≤ 30).

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In the most recent test with the reference item dimethoate (performed under IBACON Project No. 59232089 in June 2011), there were statistically significant effects on reproduction at concentrations of 4.3 mg/kg soil; the EC_{50} for reproduction was 4.33 mg dimethoate/kg soil.

III. CONCLUSIONS

In a reproduction study with *Hypoaspis aculeifer* exposed to propoxycarbazone-sodium the no observed effect concentration (NOEC) for mortality and reproduction was determined to be 1000 mg test item/kg dry soil. The LOEC was determined to be greater than 1000 mg test item/kg dry soil. The LC_{50} and EC_{50} for mortality and reproduction were determined to be greater than 1000 mg test item/kg dry soil.

Metabolite M05

Report:	[redacted]; [redacted];2012;M-466656-01-1
Title:	Effects of MKH 6561-sulfonamide on reproduction of the collembola Folsomia candida in artificial soil with 5 percent peat
Report No:	70412016
Document No:	M-466656-01-1
Guidelines:	**GLP compliant study based on OECD 232, 2009 and ISO 11267, 1999**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-sulfonamide on Collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. The test was conducted with a single concentration of 10 mg MKH 6561-sulfonamide/kg dry soil, mixed homogeneously into the soil. In addition a control group was exposed to soil mixed without test item.

After 28 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH 6561-sulfonamide/kg dry soil when compared to the control group. No effects on behaviour of the springtails were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 28 days was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-sulfonamide
Description:	White solid
Lot/Batch #:	Batch code: AE F073550-01-01; Origin Batch No: BCOO 5771-1-1
Purity:	99.4% w/w

2. Vehicle and/or positive control: n/a

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3. **Test organisms:**

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast
Acclimatisation:	Individuals were kept under breeding conditions until test start

4. **Environmental conditions:**

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 to 6.5 at test start and 6.2 at test end
Water content:	48.8% to 50.7% of the maximum WHC at test start 39.8% to 49.2% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. The test item was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-sulfonamide/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Eight replicate test containers (test item and control) each with 30 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 4, 7, 11, 14, 17, 22 and 25 by opening the lids for a short period.

2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (α = 0.05). Further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

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II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A slight mortality of 25% was observed at the single test item concentration of 10 mg/kg soil, which was not statistically significantly different compared to the control, where 19% of the collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the collembolans exposed to MKH 6561-sulfonamide was not statistically significantly different compared to the control at the single test item concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-16 Lethal and sublethal effects of MKH 6561-sulfonamide on Collembola (*Folsomia candida*)

MKH 6561-sulfonamide [mg test item/kg dry soil]	**Control**	**10**
Mortality after 28 days (%)	19	25
Mean number of juveniles after 28 days	267	255
Change of reproduction compared to control (%)	-	95
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	10	

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 19% (should be ≤ 20%)
- number of juvenile per replicate was 156 to 350 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 23.6% (should be ≤ 30).

In the most recent test with the reference item Boric acid (performed under IBACON study code 61402016 from August to October 2011), there were statistically significant effects on reproduction at concentrations of ≥ 53.7 mg/kg soil; the EC_{50} for reproduction was calculated to be 75.7 mg/kg soil. Mortality was statistically significantly higher compared to the control at 137.5 mg/kg soil and above.

III. CONCLUSIONS

In a 28 day reproduction study with *Folsomia candida* exposed to MKH 6561-sulfonamide the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

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Report:	[redacted]; [redacted];2012;M-466654-01
Title:	Effects of MKH 6561-sulfonamide on reproduction of the predatory mite hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	70411089
Document No:	M-466654-01-1
Guidelines:	**OECD 226, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-sulfonamide on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with a single concentration of 10 mg MKH 6561-sulfonamide/kg dry soil, homogenously mixed into the soil. In addition a control group was exposed to soil mixed without test item.

After 14 days, the test item caused no statistically significantly effects on mortality and reproduction at 10 mg MKH 6561-sulfonamide/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 14 days was determined to be 10 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-sulfonamide
Description:	White solid
Lot/Batch #:	Batch code: AE F073550-01-01; Origin Batch No: BCOO 5771-1-1
Purity:	99.4% w/w

2. Vehicle and/or positive control: n.a.

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 10 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 2, 4, 7, 9 and 11

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 at test start and 6.2 at test end

Water content:	47.2% to 48.9% of the maximum WHC at test start 43.1% to 46.1% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. The test item was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-sulfonamide/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Eight replicate test containers (test item and control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on day 2, 4, 7, 9 and 11 by opening the lids for a short period.

2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 7 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A slight mortality of 3% was observed in the single test item treated group, which was not statistically significantly different compared to the control, where 4% of the adult mites died (Fisher's Exact test, $\alpha = 0.05$).

Reproduction of the predatory mites exposed to MKH 6561-sulfonamide was not statistically significantly different compared to the control at the single test concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).

No differences in morphology of the mites between the test item treated groups and the control were observed.

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Table 8.4-17 Lethal and sublethal effects of MKH 6561-sulfonamide on the Predatory Mite *Hypoaspis aculeifer*

MKH 6561-sulfonamide [mg test item/kg dry soil]	Control	10
Mortality after 14 days (%)	4	3
Mean number of juveniles after 14 days	190	192
Change of reproduction compared to control (%)	-	101
Endpoints [mg test item/kg soil]		
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 226 are fulfilled:

- mortality in the control group was 4% (should be ≤ 20%)
- number of juvenile per replicate was 141 to 261 and so this validity criterion was met (should be ≥ 50 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 21.1% (should be ≤ 30).

In the most recent test with the reference item dimethoate (performed under IBACON Project No. 59232089 in June 2011), there were statistically significant effects on reproduction at concentrations of 4.3 mg/kg soil; the EC_{50} for reproduction was 4.33 mg dimethoate/kg soil.

III. CONCLUSIONS

In a 14 day reproduction study with *Hypoaspis aculeifer* exposed to MKH 6561-sulfonamide the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

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Metabolite M07

Report:	[redacted];[redacted];2012;M-466684-01
Title:	Effects of MKH 6561-saccharine on reproduction of the collembola folsomia candida in artificial soil with 5 percent peat
Report No:	70422016
Document No:	M-466684-01-1
Guidelines:	**OECD 232, 2009 and ISO 11267, 1999**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-saccharin on Collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. A 1st experiment was conducted with a single test concentration of 10 mg MKH6561-saccharin/kg dry soil. As a NOEC for reproduction could not be determined, a 2nd experiment was performed with five nominal test concentrations of 1.0, 1.7, 3.0, 5.2 and 9.0 mg test item/kg dry soil mg test item/kg dry soil. The test item was mixed homogeneously into the soil. A control was run in parallel.

The test item did not cause statistically significantly increased mortality after 28 days up to and including 10 mg test item/kg dry soil, when compared to the control group. In the 2nd experiment the test item caused no statistically significantly effects up to and including 9 mg test item/kg dry soil. At the single concentration of 10 mg test item/kg dry soil in the 1st experiment the number of juveniles was statistically significantly reduced compared to the control. No effects on behaviour of the springtails were observed during both experiments. All validity criteria according to the guidelines were fulfilled in both experiments. All validity criteria were fulfilled.

The overall no observed effect concentration (NOEC) after 28 days was determined to be 9 mg test item/kg dry soil. The overall lowest observed effect concentration (LOEC) was determined to be 10 mg test item/kg soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-saccharin
Description:	Off-White solid
Lot/Batch #:	Batch code: AE F159737 00 1 B99 0002; Origin Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control: None for the 1st experiment and acetone for the 2nd experiment

3. Test organisms:

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast

Acclimatisation: Individuals were kept under breeding conditions until test start

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	1st experiment: 6.4 at test start and 6.2 at test end 2nd experiment: 6.4 to 6.5 at test start and 6.1 at test end
Water content:	1st experiment: 20.2% - 20.8% at test start and 18.2% - 18.5% at test end 2nd experiment: 20.8% - 22.6% at test start and 19.1% - 20.1% at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. In the 1st experiment, the test item was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-saccharin/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. In the 2nd experiment, a defined amount of MKH 6561-saccharin was dissolved in acetone and a sequential dilution series was prepared. The dilutions were added to fine quartz sand and the mixture was left for approximately two hours in a fume hood until the solvent had evaporated. The sand was mixed and added to artificial soil resulting in the following nominal concentrations: 1.0, 1.7, 3.0, 5.2 and 9.0 mg MKH 6561-saccharin/kg dry soil. The control was treated with the same amount of acetone treated quartz sand as the test item groups. In both experiments, the soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

In the 1st experiment eight replicate test containers (test item and control), in the 2nd experiment, four replicate test containers (test item) and 8 replicate test containers (control) were prepared, each with 30 g ± 1.0 g artificial soil fresh weight. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 4, 7, 11, 14, 17, 22 and 25 (1st experiment) and on day 4, 7, 11, 14, 18, 21 and 25 (2nd experiment) by opening the lids for a short period.

2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller) for the 1st experiment and Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller) for the 2nd experiment. The determination of the NOEC and

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LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 20% (1st experiment) and up to 23% (2nd experiment) was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 20% (1st experiment) and 8% (2nd experiment) of the collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the collembolans exposed to MKH 6561-saccharin was statistically significantly different compared to the control at the single concentration of 10 mg test item/kg soil of the 1st experiment (Student t-test, $\alpha = 0.05$, one-sided smaller). In the 2nd experiment there were no statistical significant differences compared to the control up to and including the highest tested concentration of 9.0 mg/kg soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-18 Lethal and sublethal effects of MKH 6561-saccharin on Collembola (*Folsomia candida*)

1st experiment						
MKH 6561-saccharin [mg test item/kg dry soil]	**Control**				**10**	
Mortality after 28 days (%)	20				20	
Mean number of juveniles after 28 days	267				211 *	
Change of reproduction compared to control (%)	-				79	
2nd experiment						
Propoxycarbazone-sodium [mg test item/kg dry soil]	**Control**	**1.0**	**1.7**	**3.0**	**5.2**	**9.0**
Mortality after 28 days (%)	8	18	8	3	23	15
Mean number of juveniles after 28 days	382	349	456	421	335	379
Change of reproduction compared to control (%)	-	91	119	110	88	99
Endpoints [mg test item/kg soil]						
NOEC (mortality)	10					
LOEC (mortality)	> 10					
LC_{50} (mortality)	> 10					
NOEC (reproduction)	9.0					
LOEC (reproduction)	10					
EC_{50} (reproduction)	> 10					

* Significantly different compared to the control (Student t-test, $\alpha = 0.05$, one-sided smaller)

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 20% (1st experiment) and 8% (2nd experiment) (should be ≤ 20%)
- number of juvenile per replicate was 176 to 367 (1st experiment) and 273 to 472 (2nd experiment) and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 20.6% (1st experiment) and 19.1% (2nd experiment) (should be ≤ 30).

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In the most recent test with the reference item Boric acid (performed under IBACON study code 61402016 from August to October 2011), there were statistically significant effects on reproduction at concentrations of ≥ 53.7 mg/kg soil; the EC_{50} for reproduction was calculated to be 75.7 mg/kg soil. Mortality was statistically significantly higher compared to the control at 137.5 mg/kg soil and above.

III. CONCLUSIONS

In a 28-day reproduction study with *Folsomia candida* exposed to MKH 6561-saccharin the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 9.0 mg test item/kg dry soil.

Report:	[redacted]; [redacted]; 2012; M-466680-01
Title:	Effects of MKH 6561-saccharine on reproduction of the predatory mite hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	70421089
Document No:	M-466680-01-1
Guidelines:	**OECD 226, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-saccharin on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-saccharin/kg dry soil, homogenously mixed into the soil. In addition a control group was exposed to soil mixed without test item.

After 14 days, the test item did not cause statistically significant effects on mortality and reproduction at 10 mg test item/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 14 days was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-saccharin
Description:	Off-White solid
Lot/Batch #:	Batch code: AE F159737 00 1B99 0002; Origin Batch No: M00402
Purity:	99.9% w/w

2. Vehicle and/or positive control:

n/a

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3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 10 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 2, 4, 7, 9 and 11

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 at test start and 6.2 to 6.3 at test end
Water content:	46.9% to 48.9% of the maximum WHC at test start 43.2% to 46.1% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. The test item was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH6561-saccharin/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Eight replicate test containers (test item and control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on day 2, 4, 7, 9 and 11 by opening the lids for a short period.

2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 7 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (α = 0.05). Further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

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A. FINDINGS AND OBSERVATIONS

A slight mortality of 1% was observed in the single test item treated group, which was not statistically significantly different compared to the control, where 4% of the adult mites died (Fisher's Exact test, $\alpha = 0.05$).
Reproduction of the predatory mites exposed to MKH 6561-saccharin was not statistically significantly different compared to the control at the single test concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).

No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 8.4-19 Lethal and sublethal effects of MKH 6561-saccharin on the Predatory Mite *Hypoaspis aculeifer*

MKH 6561-saccharin [mg test item/kg dry soil]	Control	10
Mortality after 14 days (%)	4	1
Mean number of juveniles after 14 days	190	191
Change of reproduction compared to control (%)		101
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 226 are fulfilled:

- mortality in the control group was 4% (should be ≤ 20%)
- number of juvenile per replicate was 141 to 261 and so this validity criterion was met (should be ≥ 50 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 21.1% (should be ≤ 30).

In the most recent test with the reference item dimethoate (performed under IBACON Project No. 59232089 in June 2011), there were statistically significant effects on reproduction at concentrations of 4.3 mg/kg soil; the EC_{50} for reproduction was 4.33 mg dimethoate/kg soil.

III. CONCLUSIONS

In a 14 day reproduction study with *Hypoaspis aculeifer* exposed to MKH 6561-saccharin the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

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Metabolite M08

Report:	[redacted]; [redacted];2014;M-484422-01
Title:	Effects of MKH6561-4-hydroxy-saccharin on reproduction of the Collembola Folsomia candida in artificial soil with 5 percent peat
Report No:	71793016
Document No:	M-484422-01-1
Guidelines:	**OECD-Guideline for testing chemicals No. 232 Collembolan Reproduction Test in Soil (adopted September 07, 2009) ISO 11267 Soil Quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants, 1999.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-4-hydroxy-saccharin on Collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil, mixed homogeneously into the soil, and a control without test item.

After 28 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil when compared to the control group. No effects on behaviour of the springtails were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 28 days was determined to be 10 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561-4-hydroxy-saccharin
Description:	White solid
Lot/Batch #:	Batch code: AE 1364277-01-01; Origin Batch No: BCOO 6427-19-15
Purity:	99.5% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast
Acclimatisation:	Individuals were kept under breeding conditions until test start

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.0 to 6.1 at test start and 5.8 to 5.9 at test end
Water content:	56.0% to 56.4% of the maximum WHC at test start 52.5% to 53.3% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 2 days before the application. A stock solution was prepared by mixing deionised water with the test item. The solution was added to artificial soil to result in a nominal concentration of 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil. The control groups were moistened with deionised water only. The additional water required to achieve the final water content was added and the soils for each treatment group were thoroughly mixed to ensure a homogeneous distribution.

Eight replicate test containers (test item and control) each with 30 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 3, 7, 10, 14, 17, 21 and 24 by opening the lids for a short period.

2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 13% was observed in the test item treated group, which was not statistically significantly different compared to the control, where 10% of the collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

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Reproduction of the collembolans exposed to MKH6561-4-hydroxy-saccharin was not statistically significantly different compared to the control at the single test item concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-20 Lethal and sublethal effects of MKH6561-4-hydroxy-saccharin on Collembola (*Folsomia candida*)

MKH6561-4-hydroxy-saccharin [mg test item/kg dry soil]	Control	10
Mortality after 28 days (%)	10	13
Mean number of juveniles after 28 days	683	646
Change of reproduction compared to control (%)	-	95
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 10% (should be ≤ 20%)
- number of juvenile per replicate was 575 to 1021 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 22.5% (should be ≤ 30).

In the most recent test with the reference item Boric acid (performed under IBACON study code 61404016 from August to October 2013), there were statistically significant effects on mortality and reproduction at concentrations of ≥ 33.6 mg/kg soil; the EC_{50} for reproduction was calculated to be 99.6 mg/kg soil.

III. CONCLUSIONS

In a 28 day reproduction study with *Folsomia candida* exposed to MKH6561-4-hydroxy-saccharin the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

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Report:	[redacted]; [redacted];2014;M-484430-01
Title:	Effects of MKH6561-4-hydroxy-saccharin on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	71794089
Document No:	M-484430-01-1
Guidelines:	**OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, adopted October 03, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-sulfonamide on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil, mixed homogeneously into the soil, and a control without test item.

After 14 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria according to the guideline were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 14 days was determined to be 10 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561-4-hydroxy-saccharin
Description:	White solid
Lot/Batch #:	Batch code: AE 1364277-01-01; Origin Batch No: BCOO 6427-19-15
Purity:	99.5% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 11 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 3, 5, 7, 10 and 12.

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.0 to 6.1 at test start and 6.1 at test end
Water content:	56.0% to 56.4% of the maximum WHC at test start 53.9% to 54.8% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 2 days before the application. A stock solution was prepared by mixing deionised water with the test item. The solution was added to artificial soil to result in a nominal concentration of 10 mg MKH6561-4-hydroxy-saccharin/kg dry soil. The control groups were moistened with deionised water only. The additional water required to achieve the final water content was added and the soils for each treatment group were thoroughly mixed to ensure a homogeneous distribution.

Eight replicate test containers (test item and control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on day 3, 5, 7, 10 and 12 by opening the lids for a short period.

2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 7 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH.

H. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 10% was observed in the single test item concentration of 10 mg/kg soil, which was not statistically significantly different compared to the control, where 10% of the adult mites died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the predatory mites exposed to MKH6561-4-hydroxy-saccharin was not statistically significantly different compared to the control at the single test concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).

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No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 8.4-21 Lethal and sublethal effects of MKH6561-4-hydroxy-saccharin on the Predatory Mite *Hypoaspis aculeifer*

MKH6561-4-hydroxy-saccharin [mg test item/kg dry soil]	Control	10
Mortality after 14 days (%)	10	10
Mean number of juveniles after 14 days	214	212
Change of reproduction compared to control (%)	-	99
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 10% (should be ≤ 20%)
- number of juvenile per replicate was 179 to 241 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 11.7% (should be ≤ 30).

In the most recent test with the reference item dimethoate (performed under IBACON Project No. 74662089 in June 2013), there were statistically significant effects on mortality and reproduction at concentrations of 3.0 mg dimethoate/kg soil; the EC_{50} for reproduction was 4.2 mg dimethoate/kg soil.

III. CONCLUSIONS

In a 14 day reproduction study with *Hypoaspis aculeifer* exposed to MKH6561-4-hydroxy-saccharin the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

Metabolite M09

Report:	[redacted];2012;M-466718-01
Title:	Effects of MKH 6561-propoxytriazolinonamide on reproduction of the collembola folsomia candida in artificial soil with 5 percent peat
Report No:	70445016
Document No:	M-466718-01-1
Guidelines:	**OECD 232, 2009 and ISO 11267, 1999**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-propoxytriazolinonamide on collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. The test was conducted with a single concentration of 10 mg MKH 6561-propoxytriazolinonamide/kg dry soil, mixed homogeneously into the soil. In addition a control group was exposed to soil mixed without test item.

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After 28 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH 6561-propoxytriazolinonamide/kg dry soil when compared to the control group. No effects on behaviour of the springtails were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 28 days was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-propoxytriazolinonamide
Description:	White solid
Lot/Batch #:	Batch code: AE 1364275-01-01; Origin Batch No. BCSO 6405-1-3
Purity:	99.9% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast
Acclimatisation:	Individuals were kept under breeding conditions until test start

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 at test start and 6.2 at test end
Water content:	47.1% to 50.6% of the maximum WHC at test start 40.7% to 45.2% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. The test item was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-propoxytriazolinonamide/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was

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thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Eight replicate test containers (test item and control) each with 30 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 4, 7, 11, 14, 17, 22 and 25 by opening the lids for a short period.

2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (and additionally using Cochran's test, $\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A slight mortality of 21% was observed at the single test item concentration of 10 mg/kg soil, which was not statistically significantly different compared to the control, where 20% of the collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the collembolans exposed to MKH 6561-propoxytriazolinonamide was not statistically significantly different compared to the control at the single test item concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).
No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-22 Lethal and sublethal effects of MKH 6561-propoxytriazolinonamide on Collembola (*Folsomia candida*)

MKH 6561-propoxytriazolinonamide [mg test item/kg dry soil]	**Control**	**10**
Mortality after 28 days (%)	20	21
Mean number of juveniles after 28 days	276	243
Change of reproduction compared to control (%)	-	88
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 232 are fulfilled:

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- mortality in the control group was 20% (should be ≤ 20%)
- number of juvenile per replicate was 179 to 350 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 23.6% (should be ≤ 30).

In the most recent test with the reference item Boric acid (performed under IBACON study code 61402016 from August to October 2011), there were statistically significant effects on reproduction at concentrations of ≥ 53.7 mg/kg soil; the EC_{50} for reproduction was calculated to be 75.7 mg/kg soil. Mortality was statistically significantly higher compared to the control at 137.5 mg/kg soil and above.

III. CONCLUSIONS

In a 28 day reproduction study with *Folsomia candida* exposed to MKH 6561-propoxytriazolinonamide the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

Report:	[redacted]; [redacted]; 2012; M-466715-01-1
Title:	Effects of MKH 6561-propoxytriazolinonamide on reproduction of the predatory mite hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	70444089
Document No:	M-466715-01-1
Guidelines:	**OECD 226, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH 6561-propoxytriazolinonamide on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with a single concentration of 10 mg MKH 6561-propoxytriazolinonamide/kg dry soil, mixed homogeneously into the soil. In addition a control group was exposed to soil mixed without test item.

After 14 days, the test item did not cause statistically significant effects on mortality and reproduction at 10 mg MKH 6561-propoxytriazolinonamide/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria according to the guideline were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 14 days was determined to be 10 mg test item/kg dry soil.

1. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-propoxytriazolinonamide
Description:	White solid
Lot/Batch #:	Batch code: AE 1364275-01-01; Origin Batch No: BCOO 6405-1-3
Purity:	99.9% w/w

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2. **Vehicle and/or positive control:** n/a

3. **Test organisms:**

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 10 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 2, 4, 7, 9 and 11

4. **Environmental conditions:**

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.4 at test start and 6.2 to 6.3 at test end
Water content:	47.3% to 48.9% of the maximum WHC at test start 43.9% to 46.1% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat, 20% kaolin clay, 74.7% fine quartz-sand and 0.3% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. The test item was mixed with fine quartz sand and added to artificial soil, resulting in a nominal concentration of 10 mg MKH 6561-propoxytriazolinonamide/kg dry soil. The control was treated with the same amount of fine quartz sand as the test item groups. The soil for each treatment group was thoroughly mixed to ensure a homogeneous distribution and the additional water required to achieve the final water content was added.

Eight replicate test containers (test item and control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on day 2, 4, 7, 9 and 11 by opening the lids for a short period.

2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 7 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

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II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A slight mortality of 3% was observed in the single test item treated group, which was not statistically significantly different compared to the control, where 4% of the adult mites died (Fisher's Exact test, $\alpha = 0.05$).
Reproduction of the predatory mites exposed to MKH 6561-propoxytriazolinonamide was not statistically significantly different compared to the control at the single test concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).

No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 8.4-23 Lethal and sublethal effects of MKH 6561-propoxytriazolinonamide on the Predatory Mite *Hypoaspis aculeifer*

MKH 6561-propoxytriazolinonamide [mg test item/kg dry soil]	Control	10
Mortality after 14 days (%)	4	3
Mean number of juveniles after 14 days	190	184
Change of reproduction compared to control (%)	-	97
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	10	

The validity criteria according to guideline OECD 226 are fulfilled:

- mortality in the control group was 4% (should be ≤ 20%)
- number of juvenile per replicate was 141 to 261 and so this validity criterion was met (should be ≥ 50 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 21.1% (should be ≤ 30).

In the most recent test with the reference item dimethoate (performed under IBACON Project No. 59232089 in June 2011), there were statistically significant effects on reproduction at concentrations of 4.3 mg/kg soil; the EC_{50} for reproduction was 4.33 mg dimethoate/kg soil.

III. CONCLUSIONS

In a 14 day reproduction study with *Hypoaspis aculeifer* exposed to MKH 6561-propoxytriazolinonamide the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

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Metabolite M10

Report:	[redacted];2014;M-484425-01
Title:	Effects of MKH6561-N-methyl propoxytriazolinone on reproduction of the Collembola Folsomia candida in artificial soil with 5 percent peat
Report No:	71823016
Document No:	M-484425-01-1
Guidelines:	**GLP compliant study based on OECD-Guideline for testing chemicals No. 232 "Collembolan Reproduction Test in Soil" (adopted September 07, 2009) ISO 11267 Soil Quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants, 1999.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-N-methyl propoxytriazolinone on Collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil, mixed homogeneously into the soil, and an untreated control (moistened with water).

After 28 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil when compared to the control group. No effects on behaviour of the springtails were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 28 days was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Test item:	MKH6561-N-methyl propoxytriazolinone
Description:	White solid
Lot/Batch #:	Batch code: AE 1364263-01-01; Origin Batch No: NLL 5797-6-5
Purity:	99.0% w/w

2. **Vehicle and/or positive control:** n/a

3. **Test organisms:**

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast
Acclimatisation:	Individuals were kept under breeding conditions until test start

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4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	5.9 at test start and 5.7 at test end
Water content:	56.3% to 56.4% of the maximum WHC at test start 53.2% to 53.8% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. A stock solution was prepared by mixing deionised water with the test item. The solution was added to artificial soil to result in a nominal concentration of 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil. The control groups were moistened with deionised water only. The additional water required to achieve the final water content was added and the soils for each treatment group were thoroughly mixed to ensure a homogeneous distribution.

Eight replicate test containers (test item and control) each with 30 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, Collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 2, 4, 7, 11, 14, 16, 18, 21 and 25 by opening the lids for a short period.

2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 18% was observed in the test item treated group, which was not statistically significantly different compared to the control, where 15% of the collembola died (Fisher's Exact test, $\alpha = 0.05$). Reproduction of the springtails exposed to MKH6561-N-methyl propoxytriazolinone was not statistically significantly different compared to the control at the single test item concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$).

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No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-24 Lethal and sublethal effects of MKH6561-N-methyl propoxytriazolinone on Collembola (*Folsomia candida*)

MKH6561-N-methyl propoxytriazolinone [mg test item/kg dry soil]	Control	10
Mortality after 28 days (%)	15	18
Mean number of juveniles after 28 days	586	552
Change of reproduction compared to control (%)	-	94
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 15% (should be ≤ 20%)
- number of juvenile per replicate was 517 to 708 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 11.1% (should be ≤ 30).

In the most recent test with the reference item Boric acid (performed under IBACON study code 61404016 from August to October 2013), there were statistically significant effects on mortality and reproduction at concentrations of ≥ 33.6 mg/kg soil; the EC_{50} for reproduction was calculated to be 99.6 mg/kg soil.

III. CONCLUSIONS

In a 28 day reproduction study with *Folsomia candida* exposed to MKH6561-N-methyl propoxytriazolinone the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

Report:	[redacted];[redacted];2014;M-484437-01
Title:	Effects of MKH6561-N-methyl propoxytriazolinone on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	71824089
Document No:	M-484437-01-1
Guidelines:	**OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, adopted October 03, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-N-methyl propoxytriazolinone on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil, mixed homogeneously into the soil, and a control without test item.

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After 14 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria according to the guideline were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 14 days was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561-N-methyl propoxytriazolinone
Description:	White solid
Lot/Batch #:	Batch code: AE 1364263-01-01; Origin Batch No. NLL 5797-6-5
Purity:	99.0% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 14 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 2, 4, 7, 9 and 11.

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	5.9 at test start and 6.1 at test end
Water content:	56.3% to 56.4% of the maximum WHC at test start 54.4% to 57.5% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 3 days before the application. A stock solution was prepared by mixing deionised water with the test item. The solution was added to artificial soil to result in a nominal concentration of 10 mg MKH6561-N-methyl propoxytriazolinone/kg dry soil. The control groups were moistened with deionised water only. The

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additional water required to achieve the final water content was added and the soils for each treatment group were thoroughly mixed to ensure a homogeneous distribution.

Eight replicate test containers (test item and control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on day 2, 4, 7, 9 and 11 by opening the lids for a short period.

2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 7 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$). Further statistical evaluation was performed using Student t-test (pairwise comparison, $\alpha = 0.05$, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 13% was observed in the test item treated group, which was not statistically significantly different compared to the control, where 10% of the adult mites died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the predatory mites exposed to MKH6561-N-methyl propoxytriazolinone was not statistically significantly different compared to the control at the single test concentration of 10 mg/kg soil (Student t-test, $\alpha = 0.05$, one-sided smaller).
No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 8.4-25 Lethal and sublethal effects of MKH6561-N-methyl propoxytriazolinone on the Predatory Mite *Hypoaspis aculeifer*

MKH6561-N-methyl propoxytriazolinone [mg test item/kg dry soil]	Control	10
Mortality after 14 days (%)	10	13
Mean number of juveniles after 14 days	204	190
Change of reproduction compared to control (%)	-	93
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 232 are fulfilled:

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- mortality in the control group was 10% (should be ≤ 20%)
- number of juvenile per replicate was 176 to 231 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 7.8% (should be ≤ 30).

In the most recent test with the reference item dimethoate (performed under IBACON Project No. 74662089 in June 2013), there were statistically significant effects on reproduction at concentrations of 3.0 mg dimethoate/kg soil; the EC_{50} for reproduction was 4.2 mg dimethoate/kg soil.

III. CONCLUSIONS

In a 14 day reproduction study with *Hypoaspis aculeifer* exposed to MKH6561-N-methyl propoxytriazolinone the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

Metabolite M11

Report:	[redacted], 2014, M-484423-01
Title:	Effects of MKH6561-methoxy-saccharin on reproduction of the Collembola Folsomia candida in artificial soil with 5 percent peat
Report No:	71813016
Document No:	M-484423-01-1
Guidelines:	**OECD Guideline for testing chemicals No. 232 Collembolan Reproduction Test in Soil (adopted September 07, 2009) ISO 11267 Soil Quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants, 1999.**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-methoxy-saccharin on collembola *Folsomia candida* were tested in a 28-day laboratory test with regards to mortality, behavioural effects and reproduction rate in artificial soil prepared according to OECD 232 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-methoxy-saccharin/kg dry soil, mixed homogeneously into the soil, and an untreated control (moistened with water).

After 28 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH6561-methoxy-saccharin/kg dry soil when compared to the control group. No effects on behaviour of the springtails were observed during the test. All validity criteria were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 28 days was determined to be 10 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561-methoxy-saccharin
Description:	Light yellow
Lot/Batch #:	Batch code: BCS-AG71018-01-01; Origin Batch No: BCOO 6413-13-5

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Purity:	99.7% w/w

2. Vehicle and/or positive control: n/a

3. Test organisms:

Species:	*Folsomia candida* (Willem 1902)
Age:	10-12 days old
Source:	In-house culture
Diet/Food:	Granulated dry yeast
Acclimatisation:	Individuals were kept under breeding conditions until test start

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.1 at test start and 5.8 to 5.9 at test end
Water content:	55.1% to 55.7% of the maximum WHC at test start 49.4% to 54.4% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Collembola *Folsomia candida* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 2 days before the application. A stock solution was prepared by mixing deionised water with the test item. The solution was added to artificial soil to result in a nominal concentration of 10 mg MKH6561-methoxy-saccharin/kg dry soil. The control groups were moistened with deionised water only. The additional water required to achieve the final water content was added and the soils for each treatment group were thoroughly mixed to ensure a homogeneous distribution.

Eight replicate test containers (test item and control) each with 30 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 synchronized springtails per replicate were exposed for 28 days. After the introduction of the test organisms (day 0), and after 14 days, collembola were fed with approximately 2 mg of granulated dried yeast. All vessels were ventilated on day 3, 7, 10, 14, 17, 21 and 24 by opening the lids for a short period.

2. Observations

At test termination after 28 days, the number of living adult collembola and the number of juvenile adult collembola were recorded. Furthermore, surviving collembola were observed for any abnormal behaviour or conditions at day 28 after application. Water content was checked 14 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher’s Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's

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test and Levene's test (α = 0.05). Further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 10% was observed in the test item treated group, which was not statistically significantly different compared to the control, where 13% of the collembola died (Fisher's Exact test, α = 0.05, one-sided greater).
Reproduction of the collembolans exposed to MKH6561-methoxy-saccharin was not statistically significantly different compared to the control at the single test item concentration of 10 mg/kg soil (Student t-test, α = 0.05, one-sided smaller).
No behavioural abnormalities were observed in any of the treatment groups.

Table 8.4-26 Lethal and sublethal effects of MKH6561-methoxy-saccharin on Collembola (*Folsomia candida*)

MKH6561-methoxy-saccharin [mg test item/kg dry soil]	**Control**	**10**
Mortality after 28 days (%)	13	10
Mean number of juveniles after 28 days	751	779
Change of reproduction compared to control (%)	-	104
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 232 are fulfilled:

- mortality in the control group was 13% (should be ≤ 20%)
- number of juvenile per replicate was 590 to 986 and so this validity criterion was met (should be ≥ 100 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 17.0% (should be ≤ 30).

In the most recent test with the reference item Boric acid (performed under IBACON study code 61404016 from August to October 2013), there were statistically significant effects on mortality and reproduction at concentrations of ≥ 33.6 mg/kg soil; the EC_{50} for reproduction was calculated to be 99.6 mg/kg soil.

III. CONCLUSIONS

In a 28 day reproduction study with *Folsomia candida* exposed to MKH6561-methoxy-saccharin the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

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Report:	[redacted];[redacted];2014;M-484433-01
Title:	Effects of MKH6561-methoxy-saccharin on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil with 5 percent peat
Report No:	71814089
Document No:	M-484433-01-1
Guidelines:	**OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, adopted October 03, 2008**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effects of MKH6561-methoxy-saccharin on adult *Hypoaspis aculeifer* were tested in a 14-day laboratory test with regards to mortality, differences in morphology or any abnormalities and reproduction in artificial soil prepared according to OECD 226 with 5% peat. The test was conducted with a single concentration of 10 mg MKH6561-methoxy-saccharin/kg dry soil, mixed homogeneously into the soil, and a control without test item.

After 14 days, the test item did not cause statistically significantly increased mortality or decreased reproduction at 10 mg MKH6561-methoxy-saccharin/kg dry soil when compared to the control group. No differences in morphology of the mites were observed during the test. All validity criteria according to the guideline were fulfilled.

The no observed effect concentration (NOEC) for mortality and reproduction after 14 days was determined to be 10 mg test item/kg dry soil.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH6561-methoxy-saccharin
Description:	Light yellow
Lot/Batch #:	Batch code: BCS-AG71018-01-01; Origin Batch No: BCOO 6413-13-5
Purity:	99.7% w/w

2. Vehicle and/or positive control:

n/a

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini 1883)
Age:	Female adult, approximately 11 days after reaching the adult stage (from a synchronized cohort)
Source:	In-house culture
Diet/Food:	One spatula of cheese mites (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 3, 5, 7, 10 and 12

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light intensity:	Within the range of 400 lux to 800 lux
Photoperiod:	16 h light : 8 h dark
Soil pH:	6.1 at test start and 5.6 to 5.8 at test end
Water content:	55.1% to 55.7% of the maximum WHC at test start 54.3% to 54.6% of the maximum WHC at test end

B. STUDY DESIGN

1. Experimental treatments

Soil mites *Hypoaspis aculeifer* were exposed to a single concentration of the test substance in an artificial soil substrate (5% Sphagnum-peat; 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate). The artificial soil was moistened to approximately half of the final water content 2 days before the application. A stock solution was prepared by mixing deionised water with the test item. The solution was added to artificial soil to result in a nominal concentration of 10 mg MKH6561-methoxy-saccharin/kg dry soil. The control groups were moistened with deionised water only. The additional water required to achieve the final water content was added and the soils for each treatment group were thoroughly mixed to ensure a homogeneous distribution.

Eight replicate test containers (test item and control) each with 20 g ± 1.0 g artificial soil fresh weight were prepared for each treatment group. 10 adult female mites per replicate were exposed for 14 days. All vessels were ventilated on day 3, 5, 7, 10 and 12 by opening the lids for a short period.

2. Observations

At test termination after 14 days, the number of living adults and the number of juvenile mites were recorded. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Water content was checked 7 days after application and pH measurements were performed at test initiation and at test termination. Temperature and light intensity were monitored continuously.

3. Statistical calculations

Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test (α = 0.05). Further statistical evaluation was performed using Student t-test (pairwise comparison, α = 0.05, one-sided smaller). The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

A mortality of 6% was observed at the single test item concentration of 10 mg/kg soil, which was not statistically significantly different compared to the control, where 3% of the adult mites died (Fisher's Exact test, α = 0.05, one-sided greater).
Reproduction of the predatory mites exposed to MKH6561-methoxy-saccharin was not statistically significantly different compared to the control at the single test item concentration of 10 mg/kg soil (Student t-test, α = 0.05, one-sided smaller).

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No differences in morphology of the mites between the test item treated groups and the control were observed.

Table 8.4-27 Lethal and sublethal effects of MKH6561-methoxy-saccharin on the Predatory Mite *Hypoaspis aculeifer*

MKH6561-methoxy-saccharin [mg test item/kg dry soil]	Control	10
Mortality after 14 days (%)	3	6
Mean number of juveniles after 14 days	202	195
Change of reproduction compared to control (%)	-	96
	Endpoints [mg test item/kg soil]	
NOEC (mortality)	10	
LC_{50} (mortality)	> 10	
NOEC (reproduction)	10	
EC_{50} (reproduction)	> 10	

The validity criteria according to guideline OECD 226 are fulfilled:

- mortality in the control group was 3% (should be ≤ 20%)
- number of juvenile per replicate was 178 to 224 and so this validity criterion was met (should be ≥ 50 juveniles per replicate)
- the coefficient of variation of reproduction in the control was 7.4% (should be ≤ 30).

In the most recent test with the reference item dimethoate (performed under IBACON Project No. 74662089 in June 2013), there were statistically significant effects on reproduction at concentrations of 3.0 mg dimethoate/kg soil; the EC_{50} for reproduction was 4.2 mg dimethoate/kg soil.

III. CONCLUSIONS

In a 14 day reproduction study with *Hypoaspis aculeifer* exposed to MKH6561-methoxy-saccharin the overall no observed effect concentration (NOEC) for mortality and reproduction was determined to be 10 mg test item/kg dry soil.

CA 8.4.2.1 Species level testing

Further studies are not considered necessary.

CA 8.5 Effects on soil nitrogen transformation

A summary of all available relevant and compliant data for propoxycarbazone-sodium on effects to soil micro-organisms is presented in the table below. Although no longer a data requirement according to Commission Regulation (EU) No 283/2013, results for carbon transformation are also presented for completeness.

Table 8.5-1 Effects of propoxycarbazone-sodium and metabolites soil microorganisms

Test item	Study design	Endpoint	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)

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Test item	Study design	Endpoint	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
MKH 6561 70 WG	Nitrogen-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1998) AJO/174898 M-004247-01-1 KCA 8.5 /02	Yes
	Carbon-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1998) AJO/174798 M-003856-01-1 KCA 8.5 /01	Yes
M05	Nitrogen-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/197699 M-015916-01-1 KCA 8.5 /06	Yes
	Carbon-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/197599 M-014742-01-1 KCA 8.5 /05	Yes
M07	Nitrogen-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/197499 M-012596-01-1 KCA 8.5 /10	Yes
	Carbon-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/197399 M-012582-01-1 KCA 8.5 /09	Yes
M08	Nitrogen-mineralisation 28-day study	no effects > 25% up to 0.467 mg/kg soil dry weight	[redacted] (2012) 70433080 M-466704-01-1 KCA 8.5 /11	New study
M09	Nitrogen-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/199599 M-015913-01-1 KCA 8.5 /08	Yes
	Carbon-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/199499 M-014737-01-1 KCA 8.5 /07	Yes
M10	Nitrogen-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/199399 M-015942-01-1 KCA 8.5 /03	Yes
	Carbon-mineralisation 28-day study	no negative effects at 0.07 and 0.35 kg/ha, difference to control < 25%	[redacted] (1999) AJO/199299 M-014731-01-1 KCA 8.5 /04	Yes
M11	Nitrogen-mineralisation 28-day study	no effects > 25% up to 0.467 mg/kg soil dry weight	[redacted] (2012) 70467080 M-466720-01-1 KCA 8.5 /12	New study

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Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

To complete the data package, two additional studies on soil nitrogen transformation have been performed with soil metabolites M08 and M11 and are submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval. These studies are summarised below.

Metabolite M08

Report:	[redacted]; [redacted]; 2012; M-466704-01
Title:	Effects of MKH 6561-4-hydroxy-saccharin on the activity of the soil microflora in the laboratory
Report No:	70433080
Document No:	M-466704-01-1
Guidelines:	**OECD 216, 2000**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effect of MKH6561-4-hydroxy-saccharin on nitrogen transformation was investigated in a loamy sand soil enriched with lucerne meal. The test substance was applied at concentration rates of 0.093 and 0.467 mg MKH6561-4-hydroxy-saccharin/kg dry soil. A control group was added without test item and sodium chloride was applied in a separate study as reference item. Sampling of each treatment for analysis was done on day 0, 7, 14 and 28 days after treatment and NH_4-, NO_2- and NO_3-nitrogen were determined using a Dionex ion chromatography system.

No treatment related effects of MKH6561-4-hydroxy-saccharin above 25% on the activity of soil microflora were observed after 28 days of exposure when applied at concentrations up to 0.467 mg/kg soil dry weight.

Therefore it is concluded that MKH6561-4-hydroxy-saccharin has no significant long term detrimental effect on activity of soil microflora in soil at concentrations up to 0.467 mg/kg soil dry weight.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-4-hydroxy-saccharin
Description:	Light beige solid
Lot/Batch #:	Batch code: AE 1364277-01-01; Origin Batch No: BCOO 6427-19-15
Purity:	99.5% w/w

2. Vehicle and/or positive control: n/a

3. Test system:

Soil:	Biologically active agricultural soil: Loamy sand soil
Source:	Fallow grassland in [redacted]
Water content of soil:	14.2 – 12.4%
pH:	7.0
Total Org. C:	1.11%
Clay (< 0.002 mm):	8.0%
Silt (0.063 mm > 0.002 mm):	33.3%
Sand (≥ 0.063 – 2.00 mm):	58.7%

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light regime:	In the dark
Soil pH:	7.0 – 7.1
Water content:	53% - 54% of WHC max.

B. STUDY DESIGN

1. Experimental treatments

MKH6561-4-hydroxy-saccharin was tested at two treatment concentrations of 0.093 mg MKH6561-4-hydroxy-saccharin/kg dry soil (corresponding to the maximum annual application rate of the parent compound propoxycarbazone-sodium) and 0.467 mg MKH6561-4-hydroxy-saccharin/kg dry soil (corresponding to 5 times the maximum annual application rate of the parent compound propoxycarbazone-sodium) using 3 replicates each with 400 g soil dry weight. In addition a negative control (deionised water) was tested. The test concentrations were achieved by preparing a stock solution (12 mg test item and 250 mL deionised water) and mixing appropriate amounts of the stock solution into the soil. To determine the activity of soil microflora, treated and untreated soils were incubated in 0.5 L plastic boxes. The boxes were covered by perforated lids. To stimulate nitrogen transformation, the soil was amended with lucerne meal (0.5%) as a nitrogen source at the time of preparation.

2. Observations

Soil samples were taken from each treatment group within 6 hours after application and on day 7, 14 and 28 after application. Nitrogen content was determined by extraction with 0.1 M KCl-solution and subsequent determination with a Dionex ICS 1000 ion chromatopgraph. The amount of each ion was calculated from the soil extracts and the soil (mineral nitrogen content). The pH values were checked at each sampling date for one replicate of each treatment group. The soil water content was also checked at each sampling date and evaporated water was replaced.

3. Statistical calculations

Data for the soil nitrogen contents were tested for normality and homogeneity of variance using the R/S-Test ($\alpha = 0.05$) and Levene's test ($\alpha = 0.05$), respectively. The Student t-test (pairwise comparison, two sided, $\alpha = 0.05$) was used for comparison of treated and control values. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

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II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.5-2 Effects of MKH6561-4-hydroxy-saccharin on Soil Nitrogen Transformation in a Loamy Sand Soil

	NO_3 – Nitrogen Formation Rate (mg / kg soil dry weight per day) [a]				
	Control	MKH6561-4-hydroxy-saccharin			
		0.093 mg MKH6561-4-hydroxy-saccharin/kg soil dw		0.467 mg MKH6561-4-hydroxy-saccharin/kg soil dw	
Interval[1]	Nitrate-N Formation	Nitrate-N Formation	Deviation [b]	Nitrate-N Formation	Deviation [b]
Day 0 - 7	0.41	-0.16*	-139.02	-0.37*	-190.24
Day 7 - 14	3.52	3.07*	-12.78	3.14*	-10.80
Day 14 - 28	1.08	1.10	8.33	1.17	8.33

[a] related to successive intervals between samplings
[b] % deviation to control
positive values = stimulating effect
negative values = inhibitory effect
dw = dry weight
* statistically significant different from control (Student t-test; α = 0.05)

B. OBSERVATIONS

The soil nitrate content and differences in the soil nitrate formation rate deviated less than 25% from the control for both rates after 28 days.

The validity criteria according to the guideline OECD 216 were fulfilled as the coefficient of variation for the control group was < 15% and the reference item sodium chloride caused effects above 25% on soil nitrogen turnover.

III. CONCLUSIONS

After 28 days of exposure, MKH 6561-4-hydroxy-saccharin had no impact above 25% on activity of soil microflora (nitrogen transformation) when applied up to a concentration of 0.467 mg/kg soil dry weight.

Metabolite M11

Report:	[redacted];[redacted];2012;M-466720-01
Title:	Effects of MKH6561-methoxy-saccharin on the activity of the soil microflora in the laboratory
Report No:	70467080
Document No:	M-466720-01-1
Guidelines:	**OECD 216, 2000**
Deviations:	none
GLP/GEP:	**yes**

Executive Summary

The effect of MKH6561-methoxy-saccharin on nitrogen-transformation was investigated in a loamy sand soil enriched with lucerne meal. The test substance was applied at concentration rates of 0.093 and 0.467

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mg MKH6561-methoxy-saccharin/kg dry soil. A control group was added without test item and sodium chloride was applied in a separate study as reference item. Sampling of each treatment for analysis was done on day 0, 7, 14 and 28 days after treatment and NH_4-, NO_2- and NO_3-nitrogen were determined using a Dionex ion chromatography system.

No treatment related effects of MKH6561-methoxy-saccharin above 25% on the activity of soil microflora were observed after 28 days of exposure when applied at concentrations up to 0.467 mg/kg soil dry weight.

Therefore it is concluded that MKH6561-methoxy-saccharin has no significant long term detrimental effect on activity of soil microflora in soil at concentrations up to 0.467 mg/kg soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MKH 6561-methoxy-saccharin
Description:	Light yellow solid
Lot/Batch #:	Batch code: BCS-AG71018-01-01; Origin Batch No: BCO06413-13-5
Purity:	99.7% w/w

2. Vehicle and/or positive control: n/a

3. Test system:

Soil:	Biologically active agricultural soil: Loamy sand soil
Source:	Fallow grassland of [redacted]
Water content of soil:	14.2 – 12.4%
pH:	7.0
Total Org. C:	1.11%
Clay (≤ 0.002 mm):	8.0%
Silt (0.063 mm > 0.002 mm):	33.3%
Sand (≥ 0.063 – 2.00 mm):	58.7%

4. Environmental conditions:

Temperature:	18 °C – 22 °C
Light regime:	In the dark
Soil pH:	7.0 – 7.1
Water content:	53% - 54% of WHCmax

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B. STUDY DESIGN

1. Experimental treatments

MKH6561-methoxy-saccharin was tested at two treatment concentrations of 0.093 mg MKH6561-methoxy-saccharin/kg dry soil (corresponding to the maximum annual application rate of the parent compound propoxycarbazone-sodium) and 0.467 mg MKH6561-methoxy-saccharin/kg dry soil (corresponding to 5 times the maximum annual application rate of the parent compound propoxycarbazone-sodium) using 3 replicates each with 400 g soil dry weight. In addition a negative control (deionised water) was tested. The test concentrations were achieved by preparing a stock solution (12 mg test item and 250 mL deionised water) and mixing appropriate amounts of the stock solution into the soil. To determine the activity of soil microflora, treated and untreated soils were incubated in 0.5 L plastic boxes. The boxes were covered by perforated lids. To stimulate nitrogen transformation, the soil was amended with lucerne meal (0.5%) as a nitrogen source at the time of preparation.

2. Observations

Soil samples were taken from each treatment group within 6 hours after application and on day 7, 14 and 28 after application. Nitrogen content was determined by extraction with 0.1 M KCl-solution and subsequent determination with a Dionex ICS 1000 ion chromatograph. The amount of each ion was calculated from the soil extracts and the soil (mineral nitrogen content). The pH values were checked at each sampling date for one replicate of each treatment group. The soil water content was also checked at each sampling date and evaporated water was replaced.

3. Statistical calculations

Data for the soil nitrogen contents were tested for normality and homogeneity of variance using the R/S-Test (α = 0.05) and Levene´s test (α = 0.05), respectively. The Student t-test (pairwise comparison, two sided, α = 0.05) was used for comparison of treated and control values. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 8.5-3 Effects of MKH6561-methoxy-saccharin on Soil Nitrogen Transformation in a Loamy Sand Soil

	NO_3^- – Nitrogen Formation Rate (mg / kg soil dry weight per day) [a]				
	Control	MKH6561-methoxy-saccharin			
		0.093 mg MKH6561-methoxy-saccharin/kg soil dw		0.467 mg MKH6561-methoxy-saccharin/kg soil dw	
Interval[1)]	Nitrate-N Formation	Nitrate-N Formation	Deviation [b]	Nitrate-N Formation	Deviation [b]
Day 0 - 7	0.41	-0.69*	-268.29	-0.86*	-309.76
Day 7 - 14	3.52	2.85*	-19.03	2.75*	-21.88
Day 14 - 28	1.08	1.24	14.81	1.29	19.44

[a] related to successive intervals between samplings
[b] % deviation to control
positive values = stimulating effect
negative values = inhibitory effect
dw = dry weight
* statistically significant different from control (Student t-test; α = 0.05)

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B. OBSERVATIONS

The soil nitrate content and differences in the soil nitrate formation rate deviated less than 25% from the control for both rates after 28 days.

The validity criteria according to the guideline OECD 216 were fulfilled as the coefficient of variation for the control group was < 15% and the reference item sodium chloride caused effects above 25% on soil nitrogen turnover.

III. CONCLUSIONS

After 28 days of exposure, MKH 6561-methoxy-saccharin had no impact above 25% on activity of soil microflora (nitrogen transformation) when applied up to a concentration of 0.467 mg/kg soil dry weight.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

According to the data requirements for plant protection products, screening data shall only be required for plant protection products other than those exhibiting herbicidal or plant growth regulator activity. Since propoxycarbazone-sodium is an herbicide, screening data are not considered necessary.

Information on herbicidal activity of different metabolites of propoxycarbazone-sodium was submitted during the first Annex I inclusion, demonstrating that in pre- and post-emergence greenhouse trials metabolites of propoxycarbazone have no significant herbicidal activity against grasses and dicot weeds. For details please refer to report KCA 3.6.02 of the Baseline Dossier P-010245-01 ([redacted], 1999, M-022213-01-1).

CA 8.6.2 Testing on non-target plants

Studies on non-target plants have been conducted with the representative formulation ATTRIBUT SG70 (tested as MKH 6561 WG 70).
A summary of all available relevant and compliant data is presented in the table below.

Table 8.6-1 Effects of ATTRIBUT SG70 on terrestrial non-target higher plants

Test compound	Test organism	Study type	Endpoint	References	EU agreed endpoint (SANCO/4067/2001-final)
MKH 6561 WG 70	Terrestrial non-target plants; 10 species	Seedling emergence; Tier 2 dose response	lowest ER_{50} 1.57 g a.s./ha for canola (dry weight)	[redacted] et al. (1999) 108843-1 M-021505-02-1 KCA 8.6.2 /01	Yes
MKH 6561 WG 70	Terrestrial non-target plants; 10 species	Vegetative vigour; Tier 2 dose response	lowest ER_{50} 1.55 g a.s./ha for canola (shoot height)		Yes
MKH 6561 WG70 (AE 0298618 00 WG70-A103)	Canola	Seedling emergence (SE) & Vegetative vigour (VV);	ER_{50} (SE) > 7.5 g a.s./ha ER_{50} (VV) > 7.5 g a.s./ha	[redacted] & [redacted] (2004) 200994 M-059849-01-1	New

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Test compound	Test organism	Study type	Endpoint	References	EU agreed endpoint (SANCO/4067/ 2001-final)
MKH 6561 WG70 (AE 0298618 00 WG70 A104)		Tier 2 dose response	ER_{50} (SE) > 7.5 g a.s./ha ER_{50} (VV) > 7.5 g a.s./ha	KCA 8.6.2 /02	

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01). Other studies are part of the Supplemental Dossier (P-010245-02).

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

To demonstrate technical equivalence after change of specification of the active substance, a new study was designed to compare the effects of the new and the old specification to the most sensitive species in a seedling emergence and vegetative vigour test ([redacted] & [redacted] (2004), 200994, M-059849-01-1). As canola (*Brassica rapa*) was determined to be the most sensitive species in the dose response test of [redacted] et al. (1999, 108843-1, M-021505-02-1), the phytotoxicity to canola of two formulations of MKH 6561 70 WG containing either the old or the new specifications of the active substance was determined. There was no difference in the biological impact of the AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 formulations on canola for all evaluated endpoints in a pre-emergent and post-emergent exposure scenario, indicating that the specification change did not alter toxicity. The test resulted in EC_{50} values greater than the highest tested application rate of 7.5 g a.s./ha.
It is therefore considered appropriate to base the risk assessment presented in Document M-CP, Section 10, Point CP 10.6 on the current EU endpoints for seedling emergence and vegetative vigour as worst case approach.

The new study was not submitted for the first Annex I inclusion and is therefore submitted within this Supplemental Dossier P-010245-02 for the propoxycarbazone-sodium Renewal of Approval and summarised below.

Annex point	Author(s)	Year	Study title
CA 8.6.2 /02	[redacted], M.T. [redacted], C.V.	2004	Title: Tier II Seedling Emergence and Vegetative Vigor Nontarget Phytotoxicity Study on Canola Using 2 Formulations of Propoxycarbazone-sodium WG70 Company: Bayer CropScience AG, Germany Study No: 200994 Edition No: M-059849-01-1 Date: March 19, 2004 GLP: yes not published

Guideline: USEPA, FIFRA Subdivision J, Guideline 123-2
OECD 208 (draft)

Deviations: none

Testing Laboratory and Dates: Bayer CropScience [redacted], Kansas
21 January 2004 – 01 March 2004

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Executive Summary

The seedling emergence and vegetative vigour studies were conducted with two typical end use formulations of Propoxycarbazone-sodium WG70 (AE 0298618 00 WG70). The objective of this study was to determine the effects of the two formulations on canola in a seedling emergence and vegetative vigour study. The formulations consisted of a new formulation (AE 0298618 00 WG70 A103) and an old formulation (AE 0298618 00 WG70 A104). Canola was the only species tested, as this was the most sensitive species determined in the previous study ([redacted] et al. (1999), 108843-1, M-021505-02-1). The exposure of the two formulations consisted of a single application to the soil surface for the seedling emergence test and a single application to the plant canopy at the 2 to 4 true leaf stage for the vegetative vigour test.

The two formulated products of Propoxycarbazone-sodium WG70 were applied to canola at the field application rates of 0.12, 0.23, 0.47, 0.94, 1.9, 3.8, and 7.5 g a.s./ha. A control group was also included in the study design.

The seedling emergence test with two formulations of propoxycarbazone-sodium on canola resulted in EC_{50} values greater than the highest tested application rate of 7.5 g a.s./ha. There was no difference in the biological impact of the AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 formulations on canola for all evaluated endpoints in a pre-emergent and post-emergent exposure scenario.

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II. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Formulation 1:

Test item:	Propoxycarbazone-sodium; water dispersible granule; 70%
Description:	Beige granules
Code No.:	AE 0298618 00 WG70 A103
Batch No.:	Pfl. Kr. 924 438/03
Analytical content:	70.6% w/w

Formulation 2:

Test item:	Propoxycarbazone-sodium; water dispersible granule; 70%
Description:	Beige granules
Code No.:	AE 0298618 00 WG70 A104
Batch No.:	Pfl. Kr. 924 439/03
Analytical content:	69.9% w/w

2. Vehicle and/or positive control:

n/a

3. Test system:

Species: Canola

Source: The canola seeds were obtained from [redacted], Maine on 19 June 2002. Seeds were not treated with fungicides, insecticides, or repellents prior to test initiation. Seeds were stored under dark conditions in a freezer prior to use.

Soil	The soil used for this study was a mixture of a natural topsoil and sand collected from Johnson County Top Soil and Landscape Materials, [redacted], KS.

4. Environmental conditions:

Temperature:	21.9 to 25.9% (seedling emergence test) 20.5 to 23.4% (vegetative vigour test)
Humidity:	21.9 to 67.9% (seedling emergence test) 39.1 to 69.0% (vegetative vigour test)
Light regime:	12 h light / 12 hours dark
Soil pH:	6.4

B. STUDY DESIGN

1. Experimental treatments

The phytotoxicity of two formulations of Propoxycarbazone-sodium WG70 on canola was evaluated in a seedling emergence and vegetative vigour study. Test pots consisted of 4.5-inch plastic pots (10.5 cm diameter x 12 cm height) with bottom drainage holes. Test pots were filled to with test soil and seeds were planted to a suitable depth for canola. Each pot contained a stake with species, study number, and replicate number. The seeds for the seedling emergence study were planted the day prior to spray application. The seeds for the vegetative vigour study were planted four weeks prior to spray application to achieve the desired leaf stage.

Both propoxycarbazone-sodium formulations (AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104) were applied at the treatment rates of 0.12, 0.23, 0.47, 0.94, 1.9, 3.8, and 7.5 g a.s./ha. A control group was also included in the study design. The formulations were applied at the soil surface for the seedling emergence test and to seedlings at the two to four true leaf stage for the vegetative vigour test. Treatment rates in both trials were applied at approximately 30 GPA (gallon per acre) equivalent to 281 L/ha.

2. Observations

The study endpoints for the seedling emergence test were evaluated on Day 7, Day 14, and Day 21 following spray application. The endpoints evaluated on these study days were the number of emerged seedlings, number of seedlings surviving, and phytotoxicity of each replicate. Plants were excised at the soil surface on Day 21 for plant height and weight determination.

The study endpoints for the vegetative vigour test were evaluated on Day 7, Day 14, and Day 21 following spray application. The endpoints evaluated on these study days were the number of surviving plants and the phytotoxicity of each replicate. Plants were excised at the soil surface on Day 21 for plant height and weight determination.

Plant height and weight determinations were performed by the same method for the seedling emergence and vegetative vigour tests. Plant shoot height was measured by extending cut seedlings to their maximum length and recording. Plant dry weight was performed by placing all replicate plants within labelled aluminium foil sheets. The plants were placed in a drying oven and allowed to dry for at least 48 hours at approximately 70°C. Plant dry weight measurements were determined to the nearest 0.1 mg using an electronic balance.

Test temperature, relative humidity, and light intensity were recorded once per hour.

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3. Statistical calculations

Regression models used to estimate ECx values (i.e. EC_{25} and EC_{50}) with 95% confidence intervals were calculated based on the nature of the data. Continuous data such as plant height and dry weight were calculated by the four-parameter logistic or the cumulative normal models. The two models are fit using least squares regression techniques. Binary data such as emergence and survival were calculated by the probit method.

The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were identified using hypothesis testing methodology. All Day 21 data were subjected to a Shapiro-Wilk's Test to assess departures from a normal distribution and a Levene's Test to determine homogeneity of variance. To assess treatment effects for homoscedastic and normally distributed data, a Dunnett's one-way analysis of variance (ANOVA) and multiple means comparison procedure for equal replicates was used to determine those exposure concentrations exhibiting responses significantly different ($P \leq 0.05$) than the control group.

Statistical analyses were performed using SAS® Procedure NLIN (Version 6.12) 11 statistical software for personal computers.

II. RESULTS AND DISCUSSION

A. FINDINGS

The nominal propoxycarbazone-sodium concentrations for each formulation were 0 (control), 0.43, 0.82, 1.7, 3.3, 6.8, 13.5, and 26.7 mg/L.
The propoxycarbazone-sodium concentrations in the seedling emergence study were 0, 0.42, 0.83, 1.60, 3.14, 6.19, 11.7, and 24.9 mg a.s./L for the AE 0298618 00 WG70 A103 formulation representing a range of 87 to 101% of nominal. The propoxycarbazone-sodium concentrations in the seedling emergence study were 0, 0.42, 0.83, 1.64, 3.26, 6.51, 12.6, and 25.6 mg a.s./L for the AE 0298618 00 WG70 A104 formulation representing a range of 93 to 101% of nominal.
The propoxycarbazone-sodium concentrations in the vegetative vigour study were 0, 0.42, 0.76, 1.53, 2.95, 5.84, 11.8, and 23.7 mg a.s./L for the AE 0298618 00 WG70 A103 formulation representing a range of 86 to 97% of nominal. The propoxycarbazone-sodium concentrations in the vegetative vigour study were 0, 0.43, 0.85, 1.65, 3.33, 6.35, 12.2, and 23.9 mg a.s./L for the AE 0298618 00 WG70 A104 formulation representing a range of 89 to 104% of nominal.

The seedling emergence test with two formulations of propoxycarbazone-sodium on canola resulted in EC_{25} and EC_{50} values greater than the highest tested application rate of 7.5 g a.s./ha. Study endpoints determined a NOEC of 7.5 g a.s./ha and a LOEC of > 7.5 g a.s./ha.
The vegetative vigour test with the AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 formulations resulted in dry weight EC_{25} values of 4.3 g a.s./ha and 5.7 g a.s./ha, respectively. The vegetative vigour test with two formulations of propoxycarbazone-sodium on canola resulted in EC_{50} values greater than the highest tested application rate of 7.5 g a.s./ha. However, extrapolation estimates of coefficients for canola dry weight determined EC_{50} values of 8.1 g a.s./ha for AE 0298618 00 WG70 A103 and 8.4 g a.s./ha for AE 0298618 00 WG70 A104. The most sensitive endpoint was dry weight with a NOEC of 1.9 g a.s./ha and a LOEC of 3.8 g a.s./ha.

Table 8.6-2: Effects of AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 in a seedling emergence test with canola

	Seedling emergence test			
	AE 0298618 00 WG70 A103			
Growth endpoints	EC_{25} (± 95% CL)	EC_{50} (± 95% CL)	LOEC	NOEC

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	[g a.s./ha]	[g a.s./ha]	[g a.s./ha]	[g a.s./ha]
Plant length	> 7.5	> 7.5	> 7.5	7.5
Plant dry weight	> 7.5	> 7.5	> 7.5	7.5
	AE 0298618 00 WG70 A104			
Plant length	> 7.5	> 7.5	> 7.5	7.5
Plant dry weight	> 7.5	> 7.5	> 7.5	7.5

Table 8.6-3 Effects of AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 in a vegetative vigour test with canola

	Vegetative vigour test			
	AE 0298618 00 WG70 A103			
Growth endpoints	EC_{25} (± 95% CL) [g a.s./ha]	EC_{50} (± 95% CL) [g a.s./ha]	LOEC [g a.s./ha]	NOEC [g a.s./ha]
Plant length	6.69 (6.10 to 7.29)	7.5	7.5	7.5
Plant dry weight	4.25 (3.33 to 5.17)	8.1	3.8	1.9
	AE 0298618 00 WG70 A104			
Plant length	7.30 (7.28 to 7.33)	> 7.5	> 7.5	7.5
Plant dry weight	5.73 (4.83 to 6.64)	8.4 [a]	7.5	3.8

[a] Values were extrapolated estimates of coefficients therefore no 95% confidence limits were calculated.

There was no difference in the biological impact of the AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 formulations on canola for all evaluated endpoints in a pre-emergent and post-emergent exposure scenario.

B. OBSERVATIONS

Seedling emergence test

Seedling emergence and survival were not significantly affected at any treatment level in comparison to the controls for each formulation. Phytotoxicity observations for the seedling emergence test ranged from 0 to 11% and were considered minor. The phytotoxicity observed in each formulation was considered to be random and not a result of dose-response effects. Plant length and dry weight were not significantly affected at any treatment level in comparison to the controls for each formulation.

Vegetative vigour test

Plant survival was not significantly affected at any treatment level in comparison to the controls for each formulation. Phytotoxicity observations for the vegetative vigour test included chlorosis, plant mottling, plant stunting, and leaf curl. The phytotoxicity mean for the control, 0.12, 0.23, 0.47, 0.94, 1.9, 3.8, and 7.5 g a.s./ha treatments were 3, 0, 0, 0, 5, 10, and 40 for the AE 0298618 00 WG70 A103 formulation and 0, 0, 0, 0, 0, 3, 11, and 56 for the AE 0298618 00 WG70 A104 formulation. The phytotoxicity observed in each formulation was considered to be the result of a dose-response effect with the most severe effects occurring at the highest treatment of 7.5 a.s./ha. Plant lengths and plant dry weight were significantly affected for both formulations at the 7.5 g a.s./ha treatment by Dunnett's Test.

Validity criteria according to OECD 208 (2006) are fulfilled:

- seedling emergence of the control plants was 98 - 100% (should be at least 70%)

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- the seedlings did not exhibit visible phytotoxic effects
- the mean survival of emerged control seedlings was 100% (should be at least 90% for the duration of the study)

III. CONCLUSIONS

The seedling emergence test with two formulations of propoxycarbazone-sodium on canola resulted in EC_{50} values greater than the highest tested application rate of 7.5 g a.s./ha. There was no difference in the biological impact of the AE 0298618 00 WG70 A103 & AE 0298618 00 WG70 A104 formulations on canola for all evaluated endpoints in a pre-emergent and post-emergent exposure scenario.

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

No studies on other terrestrial organisms were necessary.

CA 8.8 Effect on biological methods for sewage treatment

A summary of all available relevant and compliant data on biological methods for sewage treatment for propoxycarbazone-sodium is presented in the table below.

Table 8.8-1 Effects of propoxycarbazone-sodium on biological methods for sewage treatment

Test item	Study design	Endpoint	Reference	EU agreed endpoint (SANCO/4067/ 2001-final)
Propoxy-carbazone-sodium	Activated sludge, 3 h	EC50 > 10000 mg/L	[redacted] & [redacted] (1998) 789 N/98 M-006027-01-1 KCA 8.8 /01	Evaluated during the first EU review

Studies shaded in grey have been reviewed as part of the first EU review of propoxycarbazone-sodium (in Baseline Dossier for the active substance P-010245-01).

.

For information on studies already evaluated during the first EU review of propoxycarbazone-sodium, please refer to corresponding section in the Baseline Dossier P-010245-01 included on the provided data medium and to the Monograph.

CA 8.9 Monitoring data

No monitoring data are available.

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