Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Methiocarb

Data Requirements

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

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

Date

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Version history

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

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

Table of Contents

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

Methiocarb is an insecticide and repellent active substance and was included into Annex I of Directive 91/414 on 1st October 2007 (Directive 2007/5/EC).

This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of methiocarb under Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer CropScience (BCS) for the Annex I inclusion under Directive 91/414/EEC are contained in the DAR, its Addenda and are included in the Baseline Dossier provided by BCS. These data are only mentioned in the Supplementary Dossier for the sake of completeness and only general information (e.g. author, reference etc.) is available for these data. In order to facilitate discrimination between new data and data submitted during the Annex I inclusion process under Directive 91/414/EEC, the old data are written in grey typeface. For all new studies, detailed summaries are provided within this Supplementary Dossier.

Studies with the formulation Methiocarb FS 500 G can be retrieved in the respective node and sub-nodes of CA 10 for the representative formulation.

The presented and submitted studies used different synonyms and codes for the active substance Methiocarb.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Table 8.1- 1: Endpoints Birds

Test substance	Test species	Test design	Endpoint	Reference
Methiocarb	Acute oral LD50 *Coturnix coturnix*	acute, oral	LD_{50} 5 mg a.s./kg bw	[redacted] (1983) M-012876-01-2
	Dietary test, *Colinus virginianus*	Dietary test, 21 d	NOEC 213 mg a.s./kg diet NOED 17.8 mg a.s./kg bw/d	[redacted] (2002) M-039501-01-1
	Reproductive NOEC/ NOED *Colinus virginianus*	Reproduction test, 22 w dietary	NOEC ≥ 50 mg a.s./kg diet NOED ≥ 4.45 mg a.s./kg bw/d	[redacted] (1982) M-012904-01-1
	Reproductive NOEC/ NOED *Anus platyrhynchos*	Reproduction test, 19 w dietary	NOEC 50 mg a.s./kg diet NOED 4.51 mg a.s./kg bw/d	[redacted] (1982) M-012909-01-1

CA 8.1.1.1 Acute oral toxicity to birds

For information on studies already evaluated during the first EU review of methiocarb, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.1.1.2 Short-term dietary toxicity to birds

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Report:	KCA 8.1.1.3/03 [redacted] C; [redacted]; 2002; M-039501-01-1
Title:	Technical Mesurol: A subacute dietary test with bobwhite quails
Report No.:	110982
Document No.:	M-039501-01-1
Guideline(s):	Commission Directive 96/46/EC of 16 July 1996 amending Council Directive 91/414/EEC
Guideline deviation(s):	--
GLP/GEP:	**no**

Material and methods:

- Singly housed sub-adult birds, 9 to 12 weeks of age, 10 birds per group
- Methiocarb a.s. (batch-no. 3247126701)
- 5 treatment groups (213 mg as/kg food, 470 mg as/kg food, 1033 mg as/kg food, 2273 mg as/kg food, 5000 mg as/kg food), control
- 1 week acclimation
- Exposure for 5 to 21 days
- 1 week post-exposure after switching to standard diet
- Daily feed measurements and calculation of food and chemical intake/day/bird
- Body weight measurement on day –7, 0, +7, +14, +21, 28
- Birds switched to basal diet if severe avoidance occurs
- Daily observation on signs of intoxication
- Gross necropsy

Results:

Results of the subacute dietary of bobwhite quails

Test substance:	Methiocarb technical
Test object:	Bobwhite quail (*Colinus virginianus*)
Mortality	1033 mg as/kg food: 3 birds 2273 mg as/kg food: 6 birds 5000 mg as/kg food: 3 birds
Lowest lethal concentration (mg as/kg food)	1033
No effect concentration (mg as/kg food)	213
Lethal daily dose (LD_{50})	Could be not established due to great variability in clinical history of the birds

Observations:

- Reduced food consumption above 213 mg as/kg food; severe avoidance at the higher concentrations
- Extreme loss of body weight at higher test concentration due to food avoidance
- Signs of intoxication: ataxia (from 470 mg as/kg food on), hypoactivity (from 1033 mg as/kg food on); the last one is more an indicator of general weakness than of intoxication
- All surviving birds recovered rapidly and completely
- All prematurely dead birds showed severe emaciation

- A relation between mortality and intake of a.s. was not evident.

Conclusion:

Due to the great **variability in the clinical history of the birds (no dose response) an LDD_{50} could not be evaluated.** That is not surprising for a substance which is well known for its repellency. It was observed that birds refused the food to an extent that severe signs of starvation occurred. Since all prematurely dead birds were emaciated, the starvation has to be considered the main reason for death.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.1.2.1- 1: Acute oral toxicity data for mammals exposed to methiocarb

Test substance	Exposure	Species/Origin	Endpoint		Reference
Methiocarb	Acute risk assessment	Rat	LD_{50}	19 mg a.s./kg bw[1)]	EFSA Scientific Report (2006)
		Rat	LD_{50}	50 mg a.s./kg bw	[redacted] (2005) M-261735-01-1

[1)] Figures not lowest from mammalian toxicity data package but considered most appropriate for use in wild mammal risk assessment.

Report: KCA 8.1.2.1/01 [redacted]B; 2005; M-261735-01-1
Title: Acute oral toxicity study with Mesurol Tecnico T in rats (Rattus norvegicus)
Report No.: RF-0030.305.320.05
Document No.: M-261735-01-1
Guideline(s): OECD 423
Guideline deviation(s): not applicable
GLP/GEP: **yes**

Material and methods:

Material and methods: This acute oral toxicity study in rats (*Rattus norvegicus*) was carried out in a stepwise procedure in order to evaluate the possible toxic effects of the test item Mesurol Tecnico T (Batch No 436400161) administered by the oral route. Twelve female rats, divided in four groups of three animals, were tested in four successive steps at the dose levels of 5 and 50 mg/kg of body weight. The animals were maintained under the same environmental conditions throughout the acclimatization and observation periods. The test item was diluted in com oil at a constant concentration of 20 mg/mL. The volume administered to each animal was calculated according to the body weight determined on the day of the treatment. After dosing by gavage, the animals were observed during 14 days to evaluate deaths, behavioral and clinical alterations. At the end of the

observation period for each step, all surviving animals were weighed and submitted to euthanasia and necropsy, while the animals that died during the observation period were weighed and submitted to necropsy.

Findings:
The test item did not cause any treatment-related deaths at the dose of 5 mg/kg of body weight, but caused death in three out of six animals at the dose level of 50 mg/kg of body weight. At the clinical examinations, the animals treated at the dose level of 5 mg/kg of body weight did not present any signs of toxicity. When treated at the dose level of 50 mg/kg of body weight, all six animals presented two or more of the following systemic signs of toxicity during the observation period: ventral position, muscular tremors, sialorrhea, piloerection, ataxia, and apathy. During the necropsies, no abnormalities were noted for the animals that were submitted to euthanasia at the end of the observation period. The animals found dead during the observation period had macroscopic alterations on the kidneys, liver or digestive tract that characterize possible intoxication signs.

Conclusions:
Based on the study results the derived oral LD50 is > 5 - <50 mg/kg bw/day for female rats.

Classification/labelling regarding acute oral toxicity for methiocarb:

Regulation (EC) No 1272/2008 (CLP): Acute Toxicity Category 2
H300 (Fatal if swallowed)

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience, the Monograph and the Addenda generated during the EU review. For new studies please refer to the supplemental dossier, e.g. sections CA 5.5 and CA 5.6.

Table 8.1.2.2- 1: Mammalian toxicity data of methiocarb

Test substance	Exposure	Species/Origin	Endpoint		Reference
Methiocarb	Long-term risk assessment	Rat	NOEC NOED	300 mg a.s./kg bw[1)] 15 mg a.s./kg bw/d	EFSA Scientific Report (2006)
		Rat	NOEC NOED	150 mg a.s./kg bw[1)] 14.8 mg a.s./kg bw/d	[redacted] (2002) M-064945-01-1

[1)] Figures not lowest from mammalian toxicity data package but considered most appropriate for use in wild mammal risk assessment.

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.

For methiocarb, a log P_{OW} of 3.18 (pH 7, 20°C; see methiocarb IIA, 2.8) was determined. Thus, bioaccumulation in bird prey like earthworms is considered possible. Therefore, a risk assessment for the active substance considering a generic earthworm eating bird is provided in MCP point 10.1.1

As the compound is intended to be applied as seed treatment, the exposure of aquatic organisms to methiocarb will be very limited. Therefore, risk of bioaccumulation for fish eating birds exposed to methiocarb will be presented for information only. See MCP point 10.1.1 for more details.

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

Information on effects of methiocarb on reptiles or amphibians is not available. Risk to birds and mammals is assessed in Document MCP Section 10.1

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test protocol is available, this test was developed to evaluate to potential effect on the thyroid system and not to measure population relevant effects.

Therefore no further studies can be suggested at this time for these groups of organisms.

CA 8.1.5 Endocrine disrupting properties

Wild Mammals

A detailed analysis of all the apical toxicological studies (subchronic, chronic / onco-genicity, reproduction and developmental toxicity) on Methiocarb revealed no endocrine disrupting effect. The toxicological profile of Methiocarb is governed by the well-known activity as a ChE inhibitor in animals. Therefore, based on a complete toxicological data set, there is no evidence for endocrine disrupting properties of Methiocarb in mammals.

Based on the absence of any indication of relevant effects it can be concluded that Methiocarb is not a (potential) endocrine disrupter in birds.

No further testing for endocrine disrupting properties is warranted.

Birds

The population relevant effects of Methiocarb on birds were studied in reproductive toxicity studies on Bobwhite quail and Mallard ducks. In the study on Bobwhite quails no statistically significant effects on adult birds, offspring or reproductive parameters were found at 50 mg Methiocarb/kg diet, the highest dietary concentration tested. Mallard ducks tolerated the highest dietary concentration tested of 100 mg Methiocarb/kg diet without any effect on reproductive parameters. The only effect seen in the study was an inhibition by 22% of brain cholinesterase activity at sacrifice. This effect is not caused

by an endocrine mediated mechanism but by the well-known activity of Methiocarb as a ChE inhibitor in birds.

CA 8.2 Effects on aquatic organisms

Table 8.2- 1: Endpoints used in risk assessment and additional studies for methiocarb and its metabolites

Test substance	Test species	Endpoint		Reference
Methiocarb FS 500 G	Invertebrate, acute *Daphnia magna*	EC_{50}	0.0292 mg product/L 0.0131 mg a.s./L	[redacted] (2007) M-289429-01-1 KCP 10.2.1
	Invertebrate, chronic *Daphnia magna*	NOEC	3 x 0.008 mg a.s./L	[redacted] (2007) M-295095-01-1 KCP 10.2.1
Methiocarb	Fish, acute *Oncorhynchus mykiss*	LC_{50}	1.1 mg a.s./L (nom)	[redacted] (2000) M-024375-01-1
	Fish, acute *Lepomis macrochirus*	LC_{50}	0.6 mg a.s./L (nom)	[redacted] (2000) M-021382-01-1
	Fish, chronic *Oncorhynchus mykiss*	NOEC	0.05 mg a.s./L (nom)	[redacted] (1985) M-012845-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	0.007 mg a.s./L (mm)	[redacted] (2000) M-034439-01-1
	Invertebrate, chronic *Daphnia magna*	NOEC	0.0001 mg a.s./L (mm)	[redacted] (1988) M-012825-01-1
	Chironomid, acute *Chironomus riparius*	EC_{50}	0.103 (mm)	[redacted] (2014) M-493345-01-1
	Chironomid, chronic *Chironomus riparius* (spiked water)	NOEC (emergence)	0.160 (nom)	[redacted] (2006) M-268292-01-1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	0.82 mg a.s./L (mm) 2.2 mg a.s./L (mm)	[redacted] (2000) M-024134-01-1
Methiocarb-sulfoxide (MSO)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	6.6 mg p.m./L (mm)	[redacted] (2000) M-022381-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	0.056 mg pm/L (nom)	[redacted] (2001) M-079738-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	4.64 mg p.m./L (nom)	[redacted] (2008) M-297569-01-1
	Invertebrate, chronic *Daphnia magna*	NOEC	0.00652 mg p.m./L (mm)	[redacted] (2008) M-300223-01-1
	Algae, growth inhibition	E_bC_{50}	1.31 mg p.m./L (mm)	[redacted] &

Test substance	Test species	Endpoint		Reference
	Desmodesmus subspicatus	E_rC_{50}	2.75 mg p.m./L (mm)	[redacted] (2001) M-073140-01-1
Methiocarb-phenol (MP)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	3.2 mg p.m./L (nom)	[redacted] (1999) M-016605-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	6.8 mg p.m./L (nom)	[redacted] (1999) M-016597-01-1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	6.0 mg p.m./L (nom) 11 mg p.m./L (nom)	[redacted] (1999) M-016599-01-1
Methiocarb-sulfoxide-phenol (MSOP)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	> 100 mg p.m./L (mm)	[redacted] (2001) M-056170-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	157 mg p.m./L (nom)	[redacted] (2001) M-049549-01-1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	> 100 mg p.m./L (nom) > 100 mg p.m./L (nom)	[redacted] (2001) M-073301-01-1
Methiocarb-sulfone-phenol (MSOOP)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	68.1 mg p.m./L (mm)	[redacted] (2001) M-021598-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	54 mg p.m./L (nom)	[redacted] (2001) M-047970-01-1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	105 mg p.m./L (nom) 120 mg p.m./L (nom)	[redacted] & [redacted] (2001) M-073309-01-1
Methiocarb-methoxy-sulfone (MMS)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	26.8 mg p.m./L (mm)	[redacted] (2001) M-057313-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 180 mg p.m./L (nom)	[redacted] (2001) M-049570-01-1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	97.7 mg p.m./L (nom) 137 mg p.m./L (nom)	[redacted] (2001) M-054813-01-1

mm = mean measured; nom = nominal; im = initially measured

[A] NOEC based on clinical signs of intoxication; all other NOEC and LOEC-values, based on weight, time to swim-up, hatching and survival were ≥ 0.100 mg/L.

Toxicity of the formulated product

No additional aquatic formulation tests have been performed, because due to the high toxicity of the a.s., formulation toxicity will be dominated by the a.s. alone. Also, aquatic organisms will not be exposed to the seed treatment formulation itself.

CA 8.2.1 Acute toxicity to fish

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.2 Long-term and chronic toxicity to fish

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.2.1 Fish early life stage toxicity test

See point 8.2.2. No additional studies were performed.

CA 8.2.2.2 Fish full life cycle test

See point 8.2.2. No additional studies were performed.

CA 8.2.2.3 Bioconcentration in fish

See point 8.2.2. No additional studies were performed. Studies already evaluated during the last EU review of methiocarb are presented below.

Table 8.2.2.3- 1: Bioconcentration in fish

Test substance	Test species	Endpoint	Reference
^{14}C-Methiocarb	Fish, BCF flow-through, Bluegill Sunfish (*Lepomis macrochirus*)	BCF: 60- 90	[redacted]; 1974 M-012920-01-1
^{14}C-Methiocarb-phenol	Fish, BCF flow-through, Bluegill Sunfish (*Lepomis macrochirus*)	BCF: 10.9 [A]	[redacted] et al; 2002 M-053258-02-1

[A] Normalised to 6% lipid content.

CA 8.2.3 Endocrine disrupting properties

Population relevant effects of Methiocarb on fish were studied in an early life-stage test (ELS) with rainbow trout (*O. mykiss*) under continuous exposure, resulting in a NOEC of 50 µg/L. The NOEC was based on signs of intoxication consistent with the MoA of methiocarb (erratic swimming and lying on the bottom of the chambers) at the LOEC of 100 µg/L, with no effects on other parameters like growth (weight, length) or time to swim-up. The chronic fish NOEC of 50 µg/L is orders of magnitude above the regulatory acceptable concentration, which is driven by aquatic invertebrates.

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

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

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Report:	KCA 8.2.4.1/07 [redacted]; 2008; M-297569-01-1
Title:	Acute toxicity of methiocarb-sulfoxide to the waterflea *Daphnia magna* in a water-sediment test system
Report No.:	EBMEL003
Document No.:	M-297569-01-1
Guideline(s):	Performed under principle consideration to the procedures described by OECD-Guideline No. 202 (2004)
Guideline deviation(s):	Exposure will occur in a water-sediment system similar to OECD Guideline 219 "Sediment-Water Chironomid Toxicity Test using Spiked Water" (2004). - Basins containing the water-sediment test system were not covered during any part of the study.- The water body of each study group will be artificially aerated during exposure. Daphnids containing enclosures were fitted with stainless steel grid-bottoms prevent animals from contact with air bubbles.
GLP/GEP:	**yes**

Material and methods:

Test item: Methiocarb-sulfoxide, batch ID: SES 10041-2-1, LIMS No.: 0722562, purity: 99.5 %, TOX-08014-00.

Daphnia magna (1st instars < 24 h old, 6 x 5 animals per treatment group and control), exposed in a static test system for 48 hours (without feeding) to the nominal initial concentrations of 0.10, 0.32, 1.00, 3.20 and 10.0 mg pure metabolite (p.m.) /L, freshly prepared and admixed to the overlying water at start of exposure only. Exposure concentrations of methiocarb-sulfoxide were measured only at start of the 48 hours exposure period in the overlying water phase of the whole water-sediment test system.

Results:

Study Validity:

Sensitivity of the daphnid breeding-strain used is located within the required range as verified by periodically performed acute reference substance testing. No immobilities or other effects on behaviour occurred in untreated control within 48 hours of exposure.

For water quality monitoring, temperatures, pH values and O_2 concentrations of the test solutions, as well as conductivity, hardness and alkalinity of the used dilution water were controlled during the course of the study.

Dissolved oxygen concentrations ranged in the water phase from 8.8 to 9.0 mg O2/L (8.9 mg O_2/L= 100 % O_2-saturation), the water pH values ranged from 8.4 to 8.5 and the water temperature ranged from 19.4°C to 19.5°C measured in the overlying water of each test concentration day 0 and day 2.

As measurements show, the physical / chemical properties corresponded to the recommended values.

The sediment parameters measured directly after preparation, at the start of the equilibration time (day -18) fulfilled the guideline requirements (OECD 219) with a water content of 31.9%, pH value of 6.9 and an organic carbon content of 1.9 %.

Analytical results:
The chemical analysis of methiocarb-sulfoxide spiked in the overlying water of the basins at test initiation ranged between 104 % and 118 % (mean: 111 %) of the corresponding nominal concentrations, thus all results are based on nominal initial concentrations.
Statistical significant differences compared to control findings (α = 0.05) were established for test concentrations from 0.32 to 10.0 mg p.m./L.
Observations on sub-lethal effects revealed abnormal behaviour of the exposed daphnids from test concentrations of 1.00 to 10.0 mg p.m./L.

Biological results:
Toxicity to Daphnia magna (based on nominal initial concentrations)

Test Concentration	Exposed daphnids (=100%)	Immobilised daphnids after 48 h of exposure	
mg p.m./L		n	%
control	30	0	0
0.10	30	0	0
0.32	30	6	20.0
1.00	30	10	33.3
3.20	30	12	40.0
10.0	30	18	60.0

Conclusions:
The EC_{50} for immobility of *Daphnia magna* after 48 hours of static exposure in a water sediment test system is 4.64 mg p.m./L and the 48 hours NOEC is 0.10 mg p.m. /L.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Report: KCA 8.2.4.2/01 [redacted]; 2014; M-493345-01-1
Title: Acute toxicity of methiocarb (tech.) to larvae of *Chironomus riparius* in a 48 h static laboratory test system
Report No.: EBMEN038
Document No.: M-493345-01-1
Guideline(s): OECD Guideline No. 235 (Guideline for Testing of Chemicals, "Chironomus sp., Acute Immobilisation Test, adopted July 28, 2011)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The objective of this 48 hour (h) toxicity test was to evaluate the acute immobilisation to larvae of *Chironomus riparius* (1st instar) caused by the test item. As the primary endpoint, a concentration causing 50 % immobility to larvae of *Chironomus riparius* (24 h and 48 h -EC_{50}) was determined. For this purpose, different concentrations of the test item were prepared in a geometric range in Elendt-medium. Larvae of *Chironomus riparius* were exposed under defined exposure conditions to the different concentrations of the test item and compared against control(s). Beside immobility a possible occurrence of symptoms was recorded and evaluated after 24 and 48 hours of exposure.

Material and methods:

Methiocarb (tech.), purity: 98.2 % w/w was tested, specified by origin batch-No.: NLL 9134-1-5, TOX-no. 10247-00 and specification no.: 102000005994. Larvae of *Chironomus riparius* (1st instars < 2-3 days old, 6 beakers per test concentration and control(s), with 5 animals each) were exposed for 48 hours in a static test system (water only) to concentrations of 0.05, 0.11, 0.23, 0.50 and 1.08 mg a.s./L.

Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger. Additionally water parameters (temperature, pH and oxygen) were measured in the freshly prepared test solutions of each test concentration on day 0 and on day 2 in the combined test solutions of each test concentration.

Quantitative amounts of analysed a.s. were measured in all freshly prepared test levels on day 0, and control(s). On day 2, at the end of exposure, additionally all aged test levels including control(s) were measured.

Results:

Validity of the study:

Dissolved oxygen concentrations ranged from 8.4 to 8.6 mg O_2/L (8.4 mg O_2/L = 98.2 % O_2 - saturation), the water pH values ranged from 7.8 to 8.0 and the water temperature ranged from 20.4°C to 20.7 °C over the whole period of testing, fulfilling the guideline requirements.

Analytical results:

The analysed pure metabolite found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 95 and 100 % (average 98 %). In aged test levels on day 2 there were analytical findings between 56 and 76 % (average 64 %) of nominal. Due to the recoveries of < 80 % of nominal after 2 days of exposure, all results are based on mean measured concentrations.

Biological results:

Acute toxicity of test item to first instar-larvae of *Chironomus riparius* after 48 hours (based on mean measured concentrations):

Nominal concentrations [mg a.s./L]	Mean measured concentrations [mg a.s./L]	Exposed chironomids (=100%)	Immobility			
			24 h		48 h	
			n	%	n	%

control	-	30	0	0	1	3.3
solvent-control	-	30	0	0	1	3.3
0.05	0.040	30	1	3.3	1	3.3
0.11	0.086	30	4	13.3*	10	33.3*
0.23	0.198	30	12	40.0*	29	96.7*
0.50	0.390	30	25	83.3*	30	100.0*
1.08	0.868	30	30	100.0*	30	100.0*

* statistically significant (α = 0.05)

Conclusion:
Control mortality did not exceed 15 % and measured dissolved oxygen concentrations in the control and all test concentrations did not fall below 3 mg/L during exposure, fulfilling the guideline requirements.
Statistical results of probit analysis conducted for determination of EC_{50} values (based on mean measured concentrations)

Probit analysis for data obtained after	**NOEC** [mg a.s./L] (mean measured)	**EC_{50}** [mg a.s./L] (mean measured)	lower 95% cl [mg a.s./L] (mean measured)	upper 95% cl [mg a.s./L] (mean measured)
24 hours	**0.040**	**0.200**	0.163	0.246
48 hours	**0.040**	**0.103**	0.089	0.121

Due to the results of the statistical analysis (Williams Multiple Sequential t-test Procedure, alpha = 0.05), a NOEC (No Observed Effect Concentration) of 0.040 mg a.s./L was evaluated at 24 and 48 hours of incubation.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

Report: KCA 8.2.5.1/02 [redacted]; [redacted]; 2008; M-300223-01-1
Title: Chronic toxicity of methiocarb-sulfoxide to *Daphnia magna* under flow-through conditions
Report No.: EBMEL002
Document No.: M-300223-01-1
Guideline(s): FIFRA Guideline 72-4 (b) (1982)
OPPTS Guideline 850.1300 (1996 draft)
OECD Guideline 211 (1998)
Guideline deviation(s): --
GLP/GEP: **yes**

Material and methods:
Test item: Methiocarb-sulfoxide, Purity: 99.5%, Batch code: AE 1371422-01-01.
In a 21-day chronic test first instars of Daphnia magna (< 24 h old) were exposed to nominal (mean measured) concentrations of control (<0.40), solvent control (<0.40), 3.75 (1.66), 7.50 (3.28), 15.0 (6.52), 30.0 (13.4) and 60.0 (22.8) µg a.s./L under flow-through conditions. Mean measured recoveries were within the range of 38 to 45% of the nominal concentrations. The discrepancy of measured and nominal values can be explained by the high instability of Methiocarb-sulfoxide in water even under flow-through conditions. However, analytical recoveries were consistent during the study in all test

levels and reflect actual test concentrations. The toxicity values were calculated based on mean measured concentrations.

Results:

Survival, growth and reproduction of *Daphnia magna*

Test Substance	Methiocarb-sulfoxide				
Test Object	*Daphnia magna*				
Exposure	21 -Day, Flow-Through				
	(µg a.s./L)				
Endpoint results	**Immobilization**	**Time to first brood**	**Neonates/ adult reproduction day**	**Adult body length**	**Adult dry weight**
Highest Concentration without an Effect (NOEC)	6.52	22.8	22.8	22.8	22.8
Lowest Concentration with an Effect (LOEC)	13.4	>22.8	>22.8	22.8	22.8

Observations:
No dose related behavioral effects were noted for any test level, including the control groups.

Conclusions:
The NOEC and LOEC were calculated based on mean measured concentrations. The 21-day exposure to Methiocarb-sulfoxide resulted in a NOEC of 6.52 µg a.s./L, LOEC of 13.4 µg a.s./L, and MATC (Maximum Allowable Toxicant Concentration, which equals the geometric mean of the NOEC and LOEC) of 9.35 µg a.s./L based on immobilization.

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

No chronic studies on additional aquatic invertebrate species are required.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

Report: KCA 8.2.5.3/01 [redacted], 2006; M-268292-01-1
Title: *Chironomus riparius* 28-day chronic toxicity test with Methiocarb (tech.) in a water-sediment system using spiked water
Report No.: M-268292-01-1
Document No.: M-268292-01-1
Guideline(s): OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (adopted 13 April 2004)
Guideline deviation(s): none
GLP/GEP: **yes**

Objectives:
The aim of the study was to determine the influence of the test item on emergence and development of *Chironomus riparius* for 28-days in a static water-sediment-system (spiked water exposure), expressed as NOEC, LOEC and EC_x for emergence ratio and development rate, if possible.

Material and methods:

Methiocarb (tech.), purity: 99.5 % was tested, specified by batch-no.: 436400181, TOX-No.: 7157-00 and article-no.: 0005573092). First instar of *Chironomus riparius* larvae (4 beakers per test concentration and control with 20 animals each) were exposed in a static test system for 28 days to initial nominal concentrations in the overlying medium (spiked water application) of 0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mg a.s. /L of a water-sediment system.

Dissolved oxygen concentrations ranged in the water phase from 7.2 to 8.7 mg O_2/L (7.2 mg O_2/L = 80 % O_2 - saturation), the water pH values ranged from 8.1 to 8.6 and the water temperature ranged from 20.0°C to 20.6°C measured from parallel beakers of each test concentration over the whole period of testing.

Recoveries of Methiocarb were measured three times during the study: 1 hour, 7 days and 28 days after application in one additional test container of each nominal initial test concentrations of 0.01, 0.04 and 0.32 mg a.s. /L and control (only on day 0) of the overlying water and the pore water of the sediment.

Results:

Validity of the study:

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

Analytical results:

Chemical analysis of overlying water and pore water over time reflect expected aquatic fate data with high recoveries of 85.3 % to 93.1 % (mean 89.8 %) at the beginning of the exposure period in the overlying water.

Biological results:

Start of emergence was on day 13 and 14 for the controls and test concentrations from 0.01 to 0.16 mg a.s. /L. The start of emergence was reduced for one day at the highest test concentration of 0.32 mg a.s. /L.

92.5 % of the inserted (n= 160) larvae maturated to adults in the pooled controls after 28 days, fulfilling the guideline requirements.

Influence on emergence and development rate after 28 days (based on nominal initial concentrations of the test item in the overlying water)

Concentration	Number of emerged midges	Emergence of inserted larvae			Development pooled sex
initial nominal mg a.s./L		total (%)	male (%)	female (%)	Rate (1 / d)
Controls*)	148	92.5	46.9	45.6	0.062
0.01	76	95.0	41.3	53.7	0.062
0.02	78	97.5	41.3	56.2	0.060
0.04	77	96.2	56.2	40.0	0.064
0.08	75	93.8	46.3	47.5	0.063
0.16	67	83.7	36.2	47.5	0.063
0.32	19	23.7	11.2	12.5	0.062

*) control and solvent control were pooled

The Chi^2-Test indicates no statistically different sensitivities of sexes. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.
Statistical significance (α = 0.05) on emergence ratio and development rate of females was only evaluated for 0.32 mg a.s. /L (= LOEC), resulting in an NOEC of 0.16 mg a.s./L. For the development rate of male and pooled sex, no statistical significance could be established up to the highest test concentration, resulting in an NOEC of > 0.32 mg a.s./L.

Conclusions:
Results are based on nominal initial concentrations in mg a.s./L of the test item in the overlying water.

Endpoints (mg a.s./L)	NOEC	LOEC	EC_{50}
emergence ratio (pooled sex) (95 % confidence limits)*	0.160	0.320	0.275
development rate (pooled sex)	≥ 0.32	> 0.32	> 0.32

*) due to mathematical reasons, the calculation of the confidence limits was not possible

CA 8.2.5.4 Sediment dwelling organisms

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.6 Effects on algal growth

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.6.1 Effects on growth of green algae

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.6.2 Effects on growth of an additional algal species

No additional species were tested. Not a data requirement for insecticides.

CA 8.2.7 Effects on aquatic macrophytes

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.8 Further testing on aquatic organisms

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

New studies referring to the intrinsic toxicity of methiocarb to bees, conducted since the last Annex I inclusion process are summarised in this document. For all studies submitted during the frame of the last Annex I inclusion, please refer to the corresponding section in the DAR and in the baseline dossier provided by Bayer CropScience.

The studies presented in Table 8.3.1-1 and highlighted in grey script were evaluated during the last EU review and are considered in the List of endpoints provided by EFSA (2005). As several deficiencies, compared to modern guidelines, were noted in the acute oral and contact toxicity tests on adult bees in the original submission a new test fully compliant with OECD 213 and 214 for methiocarb has been conducted and is also included in this submission ([redacted] 2009, M-308072-01-1). The findings of the new study demonstrated that the submitted previously ones were indicative of the acute toxicity to bees. Values highlighted in bold are used in the risk assessment.

Table 8.3.1- 1: Acute toxicity of methiocarb to honey bees

Test substance	Test species/ study type	Endpoint	EU agreed endpoint (EFSA Scientific Report (2006) 79	References
Methiocarb tech.	Honey bee, 48 h	LD_{50} – oral 0.47 µg a.s./bee LD_{50} – contact 0.20 µg a.s./bee	yes	[redacted] (1995) M-013166-01-1
Methiocarb tech.	Honey bee, 48 h	LD_{50} – oral 0.08 µg a.s./bee LD_{50} – contact 0.43 µg a.s./bee	New study	[redacted] (2009) M-308072-01-1

New studies for AIR:

Commission Regulation (EU) 283/2013 (1st March 2013 setting out data requirements for active substances in accordance with regulation (EC) 1107/2009 of the European Parliament and of the Council concerning the placing of Plant Protection Products on the market) requires where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, to be conducted. Consequently in addition to the standard toxicity studies performed with adult bees (OCED 213 and 214) the following additional studies are also provided:

- Acute contact toxicity to adult bumble bees under laboratory conditions
- Chronic 10 day toxicity to adult honeybees under laboratory conditions
- Acute toxicity to larva honeybees under laboratory conditions
- Semi-field feeding studies according to Testing Method with special design. One tunnel test with honey bee colonies exposed to dressed maize seeds at 5.2 g a.s./kg seeds and the other tunnel to fortified maize pollen up to 3.1 µg a.s./kg.
- Field studies simulating a dust drift exposure scenario for honey bees in flowering Phacelia at the maximum application rate for the approval renewal of methiocarb and evaluating flight intensity, mortality and colony development.

- Field study according to a tailor made study design. Honey bee colonies were exposed to guttation fluid of treated maize seeds at 1.5 mg a.s./seed and investigated in terms of mortality, colony development and subsequent overwintering performance.

Supporting study

- Semi-field studies following OEPP/EPPO Guideline No. 170(4) exposing honey bees to methiocarb-treated pollen at 48 µg a.s./kg and to treated sugar solution at 20 µg a.s./kg and evaluating flight intensity, mortality and colony development.

These studies were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the methiocarb Annex I Renewal. The studies will be summarized below.

Table 8.3.1- 2: Acute toxicity of methiocarb to bumble bees

Test substance	Test organism	Ecotoxicological Endpoints:		Reference
Methiocarb tech.	Bumble bee	48 h - LD_{50} contact	19.3 µg a.s./ bumble bee	[redacted] (2014) M-479538-01-1

Table 8.3.1- 3: Acute toxicity of methiocarb to larval bees

Test substance	Test organism	Ecotoxicological Endpoints:		Reference
Methiocarb tech.	Honey bee brood (in vitro) 72 h	NOED LD_{50}	0.064 µg a.s./larva 0.547 µg a.s./larva	[redacted] (2015) M-514260-01-1

Table 8.3.1- 4: Chronic toxicity of Methiocarb FS 500 to adult bees

Test substance	Test organism	Ecotoxicological Endpoints:		Reference
Methiocarb FS 500	Honey bee	10 d - LC_{50} 10 d - NOEC	1104.9 µg a.s./kg 420 µg a.s./kg	[redacted] (2015) M-540431-01-1

Table 8.3.1- 5: Honey bee toxicity data generated with formulated methiocarb

Test substance	Test species/study design	Ecotoxicological Endpoints:		Reference
Methiocarb FS 500	Honey bee, 48 & 72h	LD_{50} – oral LD_{50} – contact	**0.11 µg a.s./bee** **0.38 µg a.s./bee (72 h)**	[redacted] (2009) M-357085-01-1 KCP 10.3.1.1.1

Supporting study

Table 8.3.1- 6: Supporting study generated with formulated methiocarb

Bee brood feeding test				
Test substance	**Test species/study design**	**Ecotoxicological Endpoints:**		**Reference**
Methiocarb FS 500	Honey bee brood feeding test (confined conditions, forced exposure of bee colonies to methiocarb-treated sugar solution and methiocarb-treated pollen)	adult mortality, colony strength, colony and brood development	no adverse effects on mortality, colony strength, colony- and brood development, food storage and overall colony vitality up to and including about 20 ppb [µg a.s./kg] in sugar solution (nectar) and up to and including about 48 ppb [µg a.s./kg] in pollen	[redacted] (2015) M-539746-01-1 KCP 10.3.1.5

CA 8.3.1.1 Acute toxicity to bees

As previous acute oral and contact toxicity tests on adult bees in the original submission didn't comply with the new test OECD 213 and 214, new acute studies for methiocarb has been conducted and are also included in this submission. The summary can be found in following sections.

CA 8.3.1.1.1 Acute oral toxicity

Report: KCA 8.3.1.1.1/02 [redacted]; [redacted] 2008; M-308072-01-1
Title: Effects of methiocarb technical (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.: 44011035
Document No.: M-308072-01-1
Guideline(s): OECD 213 and 214 (1998)
Guideline deviation(s): none
GLP/GEP: **yes**

Material and Methods:
Test item: Methiocarb technical (Methiocarb: 100 % w/w nominal, 99.7 % w/w analytical),
Specification: Batch No.: 436700055.
Thirty worker bees per treatment were exposed for 48 hours to doses of 0.30, 0.21, 0.12, 0.07 and 0.03 µg a.i. per bee for feeding (oral, value based on the actual intake of the test item) and to doses of 1.0, 0.50, 0.25, 0.13 and 0.06 µg a.i. per bee for topical application (contact).

Results:

Contact test:

The mortality in the 1.0 and 0.50 µg a.i./bee was 100.0 % and 73.3 % at test end (48 hours), respectively. The mortality in the lower dose levels (0.25, 0.13 and 0.06 µg a.i./bee) ranged between 3.3 % and 6.7 %. No mortality occurred in the water control (water + 0.5 % Adhasit) and solvent control (acetone). During the first 4 hours test item related behavioural abnormalities (e.g. movement coordination problems and/or apathy) were observed in all dose groups except in the 0.06 µg a.i./bee dose level. 24 hours following the application, these behavioural abnormalities still occurred in the two highest dose levels (1.0 and 0.50 µg a.i./bee). During the 48 hours assessment no behavioural abnormalities were found any more in all dose levels

Oral Test:

In the oral test, the maximum nominal dose levels of the test item (0.50, 0.25 and 0.13 µg a.i./bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Oral doses of 0.30, 0.21, 0.12 and 0.07 µg a.i./bee led to dose dependent mortality ranging from 100.0 % to 10.0 % at test end (48 hours after application). No mortality occurred in the 0.03 µg a.i./bee dose group. In the solvent and water control (50 % sugar solution) no mortality occurred. During the 4 hours assessment, behavioural abnormalities (e.g. movement coordination problems and/or apathy) were observed in the four highest dose groups. No behavioural abnormalities were observed in the 0.03 µg a.i./bee dose group. After 24 hours all behavioural abnormalities had gone until test end (48 hours after application).

Toxicity to honey bees in a laboratory tests with Methiocarb technical

Test Item	Methiocarb technical	
Test object	*Apis mellifera*	
Application rate µg a.s./bee	0.30, 0.21, 0.12, 0.07 and 0.03	1.0, 0.50, 0.25, 0.13 and 0.06
Exposure	oral (sugar/acetone/water solution)	contact (solution in acetone)
LD_{50} µg a.s./bee	24 hours: 0.08 48 hours: 0.08	24 hours: 0.49 48 hours: 0.43

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.19 and 0.12 µg a.i./bee, respectively.

Conclusion:

The toxicity of Methiocarb technical was tested in both an acute contact and oral toxicity test on honey bees. The LD_{50} (48 h) was 0.43 µg a.s./bee in the contact toxicity test.

The LD_{50} (24, 48) of Methiocarb technical was determined to be 0.08 µg a.s./bee in the oral toxicity test.

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CA 8.3.1.1.2 Acute contact toxicity

In the study by [redacted] (2009) the acute oral and contact toxicity was assessed together.

Report:	KCA 8.3.1.1.1/02 [redacted],; [redacted]; 2008; M-308072-01-1
Title:	Effects of methiocarb technical (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	44011035
Document No.:	M-308072-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

This study is presented under point KCA 8.3.1.1.1.

Additionally, a contact toxicity study was performed with *Bombus terrestris* which is presented below.

Report:	KCA 8.3.1.1.2/02 [redacted]; 2014; M-479538-01-1
Title:	Methiocarb (tech.): Acute contact toxicity to the bumble bee *Bombus terrestris* L. under laboratory conditions
Report No.:	S13-05155
Document No.:	M-479538-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010) and OECD Guideline 214 (1998), and on the review article of VAN DER STEEN (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:
The contact toxicity of methiocarb (tech.) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).

Material and methods:
In the laboratory, the bumble bees were exposed to 1.2, 2.6, 5.8, 12.8 and 28.1 µg methiocarb a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after treatment. The control groups were exposed for the same period of time under identical exposure conditions to tap water and acetone, respectively.

Results:
In both control groups, treated either with tap water or acetone, no mortality was observed during the 48 h test period.
In the test item treatment group, an overall maximum mortality of 63.3 % was observed at the highest dose level of 28.1 µg methiocarb a.s./bumble bee at the final assessment after 48 hours.
In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

LD_{50} values in the bumble bees contact toxicity test with methiocarb (tech.)

Methiocarb (tech.)	Contact toxicity test [µg a.s./bumble bee]
LD_{50} (24 h)	19.3
LD_{50} (48 h)	19.3

In the test item group, no remarkable sub-lethal effects were observed until the final assessment 48 hours after start of the experimental phase.
The test item dose level of 5.8 µg methiocarb a.s./bumble bee was determined to be the NOED (No Observed Effect Dose).

Conclusion:
The 48 hour contact LD_{50} value for methiocarb (tech.) was determined to be 19.3 µg methiocarb a.s./bumble bee.

CA 8.3.1.2 Chronic toxicity to adult bees

Report: KCA 8.3.1.2/01 [redacted], 2015, M-540431-01-1
Title: Chronic oral toxicity test of methiocarb FS 500 G on the honey bee (*Apis mellifera* L.) in the laboratory
Report No.: 87471136
Document No.: M-540431-01-1
Guideline(s): GLP compliant study based on OECD 213 (1998) and CEB No. 230 with modifications and current recommendations of the ring test group (2014)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:
The objectives of this study were to determine the effects of Methiocarb FS 500 on the honey bees *Apis mellifera* L. in a 10-day chronic feeding test in the laboratory. The No Observed Effect Concentration (NOEC), the No Observed Effect Dietary Dose (NOEDD), the Lethal Concentration (LC_{50}) and the Lethal Dietary Dose (LDD_{50}) were determined at the end of the test period.

Material and Methods:
Test Item: Methiocarb FS 500 G; Methiocarb: 44.9 % w/w, 507 g/L, Sample Description: FAR01756-00, Batch ID: PH13631931, Specification No.: 102000007167, density 1.128 g/mL (at 20°C).
Over a period of 10 days, honey bees were exposed to 50% (w/v) aqueous sucrose feeding solution, with target concentrations of 3360, 1680, 840, 420 and 210 µg methiocarb/kg food [ppb] feeding solution by continuous and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution. Mortality and sub-lethal effects were assessed every day throughout the 10-day exposure period. Furthermore the daily consumption of feeding solution, the mean uptake of test item and the accumulated mean uptake of test item were determined
Samples of the feeding solutions prepared freshly every day throughout the 10-day exposure period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item. During the entire test period the bees were kept under constant darkness except during the assessments.

Reference item (nominal dose): 0.001 mg dimethoate/kg feeding solution 50% (w/v) sucrose solution.

Dates of experimental work: 24 June 2014 – 04 July 2014

Results:

10 days Chronic Oral Toxicity of Methiocarb FS 500 G to young honey bees; laboratory test

Test Object		*Apis mellifera carnica*	
Treatment Group	**Concentration [µg a.s./kg]**	**Dose Level [1] [ng a.s./bee/day]**	**Mortality at day 10 [2] [% Mean]**
Methiocarb FS 500 G	3360	97.2	100 (*)
Methiocarb FS 500 G	1680	66.5	90.0 (*)
Methiocarb FS 500 G	840	30.9	23.3 (*)
Methiocarb FS 500 G	420	14.9	3.3 (n.s.)
Methiocarb FS 500 G	210	6.8	0.0 (n.s.)
Water control	0.0	0.0	3.3
Reference Item	1000	28.7	100.0 (*)
Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC**	**NOEDD**
1104.9 µg a.s./kg	**41.5 ng a.s./bee/day**	**420 µg a.s./kg**	**14.9 ng a.s./bee/day**

[1] Mean dose per bee per day; dose measured based on consumed feeding solution
[2] Mortality at study termination 10 days after start of first feeding
Statistics: Mortality: Fisher`s Exact Test, pairwise comparison, one-sided greater, α = 0.05
NOEC/NOEDD: was estimated using Fisher`s Exact Test (pairwise comparison, one-sided greater, α = 0.05).
n.s. = no statistical significant difference compared to the control, * = statistically significant different compared to the control (α = 0.05)

Observations:

At test end, 10 days following start of exposure, 3.3 % mortality occurred in the untreated water control (50 % w/v sucrose solution). No statistically significant effect on mortality occurred up and including to 420 µg a.s./kg (corresponding to 14.9 ng a.s./bee/day). From 840 µg a.s./kg (corresponding to 30.9 ng a.s./bee/day) onwards statistically significant (Fisher`s Exact Test, α = 0.05) effects on mortality occurred.

At 1680 µg a.s./kg (corresponding to 66.5 ng a.s./bee/day) where 90.0% mortality occurred moribund, affected or apathy bees were observed from day one onwards. At 3360 µg a.s./kg (corresponding to 97.2 ng a.s./bee/day) moribund bees were observed and 100% mortality occurred on day 4.

The reference item (dimethoate) at a concentration of 1 mg dimethoate/kg sugar solution corresponding to 0.029 µg a.s./bee/day caused 100 % mortality at day 7.

Analytical Results:

The actual concentrations of Methiocarb FS 500 G in the feeding solutions were analysed in a separate study which is attached to this final report. The actual concentrations of the feeding solutions were in a range of 96 % - 122 %.

Conclusions:

The chronic oral toxicity of Methiocarb FS 500 G was tested over 10 days.

The LC_{50} value (10 days) was 1104.9 µg a.s./kg feeding solution.

The LDD_{50} value (10 days) was 41.5 µg a.s./bee/day.

The NOEC and NOEDD values (10 days) were 420 µg a.s./kg feeding solution and 14.9 µg a.s./bee/day, respectively.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	KCA 8.3.1.3/01 [redacted]; 2015, M-514260-01-1
Title:	Honey bee (*Apis mellifera* L.) larval toxicity test on methiocarb, technical, single exposure
Report No.:	87511032
Document No.:	M-514260-01-1
Guideline(s):	GLP compliant study based on the OECD TG 237 (2013)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine the acute toxicity of methiocarb, technical after a single exposure to honey bee larvae (*A. mellifera* L.) for a period of 72 hours. Assessment endpoint was mortality of the honey bee larvae during the test period.

Material and Methods:

Methiocarb technical: methiocarb: 98.2 % w/w (analytical), Origin Batch No.: NLL 9134-1-5, Customer Order No.: POX 10247-00; Spec No.: 102000005994, LIMS No.: 1325123; Material No.: 05573092.

Principle of the testing procedure: This toxicity test was performed as a dose response test with a single exposure in an *in vitro* laboratory testing design, according to the OECD Guideline No.237. 36 synchronised first instar larvae of *Apis mellifera*, obtained from three different honey bee colonies, each representing a replicate, were exposed for 72 hours to doses of 1.0, 0.4, 0.16, 0.064, 0.026 and 0.010 µg a.i. per larva *via* treated artificial diets (single exposure). An untreated, respectively a solvent control and a reference item (dimethoate, 98.5 % w/w) were included in the study. The mortality of the larvae was determined 24, 48 and 72 hours after application (each ± 2 hours, respectively). The endpoint of the study was 72 hours after application. The presence of uneaten food was assessed qualitatively at the last assessment date, 72 hours after application.

Dates of experimental work: 02 June 2014 – 05 June 2014

Results:

Toxicity of methiocarb, technical to honey bee larvae; laboratory test, single exposure

Test Item	Methiocarb, technical		
Test Species	Larvae of *Apis mellifera*		
Exposure	Single application *via* treated feeding solutions		
Application rate µg a.i./bee	1.0, 0.4, 0.16, 0.064, 0.026 and 0.010 µg a.i./larva		
	24 h	48 h	72 h
LD_{50} µg a.i./bee	n.d.	0.656	0.547
LD_{20} µg a.i./bee	0.751	0.142	0.103
LD_{10} µg a.i./bee	0.313	0.064	0.043
NOED µg a.i./bee	0.4	0.064	0.064

LD_x values were estimated with Probit Analysis (according to Finney 1971).
The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$)
n.d. = not determined

Observations:

At test end (72 hours following dosing) 58.3 %, 44.4 %, 33.3 %, 13.9 %, 2.8 % and 2.8 % mortality occurred in the test item treated dosing groups of 1.0, 0.4, 0.16, 0.064, 0.026 and 0.010 µg a.i./larva, respectively. The three highest dosing groups were found to be statistically significant compared to the solvent control group (Fisher's exact test, pairwise comparison, one-sided greater, $\alpha = 0.05$).
No mortality occurred in the untreated control and solvent control groups until test end (72 hours).
The reference item (dimethoate) at a dose of 8.8 µg a.i./larva caused 97.2 % mortality after 72 hours.

Analytical Results:

Samples of the stock solution were taken to conduct an analytical determination of the content of the active ingredient on the day of application. The analytical determination of the samples was conducted via HPLC-UV and resulted in 1.004 g/L, corresponding to 103 % of the nominal concentration of the stock solution.

Conclusions:

The toxicity of methiocarb technical was tested in a honey bee larval toxicity test.
The LD_{50} values (48 h + 72 h) were 0.656 and 0.547 µg a.i./larva, respectively.
The NOED values (24 h, 48 h + 72 h) were 0.4, 0.064 and 0.064 µg a.i./larva, respectively.

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Report:	KCA 8.3.1.3/02 [redacted],;[redacted];[redacted]; 2015; M-534766-01-1
Title:	Assessment of potential side-effects on honeybee colonies from exposure to guttation fluid of maize, seed-treated with methiocarb FS 500 G in Germany in 2014/2015
Report No.:	203
Document No.:	M-534766-01-1
Guideline(s):	Taylor made study design, no official test guideline(s) available at present
Guideline deviation(s):	none
GLP/GEP:	**yes**

This field study was conducted in order to investigate the potential effects on honeybee colonies from their exposure to guttation fluid of Methiocarb FS 500 seed-treated maize.

Material and Methods:

Test item: Methiocarb FS 500 G (Spec. No.: 102000007167; Batch No.: PH13633628).

Seed treatments (treatment group and control group): Maize seeds used for the test item treatment group were seed-treated at an nominal application rate of 75 g methiocarb a.s./ 50,000 seeds which corresponds to 1.5 mg methiocarb a.s./seeds while maize seeds used for the control group did not received any insecticidal seed treatment.

Study sites and sowing:

The study was conducted on eight commercially operated maize fields at the vicinity of [redacted], in the North of the Federal State North Rhine-Westphalia, Germany. On four study fields, five study plots were established for maize seeds which were seed treated with the insecticidal seed-treatment product Methiocarb FS 500 G (treatment group comprising study plots T1 to T5), while on the other four study fields five plots were established for maize seeds that received no insecticidal seed-treatment (control group comprising study plots C1 to C5). The airline distances between the treatment plots and the control plots where more than 10 km except for one control and one treatment plot. The airline distances between those study plots were 1.5 km. The average field size was 5.5 ha for the control group and 5.3 ha for the treatment group.

All fields were sown in April 2014 during two sowing days with a three day interval with deflected vacuum-pneumatic sowing machines under typical commercial use conditions. The sowing machines were used according to Good Agricultural Practice (GAP). The target sowing rate was 100,000 maize seeds/ha, which corresponds to 150.0 g methiocarb a.s. per ha in the treatment group.

Honeybee colonies used for the study:

Healthy honeybee colonies were provided by the [redacted]-University [redacted]. Each colony consisted of two bodies and ten frames (Zander type) per body. The colony strength of every colony at the time of set-up was approximately 10,000 adult honeybees. Healthy honeybee colonies were prepared as homogenous as practically possible regarding the number of adult honeybees, brood cells, food storage and brood status. The allocation and set-up of the colonies per study plot was randomised. Honeybee sister queens were hatched in 2013.

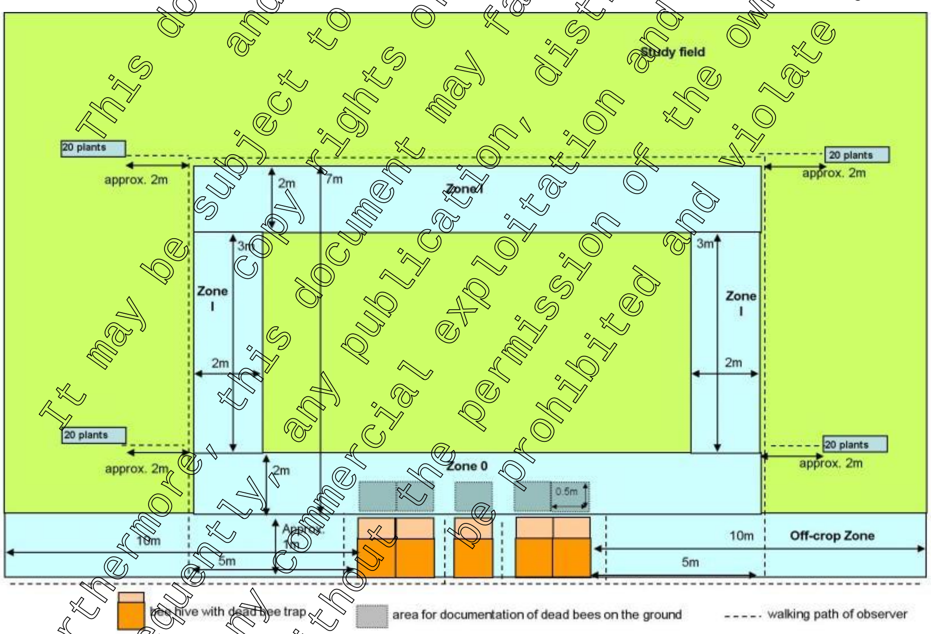
Set-up of honey bee hives:

At each of the ten study plots (five treatment and five control plots, respectively), five honey bee colonies were placed (see the figure below) four to seven days before sowing either within the herbaceous off-crop area directly adjacent to the maize fields or in a distance of 3 m to the field border on the respective study plots. The treatment and the control group comprised each of 25 honey bee colonies.

Assessment area:

An assessment area was defined on each study plot. This area was located in front of the honey bee colonies and it was divided in two in-crop zones (Zone 0 and 1, approximately 60 m², shaped rectangular) and one Off-crop Zone (approximately 25m²). The assessment area stretched up to 7 m (measured from the study field border) perpendicularly to the arranged line of beehives towards the maize crop and encompassed a horizontal distance of 5 m from the left and right border of the outer beehives and of 10 m from the left and right border of the outer beehives (Off-crop Zone).

Adjacent to every study plot, a group of five beehives were set-up parallel to the field margin, with their entrances directed towards the maize crop. The five hives were set up in the following order: two groups of two hives directly adjacent to each other and one single hive. The single hives were positioned in between the two pairs in a distance of approximately 1 m to each side.
Each assessment area had additionally four screening areas, segregated and clearly marked, at the outer corners of Zone 1. Each of these screening areas consisted of 20 maize plants, which were used to record the proportion of maize plants displaying guttation and/or dew.



Example scheme of the assessment area on a study plot with beehives.

Honey bee mortality:

Each hive was equipped with a dead bee trap. The traps were emptied daily in the evening to record the number of dead honey bees. Additionally, also the number of dead bees located on a small plot of 0.5 x 0.5 m² in front of each dead trap was recorded. Mortality assessments started on 11 April 2014, two days after the colonies had been set-up at their respective study plots and were carried out until 13 July 2014 before transport of beehives to hibernation site.

Guttation fluid sampling and estimation of the amount of guttation fluid:

In case guttation was observed in the morning at a respective treatment plot, up to three samples of guttation fluid, each with a volume of approximately 1 ml were collected from various maize plants outside of the established assessment area. The samples were thereafter stored deep frozen (≤ -18 °C) for later residue analysis. Sampling of guttation fluid lasted up to early bloom (BBCH 60) of the maize plants.

The amount of guttation fluid in the In-crop Zone and in the Off-crop Zone was compared by visual estimation whether there was more amount of guttation fluid available on all plants in the Off-crop Zone or on maize plants the In-crop Zones.

Monitoring of occurrence and frequency of guttation as well as observation of honey bees:

On each assessment day, the main prevailing BBCH code of maize plants of the field was determined. Guttation monitoring in the different zones of the assessment area on the respective study plots was started as soon as the maize plants had emerged on the study fields and carried on up to early bloom (BBCH 60).

During morning and evening hours, the respective screening areas on the study plots under investigation were systematically checked for the occurrence of guttation fluid and/or dew.

The beginning of bee flight activity at the beehives in the morning was recorded. The time when at least one beehive started its flight activity was regarded as starting point for the monitoring sessions.

If guttation was still present at the start of honey bee flight activity, the numbers of honey bees resting or walking on the ground or on the maize plants were counted and any potential uptake of guttation fluid or dew by the bees as well as any conspicuous bee behaviour was recorded. During the Assessment Phase, honeybees and guttation were investigated by a series of repeated 'monitoring sessions'. One 'monitoring session' was defined as one complete observation cycle of the assessment areas and their associated segregated areas during the overlapping of presence of guttation and honeybee flight activity. When guttation fluid was still observed at 13:00, the morning sessions were continued once every hour until the end of guttation.

The above mentioned observations lasted until the majority of maize plants on a specific study field reached BBCH 37. At that growth stage honey bee observations could not be conducted due to the height of the plants and increase in their foliage level. Thus, from that growth stage on, the frequency and proportion of guttation were only evaluated once in the morning with the start of bee flight activity. Also, both prevailing BBCH codes and occurring off-crop and in-crop guttation were determined with morning arrivals at treatment study fields. The monitoring period stopped at early bloom of the maize plants (BBCH 60).

One "monitoring session" lasted approximately 35 minutes and was defined as one complete observation cycle of the assessment area and its associated four segregated areas, at which guttation- and honey bee assessments were conducted during the presence of guttation fluid on the maize plants.

Honey bee colony strength and health assessment

The colony strength and the colony development were assessed according to the [redacted] method (Imdorf et al. 1987). The first colony assessment was conducted on 9 April 2014 (four days before the first maize fields were drilled), in order to define the exact starting conditions of the colonies. Afterwards, colony assessments were performed approximately in a 3-weeks interval until the end of October 2014 (i.e. six subsequent assessments during the Assessment Phase at the study fields, followed by five colony assessments at the remote hibernation sites). One colony assessment was performed in April 2015 to evaluate the hibernation ability of the colonies.

To determine the *Varroa* infestation in the honeybee colonies, the natural mite fall was regularly controlled. For this purpose, mite boards ('varroa boards') were placed under the colonies 2 - 4 days before every colony assessment and were controlled during each colony assessment until the end of autumn 2014. In order to account for the transport-related stress of the colonies, the first assessment of the *Varroa* infestation of the colonies was conducted after the first colony assessment, but before maize drilling.

Residue analysis:

Guttation fluid as collected throughout the Assessment Phase on the treatment plots was analysed for residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone by using High Performance Liquid Chromatography (HPLC), chromatographied under isocratic reversed phase conditions and coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

Dates of experimental work: 08 April 2014 – 26 March 2015

Results:

Sowing rates:

The sowing rates in the control group ranged from 95,839 maize seeds/ha to 104,335 maize seeds/ha. The average sowing rate was 100,664 maize seeds/ha. The sowing rates in the treatment group ranged from 99,887 maize seeds/ha to 111,760 maize seeds/ha which corresponds to 163.8 g to 183.3 g methiocarb a.s./ha. The average sowing rate was 105,984 maize seeds/ha (173.8 g methiocarb a.s./ha).

Monitoring of occurrence and frequency of guttation:

Guttation was a frequent phenomenon during the complete assessment phase. In total 372 days (defined as one observer on one study field) were spent for the observation of honeybees and guttation during the morning monitoring sessions. On 314 out of these monitoring days guttation occurred in the morning, resulting in on average 3 h 38 m bee flight activity and guttation occurrence overlap in the morning on these days. Taken as well the monitoring days without morning guttation occurrence and bee flight activity overlap into account, the average overlap duration was 3 h 03 min per morning for the whole exposure period.

Guttation fluid in the morning was observed at 94% of all observation days in the In-crop Zones on maize and at 92% in the herbaceous Off-crop Zones. During the course of the observation days, the presence of guttation was highest in the early morning hours and declined until the end of the regular assessment phase at 13:00 o’clock.

Occurrence of guttation was less in the evening compared to the morning. Here, guttation fluid was observed in the In-Crop Zones on maize at 25% and at 16% of all monitoring days in the herbaceous Off-Crop Zones. On 87 out of 341 days of evening monitoring guttation occurred, resulting in on average 1 h 03 m bee flight activity and guttation occurrence overlap in the evening on these days. Taken as well the evening monitoring days without guttation and bee flight overlap into account, the average overlap duration was 16 min per evening for the whole exposure period.

Approximately two thirds of all recordings the amount of guttation fluid was higher off-crop than in-crop.

Observations of honey bees during guttation monitoring

During 1,657 monitoring sessions in the morning in total 16,328 honey bees have been observed. In total 98.2% of the bees have been observed while sitting on soil surface or on plants while only 0.5% (55 in the control group, 25 in the treatment group) and 1.3% (132 in the control group, 76 in the treatment group) of the bees have been observed while taking up guttation liquid or dew respectively in the In-Crop Zones or in the herbaceous Off-Crop Zones.

A much lower number of honeybees could be observed during the evening monitoring sessions. During 228 monitoring sessions in the evening only 579 honey bees have been observed. In total 82.9% of the bees have been observed while sitting on soil surface or on plants while only 0.2% (0 in the control group, 1 in the treatment group) and 16.9% (5 in the control group, 93 in the treatment group) of the bees have been observed while taking up guttation liquid or dew, respectively, in the In-Crop or in the herbaceous Off-Crop Zones.

Weather conditions

Except for small spikes due to specific microclimatic conditions, weather conditions were similar for all study plots during the entire Assessment Phase from April to July 2014. At the hibernation site, precipitation and temperature developed as expected for the respective time of the year.

Soil characterisation

The different study plot soil samples were very diverse in their grain-size distributions and thus in their soil types, ranging from pure sand to clayey loam.

Honeybee mortality

In the control and treatment group, honey bee mortality was on the same, generally low level, mostly around ten dead bees per day in mean in the dead bee traps (mean daily mortality control group: 10.18 ± 14.17; treatment group: 12.43 ± 15.96 after sowing until end of recording) and around one dead bee on the 0.25 m² areas (0.86 ± 1.77 respective 0.90 ± 2.12). The mean daily mortality of the worker bee brood was on a very low level in the control and the treatment group, both in the dead bee traps (0.73 ± 2.63 respective 0.61 ± 1.85) and particularly in the 0.25 m² areas (0.02 ± 0.16 respective 0.05 ± 0.31). There was quite some variability in mortality, even amongst colonies at the same study plot, indicating that mortality of adult honey bees can be influenced by several factors as weather, colony strength, location and treatment. The variability ranged in the control group between 0 to 158 dead bees and in the treatment group between 0 to 153 dead bees in the dead bee trap per hive and day.

Considering that there is no clear difference in the number of dead honeybee workers between the control and the treatment group as well as the high variability in mortality, no test item related effect could be detected (irrespective whether the colonies were set-up directly adjacent to the field margins or at a distance of approximately 3 m to the crop).

Honeybee colony development

During the course of the study, the control and the treatment group developed in a normal and similar way, no distinct, biologically relevant differences could be detected in both, the number of adult bees and brood cells. There were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly adjacent to the field margins or at a distance of approximately 3 m to the crop). This conclusion is supported by statistical analysis.

Throughout the study, queens were replaced in 20 colonies (11 in control, 9 in treatment group) for different reasons. Beside of that, ten colonies did not survive until the end of the Field Phase in April 2015. Since seven of them were from the control group and only three from the treatment group, a test item related effect can be excluded.

Varroa destructor infestation

Natural daily mite fall was on a generally low and equal level, no significant differences between control and treatment group were detectable. The treatments with formic acid, lactic acid and oxalic acid during late summer and winter were successful and reduced the *Varroa* infestation clearly. Also here, no significant differences between control and treatment group were detectable.

Residue analysis:

Residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone in Maize guttation liquid samples

Treatment group	Residue of Methiocarb [µg/L]	Residue of Methiocarb-sulfoxide [µg/L]	Residue of Methiocarb-sulfone [µg/L]
T1	< LOD – 59	< LOD – 35,100	< LOD – 1,100
T2	< LOD – 31	< LOD – 19,600	< LOD – 506
T3	< LOD – 38	< LOD – 14,600	< LOD – 529
T4	< LOD – 66	< LOD – 26,300	< LOD – 1,060
T5	< LOD – < LOQ	< LOD – 10,700	< LOD – 348

LOQ = Limit of Quantitation = 10 µg/L for guttation liquid samples (all analytes)
LOD = Limit of Detection = 2 µg/L for guttation liquid samples (all analytes)

Residue analysis of guttation fluid revealed that methiocarb, methiocarb-sulfoxide and methiocarb-sulfone-residues were generally highest at the beginning of the assessment phase. Residues of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone declined throughout the assessment phase until its end. The maximum residue level of methiocarb was 0.066 mg/L (study field T4, first sampling event). The maximum residue level of methiocarb-sulfoxide was 35.1 mg/L (study field T1, first sampling event). The maximum residue level of methiocarb-sulfone was 1.1 mg/L (study field T1, first sampling event).

Conclusion:

Guttation of maize plants was a regular occurring phenomenon during the growth period of the investigated maize crop. Time overlap between presence of guttation fluid and bee flight activity was a common phenomenon during morning hours, but less observed in the evening.

Accounting for all honey bees, observed during the individual assessments on the study plots throughout the entire field observation period in both, treatment and control, only a small proportion of bees were directly observed taking up guttation fluid.

Residue analysis of guttation fluid, as collected throughout the duration of the study on the treatment plots, revealed that residues of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone generally peaked shortly after emergence of the dressed maize crop and declined in the further progress of the growth. The maximum residue level of methiocarb was 0.066 mg/L, the maximum residue level of methiocarb-sulfoxide was 35.1 mg/L and the maximum residue level of methiocarb-sulfone was 1.1 mg/L. Each peak value was measured during the first sampling event and declined during the course of the study.

Regarding honey bee mortality, brood and colony development, colony strength and *Varroa destructor*

infestation levels, there were no distinct, biologically relevant differences between treatment and control (irrespective whether the colonies were set-up directly at the field border or at distance of approximately 3 m to the crop). This conclusion is supported by statistical analysis. There were also no distinct, biologically relevant (or statistically significant) differences between treatment and control regarding overwintering performance. No treatment related adverse effects were observed during the entire Assessment Phase and throughout the study.

Overall, it can be concluded that guttation fluid, excreted by maize seed-treated with Methiocarb FS 500 G, does not have unacceptable effects on honey bee colonies under typical commercial use conditions, as there were no adverse acute, short-term or long-term effects on colony strength and development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality, colony health, or on overwintering performance.

Report: KCA 8.3.1.3/03 [redacted]; 2015; M-534762-01-1
Title: Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of methiocarb FS 500 G - Treated maize with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering *Phacelia tanacetifolia* in Germany
Report No.: R12261
Document No.: M-534762-01-1
Guideline(s): ENV/MC/Chem(98)17
ENV/JM/MONO(2002)9
ENV/JM/MONO(99)22
Guideline deviation(s): not specified
GLP/GEP: **yes**

Objective:

According to the Regulation (EC) 1107/2009 (2009), the potential adverse effects of crop protection products on honeybees need to be assessed. Therefore this study aimed to assess potential effects on honeybee colonies during and after vacuum-pneumatic sowing operation of maize seeds, sown directly adjacent to full-flowering *Phacelia tanacetifolia*. The employed maize seeds were commercially treated with Methiocarb FS 500 G (nominal rate: 1.5 mg methiocarb a.s./seed). Moreover, dust drift deposits during the sowing operation of the treated maize seeds were concurrently measured.

The study comprised in total four study fields, two treatment fields and two control fields, all of similar size (in average approximately 5.4 ha sown with *Phacelia* plus approximately 2.6 ha sown with maize). The Methiocarb FS 500 G-treated maize seeds were also dressed with the standard fungicide Thiram SC 700 and drilled on treatment fields only, while maize seeds dressed with Thiram SC 700 only were drilled on the control fields.

Both treatment and control fields were sown with different machines but of the same model of a deflected typical vacuum-pneumatic sowing machine. Potential impacts on the colony development and their hibernation performance were assessed. All assessments made on bee colonies were the same for both treatment groups, i.e. hives placed at the two treatment fields and hives placed at the two control fields. A comparison between the assessments of both groups was made.

Furthermore, concurrent dust drift measurements of the active substance of Methiocarb FS 500 G (a.s. methiocarb) were performed by placing vertical gauze-netting-covered construction fences directly adjacent to the sowing area on the two treatment fields during the sowing operation of the treated maize seeds in each field.

Material and Methods

Test item

Conventional maize seeds, dressed with Methiocarb FS 500 G, at a nominal treatment rate of 1.50 mg a.s. methiocarb/seed).

The maize seeds were treated and bagged at the Seed Growth Competence Center (former Seed Treatment Application Centre) of Bayer CropScience AG in D-[redacted], Germany (non-GLP). The seed treatment was done according to the typical seed-treatment and bagging practises. The seeds received a conventional seed treatment and were dressed in addition to Methiocarb FS 500 G also with the standard fungicide Thiram® SC 700 (active substance; thiram). The seeds were bagged into 1 Unit (=50,000 kernel) paper bags, and are labelled with a unique label for conventional seed bags.

Study sites and GLP-sowing

The study was conducted in the vicinity of [redacted], Eastern Germany, on four different study fields, each treatment group with two fields. To ensure exposition of the honey bees to the potential arising dust drift deposits after the sowing operation, each of the maize fields was surrounded by approximately 5.4 ha flowering Phacelia tanacetifolia, a highly bee attractive crop. The dimension of the maize-drilled area inside the Phacelia tanacetifolia fields on each individual field was approximately 2.6 ha (actual 2.46 to 2.66 ha, Figure S 1). The target drilling rate was 100,000 seeds/ha (actual 97,482 to 98,900 seeds/ha on the treatment fields) which corresponded to nominally 150 g methiocarb/ha (actual 146.22 to 148.35 g methiocarb/ha). For the sowing of the maize seeds on 06 July 2013, two vacuum-pneumatic sowing machines (one for the control, one for the treatment fields, manufacturer: Amazone) were used. Both were equipped with similar deflector technology. All maize seeds were filled in a driving distance of 1km from the study fields into the hoppers of the corresponding sowing machines. This measure was taken to ensure comparable mechanical abrasion of the seeds until the end of sowing of each field.

Prior sowing, mortality and behaviour were assessed daily for eight days (29 June 2013 to 06 July 2013) and the population strength once (01/02 July 2013). After the sowing operation in each field, a period of exposure, the honey bee hives were monitored for 17 days (07 July 2013 to 23 July 2013). During this period mortality and behaviour were assessed daily and the population strength and development once (22/23 July 2013).

After the exposure period the honey bees were relocated to three monitoring sites for further monitoring and hibernation in a region of North-Rhine-Westphalia near [redacted], with no intensive agricultural activities and no major crop in the flowering period. The 64 honey bee hives were set up evenly distributed (one third of the hives of each study field randomly selected to each hibernation location) on three hibernation locations at the monitoring site to avoid potential impacts due to a high density of honey bee hives, like a lack of food due to food concurrence or Varroa destructor infestation. To avoid local factors influencing the results of this study, honey bee hives from the study fields were relocated randomly to the monitoring sites.

Set-up of honey bee hives

In total 64 honeybee colonies were monitored in the study, 16 on each study field. The honeybee colonies were placed in the assessment plots on 27 June, 2013 approximately 3 m from the edge of the maize field (sowing area). The entrance of each hive was directed to the Phacelia areas to recreate the regular apicultural practise. The hives were relocated to the monitoring and hibernation sites in the night between 23 July 2013 and 24 July 2013

Honey bee mortality and behaviour assessments

The mortality of honeybees (e.g. workers, pupae, drones) was recorded daily for 17 days using dead bee traps during the time of exposure (07 July 2013 to 23 July 2013) and a period of eight days prior to the exposure period (29 June 2013 to 06 July 2013) in which the hives were located at the study fields. If on an assessment day ten or more dead bees were found in one dead bee trap of a hive during the exposure period, they were placed in a sample bottle and labelled individually (colony number, date) to preserve the possibility of further residue analysis. Although there were some colonies with more than ten dead bees on single days the mortality was generally inconspicuous and therefore no such analysis was performed. In parallel, observations on behavioural abnormalities of the honeybees were recorded at the entrance hole of the hives during the mortality assessments. When a queen died or showed significant reduced egg-laying capacity, it was replaced by another sister queen. This happened altogether six times (four times in colonies of the control group and two times in colonies of the treatment group).

Honey bee colony strength and health assessment

Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed using the estimation method developed by the Bee Institute [redacted] (Imdorf, Buchlmann et al 1987). The first colony assessment was done shortly after the hives were set up on the edge of the fields but before sowing. This first colony assessment (pre-assessment) defined the starting conditions of the hives before exposure. Three weeks after the pre-assessment, the next colony assessment took place at the end of the exposure period on the study fields. After this assessment, the hives were relocated to the monitoring sites, where four further colony assessments were done before hibernation every three weeks until mid of October 2013. In March 2014, the last colony assessment took place to evaluate the hibernation success of the honey bee hives.

Sampling method

At the time of bagging of the maize seeds at the Seed Treatment Application Centre of Bayer CropScience AG in D-40789 [redacted], Germany, seed samples for Heubach analysis (non-GLP) and seed loading (non-GLP) were taken (non-GLP).

Additionally, field fortification samples (0 µg, 1 µg, 100 µg clothianidin/beta-cyfluthrin/imidacloprid/methiocarb fortified gauze sample) were established just before the start of drilling in order to investigate the stability of the samples during transport and storage.

To measure aerial drift deposits vertically erected gauze-netting-samplers were set up on each assessment plot at the treatment fields. Each sowing operation per row was only performed when the wind speed was below 5 m/s, measured in the middle of the respective study field.

A total of eight units of gauze-netting-samplers (effective sampling area of 2 m x 3.3 m (6.6 m2) each, were set up alternately at a distance of approx. 3 m from the zero line. Shortly before the beginning of the sowing the gauze-netting-samplers were wetted with a 1:1 (v/v) glycerol/water mixture. Soil samples for water content (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing.
30 minutes after the completion of sowing, the gauze samples (five 50 x 50 cm squares, 0.25 m² each) were cut out of each netting unit and immediately transferred into separate polyethylene flasks.

Residue analysis
Methiocarb residues in the gauze samples were determined at the Analytical Test Site Bayer CropScience AG.

Results
Honey bee mortality
In both control and treatment groups, honey bee mortality was on the same low level. In average ten dead bees per day were found during the assessments. Regarding to the mortality, no test item related adverse effect could be detected during the whole field phase. The mortality of the brood was on a very low level (mean control group: 0.52 ± 1.91; mean treatment group: 0.45 ± 1.08). On most days, no dead pupae or larvae was found in the dead bee trap.

Honey bee colony development
Honey bee colony strength showed a similar development in control and treatment group. It was constant during the first three weeks after setup of the bee colonies on the study fields, both in control and treatment group. The amount of brood increased in the same period. This led to a strong increase of the colony strength from the first to the second colony assessment, in colonies of both control and treatment group. From the second assessment (mid of August), the colony strength decreased towards winter and stagnated on a stable level at the 4th and 5th colony assessment. Due to the normal reduction of the breeding activity during winter, the number of worker bees reduced towards spring. Throughout the Field Phase, no significant difference between the mean colony strength of the control and the treatment groups was observed. The slightly but not significant higher colony strength observed in the control group can be explained by the influence of one single hive (colony 90), that developed to a much larger colony size (up to 50,565 worker bees) than the mean colony size (up to 25,289 worker bees (control group, 2nd Assessment on 13/14 August 2013)).
The mean amount of honey bee brood in both treatment groups was in all assessments on the same level. After an increase between the pre- and first assessment the amount of brood decreased rapidly in all hives in both groups to a very low level at the last assessment (shortly before winter). This is a normal development for honey bee colonies, which typically reduce their brood amount towards winter.

Varroa destructor infestation
The infestation with Varroa mites was on approximately the same level in all colonies of both control and treatment group. Statistical analysis (Kruskal-Wallis-test, followed by Mann-Whtiney U-test) revealed significant differences regarding the number of dead mites after both formic acid and the first oxalic acid treatment between the hibernation locations with each 20 to 22 hives, randomly selected

from both groups. There were no significant differences between the locations [redacted] 1 and [redacted] 2, but between these two locations and the location [redacted] in almost all cases.
Since all honey bee colonies that did not survived the winter (three in the control group, one in the treatment group), were located at the location [redacted], it can be concluded that the losses were based on local factors like different Varroa infestation and not by test item related factors.

Residues

No residues were found in the control gauze samples (no fortification). In the field spike samples, the mean recovery at study field T1 was 94 % ± 1.6 for 1 µg methiocarb/gauze sample and 98 % ± 1.6 for 100 µg methiocarb/gauze sample. At study field T2 the mean recovery was 89 % ± 0.0 for 1 µg methiocarb/gauze sample and 99 % ± 2.3 for
100 µg methiocarb/gauze sample.

The Limit of Quantification (LOQ) referring to the determination of methiocarb from gauze netting samples was 1 µg methiocarb/L on/from gauze netting samples, equivalent to 0.04 g a.s./ha. The corresponding Limit of Detection (LOD) was 0.1 µg methiocarb/L on/from gauze netting sample, equivalent to 0.004 g a.s./ha.

On study field T2, a clear wind-depending distribution of residues could be shown. On downwind assessment plots (i.e. assessment plot 1, 2 and 7, main wind direction northeast) the residues on the gauze samples (up to average 10.34 µg methiocarb/0.25 m², equivalent to 0.41 g a.s./ha) were distinctly higher compared to those determined on the upwind assessment plots. Due to changing wind conditions, no clear association of the assessment plots at study field T1 to upwind and downwind was possible. This was also demonstrated by relatively uniform residues on most assessment plots.

Conclusion

To assess the potential effects of a sowing operation of Methiocarb FS 500 G-treated maize seeds on the colony development of honeybees (*Apis mellifera* L.), Methiocarb FS 500 G – treated maize seeds (1.5 mg methiocarb a.s./seed) were sown during bee flight in summer 2013. To increase the possible exposition of the bees to dust, the maize was sown inside adjacent areas of flowering Phacelia tanacetifolia, a highly bee attractive crop, were bees were actively foraging.
The dust drift measurements made during the sowing operation of methiocarb-treated maize seeds on the treatment fields (1.5 mg methiocarb a.s./kernel) indicate that seed-treatment dust, abraded and released during the sowing operation with modified (deflected) vacuum-pneumatic sowing equipment, resulted in a measurable off-crop exposure, which was distinctly higher at the downwind borders of the maize sowing area as compared to the corresponding upwind borders. The maximum vertical dust deposition, as measured by vertically erected gauze-netting units, directly adjacent to the maize sowing area, corresponded to a maximum drift rate of 0.41 g a.s./ha (mean values per sampling plot).
The application of Methiocarb FS 500 G did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength as well as on the bee brood and the hibernation success.

CA 8.3.1.4 Sub-lethal effects

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

CA 8.3.2 Effects on non-target arthropods other than bees

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

Studies on non-target arthropods have been performed with the representative formulation Methiocarb FS 500 and are presented in MCP; Annex point 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No additional studies were conducted. Please refer to point 8.3.2.

CA 8.3.2.2 Effects on Typhlodromus pyri

No additional studies were conducted. Please refer to point 8.3.2.

CA 8.4 Effects on non-target soil meso and macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.
In order to address new data requirements according to Regulation (EC) No 1107/2009, several additional studies on chronic exposure to earthworm have been performed and are submitted within this Supplemental Dossier:

Table 8.4.1- 1: Ecotoxicological endpoints – additional earthworm reproduction studies with active substance methiocarb and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint	Reference
Methiocarb FS 500	*Eisenia fetida* reproduction 56 d, mixed	**NOEC ≥1 mg prod./kg dws [A] ≥0.447 mg a.s./kg dws [A]**	[redacted] (2013) M-465336-01-1
Methiocarb FS 500	*Eisenia fetida* reproduction 56 d, treated seeds	NOEC ≥500,000 treated seeds/ha ≥1.983 mg a.s./kg	[redacted] (2001) M-038648-02-1 KCP 10.4.1
Methiocarb-sulfoxide-phenol	*Eisenia fetida* reproduction 56 d,	**NOEC ≥100 mg pm/kg dws**	[redacted] (2013) M-474567-01-1
Methiocarb-sulfoxide	*Eisenia fetida* reproduction 56 d,	**NOEC 1.12 mg pm/kg dws [C]**	[redacted] (2013) M-469958-01-1
Methiocarb-methoxy-sulfone	*Eisenia fetida* reproduction 56 d,	**NOEC ≥100 mg pm/kg dws**	[redacted] (2013) M-474553-01-1
Methiocarb-sulfone-phenol	*Eisenia fetida* reproduction 56 d,	**NOEC ≥100 mg pm/kg dws**	[redacted] (2013) M-474560-01-1

dws = dry weight soil; a.s. = active substance; pm = pure metabolite; prod. = product
Bold values: endpoints used for risk assessment
[A] corrected by a factor of 2 to address log P_{ow} >2 of methiocarb and the high peat content of 10% in artificial soil
[B] calculated based on test substrate of 3 kg dry weight per test vessel, maximum test rate of 5 treated corn seeds per test vessel and actual loading rate of 1.19 mg a.s./corn seed
[C] Study endpoint derived from 28-d biomass endpoint

Report: KCA 8.4.1/01 [redacted], 2013, M-465336-01-1
Title: Methiocarb FS 500 (S, Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Report No.: 82592022
Document No.: M-465336-01-1
Guideline(s): 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 earthworm (Eisenia fetida) - Part 2: Determination of effects on reproduction, International Organization for Standardization, 1998
Guideline deviation(s): none
GLP/GEP: **yes**

Material and Methods:

Test item: Methiocarb FS 500 G; short code: MTC FS 500 G; batch ID: EDFL012778; specification no.: 102000007167-03; sample description: TOX10071-00; content of a.s.: Methiocarb (H 321) 44.7 % w/w (503.2 g/L) analysed; density: 1.125 g/ml.

Test conditions: Artificial soil according to OECD 222; initial pH 6.3, pH at experimental end 6.0; water content 27.6% to 28.3% (53.0% to 54.5% of maximum water holding capacity, WHC) at experimental start and 29.6% to 31.3% (56.9% to 60.2% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.

Test design: 9 to 10 months old earthworm *Eisenia fetida* (with clitellum and weight range 300 to 564 mg) were obtained from an in-house culture. For each treatment, 10 earthworms were exposed for 56-days in treated artificial soil prepared according to OECD 222. Test concentrations were control, 0.20, 0.36, 0.63, 1.12 and 2.00 mg Methiocarb FS 500 G/kg soil dry weight. 4 replicates for the test item treatments and 8 replicates for the control were conducted. Mortality, weight change, feeding activity and reproduction rate were determined.

As reference item, Luxan Carbendazim 500 FC (Carbendazim 500 g/L nominal) was used. The effects of the reference item were investigated in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	225 – 286
Coefficient of variation of reproduction	≤ 30%	8.0%

All study validity criteria were met.

No mortality was observed in any treatment group.

The body weight changes of the earthworms after 4 weeks exposure to Methiocarb FS 500 G were not statistically significantly different compared to the control up to and including the highest test concentration of 2.00 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, two-sided).

The reproduction rates were not significantly different compared to the control up to and including the highest test concentration of 2.00 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the highest test concentration one worm was found with a detached part of his body. No further behavioural abnormalities were observed in the remaining treatment groups. The feeding activity in all the treated groups was comparable to the control (see table below).

Effect of Methiocarb FS 500 G (MTC FS 500 G) on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Methiocarb FS 500 G (MTC FS 500 G) [mg/kg soil dry weight]	**Control**	**0.20**	**0.36**	**0.63**	**1.12**	**2.00**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Significance	-	-	-	-		
Weight change (day 28) [%]	32.9	36.1	34.4	28.6	22.9	26.4
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	262	263	307	273	254	241
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 56)	-	100.4	117.1	104.1	96.9	92.2
Food consumption [g]	24.9	25.0	25.0	24.8	24.5	24.5
Endpoints [mg test item/kg soil dry weight]						
NOEC (day 28 mortality and weight)	**≥2.00**					
NOEC (day 56 reproduction)	**≥2.00**					
LOEC (day 56 reproduction)	**>2.00**					

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

Reference Item Test: In the most recent test with the reference item Lixan Carbendazim 500 FC (performed under IBACON Study Number 46045022 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 dry weight. The results are shown in Appendix 2.

Conclusion:
In an earthworm reproduction and growth study with Methiocarb FS 500 G (MTC FS 500 G) the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥2.00 mg test item/kg soil dry weight, *i.e.* the highest concentration tested.

<u>Metabolite Methiocarb-sulfoxide phenol</u>

Report:	KCA 8.4.1/02 [redacted]; 2013; M-474567-01-1
Title:	Methiocarb-sulfoxide-phenol:Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Report No.:	82612022
Document No.:	M-474567-01-1
Guideline(s):	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 earthworm (Eisenia fetida) - Part 2: Determination of effects on reproduction, International Organization for Standardization, 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and Methods:

<u>Test item:</u> Methiocarb-sulfoxide phenol; batch code: AE 1371423-01-01; origin batch: SES 10015-1-4; purity: AE 1371423: 99.6% w/w, BCS-Code: BCS-AA50184.

<u>Test conditions:</u> Artificial soil according to OECD 222; initial pH 6.3, pH at experimental end 6.1 to 6.2; water content 28.5% to 30.1% (51.9% to 54.8% of maximum water holding capacity, WHC) at experimental start and 31.9% to 32.6% (58.0% to 59.2% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.

<u>Test design:</u> Approx. 10 months old earthworm *Eisenia fetida* (with clitellum and weight range 323 to 600 mg) were obtained from an in-house culture. For each treatment, 10 earthworms were exposed for 56-days in treated artificial soil prepared according to OECD 222. Test concentrations were control and 100 mg Methiocarb-sulfoxide phenol/kg soil dry weight. Eight replicates for the test item treatment and eight replicates for the control were conducted. Mortality, weight change, feeding activity and reproduction rate were determined.

As reference item, Luxan Carbendazim 500 EC (Carbendazim, 500 g/L nominal) was used. The effects of the reference item were investigated in a separate study.

Results:

Validity Criteria	**Recommended**	**Obtained**
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	240 – 361
Coefficient of variation of reproduction	≤ 30%	13.4%

All study validity criteria were met.

No mortality was observed in any treatment group.

The body weight changes of the earthworms after 4 weeks exposure to Methiocarb-sulfoxide phenol was not statistically significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil (Student t-test, á = 0.05, two-sided).

The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Student t-test, $\alpha = 0.05$, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control (see table below).

Methiocarb-sulfoxide phenol: Effects on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Methiocarb-sulfoxide phenol [mg/kg soil dry weight]	**Control**	**100**
Mortality (day 28) [%]	0.0	0.0
Significance	-	-
Weight change (day 28) [%]	33.6	35.7
Significance [1)]	-	n.s.
Mean No. of juveniles (day 56)	305	339
Significance [1)]	-	n.s.
Reproduction in [%] of control (day 56)	-	111.0
Food consumption [g]	25.0	25.0
Endpoints [mg/kg soil dry weight]		
NOEC (day 28 mortality and weight)		**≥100**
NOEC (day 56 reproduction)		**≥100**
LOEC (day 56 reproduction)		**>100**

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

Reference Item Test: In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil dry weight and higher; the EC_{50} for reproduction was calculated as 1.7 mg carbendazim/kg soil dry weight. The results are shown in Appendix 2.

Conclusion:
In an earthworm reproduction and growth study with Methiocarb-sulfoxide phenol the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥100 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) was determined to be >100 mg test item/kg soil dry weight.

Metabolite Methiocarb-sulfoxide

Report:	KCA 8.4.1/03[redacted]; 2013; M-469958-01-1
Title:	Methiocarb -sulfoxide: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Report No.:	82602022
Document No.:	M-469958-01-1
Guideline(s):	GLP compliant study based on OECD 222, 2004 and ISO 11268-2, 1998
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and Methods:

Test item: Methiocarb-sulfoxide; origin batch no.: SES 1004-2-1; BCS-Code: BCS-AA59439; batch code: AE 1371422-01-01; purity: 99.3% w/w AE 1371422.
Test conditions: Artificial soil according to OECD 222; initial pH 6.3, pH at experimental end 5.9 to 6.0; water content 29.3% to 30.9% (53.3% to 56.1% of maximum water holding capacity, WHC) at experimental start and 31.7% to 34.4% (57.7% to 62.6% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C, photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Test design: 6-7 months old earthworm *Eisenia fetida* (with clitellum and weight range 302 to 600 mg) were obtained from an in-house culture. For each treatment, 10 earthworms were exposed for 56-days in treated artificial soil prepared according to OECD 222. Test concentrations were control and 0.20, 0.36, 0.63, 1.12 and 2.00 mg Methiocarb-sulfoxide/kg soil soil dry weight. Four replicates for the test item treatments and eight replicates for the control were conducted. Mortality, weight change, feeding activity and reproduction rate were determined.
As reference item, Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal) was used. The effects of the reference item were investigated in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	213 – 402
Coefficient of variation of reproduction	≤ 30%	22.6%

All study validity criteria were met.

No statistically significantly increased mortality was observed in any treatment group.

The body weight changes of the earthworms after a 4 week exposure to methiocarb-sulfoxide were statistically significantly reduced compared to the control at the test concentrations of 0.36 and 2.00 mg test item/kg soil dry weight. Since at the test concentrations of 0.63 and 1.12 mg test item/kg soil dry weight no significant effects were observed, the effect at 0.36 mg test item/kg soil dry weight was not considered to be test item related (Dunnett´s t-test, $\alpha = 0.05$, two-sided).

The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 2.00 mg test item/kg soil dry weight (Williams 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 (see table below).

Effect of Methiocarb-sulfoxide on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Methiocarb-sulfoxide [mg/kg soil dry weight]	**Control**	**0.20**	**0.36**	**0.63**	**1.12**	**2.00**
Mortality (day 28) [%]	0.0	2.5	0.0	0.0	0.0	0.0
Significance 1)	-	n.s.	n.s.	n.s.	n.s.	n.s.
Weight change (day 28) [%]	31.7	32.9	19.9	25.1	20.5	18.2
Significance 2)	-	n.s.	*	n.s.	n.s.	*
Mean No. of juveniles (day 56)	297	352	371	361	343	259
Significance 3)	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 56)	-	118.3	124.8	121.5	115.3	87.1
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
	Endpoints [mg/kg soil dry weight]					
NOEC (day 28 mortality)	**≥ 2.00**					
NOEC (day 28 weight)	**1.12**					
NOEC (day 56 reproduction)	**≥ 2.00**					
EC Values (reproduction) 4)	EC_{10}			EC_{20}		
	2.3			9.6		

- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
1) Fisher's Exact Test, α = 0.05, one-sided greater
2) Dunnett´s t-test, α = 0.05, two-sided
3) Williams t-test, α = 0.05, one-sided smaller
3) Probit Analysis

<u>Reference Item Test:</u> 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 dry weight and higher; the EC_{50} for reproduction was calculated as 1.7 mg carbendazim/kg soil dry weight. The results are shown in Appendix 2.

Conclusion:
In an earthworm reproduction and growth study with Methiocarb-sulfoxide the No Observed Effect Concentration (NOEC) for mortality, feeding activity and reproduction of the earthworm *Eisenia fetida* was determined to be ≥ 2.00 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) for mortality, feeding activity and reproduction was determined to be > 2.00 mg test item/kg soil dry weight.

The No Observed Effect Concentration (NOEC) for body weight changes was determined to be the concentration of 1.12 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) for body weight changes was determined to be 2.00 mg test item/kg soil dry weight. The EC_{10} was determined to be 2.3 mg test item/kg soil dry weight; the EC_{20} was determined to be 9.6 mg test item/kg soil dry weight.

Metabolite Methiocarb-methoxy-sulfone

Report:	KCA 8.4.1/04[redacted]; 2013; M-474553-01-1
Title:	Methiocarb-methoxy-sulfone: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Report No.:	82622022
Document No.:	M-474553-01-1
Guideline(s):	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 earthworm (Eisenia fetida) - Part 2: Determination of effects on reproduction, International Organization for Standardization, 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and Methods:

Test item: Methiocarb-methoxy-sulfone; batch code: AE 1371424-PU-01; origin batch: M02546; purity: 98.3% w/w, BCS-Code: BCS-AH64745

Test conditions: Artificial soil according to OECD 222; initial pH 6.3, pH at experimental end 6.1; water content 29.0% to 30.1% (52.7% to 54.8% of maximum water holding capacity, WHC) at experimental start and 30.9% to 31.9% (56.3% to 58.0% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.

Test design: Approx. 10 months old earthworm *Eisenia fetida* (with clitellum and weight range 323 to 600 mg) were obtained from an in-house culture. For each treatment, 10 earthworms were exposed for 56-days in treated artificial soil prepared according to OECD 222. Test concentrations were control and 100 mg Methiocarb-methoxy-sulfone/kg soil soil dry weight. Eight replicates for the test item treatment and eight replicates for the control were conducted. Mortality, weight change, feeding activity and reproduction rate were determined.

As reference item, Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal) was used. The effects of the reference item were investigated in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	240 – 361
Coefficient of variation of reproduction	≤ 30%	13.4%

All study validity criteria were met.
No mortality was observed in any treatment group.

The body weight changes of the earthworms after 4 weeks exposure to Methiocarb-methoxy-sulfone was not statistically significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Student t-test, $\alpha = 0.05$, two-sided).
The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil dry weight (Student t-test, $\alpha = 0.05$, 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 (see table below).

Methiocarb-methoxy-sulfone: Effects on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Methiocarb-methoxy-sulfone [mg/kg soil dry weight]	**Control**	**100**
Mortality (day 28) [%]	0.0	0.0
Significance	-	-
Weight change (day 28) [%]	33.6	37.2
Significance [1)]	-	n.s.
Mean No. of juveniles (day 56)	305	310
Significance [1)]	-	n.s.
Reproduction in [%] of control (day 56)	-	101.6
Food consumption [g]	25.0	25.0
Endpoints [mg/kg soil dry weight]		
NOEC (day 28 mortality and weight)	**≥100**	
NOEC (day 56 reproduction)	**≥100**	
LOEC (day 56 reproduction)	**>100**	

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

Reference Item Test: In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil dry weight and higher; the EC_{50} for reproduction was calculated as 1.7 mg carbendazim/kg soil dry weight. The results are shown in Appendix 2.

Conclusion:
In an earthworm reproduction and growth study with Methiocarb-methoxy-sulfone the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥100 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) was determined to be >100 mg test item/kg soil dry weight.

Metabolite Methiocarb-sulfone-phenol

Report:	KCA 8.4.1/05 [redacted]; 2013; M-474560-01-1
Title:	Methiocarb-sulfone-phenol:Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil
Report No.:	M-474560-01-1
Document No.:	M-474560-01-1
Guideline(s):	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 earthworm (Eisenia fetida) - Part 2: Determination of effects on reproduction, International Organization for Standardization, 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and Methods:

Test item: Methiocarb-sulfone-phenol; batch code: AE 1371425-01-01; origin batch: SES 10016-1-5; BCS code: BCS-AA50214, purity: 99.6% w/w.

Test conditions: Artificial soil according to OECD 222; initial pH 6.3, pH at experimental end 6.1; water content 25.0% to 30.1% (45.5% to 54.8% of maximum water holding capacity, WHC) at experimental start and 31.9% to 32.6% (58.0% to 59.3% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.

Test design: Approx. 10 months old earthworm *Eisenia fetida* (with clitellum and weight range 323 to 600 mg) were obtained from an in-house culture. For each treatment, 10 earthworms were exposed for 56-days in treated artificial soil prepared according to OECD 222. Test concentrations were control and 100 mg Methiocarb-sulfone-phenol/kg soil soil dry weight. Eight replicates for the test item treatment and eight replicates for the control were conducted. Mortality, weight change, feeding activity and reproduction rate were determined.

As reference item, Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal) was used. The effects of the reference item were investigated in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	240 – 361
Coefficient of variation of reproduction	≤ 30%	13.4%

All study validity criteria were met.
No mortality was observed in any treatment group.

The body weight changes of the earthworms after 4 weeks exposure to Methiocarb-sulfone-phenol was not statistically significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil (Student t-test, α = 0.05, two-sided).

The reproduction rate was not significantly different compared to the control at the single test item concentration of 100 mg test item/kg soil (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 (see table below).

Methiocarb-sulfone-phenol: Effects on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Methiocarb-sulfone-phenol [mg/kg soil dry weight]	**Control**	**100**
Mortality (day 28) [%]	0.0	0.0
Significance		
Weight change (day 28) [%]	33.6	32.1
Significance [1)]	-	n.s.
Mean No. of juveniles (day 56)	305	310
Significance [1)]	-	n.s.
Reproduction in [%] of control (day 56)		101.8
Food consumption [g]	25.0	24.9
	Endpoints [mg/kg soil dry weight]	
NOEC (day 28 mortality and weight)	**≥100**	
NOEC (day 56 reproduction)	**≥100**	
LOEC (day 56 reproduction)	**>100**	

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

Reference Item Test: In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 4664502 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil dry weight and higher, the EC_{50} for reproduction was calculated as 1.7 mg carbendazim/kg soil dry weight. The results are shown in Appendix 2.

Conclusion:
In an earthworm reproduction and growth study with Methiocarb-sulfone-phenol the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥100 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) was determined to be >100 mg test item/kg soil dry weight.

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

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

Testing on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) was performed with the representative formulation and four soil metabolites of methiocarb. The corresponding summaries are provided below under point 8.4.2.1.

Table 8.4.2- 1: Ecotoxicological endpoints – Collembola and soil mites reproduction studies with active substance methiocarb and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Methiocarb FS 500	*Folsomia candida* reproduction 28 d, mixed	NOEC	84.7 mg prod./kg dws **37.5 mg a.s./kg dws**	[redacted] (2002) M-062852-01-1
Methiocarb-sulfoxide-phenol	*Folsomia candida* reproduction 28 d, mixed	NOEC	**≥100 mg p.m./kg dws**	[redacted] (2001) M-061346-01-1
Methiocarb-sulfoxide	*Folsomia candida* reproduction 28 d, mixed	NOEC	**50 mg p.m./kg dws**	[redacted] (2001) M-075368-01-1
Methiocarb-methoxy-sulfone	*Folsomia candida* reproduction 28 d, mixed	NOEC	**10 mg p.m./kg dws**	[redacted] & [redacted] (2001) M-088567-01-1
Methiocarb-sulfone-phenol	*Folsomia candida* reproduction 28 d, mixed	NOEC	**≥1000 mg p.m./kg dws**	[redacted] (2001) M-087513-01-1
Soil mites, reproduction				
Methiocarb FS 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	45 mg prod./kg dws **20.12 mg a.s./kg dws**	[redacted] (2013) M-469819-01-1
Methiocarb-sulfoxide-phenol	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg p.m./kg dws**	[redacted] (2013) M-469826-01-1
Methiocarb-sulfoxide	*Hypoaspis aculeifer* reproduction 14 d,	**NOEC**	**10 mg p.m./kg dws**	[redacted] (2013) M-469961-01-1
Methiocarb-methoxy-sulfone	*Hypoaspis aculeifer* reproduction 14 d,	**NOEC**	**≥100 mg p.m./kg dws**	[redacted] (2013) M-469618-01-1
Methiocarb-sulfone-phenol	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg p.m./kg dws**	[redacted] (2013) M-469625-01-1

dws = dry weight soil; a.s. = active substance; p.m .= pure metabolite, prod. = product
Bold values: e

ndpoints used for risk assessment

CA 8.4.2.1 Species level testing

Methiocarb FS500

Report:	KCA 8.4.2.1/05[redacted],; 2002; M-062852-01-1
Title:	Methiocarb FS 500: Effects on reproduction of the collembola *Folsomia candida* in artificial soil
Report No.:	13151016
Document No.:	M-062852-01-1
Guideline(s):	ISO 11267 ISO Soil Quality - Inhibition of reproduction of Collembola (Folsomia Candida) by soil pollutants, 1999
Guideline deviation(s):	the temperature reached 23°C instead of a required maximum of 22°C due to technical reasons
GLP/GEP:	**yes**

Material and methods:

Test item: Methiocarb FS 500, Development No.: 300016791 9, Tox. No.: 5941-00, Batch No.: 233026238, Purity: 496 g/L

Test design: Methiocarb FS 500 was mixed into the soil at 37.5, 75, 150, 300, 600 mg as/kg dry weight soil to which Collembola (*Folsomia candida*) (50 Collembola per treatment group) were exposed at 20 - 23 °C, light 430 - 780 lux, 16 h light : 8 h dark, fed with dried yeast after 14 days, initial soil water content 33 to 34%, initial pH 5.9 to 6.1.

Endpoints were mortality and reproduction.

Toxic standard: Betosip, active ingredient: 166 g/L Phenmedipham, tested concentration: 200 mg Betosip / kg artificial soil, control treated with deionised water.

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	16.0 %
Mean number of juveniles per pest vessel	≥ 100	787
Coefficient of variation of reproduction	≤ 30%	6.6 %

All validity criteria for the study were met.

Effects on collembolan reproduction after 28 days

Test substance	Methiocarb FS 500		
Test object	Folsomia candida		
Exposure	Artificial soil		
Concentration [mg test item/kg soil (dw)]	Adult mortality [%]	Reproduction [% of control]	Mean number of juveniles ± SD
control	16	100	787.0 ± 52.1
37.5	10	91.0	715.8 ± 68.1
75	26	6.8	53.4 ± 24.2
150	50	0.0	0.2 ± 0.4
300	100	0.0	0.0
600	100	0.0	0.0

	Adult mortality	Reproduction
LOEC [mg as/kg artificial soil (dry weight)]	150	75
LC_{50}/EC_{50} [mg as/kg artificial soil (dry weight)]	250.2	86.7
NOEC [mg as/kg artificial soil (dry weight)]	75	37.5

Conclusion:

A statistically significant mortality occurred at the concentration of 150 mg a.s./kg artificial soil (dry weight), NOEC (mortality) was determined to be 75 mg/kg substrate. The LC_{50} (mortality) was determined to be 250.2 mg a.s./kg artificial soil (dry weight).

Reproduction was significantly affected at all tested concentration of Methiocarb FS 500. Based on the evaluated data the NOEC (reproduction) was determined to be 37.5 mg a.s./kg artificial soil (dry weight), the LOEC was determined be 75 mg a.s./kg artificial soil (dry weight). The EC_{50} was estimated to be 86.7 mg a.s./kg artificial soil (dry weight).

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Report: KCA 8.4.2.1/06 [redacted] 2013; M-469819-01-1
Title: Methiocarb FS 500 G: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Report No.: 82591089
Document No.: M-469819-01-1
Guideline(s): OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in artificial soil, adopted October 03, 2008
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods:

Test item: Methiocarb FS 500 G; short code: MTC FS 500 G; batch ID: EDFL012778; specification no.: 102000007167-03; sample description: TOX10071-00; content of a.s.: Methiocarb (H 321): 44.7 % w/w (503.2 g/L) analysed; density: 1.125 g/ml.
Test conditions: Artificial soil based on OECD 226; initial pH 5.7 to 5.9, pH at experimental end 5.5 to 5.6; water content at experimental start 19.6 % to 20.4 % (53.0 % to 55.1 % of the maximum water holding capacity); at experimental end 19.2 % to 19.9 % (51.9 % to 53.8 % of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).
Test design: Predatory mite *Hypoaspis aculeifer* were cultured by IBACON and adult females, approximately 10 days after reaching the adult stage, were exposed to treated artificial soil for 14 days. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil. Eight concentrations (2.5, 4.5, 8.0, 14.2, 25.3, 45.0, 80.0 and 142.2 mg Methiocarb FS 500 G/kg soil dry weight) and one control were tested. Four replicates/concentration and 8 replicates for the control with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 4, 7, 9 and 11. Assessment of adult mortality and reproduction performed after 14 d.
As reference item BAS 152 11 I (a.s. dimethoate, 400 g/L, nominal). The effects of the reference item are investigated at least once a year in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	11.0 %
Mean number of juveniles per pest vessel	≥ 50	253 - 335
Coefficient of variation of reproduction	≤ 30%	10.5%

All validity criteria for the study were met.

The mortality was not significantly different compared to the control up to and including the concentration of 80.0 mg test item/kg soil dry weight. At 142.2 test item/kg soil dry weight mortality was statistically significantly increased (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

Reproduction of the predatory mites exposed to Methiocarb FS 500 G was not statistically significantly different compared to the control up to and including 45.0 mg test item/kg soil dry weight. At the concentration of 80.0 mg test item/kg soil dry weight and higher a statistically significant decrease of reproduction was observed (Williams t-test, $\alpha = 0.05$, one-sided smaller). The results are shown in the table below.
The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 2.0 mg dimethoate/kg soil dry weight and above. The EC_{50} for reproduction was 4.2 mg dimethoate/kg soil dry weight.

Effect of Methiocarb FS 500 G on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study

Methiocarb FS 500 G [mg/kg soil dry weight]	Control	2.5	4.5	8.0	14.2	25.3	45.0	80.0	142.2
Mortality (day 14) [%]	11	5	8	8	8	3	13	8	58
Statistical significance[1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
No. of juveniles (day 14)	286	278	278	286	265	273	260	253	0
Reproduction in [%] of control (day 14)	-	97	97	100	92	95	91	89	0
Statistical significance[2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
Endpoints [mg/kg soil dry weight]									
NOEC (mortality)	80.0								
NOEC (reproduction)	45.0								
EC Values (reproduction)[3)]	EC_{10}			EC_{20}			EC_{50}		
	79.3			83.2			91.2		

n.s. = not significantly different compared to the control   * = significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater   [2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] Probit Analysis

Conclusion:

Methiocarb FS 500 G caused no significant effects on mortality of *Hypoaspis aculeifer* up to and including the concentration of 80.0 mg test item/kg soil dry weight. For reproduction no statistical significance was observed up to and including the concentration of 45.0 mg test item/kg soil dry weight.

Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 45.0 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was determined to be 80.0 mg test item/kg soil dry weight. The EC_{50} was determined to be 91.2 mg test item/kg soil dry weight.

<u>Metabolite Methiocarb-sulfoxide phenol</u>

Report: KCA 8.4.2.1/07 [redacted].; 2013; M-469826-01-1
Title: Methiocarb-sulfoxide phenol: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Report No.: 82611089
Document No.: M-469826-01-1
Guideline(s): OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in artificial soil, adopted October 03, 2008
Guideline deviation(s): none
GLP/GEP: **no**

Material and methods:

Test item: Methiocarb-sulfoxide phenol; batch code: AE 1371423-01-01; origin batch: SES 10015-1-4; purity: AE 1371423: 99.6% w/w, BCS-Code: BCS-AA50184.

Test conditions: Artificial soil based on OECD 226; initial pH 5.9 to 6.1, pH at experimental end 5.8 to 5.9; water content at experimental start 21.3% to 21.5% (50.8% to 51.3% of the maximum water holding capacity); at experimental end 20.3% to 20.5% (48.3% to 48.8% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).
Test design: Predatory mite *Hypoaspis aculeifer* were cultured by IBACON and adult females, approximately 9 days after reaching the adult stage, were exposed to treated artificial soil for 14 days. One concentration of the test item was mixed homogeneously into the soil which was filled in glass vessels bfore the predatory mites were introduced on top of the soil. One concentration (100 mg Methiocarb-sulfoxidephenol/kg soil dry weight) and one control were tested. Eight replicates/concentration and 8 replicates for the control with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 2, 5, 7, 9 and 12. Assessment of adult mortality and reproduction performed after 14 d.
As reference item perfekthion (a.s. dimethoate, 400 g/L, nominal). The effects of the reference item are investigated at least once a year in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	11.0 %
Mean number of juveniles per pest vessel	≥ 50	226 - 268
Coefficient of variation of reproduction	≤ 30%	5.5%

All validity criteria for the study were met.

No statistically significant mortality was observed in the single test item treated group compared to the control, where 11% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

Reproduction of the predatory mites exposed to methiocarb-sulfoxide phenol was not statistically significantly different compared to the control at the single test concentration of 100 mg test item/kg soil dry weight (Student t-test, $\alpha = 0.05$, one-sided smaller). The results are shown in the table below.

The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 3.0 mg dimethoate/kg soil dry weight and above. The EC_{50} for reproduction was 4.2 mg dimethoate/kg soil.

Methiocarb-sulfoxide phenol: Effect on the Predatory Mite *Hypoaspis aculeifer* in a14-day reproduction study

Methiocarb-sulfoxide phenol [mg test item/kg soil dry weight]	**Control**	**100**
Mortality (day 14) [%]	11	1
Statistical significance [1)]	-	n.s.
No. of juveniles (day 14)	254	256
Reproduction in [%] of control (day 14)	-	101
Statistical significance [2)]	-	n.s.
Endpoints [mg/kg soil dry weight]		
NOEC (mortality and reproduction)		100
LOEC (mortality and reproduction)		>100
EC_{50} (reproduction) [3)]		>100

n.s. = not statistically significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater [2)] Student t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value

Conclusion:
Methiocarb-sulfoxide phenol caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* at the single test concentration of 100 mg test item/kg soil dry weight.
Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥100 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be greater than 100 mg test item/kg soil dry weight.

Metabolite Methiocarb-sulfoxide

Report: KCA 8.4.2.1/08 [redacted]; 2013; M-469961-01-1
Title: Methiocarb -sulfoxide: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Report No.: 82601089
Document No.: M-469961-01-1
Guideline(s): GLP compliant study according to OECD 226, 2008
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods:

Test item: Methiocarb-sulfoxide; origin batch no.: SES 10041-2-1; BCS-Code: BCS-AA50439; batch code: AE 1371422-01-01; purity: 99.3% w/w AE 1371422.
Test conditions: In both experiments: artificial soil according to OECD 226; temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark, light intensity within the range of 400 to 800 lux.

1st experiment: pH at experimental start 5.9 to 6.1, pH at experimental end 5.6 to 6.1; water content at experimental start 21.4% to 21.8% (54.9% to 55.9% of the maximum water holding capacity), at experimental end 20.4% to 21.1% (52.3% to 54.2% of the maximum water holding capacity);
2nd experiment: pH at experimental start 6.0 to 6.1, pH at experimental end 5.7 to 5.8; water content at experimental start 21.2% to 22.3% (54.4% to 57.2% of the maximum water holding capacity), at experimental end 20.2% to 21.1% (51.8% to 54.2% of the maximum water holding capacity).

Test design: Predatory mite *Hypoaspis aculeifer* were cultured by IBACON and adult females, approximately 12 days (1st experiment) and 7 days (2nd experiment) after reaching the adult stage, were exposed to treated artificial soil for 14 days. Since the first experiment did not provide a final result, a second experiment was performed studying lower test concentrations.
Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels bfore the predatory mites were introduced on top of the soil. Five concentrations (1st experiment: 18, 32, 56, 100 and 178 mg Methiocarb-sulfoxide/kg artificial soil dry weight, 2nd experiment: 1.0, 1.8, 3.2, 5.6 and 10.0 mg Methiocarb-sulfoxide/kg artificial soil dry weight) and one control were tested; 4 replicates/concentration and 8 replicates for the controls with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 2, 5, 7, 9 and 12 (1st experiment) and on day 2, 4, 7, 9 and 11 (2nd experiment). Assessment of adult mortality and reproduction performed after 14 d.
As reference item perfekthion (a.s. dimethoate, 400 g/L, nominal) The effects of the reference item are investigated at least once a year in a separate study.

Results:

Validity Criteria	Recommended	Obtained (1st experiment)	Obtained (2nd experiment)
Mean adult mortality	≤ 20%	4%	6%
Mean number of juveniles per pest vessel	≥ 50	203 to 267	236 to 331
Coefficient of variation of reproduction	≤ 30%	11.3%	12.7%

All validity criteria for the study were met.

Mortality of *Hypoaspis aculeifer* was not statistically significantly different compared to the control up to and including the test concentration of 10.0 mg test item/kg soil dry weight. At the test concentration of 18 mg test item/kg soil dry weight and above mortality was statistically significantly increased (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).
Reproduction of the the predatory mites exposed to methiocarb-sulfoxide was not statistically significantly different compared to the control up to and including the concentration of 10.0 mg test item/kg soil dry weight. At the concentration of 18 mg test item/kg soil dry weight and above a statistically significant reduced reproduction was observed (Williams t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The results are shown in
The tables below

The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 3.0 mg dimethoate/kg soil dry weight and above. The EC_{50} for reproduction was 4.2 mg dimethoate/kg soil dry weight.

Methiocarb-sulfoxide: Effect on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study – 1st experiment

Methiocarb-sulfoxide [mg/kg soil dry weight]	Control	18	32	56	100	178
Mortality (day 14) [%]	4	18	23	18	65	63
Significance [1)]	-	*	*	*	*	*
No. of juveniles (day 28)	230	122	168	140	54	20
Significance [2)]	-	*	*	*	*	*
Reproduction in [%] of control (day 14)	-	53	73	61	23	9

n.s. = not significantly different compared to the control * = significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater [2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] Probit analysis (based on the mean reproduction values in % of the control of both experiments)
- not applicable

Methiocarb-sulfoxide: Effect on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study – 2nd experiment

Methiocarb-sulfoxide [mg/kg soil dry weight]	Control	4.0	1.8	3.2	5.6	10.0
Mortality (day 28) [%]	6	8	3	8	8	8
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	275	300	303	288	302	282
Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 28)	-	109	110	105	110	102

Endpoints [mg test item/kg soil dry weight]		
NOEC (mortality)	10.0	
NOEC (reproduction)	10.0	
EC Values (reproduction) [3)]	EC_{10}	EC_{20}
	10.34	17.82
95% Confidence Limits	1.33 – 19.95	4.35 – 29.84

n.s. = not significantly different compared to the control * = significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater [2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] Probit analysis (based on the mean reproduction values in % of the control of both experiments)
- not applicable

Conclusion:

Methiocarb-sulfoxide caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the concentration of 10.0 mg test item/kg soil dry weight.

Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 10.0 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was determined to be 18 mg test item/kg soil dry weight. The EC_{10} was determined to be 10.34 mg test item/kg soil dry weight (95% confidence limits 1.33 to 19.95 mg test item/kg soil dry weight) and the EC_{20} was

determined to be 17.82 mg test item/kg soil dry weight (95% confidence limits 4.35 mto 29.84 mg test item/kg soil dry weight).

Metabolite Methiocarb-methoxy-sulfone

Report:	KCA 8.4.2.1/09[redacted],; 2013; M-469618-01-1
Title:	Methiocarb-methoxy-sulfone: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Report No.:	82621089
Document No.:	M-469618-01-1
Guideline(s):	OECD 226: Guidelines for the testing of chemicals, Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in artificial soil, adopted October 03, 2008
Guideline deviation(s):	none
GLP/GEP:	**no**

Material and Methods:

Test item: Methiocarb-methoxy-sulfone; batch code: AE 137424-PU-01; origin batch: M02546; purity: 98.3% w/w, BCS-Code: BCS-AE64745

Test conditions: Artificial soil based on OECD 226; initial pH 5.9 to 6.1, pH at experimental end 5.8 to 5.9; water content at experimental start 21.2% to 21.3% (50.4% to 50.8% of the maximum water holding capacity); at experimental end 20.3% to 20.5% (48.3% to 48.9% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).

Test design: Predatory mite *Hypoaspis aculeifer* were cultured by IBACON and adult females, approximately 9 days after reaching the adult stage, were exposed to treated artificial soil for 14 days. One concentration of the test item was mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil. One concentration (100 mg Methiocarb-methoxy-sulfone/kg soil dry weight) and one control were tested. Eight concentrations and control with 10 female predatory mites each. Feeding of mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 2, 5, 7, 9 and 12. Assessment of adult mortality and reproduction performed after 14 d.

As reference item perfekthion (a.s. dimethoate, 400 g/L, nominal). The effects of the reference item are investigated at least once a year in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	11.0 %
Mean number of juveniles per test vessel	≥ 50	226 - 268
Coefficient of variation of reproduction	≤ 30%	5.5%

All validity criteria for the study were met.

No statistically significant mortality was observed in the single test item treated group compared to the control, where 11% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).
Reproduction of the predatory mites exposed to methiocarb-methoxy-sulfone was not statistically significantly different compared to the control at the single test concentration of 100 mg test item/kg soil dry weight (Student t-test, $\alpha = 0.05$, one-sided smaller). The results are shown in the table below.

The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 3.0 mg dimethoate/kg soil and above. The EC_{50} for reproduction was 4.2 mg dimethoate/kg soil.

Methiocarb-methoxy-sulfone: Effect on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study

Methiocarb-methoxy-sulfone [mg test item/kg soil dry weight]	**Control**	**100**
Mortality (day 14) [%]	11	3
Statistical significance [1)]	-	n.s.
No. of juveniles (day 14)	254	248
Reproduction in [%] of control (day 14)	-	98
Statistical significance [2)]	-	n.s.
Endpoints [mg/kg soil dry weight]		
NOEC (mortality and reproduction)		≥100
LOEC (mortality and reproduction)		>100
EC_{50} (reproduction) [3)]		>100

n.s. = not statistically significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater [2)] Student t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value

Conclusion:
Methiocarb-methoxy-sulfone caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* at the single test concentration of 100 mg test item/kg soil dry weight.
Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥100 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be greater than 100 mg test item/kg soil dry weight.

Metabolite Methiocarb-sulfone phenol

Report:	KCA 8.4.2.1/10[redacted],; 2013; M-469625-01-1
Title:	Methiocarb-sulfone-phenol: Effects on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil
Report No.:	82651089
Document No.:	M-469625-01-1
Guideline(s):	OECD 226: Guidelines for the testing of chemicals - Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in artificial soil, adopted October 03, 2008
Guideline deviation(s):	none
GLP/GEP:	**no**

Material and methods:

Test item: Methiocarb-sulfone-phenol; batch code: AE 1371425-01-01; origin batch: SES10016-1-5; BCS code: BCS-AA50214, purity: 99.6% w/w

Test conditions: Artificial soil based on OECD 226; initial pH 5.9 to 6.1; pH at experimental end 5.8 to 5.9; water content at experimental start 21.2% to 21.3% (50.6% to 50.8% of the maximum water holding capacity); at experimental end 20.3% to 21.0% (48.3% to 49.9% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).

Test design: Predatory mite *Hypoaspis aculeifer* were cultured by IBACON and adult females, approximately 9 days after reaching the adult stage, were exposed to treated artificial soil for 14 days. One concentration of the test item was mixed homogeneously into the soil which was filled in glass vessels bfore the predatory mites were introduced on top of the soil. One concentration (100 mg Methiocarb-sulfone-phenol/kg soil dry weight) and one control were tested. Eight replicates/concentration and 8 replicates for the control with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) ad libitum at test start and on day 2, 5, 7, 9 and 12. Assessment of adult mortality and reproduction performed after 14 d.

As reference item perfekthion (a.s. dimethoate, 400 g/L, nominal). The effects of the reference item are investigated at least once a year in a separate study.

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	11.0 %
Mean number of juveniles per pest vessel	≥ 30	226 - 268
Coefficient of variation of reproduction	≤ 30%	5.5%

All validity criteria for the study were met.

No statistically significant mortality was observed in the single test item treated group compared to the control, where 11% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

Reproduction of the predatory mites exposed to methiocarb-sulfone-phenol was not statistically significantly different compared to the control at the single test concentration of 100 mg test item/kg soil dry weight (Student t-test, $\alpha = 0.05$, one-sided smaller). The results are shown in the table below.

The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 3.0 mg dimethoate/kg soil and above. The EC_{50} for reproduction was 4.2 mg dimethoate/kg soil.

Methiocarb-sulfone-phenol: Effect on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study

Methiocarb-sulfone-phenol [mg test item/kg soil dry weight]	**Control**	**100**
Mortality (day 14) [%]	11	6
Statistical significance [1)]	-	n.s.
No. of juveniles (day 14)	254	268
Reproduction in [%] of control (day 14)		106
Statistical significance [2)]	-	n.s.
	Endpoints [mg/kg soil dry weight]	
NOEC (mortality and reproduction)		≥100
LOEC (mortality and reproduction)		>100
EC_{50} (reproduction) [3)]		>100

n.s. = not statistically significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater [2)] Student t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value

Conclusion:
Methiocarb-sulfone-phenol caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* at the single test concentration of 100 mg test item/kg soil.
Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥100 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be greater than 100 mg test item/kg soil dry weight.

CA 8.5 Effects on nitrogen transformation

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

Table 8.5- 1: Additional studies on nitrogen transformation with methiocarb and its metabolites

Test substance	Test species/study type	Endpoint	References
Mesurol FS 500	Study duration 28 d	no unacceptable effects ≥3.9 mg prod./kg dws **≥1.7 mg a.s./kg dws**	[redacted] (1988) M-013195-01-2
Methiocarb-sulfoxide-phenol	Study duration 28 d	no unacceptable effects **≥1.09 mg/kg dws**	[redacted] (2009) M-023228-01-1
Methiocarb-sulfoxide	Study duration 28 d	no unacceptable effects **≥1.47 mg/kg dws**	[redacted] (2009) M-026518-01-1
Methiocarb-methoxy-sulfone	Study duration 28 d	no unacceptable effects **≥1.33 mg/kg dws**	[redacted] (2009) M-026516-01-1
Methiocarb-sulfone-phenol	Study duration 28 d	no unacceptable effects **≥1.20 mg/kg dws**	[redacted] (2001) M-033536-01-1

grey script = study is part of the Baseline Dossier (Annex I inclusion)

CA 8.6 Effects on terrestrial non-target higher plants

For information on studies already evaluated during the first EU review of methiocarb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.6.1 Summary of screening data

According to the data requirements for plant protection products (Commission Regulation No 284/2013), screening data shall be required for plant protection products other than those exhibiting herbicidal or plant growth regulator activity. For methiocarb screening studies and tier 1 limit tests were conducted with the representative formulation Methiocarb FS 500 and another straight formulation, i.e. Methiocarb SC 500. Details are presented in MCP, Annex point 10.6.

CA 8.6.2 Testing on non-target plants

Please, refer to CA 8.6.1 above.

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

No studies on other terrestrial organisms were necessary.

CA 8.8 Effects on biological methods for sewage treatment

For information on studies already evaluated during the first EU review of methiocarb, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

The study from which the endpoint will be used for risk assessment is summarised below from the original DAR of methiocarb.

Table 8.8- 1: Additional studies on sewage treatment with methiocarb

Test substance	Test species/ study type	Endpoint	References
Methiocarb, tech.	Activated sludge, 3 h	EC_{50} > 10000 mg a.s./L	[redacted] (1999) M-010457-01-1

CA 8.9 Monitoring data

No monitoring data are available.