Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Propineb

-Amendment-

Data Requirements

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

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

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2014-05-30	Original version of document	M-490952-01-1
2015-04-21	Order of references corrected (p. 70) and study summary added for PTU (pp. 75 ff).	M-490952-02-1

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

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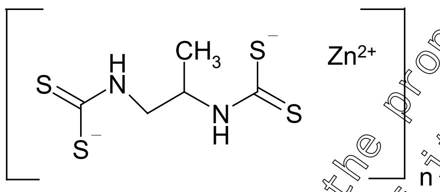
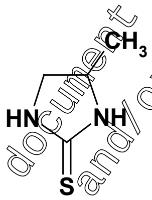
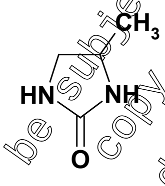
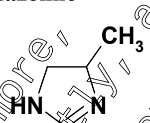
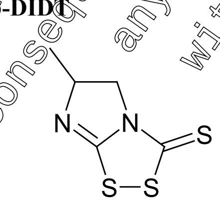
CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Data on the ecotoxicological profile of propineb and its major metabolites had been submitted within the EU Dossier (Baseline Dossier), which resulted in the Annex inclusion under Directive 91/414/EEC in 2003. In the Supplemental Dossier for renewal of approval of propineb presented here only those ecotoxicological studies are described, which had not been submitted within the Baseline Dossier. However, studies which were already evaluated during the first EU review of propineb and which are needed for the ecotoxicological risk assessment are presented in this Supplemental Dossier marked in grey letters in the endpoint tables.
The codes and structures of propineb and its metabolites are presented in Table 8 – 1.

Table 8 - 1: List of codes and structures

Formula Report name used in summaries	**Codes used** *IUPAC index name* / Other names / codes
Propineb  n > 1	Stoichiometric formula: $(C_5H_8N_2S_4Zn)_n$ Molecular mass: 289.76 g/mole (monomer) IUPAC: polymeric zinc propylene-bis(dithiocarbamate) Parent compound, Antracol U, Propineb TK83, LH 30 Z
PTU 	Stoichiometric formula: $C_4H_8N_2S$ Molecular mass: 116.19 g/mole IUPAC: 4-Methyl-imidazolidine-2-thione PTU, Propylene thiourea AE B007299 BCS-AA66386
PU 	Stoichiometric formula: $C_4H_8N_2O$ Molecular mass: 100.12 g/mole IUPAC: 4-Methyl-imidazolidin-2-one BNF 5569 BNF 5569A WAK 5599 PU, Propylene urea AE 1379609 BCS-AA17927
4-Methyl-imidazoline 	Stoichiometric formula: $C_4H_8N_2$ Molecular mass: 84.12 g/mol IUPAC: 4-Methyl-4,5-dihydro-1H-imidazole 4-MI, MI BCS-AB78877 BCS-CT29489 = Cl-salt of 4-Methyl-imidazoline
Propineb-DIDT 	Stoichiometric formula: $C_5H_6N_2S_3$ molecular mass: 190.31 g/mol IUPAC: 6-methyl-5,6-dihydroimidazo[2,1-c][1,2,4]dithiazole-3-thione Methyl-DIDT, methyl-dihydro-imidazo-dithiazole-thione BCS-CU99534

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	Endpoint		Reference
Propineb	Acute oral LD50 *Coturnix coturnix japonica*	LC_{50}	> 5000 mg/kg bw	[redacted] (1985) M-017018-01-2 KCA 8.1.1.1 /01
	Sub-acute dietary LC50 *Coturnix coturnix japonica*	LC_{50}	> 5000 ppm (923.3 mg/kg bw/d)	[redacted] (1994) (amended 2004) M-017011-02-1 KCA 8.1.1.2 /01
	Reproductive NOAEL *Coturnix coturnix japonica*	NOAEL repro (pop.)	2130 ppm (234.8 mg/kg bw/d)	Amended report [redacted] (2004) M-017014-02-1 KCA 8.1.1.3/01
	Reproductive NOAEL *Coturnix coturnix japonica*	NOAEL Repro (ind.)	≥ 550 ppm (64.7 mg/kg bw/d)	[redacted] (2014) M-487532-01-1 KCA 8.1.1.3/02

CA 8.1.1.1 Acute oral toxicity to birds

For information on studies already evaluated during the first EU review of propineb, 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 propineb, 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

In the addendum to the monograph (July 2002), the RMS evaluated the 6-week reproduction study (KCA 8.1.1.3/01; [redacted], 2004: M-017014-02-1) with regard to the protection at the population level as aim of the ecotoxicological risk assessment rather than individuals of a species.
Therefore, the overall reproductive success was considered as the appropriate endpoint, rather than intermediate effects (e.g. the cracked eggs in week 4) that did not affect the overall reproductive success. For this reason, the reproductive toxicity NOEC was set to >2130 ppm (equivalent to >234.8 mg/kg bw/day). Effects observed included temporarily decreased feed intake associated with lower egg weights predominantly at the beginning of the exposure period. As these effects coincided and they had disappeared at the latest after four weeks of treatment they were considered not to be signs of toxicity but to be due to an initial food avoidance with an adjustment to the treated diet over time. Moreover the effects observed at the concentrations 472 and 2130 mg a.s./kg diet had obviously no influence on the overall reproductive output. Therefore, an adverse effect on the population was not observed even at the highest treatment level.

This evaluation by the RMS was supported by the results of an evaluation of the 6-week avian reproduction study reported as amendment to the study report (author of the amendment: [redacted]

2004, see below). The evaluation consisted of a new conceptual and statistical evaluation of the study results on a "per pen" basis over the whole study period. Additionally it was analyzed whether the slightly increased mortality at 2130 ppm may have a relevant influence on the population level. Under the assumption that none of the prematurely dead females at 2130 ppm would rear any healthy chick, the number of all hatched chicks of these females were set to 0, independently whether or not the respective pair had produced any healthy chicks before death or not. The number of chicks per pair was again statistically analyzed. This analysis revealed no differences between any of the test groups and the control. Therefore the overall long-term and reproductive NOEC (on population level) was confirmed at 2130 ppm.

Table 8.1.1.3- 1: Evaluation of the avian reproduction study of propineb (1994 / amendment 2004)

Test substance	Exposure	Species	Endpoint	Reference
Propineb	Reproductive risk assessment	Japanese quail	NOAEL repro (population)	2130 ppm 234 mg a.s./kg bw/d	Amended report [redacted] (2004) M-017014-02-1 KCA 8.1.1.3/02 EU, evaluated

Report:	KCA 8.1.1.3 /01; [redacted], R.;M-017014-02; Amended: 2004-07-16
Title:	Effects of a subchronic dietary exposure of Antracol (80 WG) on japanese quail including effects on reproduction and health
Report No:	SXR/REP/02
Document No:	M-017014-02-1
Guidelines:	**OECD 206 (adopted 04 April 1984)**
GLP/GEP:	**Yes**

Objective: a new evaluation was conducted with statistics on a "per hen" basis according to OECD TG 206 (1984) which is still the officially adopted and thus valid guideline, and derivation of a sound endpoint for population level risk assessment.

Methods: Statistical re-analysis of data on reproductive performance. Data from adults which had to be removed from the cages as a consequence of physical injuries were discarded. In addition, weight data of eggs without a calcium-shell were omitted. Prior to analysis all ratio data (i.e., percentage data) were transformed using a square root arcsine transformation. Statistical analyses were conducted on the transformed data for all parameters that were calculated as a percentage. Bartlett's test of equal variance was performed on a 0.1% level of significance for each parameter to determine if the dose groups have equal variances. If the variances were equal, subsequent analysis was conducted using parametric techniques; otherwise, non-parametric techniques were used.
Parametric procedures involved subjecting study parameters to a standard one-way analysis of variance (ANOVA, a < 0.05). If significant differences among the means were indicated, a Multiple Range Test, e.g. a LSD (Least Significant Difference) - Test on a 5% Level (a < 0.05), was used to determine which treatment groups differed significantly from control.

Results: For reproductive parameters relative to eggs laid, statistically significant (a < 0.05) differences to control birds were found at the 2130 ppm dietary level. The average number of cracked eggs per hen and week was increased during week No.4. Consequently, a reduced number of eggs was set into the breeder, causing other parameters which were related to the mean number of laid eggs to be also significantly decreased in week No.4. Relative to the mean number of incubated eggs, no significant effects were found on a 5% confidence level.
In the 472 ppm group, significant effects on egg fertility (week No.1) and viability of embryos (week No.3) relative to the number of eggs set were found. The probability of errors in estimating the viability by candling eggs of Japanese quail is very high because of the shadows from their dark spotted shells. This made it difficult to reliably interpret these findings. Moreover the significance was only given for two weeks and a clear dose response relationship is not realized. Therefore, these effects are considered as biologically not significant.
The average weight of laid eggs per hen during week No. 2, was significantly decreased for all concentration levels relative to the control (for 95.3 ppm level additionally in week No.1). The egg weights of the 95.3 ppm and the 472 ppm group returned to control level in the third week of exposure. In the 2130 ppm the decrease in egg weight was significant throughout the study. These effects on egg weight correspond directly to the significantly decreased feeding rates, with a time shift of approx. one week which is considered to be associated with the egg development inside females oviduct. Therefore a reduced feeding rate as a consequence of food aversion can be assumed to be responsible for the effects on egg weights.
Aside from the described effects, no significant differences between treated and untreated birds in any other reproductive parameters were observed.

Conclusions: During a 6-week dietary exposure of propineb (presented as Antracol® premix (80VM), results given as mg propineb/kg diet (ppm)), to Japanese quail, significant differences in feed consumption between control and treatment groups were observed. While the food aversion lasted only 1 week in the 95.3 ppm and 472 ppm group, it remained for a period of 4 weeks in the 2130 ppm group.
At the 2130 ppm dietary level, 3 birds died prematurely, showing severe signs of emaciation at gross necropsy, which has to be referred to the reduced food intake. At 472 ppm, 95.3 ppm and in the control, slight anomalies were found at gross necropsy in few birds, often combined with injuries caused by the mating. Birds without injuries showed only very rarely anomalies at necropsy (1 female of the control; 1 female and 1 male at 95.3 ppm; 2 females at 472 ppm; 1 female and 2 males at 2130 ppm).
Based on these results, the NOEC of parental toxicity is 472 ppm. The LOEC for adults is 2130 ppm, based on a slightly increased mortality in combination with emaciation and food avoidance.
During one single week the percentage of cracked eggs was significantly increased at 2130 ppm and in consequence the percentage of living hatchlings related to eggs laid was reduced. Similar numbers of broken eggs were recorded during other weeks also in the control group. Therefore the conspicuous results from week 4 is not considered treatment related.
At 472 ppm the percentage of viable embryos on day 11 related to eggs set was reduced in week 1 and the percentage of viable embryos before hatch related to eggs set was reduced in week 3, both significantly if compared with the control. These results were only found in these particular weeks

without any indication of a dose response. In those weeks the respective data at 2130 ppm were higher and without any statistical differences to the control.
In single weeks the egg weights of dose groups 95.3 pm and 472 ppm were slightly increased if compared with control. Hatchling weights were not affected.
Based on these results the NOEC for reproduction is 2130 ppm.
Additionally it was analyzed whether the slightly increased mortality, which defines the parental NOEC of 472 ppm may have a relevant influence on the population level. Under the assumption that a prematurely dead bird will not rear any healthy chick, the number of all hatched chicks were set to 0, independently whether or not the respective pair had produced any healthy chicks before death or not. The number of chicks per pair was again statistically analysed. This analysis revealed no differences between any of the test groups and the control.
Therefore the overall NOEC (on population level) can be considered to be 2130 ppm (234 mg/kg bw/d).

During the peer-review process it was suggested to conduct a study targeted to evaluate the avoidance induced by propineb in birds. No regulatory study to this purpose was conducted by the notifier, however in the meantime a patent application has been submitted by Bayer CropScience (http://www.google.com/patents/WO2010121733A1?cl=en) for the efficient use of propineb as bird repellent in maize seed treated at 800 and 1600 ppm.

However, another bird reproduction study with propineb has been performed recently in Japanese quail and is submitted within this Supplemental Dossier for renewal of approval of propineb. This new study aimed to overcome the interference of the dose-related initial decrease in food consumption with egg-laying, as seen in the old study amended by [redacted], 2004: M-017014-02-1.
Therefore the test level in the new study were selected at concentrations (250, 400 and 550 ppm) where strong avoidance was not expected, and photo-stimulation for egg-laying was induced after 2 weeks of treatment, when food consumption (in the eventual case of initial avoidance) would have stabilized.

Table 8.1.1.3-2: Additional avian long-term study of propineb

Test substance	Exposure	Species	Endpoint	Reference
Propineb	Reproductive risk assessment	Japanese quail	NO(A)EL 64.7 mg a.s./kg bw/d	[redacted] (2014) M-487532-01-1 KCA 8.1.1.3 /03

Report:	[redacted]h;[redacted];2014;M-487532-01
Title:	Avian reproduction toxicity test of propineb on Japanese quail (Coturnix coturnix japonica)
Report No:	13/069-206FÜ
Document No:	M-487532-01-1
Guidelines:	**OECD 206** (adopted 04 April 1984) **modification of treatment duration according to the request of the sponsor**
GLP/GEP:	**Yes**

Objective: investigate the effect of dietary exposure of propineb on reproduction of Japanese quails.

Material and Methods: Japanese quail were fed a diet containing the test item in concentrations of 0, 305, 487 and 670 ppm propineb technical corresponding to 0, 250, 400 or 550 ppm propineb pure a.s., for a period of 13 weeks. The evaluation of the data is based on ppm of propineb pure a.s. and results are expressed throughout the report in terms of nominal concentrations (except for calculation of the daily dietary dose in mg/kg bw/d which is based on measured data).
Analytical determination of the content of propineb in the quail diet samples was performed by PTRL Europe GmbH as an OECD Multisite activity.

Each dose group was set up with of 20 male and 20 female birds, which were 9 weeks old at the beginning of the treatment. Test birds were housed indoors by dosage groups in pens. Each pen consisted of one male and one female.
After 2 weeks of treatment, birds were induced, by photoperiod manipulation, to start laying eggs. Eggs were collected over a ten-week period, artificially incubated and hatched, and the young maintained for 14 days.
The following parameters were monitored and statistically evaluated:

- Adults: body weight, food consumption, pathological changes.
- Egg parameter: eggs laid, eggshell thickness, eggs cracked/broken, egg weight, egg fertility, viability and embryonic death.
- Offspring parameter: number and body weight of hatchlings and of 14-day old survivors, food consumption and general state of health.

Results:

Chemical analysis

Chemical analysis confirmed the nominal target concentrations of propineb pure a.s., i.e low dose 250 ppm (mean measured: 235 ppm; 94% of nominal), mid dose level 400 ppm (mean measured: 388 ppm, 97% of nominal) and 550 ppm (mean measured: 532 ppm; 97% of nominal), respectively.

Adult birds

Achieved dose: The overall test item intake over 13 weeks of treatment was calculated based on mean body weight, mean food consumption and analytically determined exposure concentrations of pure propineb as 27.9, 45.5 and 64.7 mg a.s./kg bw/day in the low, mid and high dose groups, respectively.

Mortality and clinical signs: One animal died in the control (6166, female), and two animals in the high dose group (6018, male and 6181, female). In the case where one member of a pair died during the treatment period, the other member of the pair was also euthanized (more details in Results section).No mortality or clinical signs considered related to test item exposure was observed during the study.

Body weights: no statistically significant body weight gain differences were observed at any dose level when compared to the control group.

Food consumption: no statistically significant differences were observed after 13 weeks treatment.

Gross-macroscopic post mortem examinations: No test item-related macroscopic changes were detected. No test item related effect on organ weight was observed during the exposure period.

Reproductive Data:

Egg production: No significant differences were observed compared to the control.

Egg weight: No treatment related egg weight differences were observed compared to the control.

Abnormal eggs: No test item related changes were observed in the percentage of abnormal eggs compared to the control.

Eggshell thickness: No test item related effect was observed during the exposure period.

Viable embryos of eggs set (viability) and percentage hatchlings of eggs set: Significant differences were observed between the treated groups and the control. However deviations in the treated groups were in positive direction, and therefore not considered as adverse effect from treatment.

14-Day survivors: Statistically significant differences were observed at the low and at the mid dose groups when compared to the control. However there was no dose-related effect, as the deviation was in negative direction in case of the low dose, while it was in positive direction in the mid dose group, and therefore not considered to be related to treatment with test item.

Body weights of hatchlings and of 14-day survivors: Initial hatchling mean body weight in the test groups was similar to the control. Mean body weight of the 14-day survivor hatchlings was below the control value in the mid dose group, while it was above control in the low and in the high dose groups during the experiment.

Food consumption of the hatchlings: No test item related differences were observed in food consumption between the hatchlings of exposed and control groups.

Mortality and symptoms of the chicks: There were no symptoms of the chicks in the control or in the test item treated groups.

Overall reproduction results (10 weeks of egg-laying)

	Experimental group			
Nominal concentration (ppm)	0 (control)	250	400	550
Mean measured concentration (ppm)	0 (control)	235	388	532
Dietary dose (mg a.s./kg bw/d]	0 (control)	27.9	45.5	64.7
Number of hens (cage)	20	19	19	17
Eggs laid per hen	63	64	64	59
Eggs abnormal per hen	3.65	3.42	3.74	3.00
Eggs not abnormal/eggs laid (%)	94.2	94.7	94.1	94.9
Eggs set per hen	56	57	57	53
Eggs set/eggs laid (%)	88.5	89.2	89.0	89.4
Egg weight (g)	12.82	12.70	12.93	12.67
Eggshell thickness (mean) (mm)	0.205	0.203	0.203	0.196
Viable embryos per hen	52.7	55.1	54.4	50.2
Viable embryos/eggs set (%)	94.3	96.6 [a]	95.7 [a]	94.8 [a]
Live embryos per hen	51.3	54.5	53.9	48.9
Live embryos/viable embryos (%)	97.3	98.9	99.0	97.3
Hatchlings per hen	48.6	52.8 [a]	51.6 [a]	46.2
Initial hatchling body weight (g)	8.96	8.93	9.09	8.93
Hatchlings survivor per hen	37.7	38.0	43.8	37.5
Hatchlings/eggs set (%)	86.9	97.5	90.7	87.2
Hatchlings/eggs laid (%)	76.9	82.6	80.8	78.0
14-d Survivor body weight (g)	60.25	62.36 [a]	55.48 [a]	68.13 [a]
Hatchlings/live embryos (%)	94.6	96.9	95.7	94.6
Hatchling survival/no. hatched (%)	77.1	72.0	84.4	81.2

[a] statistically significant difference, not considered adverse / treatment related

Given the lack of adverse effects in a dose-response pattern, calculation of EC10 or EC20 was not considered meaningful and therefore not conducted.

Conclusion:

Under the conditions of this study the NOEC of Propineb administered in diet to Japanese quails in 13-weeks exposure is considered to be 550 ppm propineb pure active substance, corresponding to an achieved daily dietary dose of 64.7 mg a.s./kg bw/d.

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 propineb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

New LD50 studies submitted with the supplemental dossier

Test item	Species	Test Guidance	Endpoint [mg/kg bw]	Source
Propineb	Rat M+F	OECD 423	LD_{50} > 2000	[redacted] ; [redacted];2010;M-370055-01

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

For information on studies already evaluated during the first EU review of propineb, 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 5.6.1, 5.6.2, or 5.7.1.

The effects of propineb on reproduction and development of litters in rats were studied by [redacted] et al. 1973 (baseline dossier: KCA 5.6.1 /01; [redacted].; et al.; 1973; M-075529-01-1) and recently by [redacted] (2010) (Supplementary Dossier: KCA 5.6.1 /02; [redacted], A. D.; 2010; M-370252-01-1.

In the first study ([redacted] et al. 1973) the rats were exposed to diets containing propineb at concentrations of 0, 20, 60, 200 and 600 ppm for 70 days (pre-mating period) followed by 20-days exposure during the mating periods, and during the pregnancy (21-days) and lactation period (28-days).

In the addendum to the DAR (2003), the wild mammal reproductive risk assessment was based on the treatment level of 200 ppm from [redacted] et al (1973), since at that level there were no effects on the reproductive performance of the parent generation (LOAEL: 600 ppm). Effects on reproductive performance of the second generation were not considered relevant for wild mammal risk assessment, since continuous exposure in the field is limited to only one wild mammal generation, due to the rapid dissipation of propineb. Since the achieved dose was not reported in the study by [redacted] et al. (1973), the 200 ppm level was originally associated in the addendum to the DAR with a dose of 10 mg/kg bw/d. However, nowadays it is considered more appropriate to employ the food conversion factor of 0.08 provided in the EFSA GD 2009 (section 2.3.1.1) for effects seen to relate to the pre-mating phase of a reproduction study, which is also in agreement with the typical food consumption reported for reproduction studies in that laboratory (e.g. [redacted] 2005).

Therefore it is proposed to employ this food conversion factor of 0.08 from the EFSA GD 2009 to the results of [redacted] et al. 1973, resulting in dietary doses of 1.6, 4.8, 16 and 48 mg/kg bw/d at the test concentrations of 20, 60, 200 and 600 ppm, respectively.

In the second reproductive toxicity study ([redacted], 2010) the rats were exposed to diets containing propineb at concentration of 0 30, 60, and 180 ppm. The diets were analysed and the dietary levels of 0, 30, 60 and 180 ppm were equivalent to an achieved intake of propineb in males of approximately

1.6, 3.2 and 10.0 mg/kg bw/day, and in females of approximately 2, 4 and 12.5 mg/kg bw/day during the parental premating period of about 70 days).
Apart from minor yet statistically significant effects on female body weight (5.1% day 35, 7.9% day 63, 7.5% day 70) no effects were observed in the parental generation during pre-mating. Despite the difference in female body weight further increasing with exposure duration, no effects at all resulted in the reproductive performance when producing the F1 offspring.
Given the higher sensitivity of female rat than the male rat to effects induced by propineb, and the much higher relevance for small mammal populations, it is considered appropriate to select the dose achieved by the females in the reproduction studies as endpoint for the risk assessment.

For the reproductive risk assessment, results of both studies ([redacted] et al., 1973; [redacted], 2010) are considered relevant to assess the toxic effects of propineb on the reproductive performances. Therefore their results are combined according to the recommendations of the EFSA GD 2009.
In the reproductive toxicity study run by [redacted] et al. (1973), treatment with 600 ppm in the diet provoked severe clinical signs in the females which were affected by myasthenia of the hind extremities with mobility impairment: the affected animals could hardly reach their feed bowl and feed adequately. As a consequence body weights were decreased and survival was affected during the 70-day premating period.
Of the F0 generation at 600 ppm, only 14/20 female rats were still alive at the first mating and only 13/20 after the second mating. The main consequences of the toxic effects were the reduction of mating success (percent pregnant females) and of live pups per litter at the highest concentration of 600 ppm (Table 8.1.2.2- 1).

Table 8.1.2.2- 1: Effects of propineb on reproductive performances in rat (F1 litter generation)

Concentration [ppm]	Dose [mg a.s./kg bw/d]	Performance [% of resp. control]	Reference
Mating success (proportion pregnant females)			
0	0	100.00	[redacted] et al. (1973), [redacted] (2010)
20	1.6	95.00	[redacted] et al. (1973)
30	2	92.92	[redacted] (2010)
60	4	96.40	[redacted] (2010)
60	4.8	95.00	[redacted] et al. (1973)
180	12.5	96.52	[redacted] (2010)
200	16	95.00	[redacted] et al. (1973)
600	48	50.00 *	[redacted] et al. (1973)
Live pups per litter			
0	0	100.00	[redacted] et al. (1973), [redacted] (2010)
20	1.6	98.04	[redacted] et al. (1973)
30	2	89.57	[redacted] (2010)
60	4	96.60	[redacted] (2010)
60	4.8	97.06	[redacted] et al. (1973)
180	12.5	97.29	[redacted] (2010)
200	16	99.02	[redacted] et al. (1973)
600	48	48.04 *	[redacted] et al. (1973)

* statistically significant effect on reproductive performance of P generation

The effects on the hind limbs were also observed in other toxicity studies with rat. In the chronic toxicity study ([redacted] et al., 1974) (baseline dossier KCA 5.5 /03 [redacted].; et al.; 1974; M-050009-01-1) dietary administration of propineb at 1000 and 2000 ppm (equivalent to approximately to 60 and 120 mg/kg bw/day) produced increased weakness of the muscles in the trunk and hind extremities. This myasthenia gradually resulted in immobility and mortality in the 2000 ppm group after 5 weeks of treatment. The effects were more pronounced in the females.

In the second reproductive toxicity study ([redacted], 2010) the test concentrations were selected in order to avoid the onset of the clinical signs even after multi-generation exposure, and there were no effects on any of the reproductive parameters and pup development in both sexes in any generation.

Therefore, it can be assumed that reproduction in rat is hindered at concentrations provoking severe systemic toxicity and affecting the possibility of adequately feeding.

In the dietary subchronic toxicity study by [redacted] et al. (2003) (EU Addendum KCA 5.3.2 /06; [redacted].;2003;M-108777-01-1) propineb was administered to groups of 10 male and 10 female Wistar rats each at dietary levels of 0, 10, 25, 100 and 400 ppm (equivalent to 0.73, 1.91, 7.60 or 31.52 and 0.89, 2.42, 10.25 or 40.61 mg/kg bw/day in males and females, respectively) up to 14 weeks. Adverse effects were observed on the skeletal muscle of hind limbs in both sexes, females being affected more severely. Observed clinical signs (stepping gait, slow hind limb retraction and dragged hind limbs) were correlated with gross and histopathology findings of the

skeletal muscle in the thigh (fiber atrophy, increased fatty tissue, nerve fiber swelling) and in the skeletal muscle adjacent to the spinal cord, the sternum and in the skin. The study LOAEL was 400 ppm, equivalent to 31.52 and 40.61 mg/kg bw/day in males and females, respectively.
In the 90-day dietary neurotoxicity studies ([redacted] et al., 2004) (EU Addendum KCA 5.7.1 /02; [redacted].;2004;M-066913-01-1) propineb was administered in the diet to young-adult Wistar rats (12/sex/dietary level), using nominal concentrations of 0, 30, 150 and 300 ppm (equivalent to 0, 1.45, 7.63 or 17.11 and 0, 1.90, 9.36 and 21.21 mg/kg bw/day in males and females, respectively) for 13 weeks. All 12 rats/sex/dietary levels were used for neurobehavioral evaluation, with six/sex/dose used for micropathology. Only females were affected and showed decreased body weight and food consumption, clinical signs, decreased motor activity and micropathology findings at the 300 ppm dietary level. The NOAEL is 150 ppm (equivalent to 9.36 mg/kg bw/day) in the females and ≥ 300 ppm (equivalent to 17.11 mg/kg bw/day) in the males.

Also taking the observations from these 90d studies into account, a dietary exposure level of 200 ppm as included in the reproduction study from [redacted] et al (1973) can be considered safe for exposure of the most sensitive gender (females) in the most sensitive species (rat) over an exposure period corresponding to the premating period and first litter production, which significantly exceeds the time period of realistic worst case exposure in the field (2 applications, 10-14 d interval, rapid residue dissipation from foliage or invertebrates).

It is therefore proposed to retain this test level of 200 ppm ([redacted] et al., 1973) that had been selected for the purpose during the Annex 1 listing process for propineb also for the Annex 1 renewal of propineb, for the Tier 1 wild mammal reproductive risk assessment.

There are no new findings contradicting this endpoint assignment. With the appropriate factor of 0.08 for converting endpoints from mammalian toxicity studies from ppm to mg as/kg bw/d (EFSA GD 2009, Table 2), the daily dietary dose for Tier 1 reproductive risk assessment can be established at 200 x 0.08 = 16 mg/kg bw/d.

For higher tier wild mammal risk assessment it is important to note that the high sensitivity to gait abnormalities from myopathic effects after exposure to propineb has consistently been reported at moderate treatment levels in rats (> 20 mg/kg bw/day), with females being more susceptible than males. In the rabbit developmental toxicity study neurological effects (ventro-lateral recumbency, inability to sit or stand, abnormal head position and inability to move the extremities) were observed in the rabbit at 100 mg/kg bw/day but not at 30 mg/kg bw/day (baseline dossier KCA 5.6.2/2; [redacted]., et al; 1988; M-050184-02-1) . No similar neurological effects were observed after dietary exposure in other mammals like mice ([redacted] et al. 1980: baseline dossier KCA 5.5 /06 [redacted].; et al.; 1980; M-056652-01-1) or dogs ([redacted] 1999: Baseline Dossier KCA 5.3.2 /04 [redacted], R. D.; 1999; M-009667-01-1).

Also in targeted studies on the focal species common vole, these kinds of effects were not observed up to concentrations of 1050 ppm, equivalent to ca. 100 mg/kg bw/day (supplementary dossier KCA

8.1.1.2.2 /01; [redacted]; 2013; M-476238-01-1; Supplementary Dossier KCA 8.1.1.2.2 /02; KCA 8.1.1.2.2 /02; [redacted], R.; 2014; M-487560-01-1).

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Report:	[redacted]2; [redacted]; [redacted];2013;M-476238-01
Title:	Toxicity of propineb to the common vole (Microtus arvails)
Report No:	EnSa-13-0672
Document No:	M-476238-01-1
Guidelines:	**Method specifically developed for the test (non-standard species)**
GLP/GEP:	**No**

Objective:

The purpose of this test was to evaluate the relative sensitivity of adult Common voles (*Microtus arvalis*) when exposed to propineb in the diet at doses known to affect locomotion in toxicological studies with rat (*Rattus norwegicus*).

Daily dietary doses of ≥ 40 mg/kg bw/d elicited hindlimb paresis in 8 out of 10 female rats within 4-6 weeks of treatment (EU Addendum KCA 5.3.2 /06; [redacted].;2003;M-108777-01-1). This neurotoxic sign was the most sensitive parameter in rats, whilst such paresis was not observed in mouse *Mus musculus* (Baseline dossier KCA 5.5 /06 [redacted]; et al.; 1980; M-056652-01-1) which was also less sensitive than the rat in a range of other toxicological effects from propineb.

Hindlimb paresis can therefore be considered both as a sensitive marker for toxicity of propineb (indicative of whether other toxic effects on e.g. reproductive performance would be expected at that exposure level), and as a relevant toxic effect itself for wild mammal risk assessment.

The objective of the current investigation was therefore to determine whether hindlimb paresis effects would be induced at a considerably high level of exposure (target: ≥ 100 mg/kg bw/d) in female Common voles.

The Common vole was chosen for this test as representative species behind the generic focal species scenario "small herbivorous mammals – vole" in the EFSA GD 2009.

Material and methods:

In this test, 14 adult Common voles were acclimated under laboratory conditions for 4 weeks to feed on untreated pellets.

After acclimation, a treatment group consisting of 10 voles was selected with regard to minimum fluctuations in the food consumption over the acclimation phase. These voles were initially offered feed pellets containing technical grade propineb (TGAI) at a nominal concentration of 800 ppm (equivalent to a nominal concentration of 643 ppm pure propineb). Four voles received untreated control pellets.

Since bodyweight and actual food consumption in the treatment group still varied considerably between the individuals and over the study period, the treatment level of four voles with low food consumption needed an adjustment in order to achieve in average at least a dose of 100 mg pure propineb/kg bw/d. Following day 8 (two voles) and day 21 (two further voles), these four individuals

therefore received diet containing 1200 ppm technical grade (TGAI) propineb (965 ppm pure propineb) for the remainder of the study.
Throughout the test, behavior and feed consumption were observed daily. Initially, body weight was determined daily, after day 10 body weight was measured 2-3 times per week.
Chemical analysis was conducted to confirm that nominal concentrations were maintained under the conditions of the test.

Results:
During the test, 10 female voles exposed to propineb in the diet achieved mean doses of pure active substance in the target range of 100 mg/kg bw/d over 4 weeks.

Vole	Treatment [ppm] TGAI (pure)	mean FIR/bw	achieved dose [mg pure a.s/kg bw/d]	Observations
Cage 5	800 (643) ppm DAT1-29	0.17	*ca.* 107	no effect on survival, bodyweight, behavior or locomotion
Cage 6	800 (643) ppm DAT1-7 1200 (965) ppm DAT8-29	0.10	*ca.* 86	
Cage 7	800 (643) ppm DAT1-29	0.20	*ca.* 129	
Cage 8	800 (643) ppm DAT1-18 1200 (965) ppm DAT19-29	0.15	*ca.* 111	
Cage 9	800 (643) ppm DAT1-29	0.16	*ca.* 103	
Cage 10	800 (643) ppm DAT1-7 1200 (965) ppm DAT8-29	0.18	*ca.* 156	
Cage 11	800 (643) ppm DAT1-29	0.19	*ca.* 123	
Cage 12	800 (643) ppm DAT1-18 1200 (965) ppm DAT19-29	0.16	*ca.* 143	
Cage 13	800 (643) ppm DAT1-29	0.16	*ca.* 104	
Cage 14	800 (643) ppm DAT1-29	0.20	*ca.* 127	

All voles behaved normally and did not show any signs of impairment, decrease of body weight, behavioural changes or other visually observable symptoms of intoxication.

Conclusion:
These results suggest that Common voles are significantly less sensitive to propineb than rat.

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Report:	[redacted]*; [redacted]; [redacted];2014;M-487560-01
Title:	Common vole (Microtus arvalis): 28-day feeding study with propineb
Report No:	E 2074599-6
Document No:	M-487560-01-1
Guidelines:	**The test is especially designed for testing propineb mixed into feed pellets, with voles**
GLP/GEP:	**Yes**

Material and methods:

The purpose of this test was to evaluate the relative sensitivity of adult Common voles (*Microtus arvalis*) when exposed to propineb in the diet at doses known to affect locomotion in toxicological studies with rat (*Rattus norwegicus*) but not in mice (*Mus musculus*). The Common vole was chosen for this test as representative species behind the generic focal species scenario "small herbivorous mammals – vole" in the EFSA GD 2009.

In this test, 20 adult Common voles were acclimated under laboratory conditions for 4 weeks to feed on untreated pellets.

After acclimation, 10 voles were offered feed pellets containing technical grade Propineb (Batch Code AE F074263-01-03, Spec.-No.: 102000008955, Customer Order No.: TOX 09935-00) at nominal concentrations of 800 or 1250 mg/kg (equivalent to measured concentrations of 551 or 834 mg/kg pure propineb), targeting an average achieved dose of 100 mg/kg bw/d of pure propineb.

Throughout the test, behavior was observed daily.

Feed consumption was measured 3-, and body weight 2 times per week.

Results:

The exposed voles achieved a mean daily dietary dose of 99.5 mg a.s/kg b.w. over the 4 exposure weeks.

The exposed voles did not show any differences to the control voles with regard to behaviour, body weight, food consumption and mobility.

Conclusion:

These results confirm that Common voles are significantly less sensitive to propineb than rat.

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Report:	[redacted]5;[redacted];[redacted];2012;M-428256-02; Amended: 2012-04-10
Title:	Propylene thiourea (PTU) - Selection of the relevant NOAEL for wild mammal long-term, reproductive risk assessment
Report No:	M-428256-02-1
Document No:	M-428256-02-1
Guidelines:	**n.a. (expert evaluation)**
GLP/GEP:	**No**

PropyleneThiourea (PTU) is a mammal and plant metabolite of the fungicide propineb.

For metabolites which were detected in animals at levels above 10%, it is assumed that these metabolites are included in the NOAEL-values of the chronic and reproduction studies conducted with the active substance. Hence the risk assessment for propineb based on the chronic toxicity of this active substance should cover the potential risk deriving from PTU. However, the Scientific Committee on Plants (1) suggested that for PTU an assessment should be made for wild mammals of

the possible effects on the thyroid and the results of these effects on the survival and reproduction rate of wild mammals.

Therefore, the purpose of this document is to evaluate the available toxicity studies carried out with PTU for selecting the relevant toxicological endpoints to be used in wild mammals risk assessment.

The proposal has been developed by taking into account the recommendation provided in the last EFSA guidance documents for the wild mammal risk assessment. Accordingly, results from the toxicity database, in particular reproductive and developmental toxicity studies must be considered.

In addition, for PTU it is important also to consider the carcinogenicity studies in rat and mouse since they add valuable information on the different toxicity effects elicited by different dosing methods (drinking water vs. gavage vs. dietary).

This distinction is important since the exposure pathways for wild mammals to be considered according to EFSA GD (2009) include (i) a dietary exposure scenario and (ii) a drinking water exposure scenario.

One objective of this evaluation was therefore to identify and compare the respective toxicity thresholds, with **clear effects on body weight as benchmark** for potentially relevant wild mammal risk assessment endpoints, between the following PTU studies:

Study	**Administration**	**Effect observation**	**MTD$_{ETX}$**
Rat 2-generation reproduction	given in drinking water	bw ↓ 12% after 21d at **19** mg/kg bw/d	**2.3 mg/kg bw/d** no effects on survival, overall health status or litter development (> 3 months)
Rat developmental toxicity	given by gavage	bw ↓ 12% after 13d at **50** mg/kg bw/d	**7.0 mg/kg bw/d** no effect on dam survival, overall health status or developmental effects critical for the progeny (2 weeks)
Rat chronic / carcinogenicity	mixed in the diet	bw ↓ 20% after 14d at **123** mg/kg bw/d	**7.26 mg/kg bw/d** no effect on survival, health status and thyroid morphology (24 months)
Mouse chronic / carcinogenicity	mixed in the diet	no bw ↓ after 365d at **184** mg/kg bw/d	**21.15 mg/kg bw**/d no effect on survival, health status and thyroid morphology (12 months)

Comparative evaluation of these studies demonstrates that effects on body weight as benchmark, and the relevant toxicity thresholds (MTD: maximum tolerated dose) clearly **depend on the method of administration** employed in the respective study, but also on the duration of exposure. It is therefore proposed to consider the endpoint from the drinking water study (2.3 mg/kg bw/d) only for long-term drinking water exposure scenarios.

The **reproductive toxicity endpoint for dietary exposure scenarios** for PTU can be conservatively derived from the rat developmental study where the dose level of **7 mg/kg bw/d** (after 2 weeks of gavage exposure) did not provoke severe effects, in agreement with the lack of significant effects after lifetime dietary exposure at ≥ 7.26 mg/kg bw/d in chronic rat or mouse studies.

These endpoints are also protective for wild mammal populations from potential adverse effects on the thyroid.

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

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

As the log P_{ow} of the active substance propineb and its metabolites is not above the trigger, no evaluation of secondary poisoning is needed. See MCP point 10.1.1.2 for more details.

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

Information on effects of propineb on reptiles or amphibians is not available. Risk to birds and mammals is assessed in CP section 10.1.

CA 8.1.5 Endocrine disrupting properties

Wild Mammals

Propineb exerts an activity on the thyroid, but is not classified for reproductive toxicity. New data support the lack of classification for reproductive or developmental toxicity. Therefore propineb does not meet the interim criteria as endocrine disrupter.

Based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of propineb in wild mammals. The (adaptive) effects on thyroid weight are covered by the apical endpoints relevant for the wild mammal risk assessment.

Birds

The population relevant effects of propineb on birds were studied in a newly performed reproductive toxicity study on Japanese quail. No statistically significant effects on adult birds, offspring or reproductive parameters were found at 550 mg Propineb/kg diet (equivalent to 64.7 mg/kg bw/d), the highest dose tested.

As there have been established levels at which reproduction was not affected in an avian species, it is concluded that based on an appropriate risk assessment there are no population relevant adverse effects of propineb. Propineb does not fulfil the WHO/IPCS (2002) definition for endocrine disrupters.

No further testing for endocrine disrupting properties is warranted.

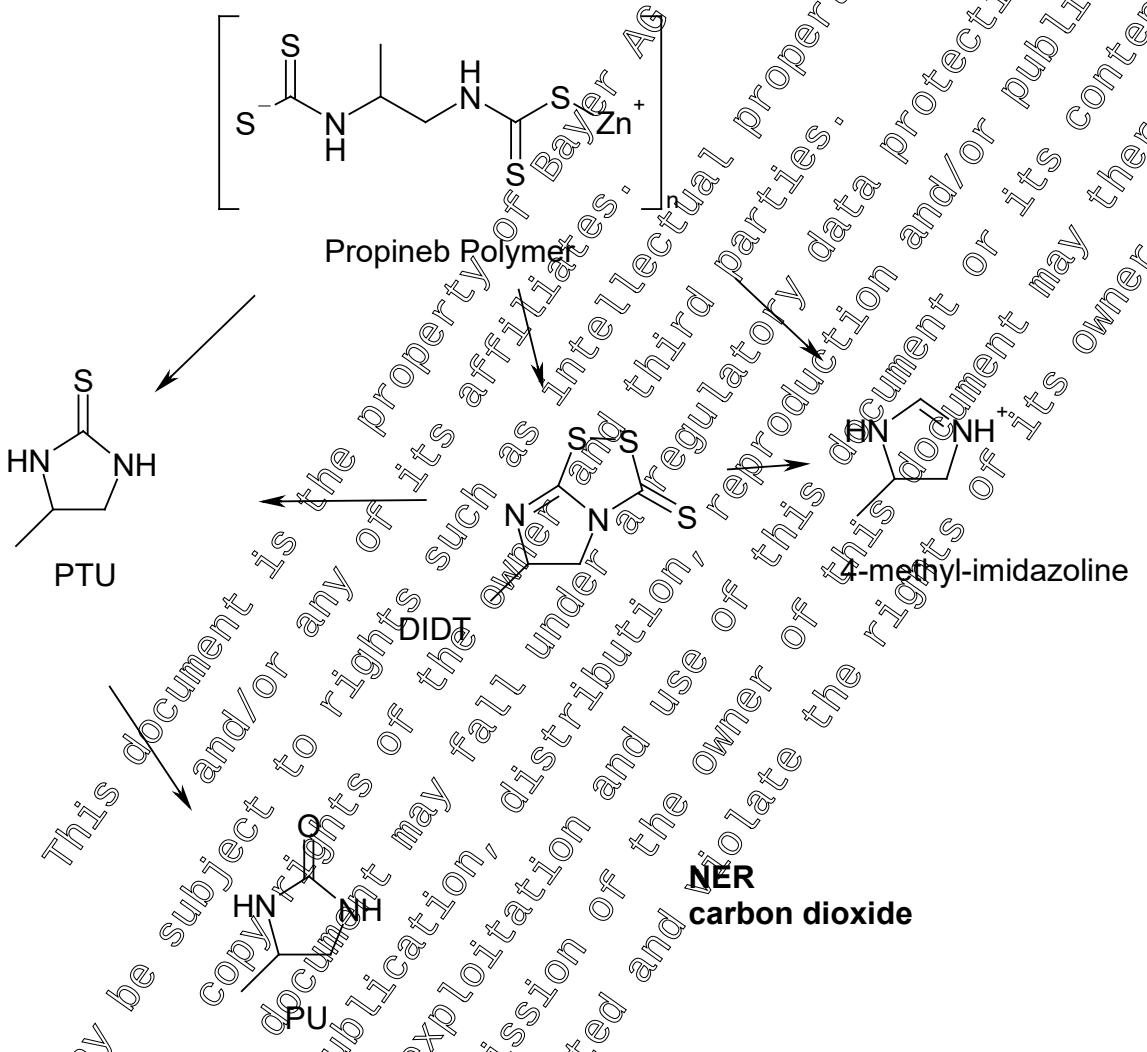
CA 8.2 Effects on aquatic organisms

In order to complete the aquatic risk assessment and to address new data requirements according to Regulation (EC) No 1107/2009, additional studies were performed. Studies that have not been evaluated during the first EU review of this compound, including those on new metabolites, will be summarized.

For studies submitted within the first Annex I inclusion, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

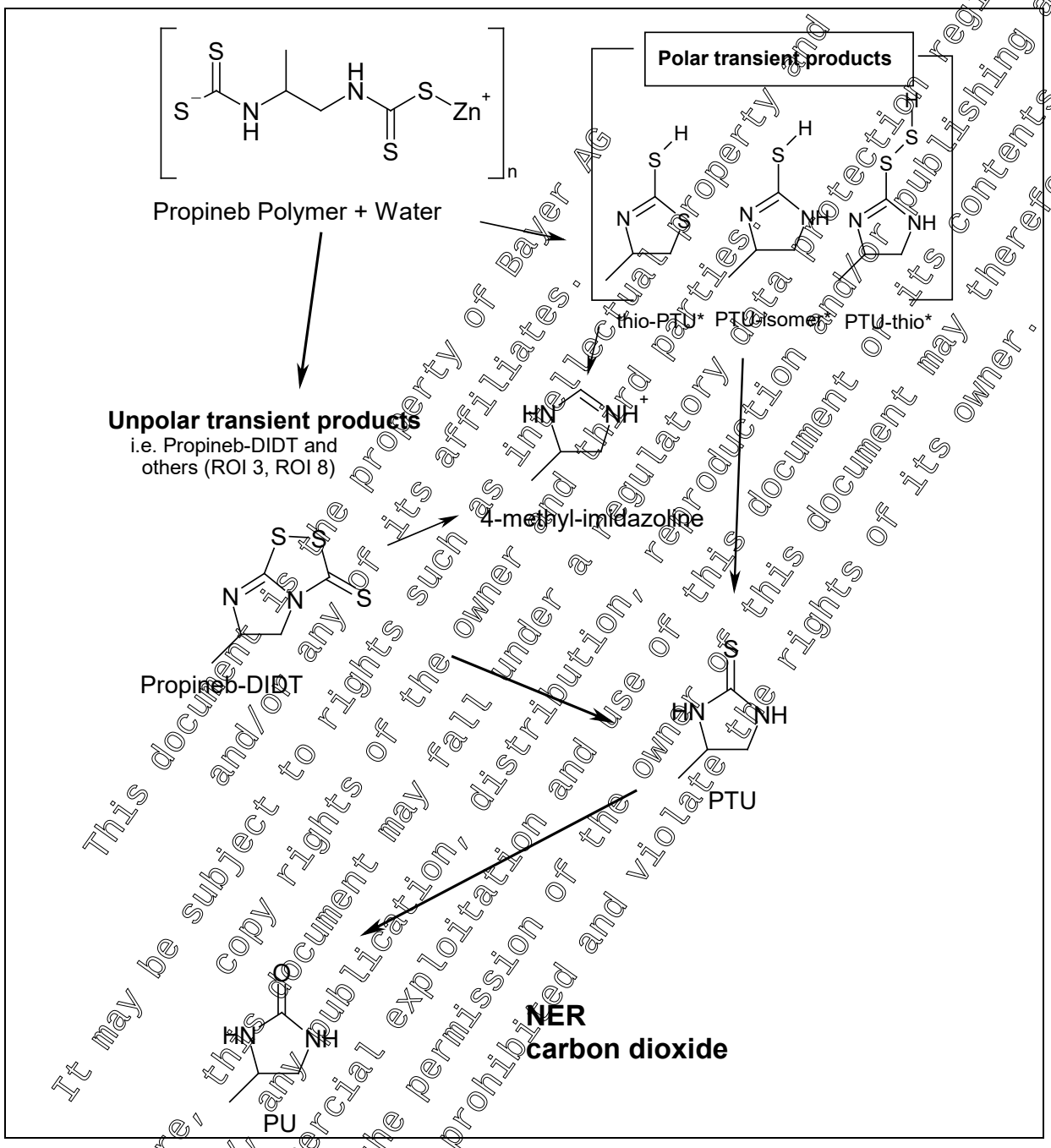
The degradation pathways in soil and water and sediment are given in the two figures below. For further details refer to Section 7: "Fate and behaviour in the environment".

Figure 8.2-1: Proposed degradation pathway of propineb in soil



Remark: In principle NER and carbon dioxide formation can result from all structures shown (either directly or indirectly).

Figure 8.2-2: **Proposed degradation pathway of propineb in natural water (i.e. water containing oxygen and organic matter (like in a water/sediment system).**



*: different isomers (position of methyl group) are possible.

Remark: In principle NER and carbon dioxide formation can result from all structures shown (either directly or indirectly).

Ecotoxicological endpoints

Table 8.2- 2: Endpoints used in risk assessment and additional studies for propineb and its metabolites

Test substance	Test species	Endpoint		Reference
Propineb WG 70	Fish, acute , *Oncorhynchus mykiss*	LC_{50}	6.81 mg product/L	[redacted] (2011) M-401282-01-1 KCP 10.2.1 /01
	Invertebrate, acute *Daphnia magna*	EC_{50}	4.10 mg product/L	[redacted] (2010) M-372880-01-1 KCP 10.2.1 /02
	Invertebrate, chronic *Daphnia magna*	NOEC	0.025 mg product/L	[redacted] (1990) M-016882-01-1 KCP 10.2.2 /01
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50}	0.239 mg product/L	[redacted] (2010) M-397379-01-1 KCP 10.2.1 /03
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	0.67 mg product/L 2.4 mg product/L	[redacted] (1989) M-016881-01-1 KCP 10.2.1 /03
Propineb VM 80	Fish, acute *Oncorhynchus mykiss*	LC_{50}	0.329 mg a.s./L 1)	[redacted] (1998) M-016891-01-1 KCA 8.2.1 /01
	Fish, acute *Cyprinus carpio*	LC_{50}	> 66.7 mg a.s./L	[redacted] (2004) M-088008-02-1 KCA 8.2.1/06
	Fish, chronic *Oncorhynchus mykiss*	NOEC	0.0823 mg a.s./L 1)	[redacted] (1998) M-016895-01-1 KCA 8.2.2 /01
	Invertebrate, acute *Daphnia magna*	EC_{50}	1.50 mg a.s./L 3)	[redacted] (2004) M-086995-01-1 KCA 8.2.3 /03
	Invertebrate, chronic *Daphnia magna*	NOEC	0.015 mg a.s./L 4)	[redacted] (1991) M-016899-01-1 KCA 8.2.5.1 /01
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50} E_rC_{50}	0.017 mg prod./L 0.055 mg prod./L	[redacted] (2004) M-088372-01-1 KCA 8.2.6.1 /04
Propineb WP 70	Invertebrate, chronic *Daphnia magna*	NOEC	0.480 mg a.s./L 5)	[redacted] (2005) M-252129-01-1 KCA 8.2.5.1 /04
	Lentic freshwater microcosm *Oncorhynchus mykiss*	NOEC	> 0.6 mg a.s./L 2)	[redacted] (2005) M-246864-02-1 KCA 8.2.8 /01
	Sediment dweller *Chironomus riparius*	EC_{15}	0.89 mg a.s./L	[redacted] (2005) M-253817-01-1 KCA 8.2.5.3 /03
Propineb-DIDT 6)	Invertebrate, acute *Daphnia magna*	EC_{50}	0.112 mg pm/L	[redacted] (2014) M-481861-01-1 KCA 8.2.4.1/04
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50}	0.114 mg pm/L	[redacted] (2014) M-485275-01-1 KCA 8.2.6.1/05

Test substance	Test species	Endpoint	Reference
PTU (propylene thiourea)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >100 mg pm/L	[redacted] (1994) M-016918-01-1 KCA 8.2.1/03
	Fish, chronic *Oncorhynchus mykiss*	NOEC ≥ 102 mg pm/L	[redacted] (1998) M-016913-01-1 KCA 8.2.2/02
	Invertebrate, acute *Daphnia magna*	EC_{50} 18.4 mg pm/L	[redacted] (1994) M-016919-01-1 KCA 8.2.4.1/02
	Invertebrate, chronic *Daphnia magna*	NOEC 3.2 mg pm./L	[redacted] (1998) M-016917-01-1 KCA 8.2.5.1/02
	Chironomid, chronic, spiked water *Chironomus riparius*	NOEC ≥ 0.1 mg pm/L	[redacted] (1998) M-016914-01-1 KCA 8.2.5.3/01 KCA 8.2.5.4/01
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50} >100 mg pm/L E_rC_{50} >100 mg pm/L	[redacted] (1998) M-016916-01-1 KCA 8.2.6.1/03
PU (propylene urea)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >100 mg pm/L	[redacted] (1994) M-016922-01-1 KCA 8.2.1/04
	Fish, chronic *Oncorhynchus mykiss*	NOEC ≥ 2.0 mg pm/L	[redacted] (1994) M-016921-01-1 KCA 8.2.2.1/01
	Invertebrate, chronic *Daphnia magna*	EC_{50} > 100 mg pm/L[7)] NOEC ≥ 100 mg pm/L	[redacted] (1994) M-016924-01-1 KCA 8.2.5.1/03
	Chironomid, chronic *Chironomus riparius*	NOEC ≥ 0.1 mg pm/L	[redacted] (1994) M-016926-01-1 KCA 8.2.5.3/02
	Algae, growth inhibition *Scenedesmus subspicatus*	E_bC_{50} > 1000 mg pm/L E_rC_{50} > 1000 mg pm/L	[redacted] (1995) M-016920-01-1 KCA 8.2.6.1/02

a.s. = active substance; pm = pure metabolite; prod. = product

1) The EU agreed endpoint refers to the tested item Propineb VM 80. The endpoint used in risk assessment is calculated on a basis of 82.3% a.s. content (nominal initial with analysis)

2) Endpoint of microcosm study is used in the refined risk assessment

3) Lower endpoint obtained with a new study ([redacted] 2004, M-086995-01-1)

4) The value of 0.026 mg as/L that appears in the EU Review Report for propineb is mistakenly attributed to the NOEC but is in reality the EC_{50} (see KCA 8.2.5). The NOEC of 0.015 mg as/L is the endpoint used in the risk assessment

5) Endpoint of the 35 d *Daphnia* population study is used in the refined risk assessment

6) Based on the low acute toxicity of propineb and the transient nature of the metabolite, no acute fish test is considered necessary.

7) The EU agreed endpoint refers to the NOEC from the chronic test, and this endpoint is also used for the acute risk assessment.

Additional studies were performed, which were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the propineb Annex I Renewal. These studies will be summarized in the respective chapters below.

The metabolite 4-methyl-imidazoline (4-MI) had already been identified as a major metabolite in soil in the baseline dossier, without a need for aquatic testing. It now also occurs in the water-sediment study at initially (0 h) 17.5 %, but not after 4 h and 24 h and at later samplings at just over 5%. Due to its transient nature and similarity with the acutely non-toxic PU, instead of aquatic tests, QSAR calculations were performed, confirming low toxicity. Accordingly, the risk assessment will only be performed for acute effects. The overall lowest aquatic endpoint of several different QSAR calculations was acute toxicity to Daphnia with an EC_{50} at 7.3 mg/L. Algae EC_{50} 49 mg/L and fish acute LC_{50} 393 mg/L (KCA 8.2.8 /02; [redacted].; M-488533-01-1).

Two spiked-water studies with Chironomus for the metabolites PTU and PU are available that indicate low chronic toxicity as for the other aquatic organisms. Since both metabolites are not major metabolites in sediment and as the endpoints derived are lower bound values (both NOECs ≥ 0.1 mg/L, from limit tests without effects), no separate risk assessment is performed using these data.

The 35-day *Daphnia* population study in a water-sediment system with exponentially growing *Daphnia* populations includes multiple applications. At the highest test concentration of 960 µg/L complete recovery of the *Daphnia* population was observed. This recovery-based endpoint is not used here to derive a NOEC. In all other concentrations no acute or chronic effects on the population were observed from propineb or its metabolites. Therefore, this chronic study has a NOEC of 480 µg a.s./L that can be used for the ecological risk assessment of the test compound to invertebrates for both exposures, chronic and acute. In conclusion, the NOEC of 480 µg/L and a chronic assessment factor of 10 can be used for the final risk assessment for aquatic invertebrates.

A 28-day higher tier study with Propineb WP 70 on rainbow trout in a microcosm enclosure under semi-field exposure conditions including multiple applications is available. It showed no effects at 600 µg a.s./L thus demonstrating that multiple exposure does not increase the toxicity of propineb.
An acute laboratory study with Propineb VM 80 resulted in a LC_{50} endpoint of 329 µg a.s./L and a NOEC of 125 µg a.s./L (KCA 8.2.1 /01, [redacted], M.; 1998; M-016891-01). This acute/chronic-ratio also underlines that the toxicity observed in the acute study does not increase in studies with prolonged exposure. Thus the 28-day outdoor enclosure study covers both exposures, chronic and acute. It is generally assumed and confirmed in a study with carp that rainbow trout is the most sensitive fish species. Thus the chronic assessment factor of 10 can be used for the final risk assessment for fish based on the 28-day microcosm enclosure.

CA 8.2.1 Acute toxicity to fish

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

Report:	[redacted];[redacted];2004;M-088008-02; Amended: 2004-09-23
Title:	Acute toxicity of Propineb to fish (Cyprinus carpio)
Report No:	DOM 24020
Document No:	M-088008-02-1
Guidelines:	**Directive 92/69/EEC, C.1 (1992); JMAFF 12 Nousan No. 8147 (2000); EPA-FIFRA § 72-1 (1982), considering also OECD No. 203 (1992), and OPPTS 850.1075 (1996)**
GLP/GEP:	**Yes**

Objective:

The aim of the study was to determine the acute toxicity of the test item to Carp (*Cyprinus carpio*), expressed as 96h-LC_{50} for mortality.

Materials and Methods:

Test item: Propineb (VM 80), purity: 82.4% w/w; batch no. EDFU310354 was tested; tox no.: 6603-00.

Test organism: Carp (*Cyprinus carpio*), mean body length 4.0 cm, mean body weight 0.8 g. The biomass loading for this test was 0.60 g fish / L test medium.

Thirty fish were exposed in a limit test for 96 h under static test conditions to a nominal (mean measured) concentration of 100 (66.7) mg test item (a.s.) / L against a water control with further 30 fish.

During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning. Within the study the pH-value, the oxygen saturation level and the temperature were measured daily. Dissolved oxygen concentrations ranged from 96% to 99% oxygen saturation, the pH values ranged from 6.9 to 7.2 and the water temperature ranged from 22.3°C to 22.6°C in all aquaria over the whole testing period. The photoperiod was 16 hours of light and 8 hours dark.

After 4, 24, 48, 72 and 96 hours of exposure the fish were inspected for the number of deaths, toxic symptoms or abnormalities. The mortality (%) after 4, 24, 48, 72 and 96 hours of exposure was calculated in each treatment group. In all groups, the concentrations of the test substance were measured at the same five time-points.

The endpoints were expressed as mean measured concentrations.

Dates of experimental work: April 05, 2004 to April 09, 2004

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality in the control	≤ 10%	0%
Constant water quality and environmental conditions during the test	Yes	Yes
Concentration of dissolved oxygen	≥ 60%	78 - 88%

All validity criteria for the study were met.

Analytical results:
Based on analytical determination (in water by HPLC - UV) of propineb measured values between 62% and 70% of nominal were found. All results are based on mean measured concentrations:
It was not possible to analyze propineb directly in the test solutions used for the exposure of fish owing to its low solubility and low hydrolytic stability in water. Therefore propineb samples were hydrolyzed totally by forming of propylene-thiourea (PTU) by further processing of water samples at 65°C for 24 hours. After this process water samples were analyzed for PTU, followed by recalculation from PTU to propineb based on molecular weight.

Biological results:
There were no behavioral observations of fish caused by the test item over the whole exposure period. There were neither any visible abnormalities nor any mortality in the control group.

LC_{50} values for carp to Propineb (VM 80) based on nominal and mean measured concentrations

Test substance:	Propineb (VM 80)
Test object:	Carp (*Cyprinus carpio*)
Exposure:	96 hours, static test design (limit)
LC_{50} 96 h:	**> 66.7 mg (mean measured) test item / L**

Conclusions:

The LC_{50} (96h) of propineb to Carp (*Cyprinus carpio*) in a static 96-hour-test was determined to be > 66.7 mg/L.

CA 8.2.2 Long-term and chronic toxicity to fish

For information on studies already evaluated during the first EU review of propineb, 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.

CA 8.2.3 Endocrine disrupting properties

Fish

As propineb has a DT_{90} in water of less than 1 d, no chronic testing is required. However a juvenile growth test and a 21 d prolonged toxicity test with rainbow trout are available and in both tests, sublethal effects (on growth) occurred only at concentrations where also mortality was observed. In an outdoor pond test, four applications of 600 µg/L resulted in no effects on survival, length, weight or growth of juvenile rainbow trout.

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

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

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

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

Report:	[redacted]; [redacted];2004;M-086995-01
Title:	Acute toxicity of propineb to the waterflea Daphnia magna
Report No:	DOM 24017
Document No:	M-086995-01-1
Guidelines:	**OECD 202; EEC Directive 92/69, Method C2; EPA / FIFRA Guideline 72-2; OPPTS Guidline 850.1010 draft 1996**
GLP/GEP:	**Yes**

Objective:

The aim of the study was to determine the influence of the test item on mobility of *Daphnia magna* over 48 hours in a static exposure, expressed as EC_{50} for immobilisation.

Materials and methods:

Test item: Propineb (specification: Batch EDFU310354, Article No.:0004804643), purity: 82.4%. *Daphnia magna* (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0, 0.15, 0.30, 0.60, 1.20, 2.40 and 4.80 mg a.s./L without feeding.

The content of propineb in exposure media was measured for verification of the test item concentrations at start and end of the exposure period.

After 24 and 48 hours, the behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements within approx. 15 seconds after gentle agitation of the test vessel. Additionally, all visible features of the test item in water as well as possible signs on sublethal affected daphnids had to be recorded.

For verification of the prepared exposure concentrations, the a.s. component propineb was analytically determined and quantified as propylenethiourea (PTU) which is the hydrolysis product of propineb. Before measurement, propineb residues were completely transferred into PTU by heating up to 65°C for 24 hours.

Dates of experimental work: June 01, 2004 to July 21, 2004

Results:

Analytical findings:

The actually dissolved and analytically determined amounts of propineb in the freshly prepared test solutions at test initiation ranged between 76% and 92% (mean: 82%) of the aspired nominal test concentrations.

The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 85% and 111% (mean: 94%) of nominal.

No contaminations of propineb were detected in samples from untreated water control.

Biological findings:

No immobilities or other effects on behaviour occurred in the untreated control within 48 hours of exposure.

Toxicity of propineb to *Daphnia magna*:

Test Concentration (mg product/L) Nominal	mg a.s./L Nominal	mg a.s./L Mean measured	Exposed daphnids (=100%)	Immobilised daphnids 24 h. n	24 h. % ± SD	48 h. n	48 h. % ± SD
Control			30	0	0	0	0
DMF Control*)			30	0	0	0	0
0.18	0.15	0.15	30	0	0	0	0
0.36	0.30	0.24	30	0	0	0	0
0.73	0.60	0.50	30	0	0	0	0
1.46	1.20	1.07	30	0	0	7	23 ± 27
2.91	2.40	2.18	30	5	17 ± 15	26	87 ± 16
5.83	4.80	4.04	30	9	30 ± 11	28	93 ± 10

*) DMF (0.1 mL/L dimethylformamide)

Conclusions:

Based on mean-measured concentrations of propineb, the EC_{50} for immobilisation after 24 and 48 hours of static exposure were assessed:

Statistical results of probit analysis conducted for determination of EC_{50} values:

Probit analysis for data obtained after	**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	**5.66**	3.48	n.d.
48 hours	**1.50**	1.28	1.76

n.d.: not determined

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Report:	[redacted]d; [redacted];2014;M-481861-01
Title:	Acute toxicity of BCS-CU99534 (propineb-DIDT) to the waterflea Daphnia magna in a static laboratory test system
Report No:	EBLHN047
Document No:	M-481861-01-1
Guidelines:	**EU Directive 91/414/EEC** **Regulation1107/2009 (Europe)** **US EPA OCSPP 850.1010**
GLP/GEP:	**Yes**

Objective:

The study was performed, to detect possible effects of the test item on mobility of *Daphnia magna* caused by 48 hours of exposure in a static laboratory test system, expressed as EC_{50} for immobilisation.

Materials and methods:

Test item: BCS-CU99534 (propineb-DIDT), batch no.: BCS-CU99534-01-01 (origin batch No. SES 11956-8-1), purity: 99.5% w/w, TOX 10290-00 (certificate mz00769).

Daphnia magna (1st instars < 24 h old, 6 × 5 animals per concentration) were exposed in a static test system for 48 hours to mean-measured concentrations of 0, 37.0, 83.8, 186, 410 and 1006 µg p.m./L without feeding.

The content of BCS-CU99534 in exposure media was analytically measured for determination of actual test item concentrations.

After 24 and 48 hours, the behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel. Additionally, all visible features of the test item in water as well as possible signs on sublethal affected daphnids had to be recorded.

Dates of experimental work: February 02, 2014 to February 12, 2014

Results:

Analytical findings:

Due to the limited solubility of BCS-CU99534 in water, the test concentration had to be prepared as sub-dilutions of a supersaturated and filtrated aqueous stock solution.

The chosen range of five geometrically spaced exposure concentrations is geared to toxicity data and the measured test concentrations, obtained from NON-GLP range finding studies.

The accompanying chemical analysis of BCS-CU99534 in the freshly prepared stock-solution filtrate revealed a maximum saturation concentration of 31.1 mg BCS-CU99534 / L Elendt M7 medium.

Corresponding measurement of test solution-concentrations at start of exposure verified correct preparation of the dilution series.

Measurement of the test solution-concentrations at the end of the 48 hours exposure period revealed a dose-depending loss of BCS-CU99534 in the test solutions, showing increasing degradation at concentrations below 1000 µg/L. No contaminations of BCS-CU99534 were detected in samples from untreated water control.

Due to the dose-depending degradation of BCS-CU99534 during 48 hours in aqueous solution (M7-medium), all reported results were related to mean-measured test concentrations.

Biological findings:

No immobilities or other effects on behaviour occurred in the untreated control within 48 hours of exposure.

Toxicity of BCS-CU99534 to *Daphnia magna* (based on mean –measured concentrations):

mean measured test concentration (µg p.m./L)	exposed daphnids (=100%)	immobilised daphnids			
		24 h.		48 h.	
		n	%	n	%
Control	30	0	0.0	0	0.0
37.0	30	0	0.0	2	6.7
83.8	30	0	0.0	7	23.3
186	30	4	13.3	25	83.3
410	30	29	96.7	30	100
1006	30	30	100	30	100

Conclusions:

Based on mean-measured concentrations of BCS-CU99534 (propineb-DIDT), the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed:

Statistical results of probit analysis conducted for determination of EC_{50} values:

probit analysis for data obtained after	**EC_{50}** µg p.m./ L (mean measured)	lower 95% cl µg p.m./ L (mean measured)	upper 95% cl µg p.m./ L (mean measured)
24 hours	**253**	214	298
48 hours	**112**	90.6	137

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

No acute studies on an additional aquatic invertebrate species are required since propineb is not an insecticide and does not show an insecticidal mode of action.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

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

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Report:	[redacted];;[redacted];2005;M-252129-01
Title:	Effect of propineb 70 WP on Daphnia populations in aquatic laboratory test systems simulating natural exposure conditions
Report No:	ALT.WB.2005.1
Document No:	M-252129-01-1
Guidelines:	**Guidance document on higher-tier aquatic risk assessment for pesticides (HARAP), SETAC-Europe Publication 1999**
GLP/GEP:	**Yes**

Objective:
The aim of the study was to assess the effect and potential recovery of repeated applications (4 ×) of the fungicide Propineb 70 WP on a mixed age population of *Daphnia magna* in aquatic laboratory test systems.

Materials and methods:
Test item: Propineb WP 70, Sponsor Article No.: 0004479696, Batch No.: PF31112692, content: 71.1% w/w.
To simulate a photolytic degradation of propineb similar to its fate under natural outdoor conditions, an artificial sunlight illumination by daylight lamps was installed above the test aquaria (approximately 270 ($\mu E/m^2/s$ for 14 h per day). The study was carried out in 18 aquaria containing 18.7 L test medium. The pre-treatment phase lasted two weeks. The treatment consisted of 4 applications of Propineb 70 WP at one week intervals. The treatment levels were: 60, 120, 240, 480, and 960 µg a.s./L in triplicate aquaria (n=3). Three aquaria served as controls. After the last application the test continued for another two weeks. In all aquaria the concentrations were measured directly (within 1 hour) after each application. In all aquaria, except for the three reference systems (0 µg a.s./L), the concentration of the active ingredient and its metabolite propylenethiourea (PTU) in the water phase were determined over time. Dynamics in alkalinity, total nitrogen content, total phosphate content, chlorophyll-a content of phytoplankton, and community metabolism (temperature, pH and dissolved oxygen content) were also followed over time in all aquaria. Effects on *Daphnia* population composition and abundance were recorded and evaluated using ANOVA and logistic regression techniques.

Dates of experimental work: February 01, 2005 to April 5, 2005

Results:

Analytical findings:

The dosed amounts were 94 + 4% (mean + SD) of the intended amounts. The concentration of the test compound decreased rapidly. Mean DT_{50} was 2.1 days after each application (minimum of all applications 1.13 + 0.52 days, maximum 2.83 + 0.83 days). Also the metabolite PTU disappeared from the systems: for the total of propineb equivalents (propineb + PTU) the mean DT_{50} was 3.7 days (minimum 2.92 + 0.58 days, maximum 4.63 ±1.27 days).

Time-weighted average concentrations of propineb over 21 days for the nominal treatment levels of 60, 120, 240, 480 and 960 µg/L were 5.6 µg/L, 19 µg/L, 47 µg/L, 110 µg/L and 256 µg/L respectively (average of three aquaria) for the period of day 7 (= day of second application) to day 28 (= 7 days after the last application). Time-weighted average concentrations of propineb over 28 days for the nominal treatment levels of 60, 120, 240, 480 and 960 µg/L were 7.8 µg/L, 20 µg/L, 46 µg/L, 103 µg/L and 239 µg/L respectively (average of three aquaria) for the period of day 0 to day 28.

In the pre-treatment period the pH increased up to almost 11 due to photosynthesis of algae. Two days after the first treatment the pH dropped to below 7 because the algae were controlled by grazing of a far more dense Daphnia population and the oxygen content dropped strongly to about 2 to 3 mg/L. Due to a slightly increased aeration the oxygen content increased thereafter to about 8 mg/L and the pH gradually increased to about 8, except for the highest treatments, where the oxygen content and pH diverged because of the reduced algae consumption.

Biological findings (*Daphnia* populations):

In all aquaria the adult, juvenile and neonate populations increased exponentially in the pre-treatment period and up to approximately one week after the first treatment. Thereafter the population abundance in all but the highest treatments had reached more or less its carrying capacity.

From day -1 onwards the maximum feeding rate of $2.5 \cdot 10^8$ algae cells/L was applied in all aquaria. Directly after feeding, the water in the aquaria was green from the applied algae. In the hours before the next feeding the water in the aquaria became clear on most occasions, indicating that most of the algae had been consumed by the daphnids. However, the water in the highest treatments was still green at the time of the next feeding at day 22, indicating that a part of the algae fed the day before had not been consumed.

At day 13 some winter eggs had been observed in all aquaria, indicating that the Daphnia populations suffered stress, probably due to overpopulation.

The development of the total numbers of Daphnia in the aquaria was dominated by the development of juveniles. Overall, juveniles were most sensitive. From day 13 onwards the juveniles in the 960 µg/L treatment decreased more than in the controls and other treatments. However, the Daphnia population at the 960 µg/L treatment level recovered from day 22 onwards. In consequence a consistent NOEC of 480 µg/L was observed on several consecutive sampling dates (univariate statistics). Since a NOEC of 240 µg/L was observed on a single isolated sampling date only for juveniles (day 6) and neonates (day 3), the overall NOEC of this study is 480 µg/L.

A consistent series of EC_{10} and EC_{50} and total abundance from day 13 to day 27. For the juveniles the geometric mean EC_{10} values was 276 µg/L and the geometric mean EC_{50} value was 650 µg/L. For total abundance the geometric mean EC_{10} value was 361 µg/L and the geometric mean EC_{50} value was 741 µg/L.

Conclusions:
Propineb disappeared from the test water with a mean DT_{50} of 2.1 days. The 960 µg a.s./L treatment level showed a consistent treatment-related effect on the abundance of juveniles, neonates and the total Daphnia population. At 480 µg a.s./L treatment-related effects had only been calculated for a single isolated sampling day after the first application for juveniles and neonates. Thus, the NOEC of the Daphnia population exposed to four treatments of Propineb WP 70 with a time interval of one week is 480 µg a.s./L (based on nominal initial treatment levels).

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 since propineb is not an insecticide and does not show an insecticidal mode of action.

CA 8.2.5.3 Development and emergence in Chironomus species

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

Propineb-WP 70

Report:	[redacted];[redacted];2005;M-253817-01
Title:	Chironomus riparius 28-day chronic toxicity test with Propineb WP70 in a water-sediment system using spiked water
Report No:	EBLHX048
Document No:	M-253817-01-1
Guidelines:	**OECD Guideline 219: "Sediment-Water chironomid Toxicity Test Using Spiked Water" (April 2003)**
GLP/GEP:	**Yes**

Objectives:
The aim of this 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.

Materials and Methods:
Propineb WP 70, a.s. content: 71.1% was tested, specified by batch-no.: PF31112692, TOX-No.: 06763-00 and article-no.: 0004479696.
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 0.176, 0.35, 0.70, 1.41, 2.81, 5.63, 11.3, and 22.5 mg form./L corresponding to 0.125, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.0 mg a.s./L of a water-sediment system. Dissolved oxygen concentrations ranged in the water phase from 6.9 to 8.7 mg O_2/L (7.8 mg O_2/L= 86 % O_2 - saturation), the water pH values ranged from 7.2 to 8.7 and the water temperature ranged from 19.8°C to 20.3°C measured from parallel beakers of each test concentration over the whole testing period.

Propineb could not be directly determined owing to the low solubility and low hydrolytic stability in water. The hydrolysis product of propineb propylenethiourea (PTU) had been analysed and the real amount of propineb was recalculated. Recoveries of propineb 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 0.125, 1.00 and 16.0 mg a.s./L corresponding to 0.176, 1.41 and 22.5 mg form./L and control (only on day 0) of the overlying water and the pore water of the sediment.

Dates of work: January 11 to March 01, 2005

Findings:

Chemical analysis of propineb (recalculated) in the overlying water and pore water over time reflect expected aquatic fate data with recoveries of 49.9 % to 59.9 % (mean 55.1 %) of nominal at the beginning of the exposure period. Taking into account the propineb analysis in the application solution (114 % of nominal), the physico-chemical properties and the expected e-fate of propineb, initial nominal concentrations of the formulation were used for reporting and evaluation of the results.

Biological results:

Start of emergence was on day 14 for the control and the lowest test concentration of 0.176 mg form./L. The start of emergence was reduced for one day at the test concentrations of 0.35 and 0.70 mg form./L, for two days at test concentration of 1.41 mg form./L, for four days at test concentration of 2.81 mg form./L, for five days at test concentration of 5.63 mg form./L and for eight days at test concentration of 11.3 mg form./L No emergence could be observed at test concentrations of 22.5 mg form./L.

96.3 % of the inserted (n= 80) larvae maturated to adults in the control 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 initial nominal mg form./L.	Number of emerged midges	Emergence of inserted larvae			Development rate (pooled sex) (1/d)
		Total (%)	Male (%)	Female (%)	
Control	77	96.3	61.3	35.0	0.064
0.176	70	87.5	46.2	41.3	0.060
0.35	66	82.5	48.7	33.8	0.058
0.70	72	90.0	46.2	43.8	0.057
1.41	59	73.8	41.3	32.5	0.055
2.81	62	77.5	35.0	42.5	0.051
5.63	54	67.5	37.5	30.0	0.046
11.3	16	20.0	12.5	7.5	0.039
22.5	0	-	-	-	-

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

The Chi²-Test indicates no statistically different sensitivities of sexes within the range of unaffected test levels. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.

Conclusions:

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

Endpoints	EC_5	EC_{10}	EC_{15}	EC_{50}
Emergence ratio (pooled sex) (95 % confidence limits)	0.55 (0.003 – 1.599)	0.869 (0.014 – 2.244)	1.25 (0.041 – 2.87)	4.99 (1.83 – 16.2)
Development rate (pooled sex) (95 % confidence limits)	0.186 (0.082 – 0.322)	0.580 (0.337 – 0.847)	1.25 (0.860 – 1.65)	31.8 (20.7 – 59.2)

CA 8.2.5.4 Sediment dwelling organisms

For information on studies already evaluated during the first EU review of propineb, 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 propineb, 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

Report:	[redacted] 7; [redacted] ;2004;M-088372-01
Title:	Pseudokirchneriella subcapitata growth inhibition test with Propineb
Report No:	DOM 24014
Document No:	M-088372-01-1
Guidelines:	**OECD Guideline 201: "Freshwater algae and cyanobacteria, growth inhibition test", DARFT (18. Febr. 2004); JMAFF Guideline (12 Nousan No. 8147, 24. Nov. 2000); OPPTS Guideline 850.5400 Draft (July 1996)**
GLP/GEP:	**Yes**

Objectives:

The aim of the study was to determine the influence of the test item on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_X for growth rate of algal biomass (cells per volume).

Materials and Methods:

Test material: Propineb (analysed purity: 82.4 % w/w was tested, specified by batch no.: EDFU310354, TOX-No.: 6603-00; article-no.: 0004804643).

Pseudokirchneriella subcapitata were exposed in a chronic multi-generation test for 3 days under static exposure conditions to the geometric mean measured concentration of 0.010, 0.031, 0.10, 0.31 and 1.0 mg a.s./L in comparison to a water and a solvent control [100 µL DMF = Dimethylformamide (including the appropriate concentration of the test item) / 1000 mL nutrient medium was added to all

concentration levels and the solvent control]. The test system consisted of three replicate vessels per test level and six replicate vessels per control. The initial cell number was 10,000 cells/mL.
The test system consisted of three replicate vessels per test level and six replicate vessels per control level. The initial cell number was 10,000 cells/mL.
Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).
The pH values ranged from 7.9 to 8.3 in the controls and the incubation temperature ranged from 24.5°C to 25.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7850 lux.
Quantitative amounts of propineb were measured in all treatment groups and in the control(s) on day 0 and day 3 of the exposure period.

Dates of experimental work: June 11 2004 to July 21 2004

Results:
Validity of the study:

Validity Criteria:	Obtained in this study:
Increase of biomass:	Biomass increased in the control by more than 16-fold within the evaluation period.
Sectional control rates:	Mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35%
Control replicate rates:	Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%

In conclusion, it can be stated that the test conditions met all validity criteria given by the mentioned guideline.

Strain material of defined sensitivity was used, as shown by reference substance testing with 3,5-dichlorophenol or potassium dichromate. Reference tests are conducted event driven *(i.e. in case of receiving new strains, introduction of new test conditions, apparatus, etc.)*. These tests are documented and archived together with strain protocols.

Analytical results:
The analytical findings of propineb in the treatment levels found on day 0 were 23% to 84% of nominal (average 49%). On day 3 analytical findings of 32% to 87% of nominal (average 56%) were found. Under test conditions propineb is virtually insoluble in alkaline nutrient medium as used for algae testing. Therefore stock solutions with DMF were used in this study. After application of these stock solutions in the aqueous test medium it had to be reckoned that most of formerly dissolved propineb would precipitate. Thus, the 0 and 72 hour aliquot samples should be handled with reservation only. Therefore reporting of nominal values is preferred.

Biological results:

Effect of propineb on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Geom. mean measured concentration [mg a.s./L]	Cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Control	493 000	--	--
Solvent control	493 000	--	--
Pooled controls	493 000	1.296	--
0.003	428 000	1.252	3.4
0.010	259 000	1.081	16.6
0.031	165 000	0.934	27.9
0.10	35 000	0.406	68.7
0.31	16 000	0.123	90.5
1.0	4 000	-0.754	158.1

test initiation with 10,000 cells/mL

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

Conclusions:

The (0 - 72h)-E_rC_{50} for propineb is 0.055 mg a.s./L.

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Report:	[redacted]9;[redacted];2014;M-485275-01
Title:	Pseudokirchneriella subcapitata - Growth inhibition test with propineb-DIDT (BCS-CU99534)
Report No:	EBLHN046
Document No:	M-485275-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009;not specified**
GLP/GEP:	**Yes**

Objectives:

The aim of the study was to determine the influence of the test item on exponentially growing populations of *Pseudokirchneriella subcapitata* expressed as EC50 for growth rate of algal biomass (cells per volume).

Materials and Methods:

Propineb-DIDT (BCS-CU99534) analysed purity: 99.5 % was tested, specified by origin batch no.: SES 11956-8-1, customer order no.: TOX10290-00 and LIMS no.: 1334297.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to geometric mean measured concentrations of 1.53, 6.14, 20.2, 62.9, 234, 839 and 2983 µg pure metabolite/L in comparison to control. The pH values ranged from 7.9 to 8.2 in the controls and the incubation temperature ranged from 22.2 °C to 22.8 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6544 lux (mean value).

Quantitative amounts of propineb-DIDT (BCS-CU99534) were measured in all treatment groups and in the controls at test start and end (day 3).

Dates of experimental work: January 31 2014 to April 09 2014

Results:

Validity of the study:

Validity Criteria:	Obtained in this study:
Increase of biomass:	Biomass increased in the control by more than 16-fold within the evaluation period.
Sectional control rates:	Mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control did not exceed 35%
Control replicate rates:	Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%

In conclusion, it can be stated that the test conditions met all validity criteria given by the mentioned guideline.

Analytical results:

The analytical findings of propineb-DIDT (BCS-CU99534) in the treatment levels found on day 0 were 93.5 % to 110 % of nominal (average 102 %). On day 3 analytical findings of 32.3 % to 90.0 % of nominal (average 57.5 %) were found. Based on the analytical findings all results are given as geometric mean measured concentrations of the test item in the test medium.

The static 72 hour algae growth inhibition test provided the following tabulated effects:

Geom. mean measured concentration [mg a.s./L]	Cell number after 72 h (means) per mL	(0-72h) average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Pooled controls	895 000	1.497	0.0
1.53	693 000	1.413*	5.7
6.14	473 000	1.285*	14.2
20.2	448 000	1.267*	15.4
62.9	200 000	0.999*	33.3
234	33 000	0.393*	73.8
839	26 000	0.311*	79.2
2983	16 000	0.150*	90.0

test initiation with 10,000 cells/mL
* significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure or Welch t-test for inhomogeneous variances with bonferroni adjustment

Conclusions:

After 72 hours the E_rC_{50} for propineb-DIDT (BCS-CU99534) is 114 µg pure metabolite/L (95 % CI: 90.2 – 145 µg pure metabolite/L) based on geometric mean measured concentrations of the test substance.

CA 8.2.6.2 Effects on growth of an additional algal species

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

CA 8.2.7 Effects on aquatic macrophytes

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

Report:	[redacted];2005;M-246864-02; Amended: 2005-04-28
Title:	Effects of propineb on rainbow trout in aquatic outdoor microcosm enclosures
Report No:	ALT.JD.2004.1
Document No:	M-246864-02-1
Guidelines:	**OECD Guidance Document "Freshwater Lentic Field Tests", 2004 (Draft) ; Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991)**
GLP/GEP:	**Yes**

Summary:

The aim of the study was to assess the effects of repeated applications (4 times) of the fungicide Propineb 70 WP on growth and survival of juvenile rainbow trout under outdoor field conditions.

The study was carried out using 10 enclosures in an experimental ditch at [redacted], the Netherlands. All enclosures contained approx. 433 dm^3 of water, some macrophytes and had a bottom layer of sediment. The treatment consisted of 4 applications of Antracol 70 WP (active ingredient: propineb) at one week intervals, simulating spray drift. Nominal treatment levels were 80 µg active ingredient l^{-1}, 150 µg active ingredient l^{-1}, 300 µg active ingredient l^{-1} and 600 µg active ingredient l^{-1}. Treatments were duplicated, using 2 enclosures per treatment level and 2 controls. The test lasted for 28 days after the first application of the test substance on 30 August 2004. The concentrations of the active ingredient and its main metabolite (propylenethiourea, PTU) in the water phase were followed over time. The weight and length of fish were determined 4 days prior to the first application of the test substance (day -4), when they were transferred to the enclosures, and at the end of the experiment (day 28). Dynamics in chlorophyll-a content of phytoplankton, macrophyte species composition and cover and community metabolism (temperature, pH and dissolved oxygen content) were followed over time in all enclosures.

Dates of experimental work: August 23, 2004 — September 27, 2004.

Findings:

Residue Analysis

The measured concentrations in the spray solutions were 106 ± 17% of nominal target concentrations, indicating that the initial concentrations in the enclosures will have been close to nominal target concentrations.

The concentration of test compound decreased quite rapidly after the application. The measured water concentrations in the enclosures measured 1 h after application were 35 - 55% of the nominal target concentrations. A very fast initial period of disappearance (usually lasting less than 1 hour) was followed by a slightly slower phase of disappearance with a half-life of approximately 0.5 days (average value over all enclosures and all applications). The DT50 for the first part of the dissipation was less than 1 hour. At all treatment levels there was a slight increase of the initial concentration after each of the treatments, i.e. a slight build up of the test substance, indicating that not all of the test substance had disappeared from the water in the interval between applications.

Time-weighted average exposure levels over the 28-day treatment period were 7.4 µg l^{-1}, 16 µg l^{-1},41 µg l^{-1} and 105 µg l^{-1}.

In all enclosures the concentration of the metabolite PTU increased rapidly after each of the applications, indicating the rapid transformation of the test substance into PTU. After each application the concentration of PTU decreases from day 1 to day 3 after application. Both PTU and the parent compound propineb disappear rather fast from the aqueous phase. In all enclosures, only 6% or less of the total (cumulative) amount of the test substance dosed is still present at the end of the test period (28 days after the first treatment), either as propineb or as PTU.

Biology

No treatment-related effects on macrophytes species composition and cover were observed, nor could any treatment-related effects be demonstrated on the measurement endpoints temperature, pH and chlorophyll-a content of phytoplankton. Oxygen content of the enclosures was slightly lowered in the highest treatment level during the first week of treatment, probably because of slight differences in macrophyte biomass and aeration between enclosures. Since decreases were small (< 1 mg l^{-1}) and oxygen content always

remained above 10 mg l^{-1} in all enclosures, these differences are considered to be of no biological relevance.

During the 28-day period of exposure to the test substance, 1 out of 100 fish had died (in one of the replicates at 600 µg l^{-1}) and 1 fish was missing at the end of the study (in one of the replicates at 300 µg l^{-1}). There was no apparent relationship between mortality and treatment with the test substance.

There were no significant differences in mean length, weight and growth of length of the fish in the various treatment levels. Growth of weight of fish was slightly, but statistically significant, larger in the highest treatment. Since the lengths and weights of the fish were not affected by the treatment, this slight increase in growth of weight is considered to be not relevant. No consistent changes in the behaviour of fish were observed in any of the treated enclosures.

Conclusion:

At all treatment levels up to and including 600 µg l^{-1} no treatment-related effects were observed on length, weight, growth of length and survival of juvenile rainbow trout. The growth of weight of juvenile rainbow trout was slightly, but statistically significant, larger at the highest treatment level.

However, the weight of the fish at the start of the study was slightly less at this treatment level. Since the lengths and weights of the fish were not affected by the treatment, this increase in growth of weight is considered to be not relevant. In addition, no consistent treatment-related effects on chlorophyll-a or community metabolism endpoints could be observed after 4 applications of the test substance in a weekly interval. Thus, the NOEC is ≥ 600 µg l^{-1}, the highest treatment level used.

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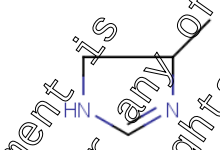
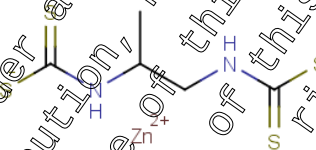
Report:	[redacted] ; [redacted] ; [redacted] ;2014;M-488533-01
Title:	Ecotoxicological predictions for 4-methylimidazoline using ECOSAR and VegaNIC
Report No:	P13096E
Document No:	M-488533-01-1
Guidelines:	**QSAR calculation based on different QSAR models**
GLP/GEP:	**No**

The current study was conducted to assess the potential ecotoxicological effect of 4-methylimidazoline [1], a metabolite of propineb [2], based on quantitative structure-activity relationship (QSARs). The ecotoxicity assessment was performed using the QSAR models ECOSAR and VegaNIC.



[1]



[2]

Structures of 4-methylimidazoline [1] and propineb [2], please note propineb is depicted as a monomer here

A summary of the aquatic ecotoxicological estimates based on the simulation models ECOSAR and VegaNIC is provided in the following table.

Predicted ecotoxicological endpoints for 4-methylimidazoline based on different QSAR models

Predicted ecotoxicity for 4-methylimidazoline based on ECOSAR and VegaNIC			**ECOSAR*)**		**VegaNIC*)**
			Endpoints mg/L	**Baseline toxicity mg/L**	**Endpoints mg/L**
Fish	96-hr	LC_{50}	393.259	5426.248	968.78 a)
Daphnid	48-hr	LC_{50}	36.68	2535.646	16.85 a) 7.31 b)
Green Algae	96-hr	EC_{50}	49.171	844.016	-

*) For details see section 3.1 ECOSAR and 3.2. VegaNIC in the report (M-488533-01-1).
a) Based on the original model inside the T.E.S.T software for US EPA.
b) Based on DEMETRA model.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

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

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:

- Chronic 10 day toxicity to adult bees under laboratory conditions
- Acute toxicity to larval bees under laboratory conditions
- Colony feeding studies (Oomen et al 2008). This is only triggered when the acute oral LD_{50} for adult bees is less than 100 µg a.s./bee which is not the situation for propineb. However a study has been conducted using a realsitic worse case spray solution concentration and covers exposure for effects on brood (eggs, young and old larvae) and their development, nurse bee on-going behaviour in brood care and colony strenght.

These additional studies on toxicity to honey bees and bee brood have been performed with technical propineb (TK 83) and the formulated product (Propineb WG 70A W) according to current, guidelines, guidance or the current understanding of the state-of-the-art of testing.

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

Table 8.3.1- 1: EU evaluated and additional studies on bee toxicity of propineb

Test substance	Test species/ study type	Endpoint	References
Propineb TK 83	Honey bee, 48 h	LD_{50} – oral > 164.6 µg a.s./bee LD_{50} – contact > 164.6 µg a.s./bee	[redacted] (1998) M-017002-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
Propineb TK 83	Honey bee, acute *Apis mellifera*	Oral. LC_{50} >107.9 µg a.s./bee Contact: LC_{50} > 100 µg a.s./bee	[redacted] (2012) 73821035 M-442120-01-1 KCA 8.3.1.1.1 /07
Propineb WG 70	Honey bee, 48 h	LD_{50} – oral >100 µg a.s./bee	[redacted] (1987) M-017013-01-2 KCP 10.3.1.1.1/02
Propineb WG 70A W	Honey bee, chronic *Apis mellifera*	NOEC >120 mg a.s./kg sucrose LC_{50} >120 mg a.s./kg sucrose	[redacted] (2014) S13-00150 M-487104-01-1 KCA 8.3.1.2 /01
Propineb WG 70A W	Honey bee brood feeding (Oomen *et al.*, 1992)	Although a small but significantly significant effect on egg termination rate was observed, Propineb WG 70A W fed at a concentration of 2.10 g a.s./L (typical for high volume spray) did not adversely affect overall honey bee brood development or success and did not increase the overall mortality rate of the colonies compared to the control.	[redacted] (2013) EBLHL033 M-454682-01-1 KCA 8.3.1.3/01
Propineb WG 70A W	Honey bee brood (in vitro) *Apis mellifera*	NOED >6.25 µg a.s./larva LD_{50} 11.1 µg a.s./larva	[redacted] (2014) S13-01495 M-488422-01-1 KCA 8.3.1.2 /03

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	[redacted];[redacted];[redacted];2012;M-442120-01
Title:	Effects of propineb TK 83B W (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	73821035
Document No:	M-442120-01-1
Guidelines:	**OECD 213 and 214 (1998)**
GLP/GEP:	**Yes**

Objective:

The purpose of this study was to determine the acute contact and oral toxicity of propineb TK 83B W to the honey bee (*Apis mellifera* L.). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Materials and Methods:

Test item: Propineb TK 83B W (Batch ID: EDFU911415, Sample Description: TOX0948-01, Specification No.: 102000008955-03, purity: 82.3 % w/w analytical).

Test organism: Honey bee (*Apis mellifera* L.), female worker bees, obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.

Under laboratory conditions *Apis mellifera* 50 worker bees per treatment were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee for topical application (contact limit test) and to a single dose of 107.9 µg a.s. per bee for feeding (oral limit test, value based on the actual intake of the test item).

Oral toxicity study

Appropriate amounts of propineb TK 83B W or reference item dilutions in acetone were mixed with syrup (ready-to-use syrup; [redacted], [redacted], D-[redacted]; composition of the sugar component: 30 % sucrose, 31 % glucose, 39 % fructose) in order to achieve the required test concentrations in a final dilution of 50 % syrup solution (45 % water, 50 % syrup and 5 % acetone (w/w)). The final concentration of sugar syrup in the aqueous test and reference item solutions offered to the bees was 50 % (w/w). For the solvent control, the same proportion of sugar syrup, water and acetone was used; for the water control tap water and sugar syrup was used at the same ratio 50 % (w/w) tap water, 50 % (w/w) ready-to-use sugar syrup.

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 3 hours 55 minutes for the test item treatments). After a maximum of 3 hours 55 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The mean target dose levels (e.g. 100 µg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar syrup solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption. The test was conducted in darkness, temperature was 25°C and humidity between 53 and 89 %. Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing. Results are based on measured concentrations of the a.s. per bee.

Contact toxicity study

The test item was applied as one 5 µL droplet of propineb TK 83B W dissolved in Acetone, placed on the dorsal bee thorax using a Burkard – Applicator. The reference item was applied as one 5 µL droplet of dimethoate dissolved in Acetone.

For the controls, one 5 µL droplet of a) tap water containing 0.5 % Adhäsit* and b) pure acetone was used. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

The test was conducted in darkness, temperature was 25°C and humidity between 53 and 89 %. Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing. Results are based on measured concentrations of the a.s. per bee.

Dates of experimental work: August 21, 2012 – August 29, 2012

Results:

The results can be considered as valid, as all validity criteria of the test were met: control mortality is < 10% in the oral and in the contact test, LD_{50} (24 h) of the toxic standard in the oral test equals 0.16 µg a.s./bee, the LD_{50} (24 h) of the toxic standard in the contact test equals 0.17 µg/bee.

Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	Mean %	Mean %	Mean %	Mean %	Mean %	Mean %
Test item						
100.0	2.0	2.0	4.0	0.0	8.0	2.0
water	0.0	0.0	0.0	0.0	4.0	0.0
solvent	0.0	0.0	0.0	0.0	4.0	0.0
Reference item						
0.30	2.0	12.0	92.0	2.0	94.0	0.0
0.20	0.0	0.0	68.0	14.0	76.0	0.0
0.15	0.0	0.0	30.0	2.0	38.0	6.0
0.10	0.0	2.0	10.0	0.0	20.0	0.0

results are averages from five replicates (ten bees each) per dosage / control
water = CO_2/water-treated control ; solvent: CO_2/solvent control

Mortality and behavioural abnormalities of the bees in the oral toxicity test

Ingested [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	Mean %	Mean %	Mean %	Mean %	Mean %	Mean %
Test item						
107.9	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
solvent	0.0	0.0	0.0	0.0	2.0	0.0
Reference item						
0.33	12.0	38.0	76.0	4.0	80.0	0.0
0.16	2.0	16.0	60.0	12.0	70.0	0.0
0.08	0.0	2.0	26.0	10.0	42.0	0.0
0.05	0.0	0.0	8.0	0.0	10.0	0.0

results are averages from five replicates (ten bees each) per dosage / control
water = water- control; solvent = solvent control

Observations:

Contact Test:

At the end of the contact toxicity test (48 hours after application), there was 8.0 % mortality at 100.0 µg a.s./bee. 4.0 % mortality occurred in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (acetone), respectively. One single bee was found apathetic during the 4 hours and 48 hours assessment, respectively.

Oral Test:
In the oral toxicity test, the maximum nominal test level of propineb TK 83B W (i.e. 100 µg a.s./bee) corresponded to an actual intake of 107.9 µg a.s./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the water control group (50 % aqueous sugar syrup solution). 2.0 % mortality occurred in the solvent control group at the end of the oral toxicity test (after 48 hours). No test item induced behavioural effects were observed at any time in the oral toxicity test.

Conclusions:

Toxicity to Honey Bees; laboratory tests

Test Item	Propineb TK 83B W	
Test object	*Apis mellifera*	
Exposure	contact (solution in acetone)	oral (sugar syrup/acetone/water solution)
Application rate (µg a.s./bee)	100.0	107.9
LD_{50} µg a.s./bee	> 100.0	> 107.9
LD_{20} µg a.s./bee	> 100.0	> 107.9
LD_{10} µg a.s./bee	> 100.0	> 107.9
NOED µg a.s./bee*	> 100.0	> 107.9

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

The contact LD_{50} (48 h) of propineb TK 83B W was > 100.0 µg a.s./bee
The oral LD_{50} (48 h) of propineb TK 83B W was > 107.9 µg a.s./bee.

CA 8.3.1.1.2 Acute contact toxicity

See point 8.3.1.1.1 above.

CA 8.3.1.2 Chronic toxicity to bees

Report:	[redacted]; [redacted]; 2014; M-487104-01
Title:	Propineb WG 70A W - Assessment of chronic effects to the honeybee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test
Report No:	S13-00150
Document No:	M-487104-01-1
Guidelines:	**No specific guideline available (however the method use is based on an adapted version of OECD 213)**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to determine the chronic effects of the test item Propineb WG 70A W on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory.

Materials and Methods:
Test item: Propineb WG 70A W (Specification No.: 102000006516-02, Sample description: FAR01501-02, Content of active ingredient (a.s.): 68.3 % w/w (683 g/kg) (analysed).

Honey bees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing nominally 120 mg a.s./kg of the test item Propineb WG 70A W over a period of 10 days by continuous and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50 % (w/v) aqueous sucrose application (feeding) solution. Mortality, sub-lethal effects and behavioural observations were assessed every day throughout the 10 days exposure period. Furthermore, the daily food uptake was determined.

Samples of the application (feeding) solutions prepared freshly every day throughout the 10 days continuous feeding period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item. The chemical analysis of the application (feeding) solutions was performed by Bayer CropScience AG, [redacted], Germany.

Dates of experimental work: 31 May 2013 – 09 July 2013

Results:

The results of the study can be considered to be valid as no mortality occurred in the control group over the duration of the test (0% mortality). A toxic reference item group was not was not included in this test, since one has not been defined or validated for this type of study. Furthermore a toxic reference is not considered necessary in the study design as the dose received by the bees of the test item was verified analytically.

After 10 days of continuous exposure, mortality at the test item treatment level of 120 mg a.s./kg of Propineb WG 70A W was not statistically significantly different when compared to the control group. The cumulative control mortality was 0.0 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg Propineb WG 70A W was 2.0 % at the final assessment.

At 120 mg a.s./kg Propineb WG 70A W, no remarkable sub-lethal effects or behavioural abnormalities were observed throughout the entire observation period of 10 days.

After 10 days of continuous exposure, by considering the actual food consumption of the honeybees, the accumulated nominal intake of the test item Propineb WG 70A W at the treatment level of 120 mg a.s./kg was 45.48 µg a.s./bee; the corresponding average daily dose was therefore 4.6 µg a.s./bee.

The overall mean daily consumption of the application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was statistically significantly different (lower) when compared to the untreated control group (37.8 mg/bee at 120 mg a.s./kg, compared to 42.9 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose application (feeding) solution was not statistically significantly different (lower) between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison), except for the 5th day of exposure.

Mean consumption of application (feeding) solution, mean nominal intake of test item accumulated over all test days, average daily dose, cumulative mortality after ten days of continuous exposure (test end) as well as the LC_{50} and NOEC

Treatment Level	Control [1]	Propineb WG 70A W at 120 mg a.s./kg (nominal) [2]
Number of bees exposed (10 replicates of 10 bees)	100	100
Number of dead bee after ten days of continue exposure	0	2
Cumulative mortality after ten days of continuous exposure [%]	0.0	2.0
Overall mean daily consumption of application (feeding) solution [mg/bee] [3]	42.9	37.8*
Mean nominal intake accumulated over ten test days [µg a.s./bee/10 d]	-	45.48
Average daily dose (nominal) throughout ten days of continuous exposure [µg a.s./bee/d]		4.6
LC_{50}	> 120 mg a.s./kg (nominal)	
NOEC[4]	120 mg a.s./kg (nominal)	

[1] Application (feeding) solution: 50 % (w/v) aqueous sucrose solution
[2] Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing Propineb WG 70A W
[3] The mean values per replicate over the test period (non-rounded values) were used for the calculation of the overall mean daily consumption of application (feeding) solution per treatment
[4] Determined to be the NOEC/NOED based on mortality (not significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided, $p \leq 0.05$)
a.s. = active substance

Analytical Results

The actual concentration of propineb in the application (stock) solution was on average 89% of the nominal concentration consequently it is acceptable to present the results as nominal values.

Conclusions

It can be concluded that the continuous ad libitum feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item Propineb WG 70A W at the treatment level of 120 mg a.s./kg caused no adverse effect regarding mortality, sub-lethal effects and behaviour.

On every single day during the 10 day continuous exposure period, the mean food consumption per bee was not statistically significantly different (lower) in the test item treatment group compared to the control group, except for the 5th day of exposure. The overall mean daily consumption of the application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was statistically significantly different (lower) when compared to the untreated control group (37.8 mg/bee at 120 mg a.s./kg, compared to 42.9 mg/bee in the control group). Overall, it can be concluded that there is a very slight anti-feeding effect (88% consumption compared to the control) of the test item at the treatment level of 120 mg a.s./kg.

The NOEC for mortality was determined at the end of the test period to be 120 mg a.s./kg (nominal).

The LC_{50} was determined to be > 120 mg a.s./kg (nominal).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	[redacted]u; [redacted];2013;M-454682-01
Title:	Study on the effects of propineb WG 70A W on honey bee brood (Apis mellifera L.) - Brood feeding test -
Report No:	71391031
Document No:	M-454682-01-1
Guidelines:	**GLP compliant study based on the method according to Oomen et al. (1992)**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to investigate the effect of the test item Propineb WG 70A W to honey bee brood when exposed by oral ingestion.

Materials and Methods:
Propineb WG 70A W: propineb (LH 30/Z): 68.4 % w/w (analytical), 70 % w/w (nominal); Batch ID.: EM20004026; Sample Description: FAR01501-01; Specification No.: 102000006516 - 02.
Propineb WG 70A W mixed with 1 L ready-to-use sugar syrup was fed to bee colonies and mortality of adult bees, pupae and larvae observed at test end (21 days after test initiation). The mixing ratio was 3.07 g Propineb WG 70A W in 1 L sugar syrup. Also bee brood development (eggs, young and old larvae) were recorded one day before test initiation and after 4, 8, 15 and 22 days. As control pure sugar syrup (30% sucrose, 31% glucose, 39% fructose) was used. 3.0 g/L syrup Insegar (25% fenoxycarb, 0.75 g fenoxycarb/L) was used as reference substance.
Bee colonies were free flying in natural field conditions, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops or flowering weeds in the surrounding area.

Dates of experimental work: May 28, 2012 to June 22, 2012

Results:

Effect of Propineb WG 70A W on honey bees (*Apis mellifera*) in a bee brood study

Test item Test object Exposure		**Propineb WG 70A W Honey bees (*Apis mellifera* L.), complete colonies Natural conditions**		
		Control	**Test item**	**Reference item**
Termination rate [%] 1)	eggs	16.0	38.4 *	97.4*
	young larvae	18.9	10.4 n.s.	83.1*
	old larvae	2.7	2.2 n.s.	16.2*
Mean brood termination rate over all stages [%]		12.5	17.0 n.s.	65.6 *
Mean mortality of worker bees/colony/day during 2)	pre-application phase	15.3	7.8 n.s.	12.7 n.s.
	during entire post application phase	22.5	14.8 n.s.	54.7 *
Mean mortality of worker pupae/colony/day during 3)	pre-application phase	0.1	0.4 n.s.	0.6 n.s.
	during entire post application phase	3.4	3.3 n.s.	11.9 n.s.
Mean number of bees before application		18165	14850	19485

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

The mean termination rate of eggs after treatment with Propineb WG 70A W was statistically significantly when compared to the values of the control group.

No effect on the development of young larvae was observed after consumption of the test item. The mean termination rate of young larvae in the test item treatment group was lower with a mean of 10.4 % compared to 18.9 % in the control group. Accordingly, this was not statistically significant compared to the control group.

There was also no significant effect on the development of old larvae after consumption of the test item.

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

No effects of the test item on honey bee pupae and larvae were observed.

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

Conclusion:

It can be concluded according to the results of this study that the administration of the test item Propineb WG 70A W resulted in an increased termination rate of eggs, but neither of young and old larvae nor of pupae. Overall, Propineb WG 70A W fed at a concentration of 2.10 g a.s./L did not adversely affect honey bee brood development or success and did not increase the overall mortality rate of the colonies compared to the control.

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Report:	[redacted];[redacted];2014;M-488422-01
Title:	Propineb WG 70A W: Honey bee (Apis mellifera L.) larval toxicity test (single feeding exposure) - Final Report -
Report No:	S13-01495
Document No:	M-488422-01-1
Guidelines:	**OECD Draft Test Guideline on Honey Bee (Apis mellifera) Larval Toxicity Test, Single Exposure (Version dated 21 February 2013); Current draft version of the Post-WNT25 Approved Larval Honey Bee Test, (Dated April 2013); OECD 237**
GLP/GEP:	**Yes**

Objective:

The purpose of this study was to determine the toxicity of propineb WG 70A W to larvae of the honey bee (*Apis mellifera* L.). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Materials and Methods:

Test item: Propineb WG 70A W (Batch ID: EM20004026, Sample description: FAR 01501-02, Content of active ingredient (a.s.): 68.3 % w/w (683 g/kg) (analysed).

The effects of the test item Propineb WG 70A W on larvae of the honey bee, *Apis mellifera* L., were assessed in two 7-day tests in the laboratory. Since the mortality in the limit test (= first test) was above 10 % a second test was performed in a dose response design with 5 doses. Only the data of the dose-response test were reported.

Per treatment group, 16 honey bee larvae (first instar, L1) from three hives were transferred onto a 48 well-plate, each hive representing a replicate. A total of 48 larvae were used per treatment group and fed a standardized amount of artificial diet. On day 4 (D4) of the test, five different doses of Propineb WG 70A W were applied to the larvae with the diet. The control group was exposed for the same period of time under identical exposure conditions to the untreated artificial diet. Mortalities were recorded on D5, D6, and D7 (i.e. 24 h, 48 h and 72 h after application, respectively). The median lethal dose (LD_{50}) was determined for the end of the test period (D7).

Samples of the stock solution prepared freshly before feeding were taken for subsequent chemical analysis in order to reveal the actual concentration of the test item. The chemical analysis of the application (stock) solution was by Bayer CropScience AG, [redacted], Germany.

Biological phase was conducted at Nds. Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES) Institut für Bienenkunde [redacted], Germany.

Fisher's Exact Test (one-sided, α=0.05) was used to evaluate whether there are significant differences between the mortality data of the test item group and the control group in order to determine the NOED

(No-observed-effect-dose) for mortality. Test item data were Bonferroni-Holms corrected prior to analysis. The reference item was not statistically evaluated. The LD_{50} was calculated using a probit analysis (max. likelihood regression). The χ^2 was used as a goodness of fit measure.

Dates of experimental work: 10 June 2013 – 07 July 2013

Results:

No mortality occurred in the control group on the first two assessment days D5 and D6. Two larvae in the control group died until the last assessment day D7 (= 4.2 % mortality).

At the assessment D5 (24 hours after exposure to food containing different levels of Propineb WG 70A W) the mortality in the test item groups was statistically significantly increased compared to the control group at dose levels of 12.5, 25 and 100 µg a.s./larva (Fisher's Exact Test, Bonferroni-Holms corrected, one-sided, $p \leq 0.05$).

At the assessment D6 (48 hours after exposure to treated food) the mortality was statistically significantly increased compared to the control group at all dose levels, except of the lowest one of 6.25 µg a.s./larva.

At the assessment D7 (72 hours after exposure to treated food) a statistically significantly increased mortality occurred at all doses and reached a maximum adjusted mortality of 100 % at the highest dose level of 100 µg a.s./larva.

In the reference item group (dose: 8.8 µg a.s./larva) the adjusted mortality was 93.5 % on the final evaluation on D7 (72 hours after application of treated food).

The LD_{50} was calculated as 11.1 µg a.s./larva with 95 % confidence intervals of 9.1 – 13.1 µg a.s./larva using a probit analysis (max. likelihood regression, $\chi^2 = 5.31$, $p(\chi^2) = 0.15$).

Adjusted mortality after 72 h of a single exposure to treated food (test end) as well as the NOED and LD_{50}

Dose Level [µg a.s./larva] 1)	Control	Propineb WG 70A W					Reference item
	0.0	6.25	12.5	25	50	100	8.8
No. tested	48	48	48	48	48	48	48
No. dead (D7)	2	8*	33*	39*	46*	48*	45**
Adjusted mortality [%]	4.2	13.0	67.4	80.4	95.7	100.0	93.5
NOED	<6.25 µg a.s./larva						
LD_{50}	13.6 µg a.s./larva						
95 % confidence limit of LD_{50}	5.59 / 33.2						

1) Refers to the nominal content of active ingredient

*Significantly increased compared to control, Fisher's Exact Test, Bonferroni-Holms corrected, one-sided, $p \leq 0.05$

**Significantly increased compared to control, Fisher's Exact Test, one-sided, $p \leq 0.05$

Analytical Results

The actual concentration of propineb in the application (stock) solution was 92% of the nominal concentration consequently it is acceptable to present the results as nominal values.

Conclusions:
With respect to the results it can be concluded that Propineb WG 70A W caused a statistically significant increase in mortality on day 7 (D7) compared to the control up to and including 100 µg a.s./larva, the highest dose tested.
The NOED of Propineb WG 70A W could thus not be determined for day 7 (D7) and is assumed to be below 6.25 µg a.s./larva, the lowest dose tested.
The LD_{50} of Propineb WG 70A W was calculated to be 11.1 µg a.s./larva (95% confidence limits 9.1 – 13.1 µg a.s./larva) for day 7 (D7).

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 propineb, 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 Propineb WG 70 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 propineb, 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 propineb and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Propineb WG 70	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**56 mg product/kg dws** **38.7 mg a.s./kg dws**	[redacted] (2014) EBLHN035 M-476355-01-1 KCA 8.4.1/04
PTU	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**178 mg pm/kg dws**	[redacted] (2014) EBLHN040 M-478183-01-1 KCA 8.4.1/05
PU	*Eisenia fetida* reproduction 56 d, sprayed	**NOEC**	**≥ 1000 mg pm/kg dws**	[redacted] (2000) MPE/RG 329 M-033580-01-1 KCA 8.4.1/02
4-MI	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**90 mg pm/kg dws**	[redacted] (2013) Kra/Rg-R-118/12 M-449101-01-1 KCA 8.4.1/07
Propineb-DIDT	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**32 mg pm/kg dws**	[redacted] (2014) EBLHN051 M-486083-01-1 KCA 8.4.1/08

dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected
Bold values: endpoints used for risk assessment;
grey script = study is part of the Baseline Dossier (Annex I inclusion)

Report:	[redacted]d; [redacted];2014;M-476355-01
Title:	Propineb WG 70A W: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No:	13 10 48 179 S
Document No:	M-476355-01-1
Guidelines:	**ISO 11268-2: 1998(E): and OECD 222: April 13, 2004**
GLP/GEP:	**Yes**

Objectives:

The purpose of this study was to determine the sublethal effects of Propineb WG 70A W on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil with 8 different test concentrations in a laboratory test.

Materials and Methods:

Test material: Propineb WG 70A W; Short name: PPB WG 70A W: (Batch ID: EDFL009314; BCS Code: BCS-AG23438, Sample description: FAR01702-0; Specification No.: 102000006516 – 02; Material No.: 05468906; pure substance content of a.s. (analysed): 69.1% w/w)

Adult earthworms (*Eisenia fetida*, about 4 months old, 8 × 10 animals for the control group and 4 × 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 5.6, 10, 18, 32, 56, 100, 178 and 316 mg test item/kg soil dry weight.

Toxic standard: 5, 10 mg Nutdazim 50 FLOW (Carbendazim, SC 500) /kg dry weight soil; control: untreated.

Artificial soil composition was 68.5% quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400-800 Lux. Earthworms were fed with dried animal manure.

The test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its WHC. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Dates of experimental work: August 21 to October 16, 2013

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	2.5%
Number of juveniles per replicate	≥ 30	94 – 143
Coefficient of variation of reproduction	≤ 30%	18.9%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Nutdazim 50 FLOW (Carbendazim, SC 500) was tested at concentrations of 5 and 10.0 mg product/kg soil dry weight. In the reference test, the number of juveniles was reduced by 39 and 100 % by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

Effects on earthworm reproduction after 56 days

	Propineb WG 70A W [mg test item /kg dws]								
	Control	**5.6**	**10**	**18**	**32**	**56**	**100**	**178**	**316**
	Mortality of adult worms after 4 weeks								
Mortality [%]	2.5	0	2.5	0	0	2.5	0	2.5	2.5
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)								
Mean [%]	30.6	28.2	29.0	31.8	26.8	27.6	26.4	28.0	27.5
	Number of juveniles per surviving adult worm after 8 weeks								
Mean	12.9	12.5	12.0	11.9	12.2	11.6	9.8	5.5	2.9
	Number of juveniles per replicate after 8 weeks								
Mean ± SD	125.9 ± 23.8	124.8 ± 16.8	117.8 ± 32.6	119.3 ± 18.1	121.8 ± 10.8	113.8 ± 16.5	89.5* ± 16.9	54.0* ± 16.9	28.0* ± 14.5
	Reproduction compared to control [%]								
% to control	100	99.1	93.5	94.7	96.7	90.4	71.1	42.9	22.2

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater)
*statistically significantly different compared to control (Williams-t-test, $\alpha = 0.05$, one-sided smaller)
SD: standard deviation

Propineb WG 70A W showed no statistically significantly adverse effects on mortality and biomass of the earthworm *Eisenia fetida* in artificial soil up to 316 mg test item/kg soil dry weight, i.e. the highest concentration tested. The test item showed statistically significantly adverse effects on reproduction at 100, 178 and 316 mg test item/kg soil d.w.

Conclusions:
The No-Observed-Effect-Concentration (NOEC) was determined to be 56 mg Propineb WG 70A W /kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 100 mg Propineb WG 70A W /kg soil d.w.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	**Propineb WG 70A W** *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>316	>316	100
NOEC	≥316	≤316	56

Metabolite Propineb- propylenethiourea (PTU)

Report:	[redacted]q; [redacted]2014;M-478183-01-1
Title:	Propineb-propylenthioharnstoff: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No:	13 10 48 180 S
Document No:	M-478183-01-1
Guidelines:	**OECD 222 (2004), ISO 11268-2 (1998)**
GLP/GEP:	**Yes**

Objectives:

The purpose of this study was to determine the sublethal effects of propineb-propylenthiourea on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil with 5 different test concentrations in a laboratory test.

Materials and Methods:

Test material: Propineb-propylenthiourea (BCS Code: BCS-AA66986, Batch code: AE B007299-01-01, LIMS No.: 1308795, Customer order No.: TOX 10062-00, Origin Batch No.: NLL 3790-7, content of a.s. (analysed): 99.3 % w/w).

Adult earthworms (*Eisenia fetida*, about 4 months old, 8 × 10 animals for the control group and 4 × 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight.

Toxic standard: 5, 10 mg Nutdazim 50 FLOW (Carbendazim, SC 500) /kg dry weight soil; control: untreated.

Artificial soil composition was 68.5% quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Dates of experimental work: September 10 to November 05, 2013

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	103 – 136
Coefficient of variation of reproduction	≤ 30%	14.8%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Nutdazim 50 FLOW (Carbendazim, SC 500) was tested at concentrations of 5 and 10.0 mg product/kg soil dry weight. In the reference test, the number of juveniles was reduced by 39 and 100 % by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

Effects on earthworm reproduction after 56 days

Propineb-propylenthiourea [mg test item /kg dws]						
	Control	**100**	**178**	**316**	**562**	**1000**
	Mortality of adult worms after 4 weeks					
Mortality [%]	0	0	2.5	0	2.5	2.5
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)					
Mean [%]	40.7	42.3	45.0	43.1	39.9	24.1
	Number of juveniles per surviving adult worm after 8 weeks					
Mean± SD	11.8	11.4	10.1	8.1	6.1	1.4
	Number of juveniles per replicate after 8 weeks					
Mean± SD	117.9 ± 17.5	113.5 ± 20.7	98.3 ± 22.1	80.8* ± 15.1	59.5*± 22.1	13.5* ± 6.8
	Reproduction compared to control [%]					
% to control	100	96.3	83.4	68.5	50.5	11.5

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, α = 0.05, one-sided greater)
*statistically significantly different compared to control (Williams-t-test, α = 0.05, one-sided smaller)
SD: standard deviation

Propineb-propylenthiourea showed no statistically significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to and including 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested. The test item caused a significant reduction in adult biomass change of the earthworm *Eisenia fetida* at 1000 mg test item/kg soil d.w. The test item showed statistically significantly adverse effects on reproduction at 316, 562 and 1000 mg test item/kg soil d.w..

Conclusions:

The overall No-Observed-Effect-Concentration (NOEC) was determined to be 178 mg propineb-propylenthiourea /kg soil d.w., and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 316 mg propineb-propylenthiourea /kg soil d.w.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	**Propineb-propylenthiourea** *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>1000	1000	316
NOEC	≥1000	562	178

Metabolite Propineb-propylenurea (PU)

Report:	[redacted]8;[redacted];2000;M-033580-01
Title:	Influence of propylenurea on the reproduction of earthworms (Eisenia fetida)
Report No:	MPE/RG 329
Document No:	M-033580-01-1
Guidelines:	-/-
GLP/GEP:	Yes

Objectives:

The purpose of this study was to determine the sublethal effects of propineb-propylenthiourea on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil with 5 different test concentrations in a laboratory test.

Materials and Methods:

Propylenurea, (a.s.-content: 99.74 %, specification: Development-No.: 3000096248, Batch-No.: KTS 9525-1-1, TOX-No.: 5177 – 00) was used in this study. Adult *Eisenia fetida* (4 × 10 animals per application rate) were exposed in an artificial soil to the test concentrations of 10, 32, 100, 316 and 1000 mg test substance/kg dry weight soil. Artificial soil composition was 69% quartz sand, 20% kaolin clay, 10% sphagnum peat and 1% $CaCO_3$ and 1% ground cattle manure. Earthworms were fed with dried animal manure. The test conditions were 20 ± 2 °C, 70- 90 % relative humidity and 16:8 hours light-dark cycle (400- 800 lux). After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offsprings was determined.

Dates of experimental work: February 03 to March 30, 2000

Results:

Effects on earthworm reproduction after 56 days

Test substance	**Propylenurea**					
Test object	***Eisenia fetida***					
Exposure	**56 d**					
Application rates (mg a.s./kg)	control	10	32	100	316	1000
Mortality of adult earthworms (%) after 28 days	0	0	0	0	0	0
Weight increase of adult earthworms (%)	28	26	36	32	38	28
Number of offsprings per surviving adult	223	221	233	217	223	230

Observations:

Mortality or a body weight reduction of adult earthworms was not observed at any application rate in this study. Also the number of offsprings was not reduced at any application rate.

Conclusions:

NOEC (56 d) ≥ 1000 mg/kg dry weight soil.

Reference Substance:

Under the same conditions, a study was carried out with the reference substance Derosal (active ingredient: 36 % Carbendazim) from 27th October 1999 to 22th December 1999 (Report HBF/Rg 323 of January 07, 2000). The following dosages had been tested by application on the soil surface at the start of the study: 0.10, 0.25 and 0.50 kg a.s./ha. Mortality or body weight reduction of adult earthworms as compared to control organisms was not observerd at any dosage. Only the highest dosage of 0.5 kg a.s./ha reduced the numbers of juvenile earthworms by 37 %. The no-observed-effect-level (NOEL) was 0.25 kg/ha (= 0.032 kg active ingredient/ha) and the lowest-observed-effect-level (LOEL) 0.5 kg/ha (= 0.079 kg active ingredient/ha).

<u>**Metabolite Propineb-4-methyl-imidazoline (4-MI)**</u>

Report:	[redacted]; [redacted]; 2013;M-449101-01
Title:	Propineb-4-methyl-imidazoline (BCS-AB78877): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No:	kra-Rg-R-118/12
Document No:	M-449101-01-1
Guidelines:	**ISO 11268-2: 1998(E): and OECD 222: April 13, 2004**
GLP/GEP:	**Yes**

Objectives:

The purpose of this study was to assess the effects of Propineb-4-methyl-imidazoline (BCS-AB78877, metabolite of propineb) on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the 1st run and 5 different test concentrations in the 2nd run.

Materials and Methods:

Test material: Propineb–4-methyl-imidazoline-BCS-AB78877 (1st run: Batch code: BCS-AB78877-01-01; Material: BCS-AB78877, pure substance; Origin Batch No.: BCOO6454-4-5; purity 96.0 % w/w; 2nd run: Batch code: BCS-AB78877-01-03; Material: BCS-AB78877; pure substance; Origin Batch No: BCOO 6454-11-4; purity: 98.8 %w/w).

Adult earthworms (*Eisenia fetida*) were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 100 mg test item/ kg dry weight soil in the 1st test run and 9, 16, 28, 50 and 90 mg test item/kg dry weight artificial soil in the 2nd test run. In the 1st test run 8 x 10 animals, approximately 5 months old, for the control as well as for the treatment group were used. In the 2nd test run 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment groups, approximately 6 months old, were used.

Toxic standard: 1.25, 2.5, 5.0 mg Carbendazim (360 g a.s./L)/ kg dry weight soil.; control: untreated.

Artificial soil composition was 73.83% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.17% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

In both test runs, the test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Dates of work: 1st run: September 12 to May 04, 2012
2nd run: November 16, 2011 to July 10, 2012

Results:

Validity Criteria	Recommended	Obtained	
		1st run	2nd test
Adult mortality	≤ 10%	0%	0%
Number of juveniles per replicate	≥ 30	282.9	271.0
Coefficient of variation of reproduction	≤ 30%	18.8%	9.3%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25 and 5.0 mg product/kg soil dry weight.

In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from February 24 to May 02, 2012. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentrations of

2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel of all test concentrations were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 2.19 mg a.s./kg dry weight artificial soil. Confidence limits (95%) could not be calculated.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

1st run: Effects on mortality, growth and reproduction of the earthworms

Propineb–4-methyl-imidazoline [mg test item /kg dws]		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	6.25
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean ± SD [%]	+73.6 ± 13.7	-67.2 ± 16.3
	Number of juveniles per surviving adult worm after 8 weeks	
Mean± SD	28.3 ± 5.8	13.0 ± 2.2
	Number of juveniles per replicate after 8 weeks	
Mean± SD	282.9±53.2	122.9± 26.7
	Reproduction compared to control [%]	
% to control	-	43.4*

* statistical significance compared to the control (STUDENT-t test, one-sided smaller, α = 0.05)

2nd run: Effects on mortality, growth and reproduction of the earthworms

Propineb–4-methyl-imidazoline [mg test item /kg dws]						
	Control	**9**	**16**	**28**	**50**	**90**
	Mortality of adult worms after 4 weeks					
Mortality [%]	0	0	0	0	0	2.5
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)					
Mean ± SD [%]	+44.12± 6.68	+47.82 ± 7.35	+43.6 ± 6.21	+52.84 ± 5.55	+54.67 ± 9.78	+60.31 ± 5.89
	Number of juveniles per surviving adult worm after 8 weeks					
Mean± SD	27.1 ± 2.5	27.7 ± 3.3	28.7 ± 2.8	28.9 ± 1.5	24.7 ± 3.1	25.4 ± 3.5
	Number of juveniles per replicate after 8 weeks					
Mean± SD	271.0±25.2	277.0±32.7	286.8±28.0	288.8±15.4	246.5±31.1	247.3 ± 31.3
	Reproduction compared to control [%]					
% to control	-	102.2	105.7	106.5	91.0	91.2

After 28 days of exposure in both test runs no worms died in the control groups. In the treatment group of the 1st test run one worm in three replicates and 2 worms in one replicate (five dead worms in total). One worm died in the highest test concentration of the 2nd test run.
Statistically significant different values for the growth relative to the control were observed only in the two highest test concentrations of the 2nd test run. Since the weight increase of the adult worms of the two highest test concentrations was higher than in the control, this is not considered as an adverse effect.
In the 1st run (limit test), statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 100 mg test item/kg dry wt. artificial soil. In the 2nd test run (dose-response-test) no statistically significantly different values for the number of juveniles per test vessel relative to the control were observed.

Conclusions:
Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 90 mg propineb–4-methyl-imidazoline /kg dry weight artificial soil. Thus, the overall LOEC is determined to be 100 mg propineb–4-methyl-imidazoline /kg dry weight artificial soil.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	Propineb–4-methyl-imidazoline *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100[1]	>100	100
NOEC	≥100[1]	≥100	90

Metabolite Propineb-DIDT

Report:	[redacted]h; [redacted];2014;M-486083-01
Title:	Propineb-DIDT (BCS-CU99534): Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No:	M-486083-01-1
Document No:	M-486083-01-1
Guidelines:	**ISO 11268-2 (1996)** **BBA, Guidelines for the Testing of Plant Protection Products Within Registration, Part VI, 2 -2, January 1994**
GLP/GEP:	**Yes**

Objectives:

The purpose of this study was to determine the sublethal effects of the test item on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil in a laboratory test.

Materials and Methods:

Test item: Propineb-DIDT (BCS-CU99534), BCS Code: BCS-CU99534, Batch code: BCS-CU99534-01-01, Origin Batch No.: SES 11956-8-1, LIMS No.: 1334297, Customer order No.: TOX 10290-00, analysed purity: 99.5 % w/w.

Adult earthworms (*Eisenia fetida*, about 4 months old) were exposed to 10 – 18 – 32 – 56 - 100 mg pure metabolite/kg dry weight (d.w.) of soil containing 68.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat, 1 % food and 0.5 % $CaCO_3$, at 18.3 – 21.9 °C and a photoperiod: light : dark = 16 h : 8 h (530 lx) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

Toxic standard: 5 and 10 mg Nutdazim 50 FLOW/kg soil d.w.; control: untreated, solvent control: none.

Dates of work: February 20 to April 17, 2014

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	1.3%
Number of juveniles per replicate	≥ 30	127, 132, 104, 90, 96, 129, 142 and 156
Coefficient of variation of reproduction	≤ 30%	19.0%

All validity criteria for the study were met.

In a reference test, the number of juveniles was reduced by 39 and 100 % by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

Effects on mortality, growth and reproduction of the earthworms after 56 days

Propineb-DIDT (BCS-CU99534) [mg pure metabolite/kg soil d.w.]						
	Control	**10**	**18**	**32**	**56**	**100**
	Mortality of adult worms after 4 weeks					
Mortality [%]	0	2.5	0.0	0.0	5.0	2.5
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)					
Mean (mg)	133.6	124.8	128.4	139.4	126.0	123.3
Mean (%)	37.8	35.6	36.8	39.7	36.1	35.3
	Number of juveniles per surviving adult worm after 8 weeks					
Mean	12.3	13.3	12.4	10.9	8.1	4.3
	Number of juveniles per replicate after 8 weeks					
Mean	122.0	129.3	123.5	108.5	77.5*	41.5*
	Reproduction compared to control [%]					
% to control	100	105.9	101.2	88.9	63.5	34.0

No statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, □ = 0.05, one-sided greater) and for biomass (Williams-t-test, □ = 0.05, one-sided smaller)
* statistically significantly different compared to the control for reproduction (Williams t test, one-sided smaller, α = 0.05)

Propineb-DIDT (BCS-CU99534) showed no statistically significantly adverse effects on mortality and adult biomass change of the earthworm *Eisenia fetida* in artificial soil up to and including 100 mg pure metabolite/kg soil dry weight, i.e. the highest concentration tested. The test item showed statistically significantly adverse effects on reproduction at 56 and 100 mg pure metabolite/kg soil d.w.

Conclusions:

Based on the statistical evaluation of the results, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 32 mg pure metabolite/kg soil d.w., and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 56 mg pure metabolite/kg soil d.w.

Effects on mortality, growth and reproduction of the earthworms

Test item **Test object** **Exposure**	**Propineb- DIDT (BCS-CU99534)** ***Eisenia fetida*** **Artificial soil**		
	Mortality	Biomass change	Reproduction
	[mg pure metabolite/kg soil d.w.]		
NOEC	≥100	≥100	32
LOEC	>100	>100	56

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

For information on studies already evaluated during the first EU review of propineb, 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 propineb. 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 propineb and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Propineb WG 70	*Folsomia candida* reproduction 28 d, mixed	NOEC	56 mg prod./kg dws **39.1 mg a.s./kg dws**	[redacted] (2011) 11 10 48 088 S M-416315-01-1 KCA 8.4.2.1/01
PTU	*Folsomia candida* reproduction 28 d, mixed	NOEC	**9.0 mg pm/kg dws**	[redacted] (2014) FRM-Coll-169/14 M-484500-01-1 KCA 8.4.2.1/10
PU	*Folsomia candida* reproduction 28 d, mixed	NOEC	**90 mg pm/kg dws**	[redacted] (2011) FRM-Coll-130/11 M-420414-01-1 KCA 8.4.2.1/04
4-MI	*Folsomia candida* reproduction 28 d, mixed	NOEC	**≥100 mg pm/kg dws**	[redacted] (2013) FRM-Coll-168/13 M-473843-01-1 KCA 8.4.2.1/06
Propineb-DIDT	*Folsomia candida* reproduction 28 d, mixed	NOEC	**≥100 mg pm/kg dws**	[redacted] (2014) 14 10 48 093 S M-481886-01-1 KCA 8.4.2.1/08
Soil mites, reproduction				
Propineb WG 50	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	56 mg prod./kg dws **39.1 mg a.s./kg dws**	[redacted] (2011) 11 10 48 087 S M-421441-01-1 KCA 8.4.2.1/02
PTU	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	**≥100 mg pm/kg dws**	[redacted] (2014) 14 10 48 096 S M-484793-01-1 KCA 8.4.2.1/03
PU	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	**≥100 mg pm/kg dws**	[redacted] (2011) kra-HR-56/11 M-415889-01-1 KCA 8.4.2.1/04
4-MI	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	**≥100 mg pm/kg dws**	[redacted] (2014) EBLHN054 M-487109-01-1 KCA 8.4.2.1/07
Propineb-DIDT	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	**≥100 mg pm/kg dws**	[redacted] (2014) 14 10 48 094 S M-487493-01-1 KCA 8.4.2.1/09

dws = dry weight soil; a.s. = active substance; pm = pure metabolite, prod. = product
Bold values: endpoints used for risk assessment

CA 8.4.2.1 Species level testing

Report:	[redacted]y; [redacted];2011;M-416315-01
Title:	Propineb WG 70A W: Effects on the reproduction of the collembolans Folsomia candida
Report No:	11 10 48 088 S
Document No:	M-416315-01-1
Guidelines:	**OECD 232 (2009): OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009): Collembolan reproduction test in soil ISO 11267 (1999): Soil quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants**
GLP/GEP:	**Yes**

Objectives:

The purpose of this study is to determine potential the effects of different concentrations of the test item on the reproductive output of the collembolans *Folsomia candida* as a representative of soil micro arthropods during a test period of 28 days.

Materials and Methods:

Test material: Propineb WG 70A W, Sample description: TOX09313-00, Batch ID: EDFL010973, Specification No.: 102000006516 - 02, Material No.: 05468906, analysed active ingredient: 69.8 % w/w Propineb (LH 30/Z), water solubility: dispersible.

Ten collembola (9-12 days old) were exposed to 10, 18, 32, 56 and 100 mg test item/kg soil dry weight containing 74.7 % quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$, at 18.0 - 22°C and a photoperiod: light : dark = 16 h : 8 h (720 lux) and were fed weekly with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

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

Dates of work: August 02, 2011 to August 30, 2011

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	6.3 %
Mean number of juveniles per pest vessel	≥ 100	436
Coefficient of variation of reproduction	< 30%	10.7 %

All validity criteria for the study were met

Effects on collembolan reproduction after 28 days

	Propineb WG 70A W [mg test item /kg soil d.w.]					
	Control	**10**	**18**	**32**	**56**	**100**
Mortality of parental collembolans after 4 weeks [%]	6.3	5.0	7.5	7.5	7.5	45.0*a
Mean number of juveniles after 4 weeks	436	421	434	429	431	185*b
CV %	10.7	15.1	26.3	4.4	5.1	31.8
% Reduction of reproduction compared to control	-	3	0	2	1	58

* statistically significant differences compared to the control (a Fisher's Exact Binomial Test with Bonferroni Correction for mortality, α = 0.05, one-sided greater; b Welch-t-test for Inhomogeneous Variances with Bonferroni Adjustment for reproduction; α = 0.05, one-sided smaller)
Calculations were done using non-rounded values
Percent reduction: (1 - Rt / Rc) * 100 %
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group
Negative values indicate a higher reproductive performance compared to control.

The test item Propineb WG 70A W showed statistically significantly adverse effects on adult mortality of the collembolans *Folsomia candida* in artificial soil at 100 mg test item/kg soil d.w. The test item caused a significant reduction of reproduction of the collembolans *Folsomia candida* in artificial soil at 100 mg test item/kg soil dry weight, i.e. the highest concentration tested.

Conclusions:

The overall No-Observed-Effect-Concentration (NOEC) was determined to be 56 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 100 mg test item/kg soil d.w.

Effects of Propineb WG 70A W on collembolans: summary of statistical analysis

	Propineb WG 70A W (mg test item/kg soil dry weight)	
	Mortality	Reproduction
LOEC	100	100
LC_{50}/ EC_{50}	128	96
95% confidence limit, lower - upper	(92 - 178)	(92 – 98)
NOEC	56	56

Reference test:

In the most recent study (BioChem project No. R 11 10 48 004 S, dated May 05, 2011) the EC_{50} was determined to be 107 mg a.s./kg soil dry weight. The LC_{50} was determined to be 193 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg a.s./kg soil dry weight, respectively. The EC_{50} value for the reduction of reproduction was close to the value

of 100 mg a.s./kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

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Report:	[redacted]y;[redacted];2011;M-421441-01
Title:	Propineb WG 70A W: Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No:	11 10 48 087 S
Document No:	M-421441-01-1
Guidelines:	**OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil**
GLP/GEP:	**Yes**

Objective:

The purpose of the study was to assess the effects of Propineb WG 70A W on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:

Test item: Propineb WG 70A W (short name: PPB WG 70A W; Sample description: TOX09313-00; Batch ID: EDFL010973; Specification No.: 102000006516 – 02; purity (analysed): 69.8 %w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each application rate) were exposed to control (water treated) and treatments. An additional 2nd test run with changed concentrations of the test item was performed because no NOEC and no EC_{50} for reproduction could be determined from the results of the 1st test run. Concentrations of 100, 178, 316, 562, 1000 mg test item/ kg dry weight soil was tested in the 1st run und 5.6, 10, 18, 32 56 mg test item/ kg dry weight soil in the 2nd run.

In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (1st run: 29 days after start of egg laying; 2nd run: 35 days after start of egg laying). During the test, they were fed with *Tyrophagus putrescentiae*. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand, 5% Sphagnum peat, 20% kaolin clay and approximately 0.2 % Calcium carbonate (CaCO3).

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen high-gradient extractor (heat/light extraction method). Extracted mites were collected in a fixing liquid.

Dates of experimental work: August 16, 2011 to November 14, 2011

Results:

Validity Criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mean adult mortality	≤ 20%	2.5%	3.8%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	271.8	273.5%
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	15.7%	7.9%

All validity criteria for the study were met.

Effect of Propineb WG 70A W on *Hypoaspis aculeifer* in a 14-day reproduction study

1st run

Test item Test object Exposure	Propineb WG 70A W *Hypoaspis aculeifer* Artificial soil		
mg test item/kg dry weight artificial soil	Adult mortality (%)	Mean number of juveniles ± SD	Reproduction (% to control)
Control	2.5	271.8 ± 42.7	-
100	7.5	110* ± 23.8	59.5
178	0	0* ± 0	100.0
316	5.0	6.0* ± 7.7	97.8
562	2.5	0.5* ± 0.6	99.8
1000	30.0*	0* ± 0	100.0
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			could not be determined
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			

* statistically significant difference compared to control (Fisher`s Exact Binomial Test for mortality, α = 0.05; Welch t-test for reproduction; α = 0.05)

2nd run

Test item Test object Exposure	Propineb WG 70A W *Hypoaspis aculeifer* Artificial soil		
mg test item/kg dry weight artificial soil	Adult mortality (%)	Mean number of juveniles ± SD	Reproduction (% to control)
Control	3.8	273.5 ± 21.7	-
5.6	2.5	275.0 ± 42.7	-0.5
10	2.5	278.3 ± 18.7	-1.7
18	0	293.0 ± 22.7	-7.1
32	7.5	274.3 ± 6.9	-0.3
56	0	241.3 ± 41.5	11.8
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			56
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			100

No statistically significant differences compared to the control were calculated (Fisher`s Exact Binomial Test for mortality, α = 0.05; Dunnett-t-test for reproduction; α = 0.05)

Mortality:

In the control group the mortality rate was 2.5 % and 3.8 % in the 1st and 2nd test run, respectively, which is below the allowed maximum of ≤ 20% mortality. The observed mortality rates for adult mortality in the test item treatment groups compared to control were not statistically significant up to

and including a test concentration of 562 mg test item/kg soil d.w. (Fisher's Exact Binomial Test, α = 0.05, one-sided greater). However, a statistically significant effect on mortality compared to control was found at 1000 mg test item/kg soil d.w. The No-Observed-Effect-Concentration (NOEC) and the Lowest-Observed-Effect-Concentration (LOEC) for mortality were determined to be 562 mg test item/kg soil d.w. and 1000 mg test item/kg soil d.w., respectively.

Reproduction:
The test item caused no statistically significant reduction of reproduction up to and including a test concentration of 56 mg test item/kg soil dry weight (Dunnett-t-test, α = 0.05, one-sided smaller). However, statistically significant effects on reproduction compared to control were found beginning at a concentration of 100 mg test item/kg soil d.w. (Welch t-test, α = 0.05, one-sided smaller).
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 56 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 100 mg test item/kg dry weight artificial soil.

Conclusion:
The $NOEC_{reproduction}$ is 56 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ 100 mg test item/kg soil d.w.

Reference test:
To verify the sensitivity of the test system, the reference item Dimethoate EC 400 was tested at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg soil dry weight.
In the most recent study (BioChem project No. R 11 10 48 003 S, dated March 29, 2011), the EC_{50} (reproduction) was calculated to be 5.4 mg a.s./ kg soil d.w.
The results of the reference test demonstrate the sensitivity of the test system.

Metabolite Propineb- propylenethiourea (PTU)

Report:	KCA 8.4.2.1/10; [redacted], U.; 2014; M-484500-01-1
Title:	Propineb-propylenthiourea (BCS-AA66386): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Report No:	FRM-Coll-169/14
Document No:	M-484500-01-1
Guidelines:	**OECD 232 (2009): OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009): Collembolan reproduction test in soil**
GLP/GEP:	**Yes**

Objectives:
The purpose of this study was to assess the effect of propineb-propylenthiourea (BSC-AA66386) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Propineb-propylenthiourea (BCS-AA66386), analytical findings: 99.3 % w/w AE B007299, origin batch no.: NLL 3790-7, customer order no.: TOX10062-00, CAS no.: 2122-19-2, batch code: AE B007299-01-01.

Since the 1st test run on the test item did not provide a final result, a 2nd test run was performed studying lower test concentrations. In the 1st test run 10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 10, 18, 32, 56, and 100 mg test item/kg artificial soil dry weight. In the 2nd test run 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 3.0, 5.2 and 9.0 mg test item/kg artificial soil dry weight. Both runs at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Dates of work: August 23, 2013 to February 19, 2014

Results:

Validity Criteria	**Recommended**	**Obtained**	
		1st run	2nd run
Mean adult mortality	≤ 20%	3.8 %	7.5 %
Mean number of juveniles per pest vessel	≥ 100	1270.8	1372.3
Coefficient of variation of reproduction	< 30%	8.3 %	4.9 %

All validity criteria for the study were met

Effects on collembolan reproduction after 28 days

Propineb-propylenthiourea (BCS-AA66386) [mg test item /kg soil d.w.]						
1st run						
	Control	**10**	**18**	**32**	**56**	**100**
Mortality of parental collembolans after 4 weeks [%]	3.8	15.0	7.5	42.5	95.0	100.0
Mean number of juveniles after 4 weeks	1270.8	1049.3	1223.8	564.8	59.3	20.3
CV %	105.6	102.1	151.4	220.9	55.5	12.7
% Reduction of reproduction compared to control *	-	82.6 s.	96.3 n.s.	44.4 s.	4.7 s.	1.6 s.
2nd run						
	Control	**3.0**	**5.2**	**9.0**	**-**	**-**
Mortality of parental collembolans after 4 weeks [%]	7.5	10.0	7.5	5.0	-	-
Mean number of juveniles after 4 weeks	1372.3	1389.0	1282.0	1285.8	-	-
CV %	67.0	76.9	116.9	34.2	-	-
% Reduction of reproduction compared to control	-	101.2 n.s.	93.4 s.	93.7 s.	-	-

* = Welch-t test for inhomogeneous variances, one-sided-smaller, α = 0.05, s. = significant, n.s. = not significant for the 1st run, William's test, one-sided-smaller, α = 0.05, s. = significant, n.s. = not significant for the 2nd run

Observations:

Mortality:

In the control group 3.8 % (1st run) and 7.5 % (2nd run) of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:

Concerning the number of juveniles in the 1st test run statistical analysis (Welch-t test for inhomogeneous variances, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group at 18 mg test item/kg artificial soil dry weight, while all other treatment groups including 10 mg test item/kg artificial soil dry weight were significantly reduced.

In the 2nd test run no statistically significant effects were observed in the treatment group 3.0 mg test item/kg artificial soil dry weight (reproduction 101.2 % of the control). In the next higher test item groups 5.2 and 9.0 mg test item/kg artificial soil dry weight the reproduction was 93.4 % and 93.7 % of the control, indicating a statistical significant difference of 6.6 % and 6.3 % (William's test, one-sided smaller, α = 0.05), respectively. Since the observed statistically significant differences at 5.2 and 9.0 mg test item/kg artificial soil dry weight are very low and no dose-response relationship was observed these differences can be regarded as not biologically relevant.

Therefore the biological relevant Lowest-Observed-Effect-Concentration (LOEC) for reproduction is considered to be 10 mg test item/kg artificial soil dry weight and the corresponding biological relevant No-Observed-Effect-Concentration (NOEC) for reproduction is considered to be 9.0 mg test item/kg artificial soil dry weight.

Conclusions:
The overall No-Observed-Effect-Concentration (NOEC) was determined to be 9.0 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 10 mg test item/kg soil d.w.

Reference test:
The most recent non-GLP-test (FRM-Coll-Ref-24/14, U. [redacted], March 13, 2014) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.
Boric acid showed an EC_{50} of 90 mg test item/kg artificial soil dry weight (95 % confidence limits from 68 mg to 119 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be <44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 44 mg Boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

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Report:	[redacted] g; [redacted] ;2014;M-484793-01
Title:	Propineb-propylenthiourea (BCS-AA66386): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No:	14 10 48 096 S
Document No:	M-484793-01-1
Guidelines:	**OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil**
GLP/GEP:	**Yes**

Objective:
The purpose of the study was to determine potential effects of the test item on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro-arthropods during a test period of 14 days.

Materials and Methods:
Test item: Propineb-propylenthiourea, BCS-code: BCS-AA66386, Batch code: AE B007299-01-01, Origin Batch No.: NLL 3790-7, CAS No.: 2122-19-2, LIMS No.: 1308795, Customer Order No.: TOX 10062-00 analysed purity: 99.3 % w/w.
Ten adult soil mites (females) were exposed to 100 mg pure metabolite/kg dry weight (d.w.) of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.2 % CaCO3, at 19.7 - 21.2 °C and a photoperiod: light : dark = 16 h : 8 h (517 lx) and were fed every 2 - 3 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.
Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg soil d.w.; control: untreated, solvent control: none.

Dates of experimental work: February 04, 2014 to March 04, 2014

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	5.0%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	244.6
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	11.9%

All validity criteria for the study were met.

Effects on reproduction of soil mites after 14 days

Endpoint	Propineb-propylenthiourea (BCS-AA66386) (mg pure metabolite/kg soil d.w.)	
	Control	**100.0**
Mortality of soil mites after 14 days (%)	5.0	7.5
Mean number of juveniles after 14 days	244.6	222.5
CV (%)	11.9	9.8
Reproduction (% to control)	100	91

No statistically significant differences compared to control were calculated (Chi[2] 2x2 Test for mortality, $\alpha = 0.05$)
Calculations were done using non-rounded values
Percent reproduction: (Rt / Rc)* 100%
Rt = mean number of juvenile mites in the treated group(s)
Rc = mean number of juvenile mites in the control group

The test item propineb-propylenthiourea (BCS-AA66386) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg pure metabolite/kg soil dry weight.

Conclusion:
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg pure metabolite/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg pure metabolite/kg soil dry weight.

Effect on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Propineb-propylenthiourea (BCS-AA66386) *Hypoaspis aculeifer* Artificial soil	
	Adult mortality (%)	**Reproduction (% of control)**
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100

Metabolite Propineb-propylenurea (PU)

Report:	[redacted]k;[redacted];2011;M-420414-01
Title:	Propineb-propylenharnstoff (AE 1379609): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-130/11
Document No:	M-420414-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil**
GLP/GEP:	**Yes**

Objective:

The purpose of this study was to assess the effect of propineb-propylenurea (AE 1379609) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Test material: propineb-propylenurea (AE 1379609) (analytical findings: 98 % w/w, origin batch no.: RDL 125-21-1, certificate no.: AZ 17285, LIMS no.: 1109639, batch code: AE 1379609-01-02.

Since the first test run on the test item did not provide a final result, a second test run was performed studying lower test concentrations. 10 collembolans (10-12 days old in the 1st test run and 11-12 days old in the 2nd test run) per replicate (8 replicates for the control group and 8 replicates for the treatment group in the 1st test run, 4 replicates for each treatment group in the 2nd test run) were exposed to control (water treated and 100 mg test item/kg artificial soil dry weight in the 1st test run and 10, 17, 30, 52, and 90 mg test item/kg artificial soil dry weight in the 2nd test run at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Dates of experimental work: July 29, 2011 to October 13, 2011

Results:

Validity Criteria	**Recommended**	**Obtained in this study**	
		1st run	2nd run
Mean adult mortality	≤ 20%	7.5%	13.8 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1491	1389
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	15.2 %	17.3 %

All validity criteria for the study were met.

Effect of PU on reproduction of *Folsomia candida* after 28 days

Test item Test object Exposure	Propineb-propylenurea (AE 1379609) *Folsomia candida* Artificial soil		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
1st run			
Control	7.5	1491.4 ± 227.1	-
100	23.8	1146.6 ± 123.0	76.9*
2nd run			
control	13.8	1389.4 ± 240.5	-
90	32.5	1250.3 ± 142.9	90.0 n.s.
52	35.0	1344.5 ± 198.7	96.8 n.s.
30	17.5	1350.5 ± 73.3	97.2 n.s.
17	20.0	1389.5 ± 56.2	100.0 n.s.
10	2.5	1433.3 ± 104.5	103.2 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight)			90
$LOEC_{reproduction}$ (mg test item/kg dry weight)			100

The calculations were performed with un-rounded values
* = statistically significant (Student-t test one-sided-smaller, α = 0.05)
n.s. = statistically not significant (William's t test one-sided-smaller, α = 0.05)

Mortality:
In the control group 7.5 % (1st run) and 13.8 % (2nd run) of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed statistically significant difference between control and the treatment group in the 1st test run. In the 2nd test run Williams-t test, one-sided smaller, α = 0.05 revealed no significant differences between control and any treatment group in the 2nd test run.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 90 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 100 mg test item/kg artificial soil dry weight.

Conclusions:
$NOEC_{reproduction}$: 90 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: 100 mg test item/kg artificial soil dry weight.

Reference test:
The most recent non-GLP-test (FRM-Coll-Ref-15/11, U. [redacted], March 08, 2011) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.
Boric acid showed an EC_{50} of 91 mg test item/kg artificial soil dry weight (95 % confidence limits from 80 mg to 104 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.

The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.
This shows that the test organisms are sufficiently sensitive.

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Report:	[redacted]::[redacted];2011;M-415889-01
Title:	Propineb-propylenharnstoff (AE 1379609): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-56/11
Document No:	M-415889-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil**
GLP/GEP:	**Yes**

Objective:
The purpose of the study was to assess the effects of propineb-propylenurea (AE 1379609) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:
Test item: propineb-propylenurea (AE 1379609); (Batch code: AE 1379609-01-02; Origin Batch No.: RDL 125-21-1; Material: AE 1379609, technical substance; Certificate No.: AZ 17285; CAS No.: 6531-31-3; purity: 98 %w/w)
Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 8 replicates for each application rate) were exposed to control (water treated) and treatments. The concentration of 100 mg test item/ kg dry weight soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2 % Calcium carbonate ($CaCO_3$).
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Dates of experimental work: July 04, 2011 to July 29, 2011

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	7.5%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	269.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	12.5%

All validity criteria for the study were met.

Effect of propineb-propylenurea (AE 1379609) on *Hypoaspis aculeifer* in a 14-day reproduction study

Test item Test object Exposure	Propineb-propylenurea (AE 1379609) *Hypoaspis aculeifer* Artificial soil		
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	7.5	269.5 ± 33.6	
100	7.5	262.8 ± 46.5	97.9
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

No statistical significance (Student-t-test, one-sided smaller, a = 0.05) was found.

Mortality:
In the control group 7.5% of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20% mortality.

Reproduction:
Concerning the number of juveniles statistical analysis revealed no significant difference between control and all concentrations up to 100 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil.

Conclusion:
The $NOEC_{reproduction}$ is ≥ 100 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ > 100 mg test item/kg soil d.w.

Reference test:
The most recent non-GLP-test (Marie-Agnes [redacted], kra/HR-O-10/11, March 21, 2011) with the reference item dimethoate was performed at test concentrations 0.990, 1.780, 3.156, 5.517 and 9.853 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 4.051 mg a.s./kg (95 % confidence limits from 3.222 to 5.313 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The $NOEC_{reproduction}$ was calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.5 mg a.s./kg dry weight artificial soil according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided. Dimethoate showed a EC_{50} of 6.445 mg a.s./kg dry weight artificial soil (95 % confidence limits from 6.02 to 8.02 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.

Metabolite Propineb-4-methyl-imidazoline (4-MI)

Report:	[redacted]3; [redacted];2013;M-473843-01
Title:	Propineb-4-methyl-imidazoline (BCS-AB78877): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-Coll-168/13
Document No:	M-473843-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable; OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to assess the effect of propineb-4-methyl-imidazoline (BCS-AB78877) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test material: propineb-4-methyl-imidazoline (BCS-AB78877), analytical findings: 98.8 % w/w, origin batch no.: BCOE 6454-11-4, certificate no.: AZ-17925, batch code: BCSAB78877-01-03, LIMS no.: 1202055.
10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (water treated), solvent control (aceton treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Dates of experimental work: August 23, 2013 to October 01, 2013

Results:

Validity Criteria	Recommended	Obtained in this study
Mean adult mortality	≤ 20%	5%
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1282
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	8.7 %

All validity criteria for the study were met.

Effect of propineb-4-methyl-imidazoline on reproduction of *Folsomia candida* after 28 days

Test item Test object Exposure	Propineb-4-methyl- imidazoline (BCS-AB78877) (AE 1379609) *Folsomia candida* Artificial soil				
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles per test vessels ± SD		Reproduction (% of control)	Significance (*)
Control	3.8	1270.8	± 105.6		
Solvent control	6.3	1293.3	± 124.0	101.8	-
Pooled control	5.0	1282.0	± 111.9		
100	3.8	1317.4	± 123.9	103.7	-
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)				≥ 100	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)				> 100	

The calculations were performed with un-rounded values
(*) = (Student-t test one-sided-smaller, α = 0.05, + = significant, - = not significant)

Since there was no statistically significant difference between control and solvent control, the pooled control values were used for further calculations.

Mortality:
In the pooled control group 5 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, α = 0.05) revealed no statistically significant difference between the pooled control and the single treatment group.

Conclusions:
$NOEC_{reproduction}$: ≥ 100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: >100 mg test item/kg artificial soil dry weight.

Reference test:

The most recent non-GLP-test (FRM-Coll-Ref-21/13, U. [redacted], March 26, 2013) with the reference item Boric acid was performed at test concentrations 44 – 67 - 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.

Boric acid showed an EC_{50} of 108 mg test item/kg artificial soil dry weight (95 % confidence limits from 98 mg to 120 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.

The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

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Report:	[redacted];[redacted];2014;M-487109-01
Title:	Propineb-4-methyl-imidazoline (BCS-AB78877): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	LAR-HR-106/14
Document No:	M-487109-01-1
Guidelines:	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: Not Applicable; OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil**
GLP/GEP:	**Yes**

Objective:

The purpose of this study was to assess the effect of propineb-4-methyl-imidazoline (BCS-AB78877) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:

Propineb-4-methyl-imidazoline (BCS-AB78777) (analytical findings: 98.8 % w/w; batch code: BCS-AB78877-01-03; certificate no.: AZ 19293; origin batch no.: BCOO 6454-11-4; material: BCS-AB78877, technical substance)

Ten adult, fertilized, female *Hypoaspis aculeife*r per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 10, 18, 32, 56 and 100 mg test item/kg artificial soil dry weight were tested. During the test, the *H. aculeife*r were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2°C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and Calcium carbonate ($CaCO_3$) for the adjustment to pH to 6.0 ± 0.5

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *H. aculeifer* were counted under a binocular.

Dates of experimental work: March 26, 2014 to April 22, 2014

Results:

Validity Criteria	Recommended	Obtained in this study
Mean adult mortality	≤ 20%	1.3%
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	269.8
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	6.0%

All validity criteria for the study were met.

Effect of propineb-4-methyl-imidazoline on reproduction of *Hypoaspis aculeifer* after 14 days

Test item Test object Exposure	Propineb-4- methyl- imidazoline (BCS-AB78877) *Hypoaspis aculeifer* Artificial soil			
mg test item/kg dry weight artificial soil	% mortality (adults)	Mean number of juveniles per test vessels ± standard deviation	Reproduction (% of control)	Significance (*)
Control	1.3	269.8 ± 16.2	-	-
10	2.5	281.3 ± 15.4	104.3	-
18	2.5	283.8 ± 38.1	105.2	-
32	0.0	297.3 ± 31.8	110.2	-
56	0.0	295.5 ± 21.4	109.5	-
100	0.0	291.0 ± 13.9	107.9	-
$NOEC_{reproduction}$ (mg test item/kg soil dry weight artificial soil)			≥ 100	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight artificial soil)			> 100	

(*)=William's-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

Mortality:

In the control group 1.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight.

Conclusions:
$NOEC_{reproduction}$: ≥ 100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: >100 mg test item/kg artificial soil dry weight.

Reference test:
The most recent non-GLP-test (Maria Ivonne [redacted], LAR/HR-O-14/14, March 11, 2014) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 3.51 mg a.s./kg (95 % confidence limits from 3.46 mg a. s./kg to 3.57 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test α = 0.05, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.28 mg a. s./kg (95 % confidence limits from 4.02 mg a. s./kg to 6.47 mg a. s./kg) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a. s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Metabolite Propineb-DIDT

Report:	[redacted] $a [redacted] ;2014;M-481886-01
Title:	Propineb-DIDT (BCS-CU99534): Effects on the reproduction of the collembolan Folsomia candida
Report No:	14 1048 093 S
Document No:	M-481886-01-1
Guidelines:	**OECD 232 (2009): ISO 11267 (1999)**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to assess the effect of Propineb-DIDT (BCS- CU99534) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test material: Propineb-DIDT (BCS- CU99534), Batch code: BCS-CU99534-01-01, Origin Batch No.: SES 11956-8-1, LIMS No.: 1334297, Customer order No.: TOX 10290-00, analysed purity: 99.5 % w/w.

Ten collembolans (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control (quartz sand) and 100 mg test item/kg artificial soil dry weight at 18.7 – 21.6°C, 520 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Dates of experimental work: February 11, 2014 to March 11, 2014

Results:

Validity Criteria	Recommended	Obtained in this study
Mean adult mortality	≤ 20%	7.3%
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	644
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	12.5 %

All validity criteria for the study were met.

Effect of Propineb-DIDT on *Folsomia candida* after 28 days

Test item Test object Exposure	Propineb-DIDT (BCS- CU09534) *Folsomia candida* Artificial soil			
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles per test vessels ± SD**	**Reproduction (% of control)**	**Significance (*)**
Control	1.3	644 ± 80		
100	1.3	631 ± 105	98	-
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥ 100	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			> 100	

The calculations were performed with un-rounded values
(*) = (Student-t test one-sided, smaller, α = 0.05, + = significant, - = not significant)

Since there was no statistically significant difference between control and solvent control, the pooled control values were used for further calculations.

Mortality:
No statistical significant effect on parental mortality was found for the concentration tested.

Reproduction:
Concerning the number of juveniles statistical analysis revealed no statistically significant difference between the control and the single treatment group.

Conclusions:
$NOEC_{reproduction}$: ≥100 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: >100 mg test item/kg artificial soil dry weight.

Reference test:
In the most recent study (BioChem project No. R 13 10 48 004 S, dated July 16, 2013) the EC_{50} was determined to be 108 mg a.s./kg soil dry weight. The LC_{50} was determined to be 192 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg a.s./kg soil dry weight, respectively.
The EC_{50} value for the reproduction was close to the value of 100 mg a.s./kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

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Report:	[redacted]2;[redacted];2014;M-487493-01
Title:	Propineb-DIDT (BCS-CU99534): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No:	14 10 48 094 S
Document No:	M-487493-01-1
Guidelines:	**OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to determine potential effects of the test item on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (Canestrini) as a representative of soil micro-arthropods during a test period of 14 days. A NOEC and a LOEC were determined. The test was performed according to the OECD guideline 226 (2008).

Materials and Methods:
Test item: Propineb-DIDT, BCS-code: BCS-CU99534, Batch code: BCS-CU99534-01-01, Origin Batch No.: SES 11956-8-1, LIMS No.: 1334297, Customer Order No.: TOX 10290-00, analysed purity: 99.5 % w/w.
10 adult soil mites (females) were exposed to 1.8 - 3.2 - 5.6 - 10 - 18 - 32 - 56 - 100 mg pure metabolite/kg dry weight (d.w.) of soil containing 74.5 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.2 % $CaCO_3$, at 19.7 - 21.1 °C and a photoperiod: light : dark = 16 h : 8 h (512 lx) and were fed every 2 - 3 days with *Tyrophagus putrescentiae* (Schrank). Mortality and reproduction were determined after 14 days of exposure.
Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.i./kg soil d.w.; control: untreated, solvent control: none.

Dates of experimental work: March 12, 2014 – April 01, 2014

Results:

Validity Criteria	Recommended	Obtained in this study
Mean mortality of adult females	≤ 20%	3.8%
Mean number of juveniles per replicate	≥ 50	187.4
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	24.0%

The validity criteria for the control group were accomplished.

Effect of Propineb-DIDT (BCS-CU99534) on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Propineb-DIDT (BCS-CU99534) *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg pure metabolite/kg soil d.w.)	
NOEC	56	32
LOEC	100	56
EC_{10}	> 56	17.5
(95 % confidence limits)	-	(7.7 – 39.7)
EC_{20}	> 56	30.1
(95 % confidence limits)	-	(17.6 – 51.4)

In the control group a parental mortality of 3.8 % could be observed. The mortality in the test item treatment groups ranged between 0.0 and 27.5 %.
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 187.4 in the control and 176.0, 188.3, 191.5, 169.8, 179.5, 159.8, 91.3 and 96.8 at concentrations of 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg pure metabolite/kg soil d.w., respectively.

The observations are summarised in the table below.

Observations:

Endpoint	Treatment group (mg pure metabolite/kg soil d.w.)								
	Control	1.8	3.2	5.6	10	18	32	56	100
Mortality of soil mites after 14 days (%)	3.8	5.0	10.0	0.0	0.0	5.0	5.0	2.5	27.5 *1
Mean number of juveniles after 14 days	187.4	176.0	188.3	191.5	169.8	179.5	159.8	91.3 *2	96.8 *2
CV (%)	24.0	21.7	26.0	25.5	34.4	18.5	11.5	75.0	70.1
Reproduction (% to control)	**100**	**94**	**100**	**102**	**91**	**96**	**85**	**49**	**52**

*1 statistically significant differences compared to the control (Fisher's Exact Binomial with Bonferroni Correction for mortality, α = 0.05, one-sided greater)
*2 statistically significant differences compared to the control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)

Calculations were done using unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group
CV (%) = Coefficient of variation

Conclusions:

The test item Propineb-DIDT (BCS-CU99534) showed no statistically significantly adverse effects on adult mortality of the predatory mite *Hypoaspis aculeifer* in artificial soil up to and including a test concentration of 56 mg pure metabolite/kg soil dry weight. However, at a test concentration of 100 mg pure metabolite/kg soil dry weight a statistically significant effect on adult mortality (Fisher's Exact Binomial Test, $\alpha = 0.05$) could be observed.

Furthermore, the test item showed no statistically significantly adverse effects on reproduction of *Hypoaspis aculeifer* up to and including a test concentration of 32 mg pure metabolite/kg soil dry weight. However, at test concentrations of 56 mg and 100 mg pure metabolite/kg soil dry weight statistically significant effects on reproduction (Williams-t-test, $\alpha = 0.05$) could be observed.

Therefore, the No-Observed-Effect-Concentration (NOEC) and Lowest-Observed-Effect-Concentration (LOEC) for mortality were determined to be 56 mg and 100 mg pure metabolite/kg soil dry weight, respectively.

Therefore, the No-Observed-Effect-Concentration (NOEC) and Lowest-Observed-Effect-Concentration (LOEC) for reproduction were determined to be 32 mg and 56 mg pure metabolite/kg soil dry weight, respectively.

Reference test:

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

The results of the reference test demonstrate the sensitivity of the test system.

CA 8.5 Effects on soil nitrogen transformation

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

Additional N-transformation studies were performed, which were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the propineb Annex I Renewal. These studies will be summarized below.

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

Test substance	Test species/study type	Endpoint		References
Propineb WG 70A W	Study duration 70 d	no unacceptable effects	≥30 kg prod./ha **≥40 mg prod./kg dws**	[redacted] (2012) M-422074-01-1 KCA 8.5 /05
PTU	Study duration 28 d	no unacceptable effects	≥8.48 kg /ha **≥11.31 mg/kg dws**	[redacted] (2014) M-480253-01-1 KCA 8.5 /06
PU	Study duration 56 d	no unacceptable effects	≥7.26 kg/ha **≥9.68 mg/kg dws**	[redacted] (2013) M-472711-01-1 KCA 8.5 /07
4-MI	Study duration 28 d	no unacceptable effects	≥6.1 kg/ha **≥8.13 mg/kg dws**	[redacted] (2013) M-472708-01-1 KCA 8.5 /08
Propineb-DIDT	Study duration 42 d	no unacceptable effects	≥13.87 kg/ha **≥18.49 mg/kg dws**	[redacted] (2014) M-485360-01-1 KCA 8.5 /09

Report:	[redacted];[redacted];2012;M-422074-01
Title:	Propineb WG 70A W: Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	11 10 48 053 N
Document No:	M-422074-01-1
Guidelines:	**OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test.

Materials and Methods:
Test item: Propineb WG 70A W (analytical findings: 69.8 % w/w propineb (CH 30/2), Specification No.: 102000006516-02, Batch ID: EDFL010973, Sample description TOX09313-00, Material No.: 05468906) was used in the test.
A loamy sand soil (DIN 4220) was exposed for 70 days to 4.00 and 40.00 mg test item/kg soil dry weight. Application rates were equivalent to 3 and 30 kg test item/ha.
Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyzer II (BRAN+LUEBBE) at different sampling intervals (0, 7, 14, 28, 42, 56 and 70 days after treatment).

Dates of experimental work: August 31, 2011 to November 09, 2011

Results:

Validity Criteria	Recommended	Obtained
coefficients of variation in the control for NO_3-N	≤ 15%	2.1%

The validity criterion for the study was met.

Effects on nitrogen transformation in soil after treatment with Propineb WG 70A W

Time Interval (days)	Application rates [Propineb WG 70A W]				
	control	4.00 mg /kg soil dry weight		40.00 mg /kg soil dry weight	
	Nitrate-N [1]	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	1.87 ± 0.06	1.64 ± 0.29	**-12.2** n.s.	-0.12 ± 0.16	**-106.4** *s.
7 - 14	0.67 ± 0.11	1.07 ± 0.14	**+60.0** *s.	0.64 ± 0.33	**-3.6** n.s.
14-28	0.70 ± 0.07	0.68 ± 0.07	**-2.4** n.s.	1.78 ± 0.14	**+154.1** *s.
28 - 42	0.63 ± 0.02	0.65 ± 0.07	**+3.0** n.s.	0.96 ± 0.39	**+52.1** n.w.
42 -56	0.31 ± 0.01	0.50 ± 0.13	**+61.1** n.w.	0.44 ± 0.04	**+40.5** *s.
56 - 70	0.31 ± 0.12	0.25 ± 0.07	**-18.9** n.s.	0.33 ± 0.10	**+4.5** n.s.

The calculations were performed with unrounded values
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided)
n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided)
*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

The test item Propineb WG 70A W caused temporary stimulations of the daily nitrate rate at a test concentration of 4.00 mg/kg dry weight soil at the time intervals 7-14 and 42-56 days after application. Temporary stimulations of the daily nitrate rate were also observed at 40.00 mg/kg dry soil beginning at time interval 14-28 until time interval 42-56 days after application. Furthermore, a temporary inhibition of the daily nitrate rate could be observed at 40.00 mg/kg dry soil at time interval 0-7 days after application.
However, no adverse effects of Propineb WG 70A W on nitrogen transformation in soil could be observed at both tested concentration at the end of the test, 70 days after application (time interval 56-70 days after application).
Only negligible differences to control of -18.9 % (test concentration 4.00 mg/kg dry soil) and +4.5 % (test concentration 40.00 mg/kg dry soil) were measured at the end of the 70-day incubation period (time interval 56-70 days after application).

Conclusion:
Propineb WG 70A W caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 70-day incubation period. The study was performed in a field soil at concentrations up to 40.00 mg test item/kg soil, which is equivalent up to an application rate of 30 kg test item/ha.

Reference test
In the most recent test, dated 05.01. - 07.02.2011, the toxic standard Dinoterb caused an effect of +42.0 %, +68.1 % and +92.3 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Metabolite propineb- propylenethiourea (PTU)

Report:	[redacted] v; [redacted] ;2014;M-480253-01
Title:	Propineb-propylenthiourea (BCS-AA66386): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	14 10 48 022 N
Document No:	M-480253-01-1
Guidelines:	**OECD 216 (2000); adopted January 21, 2000; OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test.

Materials and Methods:
Test item: Propineb-propylenthiourea, BCS-code: BCS-AA66386, Batch code: AE B007299-01-01, Origin Batch No.: NLL 3790-7, LIMS No.: 1308795, Certificate No.: MZ 00033, analysed purity: 99.3 % w/w.
A loamy sand soil (DIN 4220) was exposed for 28 days to 1.13 and 11.31 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3-and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of experimental work: 'January 10, 2014 to February 07, 2014

Results:

Validity Criteria	Recommended	Obtained
coefficients of variation in the control for NO_3-N	≤15%	4.0%

The validity criterion for the study was met.

Effects on nitrogen transformation in soil after treatment with propineb-propylenthiourea (BCS-AA-66386)

Time interval (days)	control	1.13 mg test item /kg soil dry weight equivalent to 0.85 kg test item/ha		11.31 mg test item /kg soil dry weight equivalent to 8.48 kg test item/ha	
	Nitrate-N [1]	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	3.51 ± 0.19	3.82 ± 0.50	**+8.8** n.s.	3.80 ± 0.16	**+8.3** n.s.
7 - 14	1.37 ± 0.19	1.25 ± 0.34	**-8.7** n.s.	1.46 ± 0.43	**+6.2** n.s.
14 - 28	1.16 ± 0.12	1.06 ± 0.19	**-8.8** n.s.	1.11 ± 0.14	**-4.3** n.s.

The calculations were performed with unrounded values
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

No adverse effects of propineb-propylenthiourea (BCS-AA66386) on nitrogen transformation in soil could be observed at both test concentrations (1.13 mg test item/kg dry soil and 11.31 mg test item/kg

dry soil) during the 28-day experiment. Differences from the control of -8.8 % (test concentration 1.13 mg test item/kg dry soil) and -4.3 % (test concentration 11.31 mg test item/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Conclusion:

Propineb-propylenthiourea (BCS-AA66386) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at test concentrations up to 8.48 kg test item/ha.

Reference test

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +101.8 % and +172.8 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system.

Metabolite propineb-propyleneurea (PU)

Report:	[redacted] q; [redacted] ;2013;M-472711-01
Title:	Propineb-propylenharnstoff (BCS-AA-17927): Effects on the activity of soil microflora - (Nitrogen transformation test)
Report No:	13 10 48 113 N
Document No:	M-472711-01-1
Guidelines:	**OECD 216 (2000)**
GLP/GEP:	**Yes**

Objective:

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test.

Materials and Methods:

Test item: Propineb-propyleneurea, BCS-code: BCS-AA-17927, Batch code: AE 1379609-01-02, Origin Batch No.: RDL 125-21-1, LIMS No.: 1109639, Certificate No.: AZ 17285, CAS. No.: 6531-31-3; analysed purity: 98 % w/w.

A loamy sand soil (DIN 4220) was exposed for 56 days to 0.97 and 9.68 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14, 28, 42 and 56 days after treatment).

Dates of experimental work: September 18, 2013 to November 13, 2013

Results:

Validity Criteria	Recommended	Obtained
coefficients of variation in the control for NO_3-N	≤ 15%	5.4%

The validity criterion for the study was met.

Effects on nitrogen transformation in soil after treatment with propineb-propylenurea (BCS-AA-17927)

Time interval (days)	control	0.97 mg test item /kg soil dry weight equivalent to 0.73 kg test item/ha		9.68 mg test item /kg soil dry weight equivalent to 7.26 kg test item/ha	
	Nitrate-N [1]	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	3.17 ± 0.12	3.60 ± 0.10	**+13.7** [*s.]	3.43 ± 0.22	**+8.3** [n.s.]
7 - 14	1.58 ± 0.48	1.84 ± 0.37	**+16.3** [n.s.]	2.34 ± 0.36	**+48.2** [n.s.]
14 - 28	1.01 ± 0.32	0.55 ± 0.32	**-45.5** [n.s.]	0.75 ± 0.09	**-25.8** [n.s.]
28 - 42	0.52 ± 0.24	0.71 ± 0.27	**+36.1** [n.s.]	0.79 ± 0.40	**+51.1** [n.s.]
42 - 56	0.59 ± 0.06	0.67 ± 0.14	**+13.7** [n.s.]	0.67 ± 0.21	**+13.3** [n.s.]

The calculations were performed with unrounded values
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

The test item propineb-propylenurea (BCS-AA-17927) caused temporary stimulations and inhibitions of the daily nitrate rate at both test concentrations (0.97 mg/kg and 9.68 mg/kg soil dry weight) up to time interval 28-42 days after application. However, no adverse effects of propineb-propylenurea (BCS-AA-17927) on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 56 days after application (time interval 42-56 days after application). Differences from the control of +13.7 % (test concentration 0.97 mg/kg dry soil) and +13.3 % (test concentration 9.68 mg/kg dry soil) were measured at the end of the 56-day incubation period (time interval 42-56).

Conclusion:
Propineb-propylenurea (BCS-AA-17927) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 56-day incubation period. The study was performed in a field soil at concentrations up to 9.68 mg test item/kg soil dry weight.

Reference test
In the most recent test with the toxic standard (conducted from 04.01.2013 to 01.02.2013), Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Metabolite propineb-4-methyl-imidazoline (4-MI)

Report:	[redacted]y;[redacted];2013;M-472708-01
Title:	Propineb-4-methyl-imidazoline (BCS-AB78877): Effects on the activity of soil microflora - (Nitrogen transformation test)
Report No:	13 10 48 112 N
Document No:	M-472708-01-1
Guidelines:	**OECD 216 (2000)**
GLP/GEP:	**Yes**

Objective:

The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test.

Materials and Methods:

Test item: Propineb-4-methyl-imidazoline, BCS-code: BCS-AB78877, Batch code: BCS-AB78877-01-03, Origin Batch No.: BCOO 6454-11-4, LIMS No.: 1202055, Certificate No.: AZ 17925, analysed purity: 98.8 % w/w.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.81 and 8.13 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4- nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of experimental work: October 08, 2013 to November 05, 2013

Results:

Validity Criteria	**Recommended**	**Obtained**
coefficients of variation in the control for NO_3-N	≤ 15%	3.6%

The validity criterion for the study was met.

Effects on nitrogen transformation in soil after treatment with propineb-4-methyl-imidazoline (BCS-AB78877)

Time interval (days)	control	0.81 mg test item /kg soil dry weight equivalent to 0.61 kg test item/ha		8.13 mg test item /kg soil dry weight equivalent to 6.1 kg test item/ha	
	Nitrate-N [1]	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	3.61 ± 0.21	3.95 ± 0.18	**+9.2** n.s.	3.81 ± 0.01	**+5.5** n.w.
7 - 14	1.31 ± 0.04	1.11 ± 0.14	**-14.9** n.s.	1.27 ± 0.18	**-2.9** n.s.
14 - 28	1.20 ± 0.27	1.17 ± 0.06	**-2.8** n.s.	1.27 ± 0.17	**+5.7** n.s.

The calculations were performed with unrounded values
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)
n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, p ≤ 0.05)

No adverse effects of propineb-4-methyl-imidazoline (BCS-AB78877) on nitrogen transformation in soil could be observed at both test concentrations (0.81 mg/kg dry soil and 8.13 mg/kg dry soil) during

the 28-day experiment. Differences from the control of -2.8 % (test concentration 0.81 mg/kg dry soil) and +5.7 % (test concentration 8.13 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Conclusion:
Propineb-4-methyl-imidazoline (BCS-AB78877) caused no adverse effects (difference to control <25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at test concentrations up to 6.1 kg test item/ha.

Reference test
In the most recent test with the toxic standard (conducted from 04.01.2013 to 01.02.2013), Dinoterb caused an effect of +33.7 % and +42.6 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

<u>Metabolite Propineb-DIDT</u>

Report:	[redacted]v;[redacted];2014;M-485360-01
Title:	Propineb-DIDT (BCS-CU99534): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	14 10 48 021 N
Document No:	M-485360-01-1
Guidelines:	**OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation**
GLP/GEP:	**Yes**

Objective:
The purpose of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and Methods:
Test item: Propineb-DIDT, BCS-code: BCS-CU99534, Batch code: BCS-CU99534-01-01, Origin Batch No.: SES 11956-8-1, LIMS No.: 1334297, Customer Order No.: TOX 10290-00, Certificate No.: MZ 00769, analysed purity: 99.5 % w/w.
A loamy sand soil (DIN 4220) was exposed for 42 days to 1.85 and 18.49 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14, 28 and 42 days after treatment).

Dates of experimental work: January 09, 2014 to March 07, 2014

Results:

Validity Criteria	Recommended	Obtained
coefficients of variation in the control for NO_3-N	≤ 15%	7.5%

The validity criterion for the study was met.

Effects on nitrogen transformation in soil after treatment with Propineb-DIDT (BCS-CU99534)

Time Interval (days)	Control	1.85 mg test item/kg soil dry weight equivalent to 1.39 kg test item/ha		18.49 mg test item/kg soil dry weight equivalent to 13.87 kg test item/ha	
	Nitrate-N [1]	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	3.80 ± 0.51	3.95 ± 0.20	**+4.0** [n.s.]	0.39 ± 0.29	**-89.8** [*s.]
7 - 14	1.59 ± 0.64	1.46 ± 0.51	**-8.1** [n.s.]	0.93 ± 0.08	**-41.3** [n.w.]
14-28	0.78 ± 0.11	0.99 ± 0.25	**+27.3** [n.s.]	3.72 ± 0.12	**+379.4** [*s.]
28 - 42	1.12 ± 0.31	0.92 ± 0.18	**-17.6** [n.s.]	1.00 ± 0.10	**-10.4** [n.s.]

The calculations were performed with unrounded values
[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)
*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

The test item propineb-DIDT (BCS-CU99534) caused a temporary stimulation of the daily nitrate rate at the tested concentration of 1.85 mg test item/kg dry soil at time interval 14-28 days after application.
However, no adverse effects of propineb-DIDT (BCS-CU99534) on nitrogen transformation in soil could be observed at the tested concentration of 1.85 mg test item/kg dry soil, 42 days after application (time interval 28-42 days).
Temporary inhibitions of the daily nitrate rate were observed at 18.49 mg test item/kg dry soil until time interval 7-14 days after application. Furthermore, a temporary stimulation of the daily nitrate rate could be observed at 18.49 mg test item/kg soil dry weight at time interval 14-28 days after application. However, no adverse effects of propineb-DIDT (BCS-CU99534) on nitrogen transformation in soil could be observed at the tested concentration of 18.49 mg test item/kg dry at the end of the test, 42 days after application (time interval 28-42 days).
Differences from the control of -17.6 % (test concentration 1.85 mg test item/kg dry soil) and -10.4 % (test concentration 18.49 mg test item/kg dry soil) were measured at the end of the 42-day incubation period (time interval 28-42).

Conclusion:
Propineb-DIDT (BCS-CU99534) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 42-day incubation period. The study was performed in a field soil at test concentrations up to 13.87 kg test item/ha.

Reference test
In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +101.8 % and +172.8 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14 - 28).

CA 8.6 Effects on terrestrial non-target higher plants

For information on studies already evaluated during the first EU review of propineb, 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 propineb as a fungicide, tier 1 limit tests were conducted with the representative formulation Propineb WG 70. The studies are presented in MCP, Annex point 10.6.2.

CA 8.6.2 Testing on non-target plants

Studies on non-target plants (seedling emergence and vegetative vigour) have been conducted with the representative formulation Propineb WG 70 and are presented in MCP, Annex point 10.6.2.

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 propineb, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

An activated sludge study with propineb was performed, which was not submitted during the first Annex I inclusion process and is submitted within this Supplemental Dossier for the propineb Annex I Renewal. This study will be summarized below.

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

Test substance	Test species/ study type	Endpoint		References
propineb, tech.	Activated sludge, 3 h	EC_{50}	> 1000 mg a.s./L	[redacted] (2009) M-361047-01-1 2009/0132/01 KCA 8.8/01

Report:	[redacted] ; [redacted] ;2009;M-361047-01
Title:	Activated sludge, respiration inhibition test with propineb
Report No:	2009/0132/01
Document No:	M-361047-01-1
Guidelines:	**Council Regulation (EC) No 440/2008, Method C.11 Activated sludge respiration inhibition (2008). This test method is equal to OECD Guideline 209 (1984)**
GLP/GEP:	**Yes**

Objective:
A study was performed to assess the toxicity of propineb to bacteria.

Materials and methods:
Test item: Propineb (Batch: EDFU711100, purity: 81.8%).
The activated sludge was exposed to propineb to nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L. The respiration rate of each mixture was determined after aeration periods of 3 hours.
Two controls without test item were included in the test design, one at the start and the other at the end of the test series. Each batch of activated sludge was checked using 3,5-Dichlorophenol as a reference compound.
The physico-chemical oxygen consumption control was carried out, since some substances may consume oxygen by chemical reactivity. In order to be able to differentiate between physico-chemical oxygen consumption and biological oxygen consumption (respiration), at least the maximum concentration of the test item was additionally tested without activated sludge.
To measure the oxygen consumption, 250 mL of sludge with test item (or control or reference compound) was incubated for 3 h in 300 mL closed Erlenmeyer flasks (with air inlet and outlet) and aerated through a glass tube at 50-100 L/h with clean oil-free air. For measurement, the content of the Erlenmeyer flasks was completely transferred to 250 mL BOD bottles and O_2-content was measured with an O_2-meter (redox electrode) with writer.
The respiration rate for each concentration was determined graphically from the linear part of the curve of O_2-content versus time. The inhibitory effect of the test item at a particular concentration is expressed as a percentage of the mean of the respiration rates of two controls. An EC_{50} value was calculated from the respiration rates at different test item concentrations.

Dates of experimental work: December 07, 2009 to December 12, 2009

Results:
All validity criteria of the test method were met:
- respiratory rates of the 2 controls differ less than 15 % from each other
- The EC_{50} of the reference compound 3,5-Dichlorophenol is in the range 5 – 30 mg/L

Propineb showed 17.8 % respiration inhibition of activated sludge at a test item concentration of 1000 mg/L.

Conclusions:

The EC_{50} is higher than 1000 mg/L.

The effect value relates to nominal concentration, since no analytical monitoring was performed.

CA 8.9 Monitoring data

No monitoring data are available.