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Document Title

Summary of the ecotoxicological studies for deltamethrin

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
2014-06-02	Update of Section CA 8.6.1	M-480243-02-1
2016-05-20	Update of Section CA 8.2 Inlcusion of answers to questions document M-583896-01-1	M-480243-03-1

[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

Ecotoxicological data of deltamethrin 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 the Supplemental Dossier for renewal of approval of deltamethrin only those ecotoxicological studies are described, which had not been submitted within the Baseline Dossier. The codes and structures of deltamethrin and its metabolites addressed in this section are presented in Document N3 of the dossier. To differentiate between studies already evaluated during the last Annex I listing and new studies, the references given in tables are written in grey for studies already evaluated and in bold black for new studies. Endpoints used for risk assessments highlighted in bold letters.

To facilitate the review of the public literature comprehensive summaries are provided. However all references given to secondary literature (e.g. in foot notes) are not included in the dossier.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Studies on bobwhite quail and mallard ducks have been conducted with the active substance deltamethrin and were evaluated and accepted during the Annex I inclusion.

CA 8.1.1.1 Acute oral toxicity to birds

For studies already evaluated during the first EU review of deltamethrin, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.
In addition, an acute study with a canary (*Serinus canaria*) conducted for the re-registration in the USA, which was not evaluated during the first EU Review of deltamethrin, is summarized under this point.

Report:	**KCA 8.1.1.1/03, [redacted]; [redacted]; 2013**
Title:	Toxicity of Deltamethrin Technical during an Acute Oral LD50 with the Canary (*Serinus canaria*)
Document No:	M-444452-01-1 (Rep. No: EBDAL083)
Guidelines:	OPPTS 850.2100
GLP:	Yes

Objective:
The purpose of this study was to estimate the acute oral toxicity (LD_{50}) of deltamethrin (purity 99.52%) to a canary (*Serinus canaria*).

Materials and Methods:
Adult Canary were orally dosed with Deltamethrin technical based on body weight at dose levels of 0, 125, 250, 500, 1000, and 2000 mg a.s./kg body weight. Ten birds per dose level (five males and five females) were randomized by body weight into each treatment level on experimental Day -1. Birds were capsule dosed on Day 0 and subsequently monitored for 14 days. All feed and water were provided *ad libitum*. Adult body weights were measured on experimental Day -1, Day 7, and Day 14. Feed consumption and clinical observations occurred daily.

Results:

Mortality & Clinical Observations

There were no mortalities in any of the 125, 250, 500, 1000, and 2000 mg a.s./kg body weight treatment groups. No observed effects occurred in the 125 mg a.s./kg body weight treatment. Ataxia (loss of muscular coordination) was observed in the 250 mg a.s./kg body weight treatment group. Ataxia and hypo-reactivity (lethargy) was observed in the 500, 1000, and 2000 mg a.s./kg body weight treatment groups. Due to the absence of mortalities in each treatment group, the LD_{50} was higher than the highest dose tested of 2000 mg a.s./kg body weight.

Body Weight & Feed Consumption

Body weight measurements (Day 0, Day 7 and Day 14) and changes in body weight (Day 0 to Day 7, Day 7 to 14, and Day 0 to Day 14) were compared among all surviving birds by treatment group and then by sex and treatment group.

No significant difference occurred for male bodyweight change over the three intervals. Female bodyweight change was significantly different from the control for the following intervals: Day 0 to 7 (1000 mg a.s./kg bw), Day 7 to 14 (125 mg a.s./kg bw), and Day 0 to 14 (125 mg a.s./kg bw). The significant differences in female bodyweight change were considered transient effects as there was no effect in overall bodyweight change for Day 7 to 14 for both males and females. Additionally, no significant differences were observed in daily feed consumption for males and female birds for any interval.

Conclusion:

The acute oral LD_{50} for deltamethrin technical in canary was >2000 mg a.s./kg body weight. Based on all parameters measured, the NOEC was 125 mg a.s./kg body weight and the LOEC was 250 mg a.s./kg body weight.

CA 8.1.1.2 Short-term dietary toxicity to birds

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Studies with mammals that have been conducted with the active substance deltamethrin are reported in the toxicology section MCA 5.

CA 8.1.2.1 Acute oral toxicity to mammals

No additional studies were performed. Please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

One study, additional to the first Annex I inclusion process is included in the ecotoxicological assessment. This study is presented in the toxicological section under point MCA 5.7. For all studies submitted during the frame of the first Annex I inclusion, please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.
A justification for the use of the reproductive endpoint which was used in the risk assessment presented in the MCP is given below.

Justification for the use of the reproductive endpoint:

Table 8.1.2.2- 1: Neurotoxicity and reproductive toxicity of deltamethrin a.s. for wild mammal risk assessment (please refer also for the study reports listed below to KCA 5)

Organism	Duration, Exposure	Test substance	Reference	Ecotoxicological endpoint	
Neurotoxicity					
Rat	Acute, oral (gavage in corn oil)	Deltamethrin a.s.	[redacted] (1995) M-152413-01-1	NOEL $_{neurotoxicity}$ LOEL $_{neurotoxicity}$	5 mg a.s./kg bw 15 mg a.s./kg bw
Rat	90-d, dietary	Deltamethrin a.s.	[redacted] (1998) M-152562-01-1	NOEL $_{neurotoxicity}$ LOEL $_{neurotoxicity}$	4 mg a.s./kg bw 14 mg a.s./kg bw
Rat	**Developmental neurotoxicity (dietary)**	**Deltamethrin a.s.**	**[redacted] (2006) KCA 5.7/08 M-270180-03-1 MCA 5.7/08 (summary)**	**NOAEL**	**80 mg a.s./kg diet 6.78 mg a.s./kg bw/d**
Reproduction (long-term)					
Rat	Multigen. reproduction) (dietary)	Deltamethrin a.s.	[redacted] (1992) M-149348-01-1	NOEL $_{adults/offspring}$ NOEL $_{adults/offspring}$	80 mg a.s./kg diet **4.2 mg a.s./kg bw/d**
Rabbit	Developmental toxicity (gavage)	Deltamethrin a.s.	[redacted] (2001) M-204103-01-1	NOAEL $_{maternal}$ (LOEL) NOAEL $_{offspring}$ (LOEL)	10 mg a.s./kg bw/d 32 mg a.s./kg bw/d 32 mg a.s./kg bw/d > 32 mg a.s./kg bw/d
Rat	Developmental toxicity (gavage)	Deltamethrin a.s.	[redacted] (1978) M-094134-01-1	NOAEL $_{maternal}$ (LOEL) NOAEL $_{offspring}$ (LOEL)	2.5 mg a.s./kg bw/d 5 mg a.s./kg bw/d 5 mg a.s./kg bw/d > 5 mg a.s./kg bw/d
Rat	Developmental toxicity (gavage)	Deltamethrin a.s.	[redacted] (1990) M-149353-01-1	NOAEL $_{maternal}$ (LOEL) NOAEL $_{offspring}$ (LOEL)	3.3 mg a.s./kg bw/d 7 mg a.s./kg bw/d 11 mg a.s./kg bw/d > 11 mg a.s./kg bw/d
Mouse	Developmental toxicity (gavage)	Deltamethrin a.s.	[redacted] (1978) M-094154-01-1	NOAEL $_{maternal}$ (LOEL) NOAEL $_{offspring}$ (LOEL)	3 mg a.s./kg bw/d 6 mg a.s./kg bw/d 12 mg a.s./kg bw/d > 12 mg a.s./kg bw/d

Organism	Duration, Exposure	Test substance	Reference	Ecotoxicological endpoint	
Rabbit	Developmental toxicity (gavage (cell.))	Deltamethrin a.s.	[redacted] (1990) M-149350-01-1	NOAEL mater/offspr (LOEL)	25 mg a.s./kg bw/d 100 mg a.s./kg bw/d
Rabbit	Developmental toxicity (gavage)	Deltamethrin a.s.	[redacted] (2001) M-204103-01-1	NOAEL maternal (LOEL) NOAEL offspring (LOEL)	10 mg a.s./kg bw/d 32 mg a.s./kg bw/d 32 mg a.s./kg bw/d > 32 mg a.s./kg bw/d

In bold: Endpoint selected for the long-term risk assessment in the MCP; bw = body weight

In the rat multigeneration study ([redacted], 1992; M-149348-01-1), deltamethrin did not affect the mating performance or fertility. Treatment-related effects in parent animals were limited to the high dose treated group and consisted in mortality, clinical signs, reduced body weight, reduced food consumption and gastric erosion. On that basis the dose level of 80 ppm (the average achieved dosages ranged from 4.2 to 12.4 mg/kg bw/day in the periods evaluated in this study) was considered to be the NOAEL. Treatment-related effects in offsprings were limited to the high dose group level and consisted in mortality, reduced lactation index and reduced body weight. On that basis the dose level of 80 ppm was considered to be the offspring NOAEL. Thus, the overall NOAEL from this study is 80 ppm (corresponding to dosages achieved of 4.2 to 12.4 mg/kg bw/day).

In the rabbit development study ([redacted], 2001; M-204103-01-1), deltamethrin did not induce any embryotoxicity, foetoxicity and teratogenicity. Treatment-related effects in dams were limited to the high dose treated group and consisted of slight reduced body weight and food intake. On that basis the dose levels of 10 mg/kg bw/d were considered to be the maternal NOAEL. There were no treatment-related effects in foetuses at any dose level. On that basis the dose levels of 32 mg/kg bw/d were considered to be the foetal NOAEL.

In rat development studies ([redacted] 1978; M-094154-01-1 and [redacted], 1990; M-149350-01-1), deltamethrin did not induce any embryotoxicity, foetal toxicity and teratogenicity. Treatment-related effects in dams were limited to the high dose treated group and consisted of mortality ([redacted], 1978; M-094154-01-1), clinical signs and reduced body weight. On that basis the dose levels of 2.5 and 3.3 mg/kg bw/d were considered to be the maternal NOAELs of respective studies ([redacted], 1978; M-094154-01-1 and [redacted] 1990; M-149350-01-1). Treatment-related effects in foetuses were limited to the high dose treated group of [redacted] study. There, a part of the dams were allowed to give birth and – remaining on dose - to raise pups during lactation until day 15 post-partum. Pups were afterwards reared on untreated diet until day 42 post-partum. Effects on pup body weight during pre-weaning, disappeared upon the cessation of dosing. Additional neurological investigations of locomotor activity, open field observation and righting and auditory startle reflexes showed that deltamethrin did not affect the normal development of these foetuses at any dose level. On that basis, the slight and transient early changes in body weight reported in foetuses of the high treated group were considered to be of no toxicological relevance. On that basis, the dose levels of 5 mg/kg bw/d were considered to be the foetal NOAEL.

In the rat developmental neurotoxicity study ([redacted] 2006; M-270180-03-1), deltamethrin did not affect the reproductive performance. Treatment-related effects in dams were limited to the high dose treated group and consisted of reduced body weight and food intakes. On that basis the dose level of 80

ppm (6.78 mg/kg bw/d) was considered to be the maternal NOAEL. Treatment-related effects in foetuses were limited to the high dose treated group and consisted of reduced body weight and delayed balanopreputial separation. On that basis the dose level of 6.78 mg/kg bw/d was considered to be the offspring NOAEL. The absence of pup growth retardation after the dietary administration of deltamethrin throughout gestation and lactation at dose levels up to 200 ppm (16.1 mg/kg bw/d) confirmed that the early and transient reduced body weight that were noted in the rat developmental study ([redacted]1978; M-094154-01-1) following daily oral gavage exposure were therefore of no ecotoxicological relevance.

Overall evaluation:

The long-term risk assessments are generally derived from the study which examines potential effects of a compound on populations. The rat multigeneration study, which investigates a large number of critical end-points like reproduction rate, survival rate and development of individuals following repeated dietary exposure, is usually considered the most relevant study to cover long-term risks of wild mammals in the field. Taking into account that this study showed the overall lowest NOAEL (4.2 mg/kg bw/d), Bayer CropScience considers this study to be the most appropriate to run the long-term risk assessment for wild mammals.

This approach is supported by the fact that NOAELs determined in other studies of interest are essentially comparable. In developmental studies with gavage exposure, NOAELs ranging from 2.5 to 10 mg/kg bw/d and 5 to 32 mg/kg bw/d were observed in dams and feus, respectively. All LOAELs were higher than the proposed NOAEL of 4.2 mg/kg bw/d. Also in neurotoxicity studies, with the more relevant dietary route of exposure is more relevant, NOAELs ranging from 4.0 to 6.78 mg/kg bw/d were reported in adult and growing animals.

Therefore it is proposed to apply a NOAEL of 80 ppm (4.2 mg/kg bw/d) from the reproduction study in rat (with dietary administration over a full life cycle) in the reproductive wild mammal risk assessment.

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

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds and mammals 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 deltamethrin is above the trigger, an evaluation of secondary poisoning is conducted. For the evaluation please refer to point 10.1.1.2 of the chemical product dossier.

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

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

CA 8.1.5 Endocrine disrupting properties

Wild Mammals

A detailed analysis of all the apical toxicological studies (developmental toxicity studies in rats and rabbits, reproductive toxicity study in rats, developmental neurotoxicity study in rats and long-term toxicity/carcinogenicity in mice and rats) on Deltamethrin revealed no evidence of any reproducible endocrine effect. Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of Deltamethrin in mammals.

Birds

The population relevant effects of Deltamethrin on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. For both species there were no effects on adult birds, offspring or reproductive parameters up to and including the highest test level of 450 ppm a.s. As reproduction was not affected in two avian species, it is concluded that there are no population relevant adverse effects of Deltamethrin.

Based on the absence of any indication of relevant effects it can be concluded that Deltamethrin is not a (potential) endocrine disrupter.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. In addition, tests on marine species, which were no data requirement according to the old regulation and hence were not evaluated during the first EU Review of this compound, will be summarized.

For studies already evaluated during the first EU review of deltamethrin, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

To complete the aquatic data package, several new studies were conducted with the major metabolites **alpha-R-isomer of deltamethrin**, **trans-isomer of deltamethrin**, **4'OH-deltamethrin**, **mPBacid**, **Br_2CA**, and **Serinyl-BrCA**, which can be formed in the aquatic environment or can be transported to surface water bodies via run-off and drainage. For further details reference is made to Section 7: "Fate and behaviour in the environment". Summaries of the aquatic studies are provided in the following.

The metabolite **BrCA** was also identified as major metabolite and needs to be addressed in the aquatic risk assessment. BrCA is formed from Br_2CA via elimination of a bromine atom. Other than that, the two metabolites are identical. Br_2CA showed no toxicity to aquatic organisms in acute studies, with an LC_{50} of 100 mg/L for fish and an EC_{50} > 100 mg/L for *Daphnia*, respectively. Therefore, it is not expected that the metabolite BrCA poses a risk to aquatic organisms. No studies were conducted for this metabolite.

The degradation pathways in soil and water and sediment are given in the two figures below.

Answers to questions concerning the studies on effects on aquatic organisms can be found in document M-583896-01-1

Figure 8.2 - 1: Proposed degradation pathway of deltamethrin in soil (major metabolites are highlighted in bold writing)

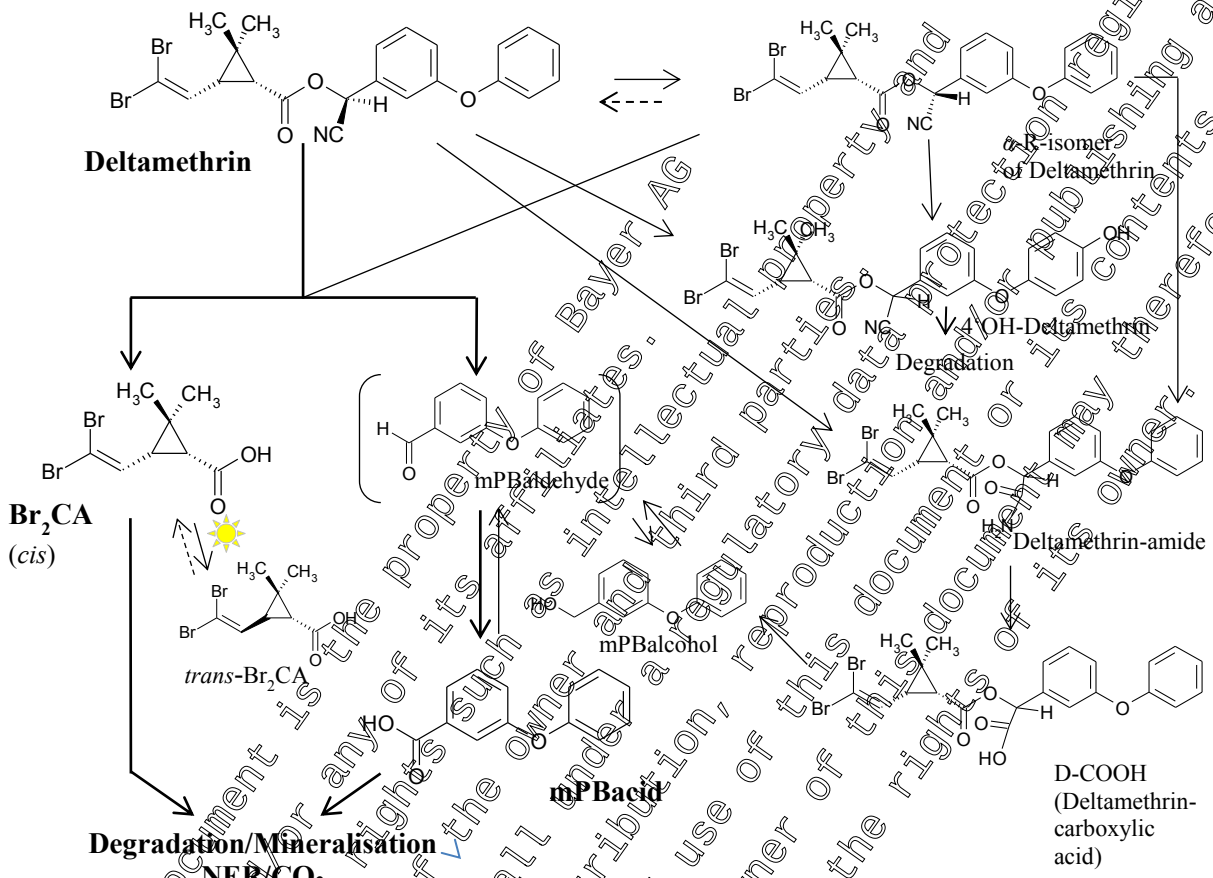
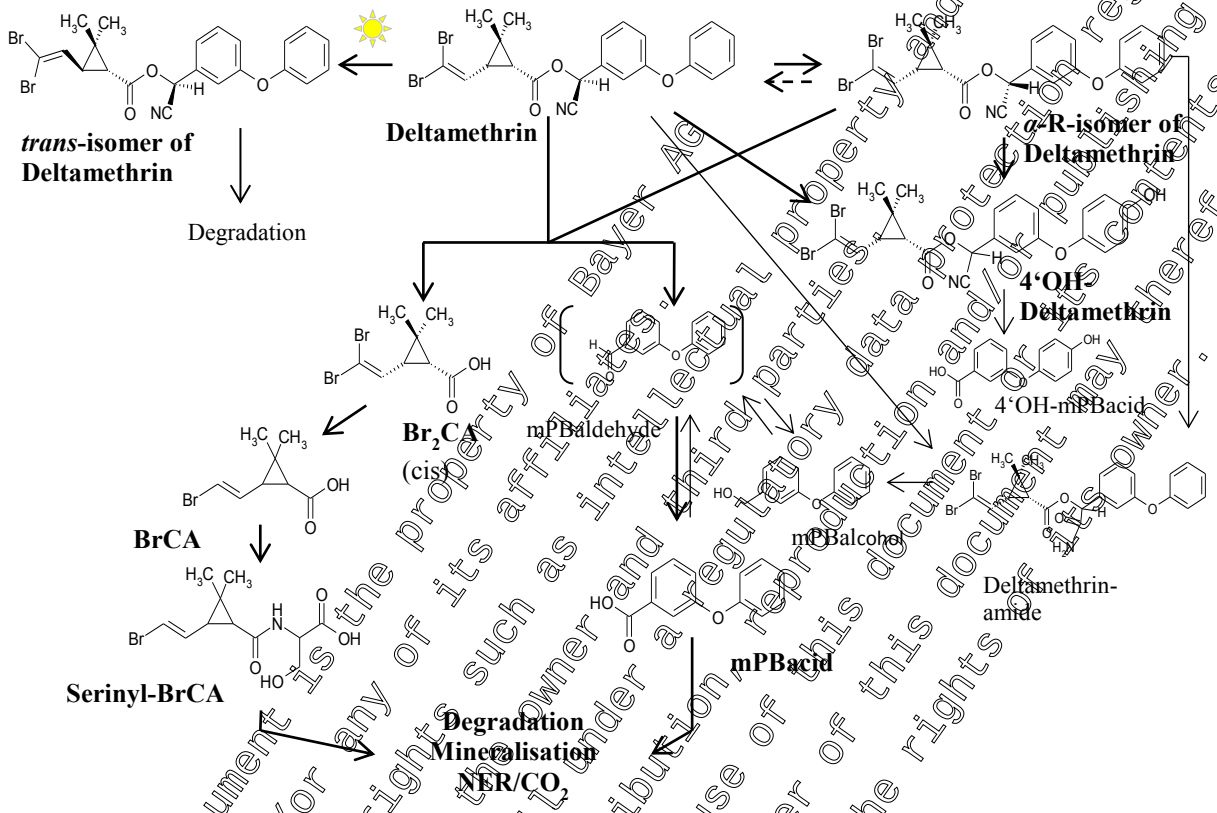


Figure 8.2 - 2: Proposed degradation pathway of deltamethrin in water and sediment (major metabolites are highlighted in bold writing)



CA 8.2.1 Acute toxicity to fish

For studies already evaluated during the first EU review of deltamethrin, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.
The endpoint from the following table was evaluated during the first EU review and is considered in the List of endpoints provided by EFSA (review report 6504/VI/99). However, the endpoint was derived from a study conducted under static conditions without chemical confirmation of test concentrations, and is therefore of limited reliability.

Table 8.2.1- 1: Acute toxicity to fish exposed to deltamethrin

Test substance	Test species	Endpoint	Reference
Deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50} 0.91 µg a.s./L (nom)	[redacted] (1986), M-149417-01-1

nom = nominal

A prolonged (28 day) acute study with deltamethrin on Rainbow trout is available ([redacted], 1990; M-135553-01-1), from which a 96 h-LC_{50} can be derived. As this study was conducted under flow-through conditions and included chemical analysis, it is considered more suitable to address the data requirement. This study was already evaluated during the first EU review of deltamethrin, but not with a focus on the 96 h data. Therefore, an adapted summary is presented below.

In addition, an acute study with the marine species *Cyprinodon variegatus* (Sheepshead minnow), which was not evaluated during the first EU Review of deltamethrin, is summarized under this point, as well as a publication on the acute toxicity of deltamethrin to fingerlings of the European catfish (*Silurus glanis*).

In order to complete the aquatic data package on fish, additional studies are provided for metabolites of deltamethrin in this Supplemental Dossier. Acute fish studies were conducted with the metabolites alpha-R-isomer and trans-isomer of deltamethrin, 4'OH-deltamethrin, mPBacid and Br_2CA. Respective study summaries are given below.

Table 8.2.8- 1: Additional acute fish endpoints of deltamethrin and its metabolites

Test substance	Test species	Endpoint		Reference
Deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50}	0.15 µg a.s./L (mm)	**[redacted] (1990) M-135553-01-1**
Deltamethrin	Fish, acute *Cyprinodon variegatus*	LC_{50}	0.48 µg a.s./L (mm)	**[redacted] (1990) M-135536-01-1**
alpha-R-isomer of deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50}	16.2 µg/L (mm)*	**[redacted] (2014) M-473954-01-1**
trans-isomer of deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50}	0.239 µg/L (mm)*	**[redacted] (2013) M-473731-01-1**
4'OH-deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50}	3.99 µg/L (mm)	**[redacted] (2013) M-473195-01-1**
Br_2CA (AE F108565)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	100000 µg/L (nom)	**[redacted] (2001) M-199816-01-2**
mPBacid (AE F109036)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	13300 µg/L	**[redacted] (1981) BL/B/2038 [redacted] number: CGA55186/0707 Letter of Access: M-479954-01-1**

mm – mean measured; nom = nominal

Tested as Decis 2.5 EC

* Results from the studies with the alpha-R-isomer and the trans-isomer of deltamethrin <u>are not suitable for the use in aquatic risk assessments</u>. In both studies, the parent compound deltamethrin was also detected at concentrations, which are lethal to fish, due to re-isomerization of the alpha-R-isomer and the trans-isomer into the parent compound deltamethrin under test conditions. Therefore, it is expected that deltamethrin contributed significantly to the toxic effects observed in these studies. In a conservative approach, endpoints were derived based on the mean measured concentrations of the respective metabolite alone. However, these endpoints overestimate the actual toxicity of the metabolites, as they do not consider the effects caused by the presence of deltamethrin. The available studies do not allow for a definite determination of the metabolite toxicity, and are therefore not considered adequate for a risk assessment. Nevertheless, these worst-case endpoints clearly demonstrate that neither the alpha-R-isomer nor the trans-isomer of deltamethrin is more toxic to aquatic organisms than the parent compound itself.

Active substance deltamethrin

The study [redacted] (1990; M-135553-01-1) was already evaluated during the last Annex I listing, but with focus on chronic results. The study is again summarised in this MCA focusing on acute data:

Report:	**KCA 8.2.1/03, [redacted]; 1990**
Title:	(LX 165-08, deltamethrin technical) – Acute (28-Day) toxicity to rainbow trout (*Oncorynchus mykiss*) under flow-through conditions.
Document No:	M-135553-01-1 (Rep. No: A47111)
Guidelines:	OECD 204, US EPA OPPTS 850.1075
GLP:	Yes

Objective:
The purpose of this study was to estimate the acute toxicity (LC_{50}) of deltamethrin (purity 99.2%) to Rainbow trout (*Oncorhynchus mykiss*) in a prolonged acute study (28 days) under flow-through conditions.

Materials and Methods:
Test item: LX 165-08 (deltamethrin technical, purity: 99.2%), Lot No. 8B 0153 B3, received from [redacted] Georgia.
Test organism: Rainbow trout (*Oncorhynchus mykiss*), mean body length 3.0 (2.0-3.5) cm, mean body weight 0.22 (0.10-0.35) g. The maximum organisms loading concentration during the initiation of the exposure period was 0.023 g of biomass per liter of flowing test solution per day (0.063 g/L at test termination).
Twenty fish (10 per replicate) were exposed in duplicate test aquaria in a flow-through system to five concentrations of deltamethrin, a solvent control (acetone) and a dilution water control. During the test, nominal concentrations of 0.044, 0.068, 0.11, 0.16, 0.25 µg test item/L (deltamethrin tech.) were maintained by introducing approximately 6.4 aquarium volumes per day of newly prepared test solution via an intermittent flow proportional diluter apparatus.
Each replicate solution was sampled and analyzed for deltamethrin concentration once prior to test initiation, at test initiation and weekly thereafter (i.e. test days -2, 0, 7, 14, 21, 28). Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and at each subsequent 24-hour interval until termination of the test.

Results:
Analytical results:
Analyses of treatment level solutions throughout the concentration range resulted in mean measured concentrations which averaged 68% (range 59% to 73%) of the nominal levels. Mean measured test concentrations were based on the analytical results from the in-life portion of the study (i.e. day 0 onwards) and were 0.032, 0.041, 0.072, 0.11 and 0.18 µg a.s./L.
The endpoints were expressed in terms mean measured concentrations.

Biological results:

Cumulative mortality [%] during the flow-through exposure of Rainbow trout to deltamethrin techn.

Mean measured deltamethrin conc.	24 h	48 h	72 h	96 h
Control	0	0	0	0
Solvent control	0	0	0	0
0.032 µg a.s./L	0	0	0	0
0.040 µg a.s./L	0	0	0	0
0.072 µg a.s./L	0	0	0	0
0.11 µg a.s./L	0	0	0	0
0.18 µg a.s./L	0	35	70	80

Surviving fish at levels ≥ 0.11 µg a.s./L showed the following symptoms after 96 hours:

- complete loss of equilibrium
- erratic behavior
- partial loss of equilibrium
- darkened pigmentation
- darkened pigmentation and rapid respiration
- complete loss of equilibrium and rapid respiration

There were neither any sub-lethal effects nor any mortality in the control and solvent control group.

LC_{50} values for rainbow trout exposed to deltamethrin technical based on mean measured concentrations

Test substance:	Deltamethrin techn. (LX 165-08)
Test object:	Rainbow trout (*Oncorhynchus mykiss*)
Exposure:	28 days, flow-through test design (dose-response)
LC_{50} 96 h (95% C.L.):	**0.15 (0.11-0.18) µg a.s./L (mean measured)**

Conclusions:

The LC_{50} (96 h) of deltamethrin (LX 165-08) to Rainbow trout (*Oncorhynchus mykiss*) in a prolonged acute test (28 d) under flow-through conditions was determined to be 0.15 µg a.s./L (mean measured).

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Report:	**KCA 8.2.1.1/04, [redacted]; [redacted]; [redacted]; 1990**
Title:	Acute toxicity of deltamethrin – active ingredient to sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions
Document No:	M-135536-01-1 (Rep. No: A47094)
Guidelines:	Protocol for Conducting a Flow-Through Acute Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*) Following FIFRA Guideline 72-3; #102387/72.3 SM-FA.
GLP:	yes

Objective:

The purpose of this study was to estimate the acute toxicity (LC_{50}) of deltamethrin (purity 99.2%) to Sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions.

Materials and methods:

Test item: deltamethrin (purity: 99.2%), Lot No. 8B 0153 B3; received from [redacted] New Jersey

Test organism: Sheepshead minnow (*Cyprinodon variegatus*), mean body length 2.7 (2.2-3.2) cm, mean body weight 0.31 (0.17-0.58) g. The biomass loading for this test was 0.032 g of biomass per liter of flowing test solution per day.

Twenty organisms were exposed in duplicate test aquaria in a flow-through system to five concentrations of deltamethrin, a solvent control and a dilution (seawater) water control. During the test, nominal concentrations of 0.27, 0.41, 0.63, 0.97 and 1.5 µg/L deltamethrin were maintained by introducing approximately 6.4 aquarium volumes per day of newly prepared test solution via an intermittent flow proportional diluter apparatus. Each replicate solution was sampled and analyzed for deltamethrin concentration on day 0 (test initiation) and on day 4 (test termination) of the exposure period. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and every 24 hours thereafter until the test was terminated.

Results:

Analytical results:

During the exposure period, no visible sign of undissolved test material (e.g. precipitate, film on solution surface) was observed in any of the treatment level or control solutions.

Mean measured concentrations of deltamethrin in the test media ranged from 55% to 60% in the various test levels. Based on these results, the mean measured test concentrations were determined to be 0.16, 0.23, 0.35, 0.53 and 0.90 µg a.s./L. Biological results were based on mean measured concentrations.

Biological results:

After 96 hours of exposure, mortality of 100% and 70% was recorded in the two highest mean measured concentrations of tested deltamethrin (0.90 and 0.53 µg/L, respectively). During the same period, < 10% mortality was observed among fish exposed to the remaining treatment levels (0.35, 0.23 and 0.16 µg/L deltamethrin). Throughout the exposure, no toxicant related sub-lethal effects were observed among the fish in test solutions < 0.35 µg/L. Based on these data, it was established that the observed effects during this study were clearly concentration-dependent.

LC_{50} values for Sheepshead minnow exposed to Deltamethrin based on mean measured concentrations

Test substance:	Deltamethrin
Test object:	Sheepshead minnow (*Cyprinodon variegatus*)
Exposure:	96 hours, flow-through test design (dose-response)
LC_{50} 96 h (95% C.I.):	**0.48 (0.35-0.90) µg a.s./L (mean measured)**

Conclusion:

The LC_{50} (96 h) of deltamethrin to Sheepshead minnow (*Cyprinodon variegatus*) in a 96-hour-test under flow-through conditions was determined to be 0.48 µg a.s./L.

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Metabolite alpha-R-isomer of deltamethrin

Report:	**KCA 8.2.1/05, [redacted]; 2014**
Title:	Acute toxicity of alpha-R-isomer of deltamethrin (tech.) to fish (Oncorynchus mykiss) under static renewal conditions
Document No:	M-473954-01-1 (Rep. No: EBDAL021)
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OCSPP 850.1075 (Public Draft, 1996) Council Regulation (EC) No 440/2008, C.1 (2008) OECD No. 203 (rev. 1992) JMAFF, 12 Nousan No. 8147
GLP:	Yes

Objective:
The aim of the study was to determine the acute toxicity of the test item to rainbow trout (*Oncorhynchus mykiss*) expressed as 96h-LC_{50}.

Material and methods:
Test item: Alpha-R-isomer of deltamethrin, purity: 94.3% w/w, specified by batch number: AE F108569-PU-02, analysis ref. code: AZ 15832. The test item also contains 0.2% of deltamethrin as an impurity.
Test organism: Rainbow trout (*Oncorhynchus mykiss*), mean body length 4.7 cm, mean body weight 1.1 g, Lot F 11 / 10 A were delivered on September 30, 2010. The biomass loading for this test was 0.28 g fish/L test medium.
Ten fish in each test level were exposed for 96 h under static-renewal conditions (daily renewal) to nominal concentrations of 0 (control), 0 (solvent control: 100 µg acetone/L), 1.30, 2.80, 6.20, 13.6 and 30.0 µg test item/L. The alpha-R isomer of deltamethrin, deltamethrin and the trans-isomer of deltamethrin were analyzed in all test levels after 0 h, and daily from day 1 to day 4 of the exposure period in new and aged test media by HPLC-MS/MS.

Findings:
Dissolved oxygen concentrations ranged from 87% to 98% oxygen saturation, the pH values ranged from 6.8 to 7.3 and the water temperature ranged from 10.6°C to 12.0°C in all aquaria over the whole testing period.

Analytical findings:
The concentrations of alpha-R isomer of deltamethrin in the stock solutions ranged from 95% to 120% of nominal.
The accompanying chemical analysis of alpha-R-isomer of deltamethrin in the freshly prepared test solutions at start of each renewal interval revealed single recoveries between 77% and 176% (mean: 125%) of the corresponding nominal concentrations.
The corresponding concentrations of the aged test solutions at the end of each 24-hour exposure period ranged from 30% to 541% (mean: 147%) of nominal. This resulted in mean measured concentrations of 0 (control), 0 (solvent control), 2.14, 3.53, 4.57, 22.5 and 34.0 µg pure metabolite (p.m.)/L.
Mean measured concentrations of deltamethrin (measured values below the LoQ) were 0.105, 0.189, 0.135, 0.494 and 0.505 µg/L for the 1.3, 2.8, 6.2, 13.6 and 30 µg test item/L concentrations, respectively. Concentrations of deltamethrin were higher in aged test media compared to fresh media. The detected concentrations of deltamethrin are within a range where lethal effects on fish can be observed. It can

therefore be assumed, that deltamethrin also contributed to the acute fish toxicity observed in this study. None of the water samples contained trans-isomer of deltamethrin above the limit of quantification (LOQ = 0.125 µg/L).

The biological results were based on mean measured concentrations of the alpha-R-isomer of deltamethrin. However, the reported endpoints **for the alpha-R-isomer of deltamethrin** are of limited reliability, as **deltamethrin** was also present in the test media at concentrations lethal to fish due to re-isomerization of the alpha-R-isomer into the parent compound deltamethrin under test conditions.

Biological findings:
In the controls no mortalities or sub-lethal findings were observed. In all test levels ≥2.14 µg p.m./L behavioral changes were observed during the entire exposure period. After 96 h of exposure towards the nominal concentration of ≥2.14 µg p.m./L fish showed the following behavioural symptoms:

- remaining for unusually long periods on the bottom of the aquarium
- showed labored respiration
- did not show any abnormal signs

Cumulative mortality was observed as follows (10 fish per test level):

Nominal test item concentrations	Mean measured conc. of alpha-R-isomer of deltamethrin	Cumulative mortality [%]				
		4 h	24 h	48 h	72 h	**96 h**
Control	-	0	0	0	0	**0**
Solvent control	-	0	0	0	0	**0**
1.30 µg/L	2.14 µg p.m./L	0	0	0	0	**0**
2.80 µg/L	3.53 µg p.m./L	0	0	0	0	**0**
6.20 µg/L	4.57 µg p.m./L	0	0	0	20	**20**
13.6 µg/L	22.5 µg p.m./L	0	0	20	60	**70**
30.0 µg/L	34.0 µg p.m./L	0	0	0	50	**70**

Conclusions:
The test conditions met all validity criteria, given by the mentioned guidelines. The following endpoints were determined based on mean measured concentrations of alpha-R-isomer of deltamethrin:

LC_{50} (96 h)	**16.2 µg p.m./L(C.I.95%: 10.2 – 29.3 µg/L)**
100 % mortality	> 34.0 µg p.m./L
NOLEC no-observed-lethal-effect-concentration	3.53 µg p.m./L
NOEC highest concentration without sub-lethal effects	< 2.14 µg p.m./L

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Metabolite trans-isomer of deltamethrin

Report:	**KCA 8.2.1/06, [redacted]; 2013**
Title:	Acute toxicity of trans-isomer of deltamethrin (tech.) to fish (*Oncorynchus mykiss*) under static condtions
Document No:	M-473731-01-1 (Rep. No: EBDAL029)
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OCSPP 850.1075 (Public Draft, 1996) Council Regulation (EC) No 440/2008, C.1 (2008) OECD No. 203 (rev. 1992) JMAFF, 12 Nousan No. 8147
GLP:	Yes

Objective:
The aim of the study was to determine the acute toxicity of the test item to Rainbow trout (*Oncorhynchus mykiss*), expressed as 96 hours LC_{50}.

Material and methods:
Test item: Trans-isomer of deltamethrin (tech.), analyzed content of active substance: 95.1 % w/w; specified by batch code: AE 0035073 00 1B97 0001, Origin batch no: 5E0551, AZ-No.: 16455. The test item also contains 1.4% of deltamethrin as an impurity.
Test organism:
Rainbow trout (*Oncorhynchus mykiss*), mean body length 4.3 cm, mean body weight 0.9 g. Lot F 6 / 11 A were delivered on February 10, 2011. The biomass loading for this test was 0.23 g fish / L test medium.
Ten fish in each test level were exposed for 96 h under static conditions to nominal concentrations of 0 (control), 0 (solvent control: 100 µg acetone/L), 0.0291, 0.0640, 0.141, 0.310, 0.682 and 1.50 µg test item/L. The trans-isomer of deltamethrin, as well as its isomers deltamethrin and alpha-R-isomer of deltamethrin were analyzed in all test levels after 0 h, on day 2 and on day 4 of the exposure period.
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 with commercial measurement devices.

Findings:
Dissolved oxygen concentrations ranged from 79% to 99% oxygen saturation, the pH values ranged from 6.8 to 7.2 and the water temperature ranged from 10.5°C to 12.6°C in all aquaria over the whole testing period.

Analytical findings:
The accompanying chemical analysis of trans-isomer of deltamethrin revealed recoveries between 112% and 256% of nominal values at test initiation. At test termination most recoveries were below the LoQ (LoQ = 0.0313 µg/L). Mean measured values over the entire test period of 96 hours ranged between 23% and 100% of nominal values for trans-isomer of deltamethrin. Geometric mean measured concentrations of the trans-isomer of deltamethrin were: 0 (control and solvent control), 0.0235, 0.0338, 0.0463, 0.0691, 0.317 and 1.43 µg p.m./L.

Deltamethrin was detected in samples of the three highest test concentrations from day 2 onwards (measured values below the LoQ). The maximum measured concentration, i.e. 0.044 µg/L, was measured on day 2 in the highest test level. At this concentration, deltamethrin is acutely toxic to fish.

Therefore, it can be assumed, that deltamethrin contributed significantly to the toxicity observed in this study.
No amounts of the alpha-R isomer of deltamethrin were found in any of the measured water samples and none of the measured compounds was detected in samples from untreated water and solvent control.

Biological findings:
The biological results are based on geometric mean measured concentrations of the trans-isomer of deltamethrin. However, the reported endpoints for the **trans-isomer of deltamethrin** are of limited reliability, as **deltamethrin** was also present in the test media at concentrations lethal to fish, due to re-isomerization of the trans-isomer into the parent compound deltamethrin under test conditions.

In the controls no mortalities or sub-lethal findings were observed. In all test levels ≥0.0609 µg p.m./L sub-lethal effects could be observed during the entire exposure period. After 96 h of exposure towards nominal concentrations of ≥0.0609 µg p.m./L fish showed the following behavioural symptoms:

- showed labored respiration
- remained for unusually long periods at the water surface
- were inactive or displayed abnormally low activity
- turned dark in coloration
- showed loss of equilibrium
- were hyperactive
- showed weaker coloration

Cumulative mortality was observed as follows (10 fish per test level):

Exposure time	4 h		24 h		48 h		72 h		**96 h**	
Geometric mean measured conc. [µg p.m./L]	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	**no. of dead**	**% dead**
control	0	0	0	0	0	0	0	0	**0**	**0**
solvent control	0	0	0	0	0	0	0	0	**0**	**0**
0.0235	0	0	0	0	0	0	0	0	**0**	**0**
0.0338	0	0	0	0	0	0	0	0	**0**	**0**
0.0463	0	0	0	0	0	0	0	0	**0**	**0**
0.0691	0	0	0	0	0	0	0	0	**0**	**0**
0.317	0	0	8	80	9	90	9	90	**9**	**90**
1.43	0	0	10	100	10	100	10	100	**10**	**100**

Conclusions:
The test conditions met all validity criteria, given by the mentioned guidelines. The following endpoints were determined based on geometric mean measured concentrations of the trans-isomer of deltamethrin:

Test substance:	trans-isomer of deltamethrin (tech.)
Test object:	Rainbow trout *(Oncorhynchus mykiss)*
Exposure:	96 hours, static test
LC $_{50}$ 96 hours (95% C.I.):	**0.239 µg p.m./L (C.I.95%: n.d.)**
LOEC: lowest concentration with an effect	0.0238 µg p.m./L
NOEC: highest concentration without toxic effects	0.0235 µg p.m./L
NOLEC: highest concentration causing no mortality	0.0691 µg p.m./L
100 % mortality:	1.43 µg p.m./L

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Metabolite 4'OH-deltamethrin

Report:	**KCA 8.2.1/07, [redacted]; 2013**
Title:	Acute toxicity to fish (*Oncorynchus mykiss*) under static-renewal conditions
Document No:	M-473195-01-1 (Rep. No: EBDAL030)
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OCSPP 850.1075 (Public Draft, 1996) Council Regulation (EC) No 440/2008, C.1 (2008) OECD No. 203 (rev. 1992) JMAFF, 12 Nousan No. 8147
GLP:	Yes

Objective:
The aim of the study was to determine the acute toxicity of the test item to Rainbow trout (*Oncorhynchus mykiss*), expressed as 96 hours LC_{50}.

Material and methods:
Test item: BCS-BY84407 (tech.), purity: 96.5 % w/w; specified by BCS-batch code: BCS-BY84407-01-01, Origin batch No.: SES 12072-8-11, LIMS No.: 1237717, tox no.: 09494-00.
Test organism:
Rainbow trout (*Oncorhynchus mykiss*), mean body length 5.4 cm, mean body weight 1.6 g. Lot F 6 /13 B were delivered on April 25, 2013. The biomass loading for this test was 0.40 g fish/L test medium.
Ten fish in each test level were exposed for 96 hours under static-renewal conditions to nominal (mean measured pure metabolite) test item concentrations of 1.30 (1.06), 2.60 (2.19), 5.18 (4.26), 10.4 (8.26) and 20.7 (17.6) µg/L against control and a solvent control (100 µg acetone/L) with further 10 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 with commercial measurement devices, daily.

Findings:

Dissolved oxygen concentrations ranged from 92% to 99% oxygen saturation, the pH values ranged from 7.0 to 7.7 and the water temperature ranged from 11.5°C to 12.5°C in all aquaria over the whole testing period. BCS-BY84407 was analyzed in all aged and freshly prepared test media.

Measured concentrations of BCS-BY84407 ranged from 99% to 144% in the fresh test solutions and from 29% and 94% in aged solutions, respectively. Therefore the biological results of this study are based on mean measured concentrations of the pure metabolite.

In the controls no mortalities or sub-lethal findings were observed.

In all test levels ≥ 2.19 µg p.m./L behavioral changes were observed during the entire exposure period.

After 96 hours of exposure the surviving fish showed the following behavioural symptoms:

- remained for unusually long periods at the water surface
- showed labored respiration

Cumulative mortality was observed as follows (10 fish per test level):

Exposure time	**4 hours**		**24 hours**		**48 hours**		**72 hours**		**96 hours**	
Test conc. [µg p.m./L]	**no. of dead**	**% dead**	**no. of dead**	**% dead**	**no. of dead**	**% dead**	**no. of dead**	**% dead**	**no. of dead**	**% dead**
Control	0	0	0	0	0	0	0	0	**0**	**0**
solvent c.	0	0	0	0	0	0	0	0	**0**	**0**
1.06	0	0	0	0	0	0	0	0	**0**	**0**
2.19	0	0	0	0	0	0	0	0	**0**	**0**
4.26	0	0	1	10	3	30	7	70	**7**	**70**
8.26	0	0	6	60	10	100	10	100	**10**	**100**
17.6	0	0	10	100	10	100	10	100	**10**	**100**

Conclusions:

Test conditions met all validity criteria, given by the mentioned guidelines. The following endpoints were determined based on mean measured concentrations of BCS-BY84407:

Test substance:	BCS-BY84407
Test object:	Rainbow trout *(Oncorhynchus mykiss)*
Exposure:	96 hours, static test
LC_{50} 96 hours (95% C.I.):	**3.99 µg p.m./L (C.I.95%: n.d.)**
LOEC: lowest concentration with an effect	2.19 µg p.m./L
NOEC: highest concentration without toxic effects	1.06 µg p.m./L
NOLEC: highest concentration causing no mortality	2.19 µg p.m./L
100 % mortality:	8.26 µg p.m./L

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Metabolite Br_2CA

Report:	KCA 8.2.1/08, [redacted]; 2001
Title:	Acute toxicity to *Oncorhynchus mykiss* (rainbow trout) AE F108565; substance, pure (Metabolite of Deltamethrin)
Document No:	M-199816-01-2 (Rep. No: EC00/074)
Guidelines:	OECD No. 203, US-EPA E, §72-1, EU C.1
GLP:	yes

Objective:

The purpose of this study was to estimate the acute toxicity (LC_{50}) of AE F108565 (metabolite of deltamethrin, purity 98.8%) to Rainbow trout (*Oncorhynchus mykiss*) under static conditions.

Materials and Methods:

Test item: AE F108565 (metabolite of deltamethrin), purity: 98.8% w/w, specified by batch code: AE F108565 00 1B99 0001 (AZ 08085).

Test organism: Rainbow trout (*Oncorhynchus mykiss*), 6 months old, mean body length 5.7cm, mean body weight 3.2 g. The biomass loading for this test was 0.64 g fish / L test medium.

Ten fish were exposed to the nominal concentrations of 10, 18, 32, 56 and 100 mg test substance/L together with an untreated control and a solvent control (0.1 mL acetone/L) for 96 hours under static conditions. During the test, fish were examined daily for mortalities and signs of poisoning. Within the study the pH-value, the oxygen saturation level and the temperature were measured with commercial measurement devices, daily. Dissolved oxygen concentrations ranged from 6.2 mg/L to 9.9 mg/L, the pH values ranged from 7.2 to 8.1 and the water temperature ranged from 12.9°C to 13.5°C in all aquaria over the whole testing period. The photoperiod was 16 hours of light and 8 hours dark.

After 24, 48, 72 and 96 hours of exposure the fish were inspected for the number of deaths, toxic symptoms or abnormalities. The mortality (%) after 24, 48, 72 and 96 hours of exposure was calculated in each treatment group. Chemical analysis of the freshly prepared and aged (96 hours old) test solutions was performed for the test item AE F108565 using HPLC/UV.

Results:

Analytical results:

Analyses of freshly prepared exposure media for AE F108565 resulted in test item concentrations ranging from 39.7% to 61.7% of nominal values due to a limited solubility during the first two test days. Analyses of aged water (96 h) for AE F108565 at experimental termination resulted in test item concentrations from 93.7% to 102.7% of nominal values. As the analyzed concentrations of AE F108565 were within ±20% of nominal at the end of the study, the biological results were based on nominal concentrations.

Biological results:

Mortality, lethargy, surfacing, ceased swimming and/or loss of equilibrium were observed as intoxication symptoms at the treatment levels of and above 32 mg/L. Therefore the concentration without mortality and without any intoxication symptoms (NOEC) was 18 mg test item/L.

LC_{50} values for rainbow trout exposed to AE F108565 based on nominal concentrations

Test substance:	AE F108565
Test object:	Rainbow trout (*Oncorhynchus mykiss*)
Exposure:	96 hours, static test design (dose-response)
LC_{50} 96 h:	**100 mg test item/L (nominal)**

Conclusion:

The LC_{50} (96h) of AE F108565 to Rainbow trout (*Oncorhynchus mykiss*) in a static 96-hour test was determined to be 100 mg/L (nominal).

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Metabolite mPBacid

Report:	**KCA 8.2.1/09, [redacted]; [redacted]; 1981**
Title:	Determination of the acute toxicity of 3-Phenoxy Benzoic Acid to Rainbow Trout (*Salmo gairdneri*)
Document No: Letter of Access:	BL/B/2038 M-479954-01-1
Guidelines:	(No guideline)
GLP:	Yes

Objective:

The study was performed to determine the acute toxicity of 3-Phenoxy Benzoic Acid to Rainbow Trout (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*) in freshwater. A static-renewal test system was used to determine 24, 48, 72 and 96 hour LC_{50} values.

Material and methods:

Test item: 3-Phenoxy Benzoic Acid, purity 99% w/w, received from [redacted], USA.

Test organism: Rainbow trout (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*), mean body length 52 mm (47 mm- 58 mm), mean body weight 1.9 g (1.31 g- 2.74 g).

Ten Rainbow Trout fry per concentration were exposed in a static-renewal system for 96 hours to six test item concentrations, a solvent control (DMSO; 2000 mg/L) and a dilution water control (freshwater control). During the test, nominal concentrations of 3.2, 5.6, 10, 18, 32, 56 mg test item/L (3-Phenoxy Benzoic Acid) were maintained by daily change of test solution.

The concentrations of 3-Phenoxy Benzoic Acid in the exposure vessels were analysed in the freshly prepared and aged (24 h old) test media of each renewal period. Biological observations and observations of the physical characteristics of the exposure solutions were made and recorded at test initiation and at each subsequent 24-hour interval until termination of the test.

Findings:

Analytical results: Measured concentrations of 3-Phenoxy Benzoic Acid ranged from 98.8% to 111.6% in the fresh test solutions and from 96.9% to 105% in aged solutions, respectively. Biological results are based on mean measured concentrations.

Biological results:

Nominal concentration of 3-Phenoxy Benzoic Acid (mg/L)	Mean measured concentration of 3-Phenoxy Benzoic Acid (mg/L)	Percentage mortality observed			
		24 hours	48 hours	72 hours	96 hours
Freshwater Control	-	0	0	0	0
DMSO Control	-	0	0	0	0
3.2	3.5	0	0	0	0
5.6	5.5	0	0	0	0
10.0	10.0	10	10	10	10
18.0	17.7	60	90	90	90
32.0	33.7	100	100	100	100
56.0	56.7	100	100	100	100

There were neither any sub-lethal effects nor any mortality in the control and solvent control group.

Surviving fish at levels ≥ 0.10 mg test item/L showed the following symptoms:

- Fish darkening
- Loss of balance
- Fish spiralling
- Weakness of fish
- Fish surfacing
- Laboured respiration spiralling

LC_{50} values for for Rainbow trout exposed to 3-Phenoxy Benzoic Acid technical based on mean measured concentrations:

Test substance:	3-Phenoxy Benzoic Acid
Test object:	Rainbow trout (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*)
Exposure:	24, 84, 72 and 96 hours, static-renewal test design (dose-response)
LC_{50} 96 h (95% C.I.):	13.3 (11.2 – 15.8) mg test item/L (mean measured)

The no observed effect concentration (NOEC) at which no toxic symptoms were observed throughout the 96 hour exposure period was found to be 5.5 mg test item/L.

Conclusions:

The LC_{50} (96 h) of 3-Phenoxy Benzoic Acid to Rainbow trout (*Oncorhynchus mykiss*, formerly known as *Salmo gairdneri*) in an acute test under static-renewal conditions was determined to be 13.3 mg test item/L based on mean measured concentrations.

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Results from literature review

Report:	**KCA 8.2.1/10; [redacted], M.; [redacted], S.; [redacted]; 2006**
Title:	Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Fingerling European Catfish, Silurus glanis L.
Source:	Bull. Environ. Contam. Toxicol., 76, 1, p. 59-65
DOI No: Document No:	10.1007/s00128-005-0889-3 M-460890-01-1
Guidelines:	APHA 19851
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

A static acute toxicity bioassay was performed to determine the toxicity of deltamethrin for European catfish fingerlings (*Silurus glanis*).
Fish were exposed for 96 hours under static renewal conditions to the seven concentrations of deltamethrin: 0.25, 0.50, 0.75, 1, 2, 3 and 4 µg/L (nominal), as well as in a control and solvent control (acetone). Mortality was assessed at 1, 24, 48, 72 and 96 h after the start. Behavioural changes of test animals were closely followed and recorded.
The 96-hour LC_{50} value for fingerlings of the European catfish (*Silurus glanis*) following exposure to deltamethrin was determined as 0.686 µg deltamethrin/L.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 2.5 EC
Active substance(s):	Deltamethrin
Chemical state and description:	Not reported
Source of test item:	[redacted]
Batch number:	Not reported
Purity:	2.5 % deltamethrin
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	Not reported
Method of preparation:	Deltamethrin was prepared from a stock solution weighed in a glass boat and transferred to a volumetric flask containing experimental water. Dilutions of the defined stock solution were used for the tests.
Evidence of unsolved material:	Not reported

[1] APHA (1985) Standard methods for the examination of water and wastewater. 16th Edition, American Public Heaith Association, Washington, DC

3. Test organism(s)

Species:	*Silurus glanis* L.
Common name:	Fingerling European catfish
Source of test species:	[redacted] Region Directorate
Size of test organisms:	Fingerlings, weight 15-18 g, length: 13-15 cm

4. Culture conditions of test organism(s)

Culture medium:	Not reported
Temperature:	17±1 °C
Photoperiod:	Not reported
Light intensity:	Fluorescent light
pH:	Not reported
Oxygen saturation:	Not reported
Food and feeding regime:	Fish were fed with pellet feeds during adaptation, but not during the last 24 h of adaptation
Acclimatisation prior to testing:	Fish were kept for 7 days in experimental aquaria with 17±1 °C water temperature and a 12 h photoperiod. Fish were fed with pellet feeds during adaptation, but they were not fed during the last 24 h of adaptation.
Observations during acclimatisation:	Not reported

B. Study design and methods

1. Test procedure

Test system:	Acute toxicity bioassay according to the standard method APHA 1985[2]
Test concentration(s):	0.25, 0.50, 0.75, 1, 2, 3 and 4 µg/L (nominal)
Control(s):	Water control and solvent control (acetone)
Number of replicates:	5 replicates, with 20 fish each
Test conditions:	280 L aquaria, filled with 270 L of dechlorinated tap water
Feeding:	No feeding
Medium renewal:	Every 12 hours
Frequency of test item application:	Not clearly specified – but presumably via new test media with each renewal
Test duration:	96 h
Endpoints:	Mortality and behavioural changes
Measurement frequency:	Mortality was assessed 1, 24, 48, 72 and 96 h after the start
Statistics:	Statistical analysis was performed with the SPSS 10.1 computer program (SPSS Inc., Chicago, Illinois). Data were evaluated using the probit analysis method. The chi-square test was employed for comparing mean mortality values using a significance level of 0.05.

2. Measurements during the test

Water/medium parameters:	Dissolved oxygen 7.2±0.4 mg/L, pH 8.4±0.1, electrical conductivity 227±9.5 µS/cm, alkalinity 148±24 mg/L and total hardness 196±15 mg/L as CaCO3.

3. Chemical analysis

[2] APHA (1985) Standard methods for the examination of water and wastewater. 16th Edition, American Public Health Association, Washington, DC

Guideline/protocol: No chemical analysis conducted.

RESULTS

1. Biological findings:

Mortality of fingerlings of the European catfish following exposure to deltamethrin is summarized in the table below.
Normal behaviour was observed for fish of the control group and 0.25 µg/L. After 48 h, some abnormalities such as less general activity and loss of equilibrium were observed in fish exposed to 0.5 µg/L.The abnormal behavioural responses observed at all concentrations higher than 0.50 µg/L were loss of equilibrium, hanging vertically in the water, rapid gill movement, erratic swimming, swimming at the water surface, air gulping from the water surface, or staying motionless on the aquarium bottom.

Cumulative mortality (n = 100 in five replicates) and lethal concentrations (LC_{50}) of deltamethrin depending on time (1-96 h) for European catfish fingerlings:

Nominal concentrations [µg deltamethrin/L]	**Number of dead fish**				
	1 h	**24 h**	**48 h**	**72 h**	**96 h**
Control	-	-	-	-	-
0.25	-	-	-	-	-
0.50	-	-	-	9	20
0.75	-	-	14	35	67
1	-	17	39	70	93
2	25	59	83	95	ND
3	70	91	ND		
4	94	ND			
LC_{50} [µg/L]	2.497 (2.350-2.634)	1.446 (1.311-1.577)	1.215 (1.126-1.325)	0.866 (0.679-1.134)	**0.686 (-)**

ND: No data because of 100% mortality, (-): Not dead

RESULTS SUMMARY

The 96-hour LC_{50} value for fingerlings of the European catfish (*Silarus glanis*) following exposure to deltamethrin was determined as 0.686 µg deltamethrin/L.

Comment by the Notifier:

The study is considered supplementary information only, as a GLP study is available to address this data point (acute fish toxicity). Reliability of the published data is limited due to missing analytical verification. However, test media were renewed every 12 hours, so a continuos exposure of fish can be assumed.
Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CA 8.2.2 Long-term and chronic toxicity to fish

For studies already evaluated during the first EU review of deltamethrin, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

The endpoints from the following table have been evaluated during the first EU review (review report of Deltamethrin 6504/VI/99-final)). In the EFSA List of endpoints, the NOEC < 0.032 µg a.s./L from a prolonged toxicity test is considered ([redacted], 1990; M-135553-01-1). However, a fish full life cycle study with deltamethrin ([redacted], 1993; M-149454-01-1) is available and was evaluated already in the first EU review. This study provides a real NOEC, which is also lower, than the current EU endpoint. Therefore, the NOEC of 0.017 µg a.s./L will be considered in the Tier 1 risk assessment. It is not clear, why this endpoint was not considered in the EU list of endpoints.

Table 8.2.2- 1: Chronic fish toxicity of deltamethrin

Test substance	Test species	Endpoint	Reference
Deltamethrin	Fish, chronic (FFLC) *Pimephales promelas*	**NOEC 0.017 µg a.s./L (mm)**	[redacted] (1993) M-149454-01-1
Deltamethrin	Fish, chronic (ELS) *Pimephales promelas*	NOEC 0.022 µg a.s./L (mm)	[redacted] (1991) M-149413-01-1
Deltamethrin	Fish, chronic (prolonged toxicity, 28 d) *Oncorhynchus mykiss*	NOEC < 0.032 µg a.s./L (mm)	[redacted] (1990) M-135553-01-1

mm = mean measured

In addition, an ELS study with the marine species *Cyprinodon variegatus* (Sheepshead minnow) was conducted to fulfill the data requirements for the registration of deltamethrin in the USA. A summary is provided under point 8.2.2.1.

Table 8.2.2- 2: Additional chronic fish endpoints for deltamethrin

Test substance	Test species	Endpoint	Reference
Deltamethrin	Fish, chronic (ELS) *Cyprinodon variegatus*	NOEC 0.024 µg a.s./L (mm)	[redacted] (2012) M-439783-01-1

CA 8.2.2.1 Fish early life stage toxicity test

Report:	**KCA 8.2.2.1/02, [redacted]; [redacted]; [redacted]; 2012**
Title:	Early life stage toxicity of deltamethrin technical to the sheepshead minnow (Cyprinodon variegatus) under flow-through conditions
Document No:	M-439783-01-1
Guidelines:	FIFRA 72-4 (1982) OPPTS Guideline 850.1400 (1996 draft) OECD Guideline 210 (1992)
GLP:	yes

Objective:
The purpose of this study was to estimate the chronic toxicity of deltamethrin to Sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions.

Materials and methods:

Test item: deltamethrin techn. (purity: 99.52%), batch no. EGDLTK113
Test organism: Sheepshead minnow (*Cyprinodon variegatus*) were exposed in artificial sea water to nominal test concentrations of 0.0063, 0.0125, 0.025, 0.05 and 0.1 µg a.s./L plus a control and solvent control (10 µL/L DMF) for 35 days under flow-through conditions. At test initiation, 35 eggs (24-48 h old, in the nerula stage) were exposed in each replicate (4 replicates per concentration) in egg cups placed in individual test vessels. When hatch was completed, the alevins were impartially thinned to 20 individuals per replicate and released to the test vessel, in which the egg cup had been suspended.
Concentrations were analytically verified at test initiation and at least once a week thereafter including experimental finish of the exposure period.
Biological parameters assessed were sublethal effects (daily), fish hatchability (daily during hatching phase), survival (daily) and growth (length and dry weight of surviving fish on day 35).

Results:

Analytical results:

During the exposure period, no visible sign of undissolved test material (e.g. precipitate) was observed in any of the treatment level or control solutions. Mean measured concentrations of deltamethrin in the test media ranged from 48% to 49% in the various test levels. Based on these results, the mean measured test concentrations were determined to be 0.003, 0.006, 0.012, 0.024, and 0.049 µg a.s./L. Biological results were based on mean measured concentrations.

Biological results:

Fish in the controls and all other test levels appeared normal during the course of the study.
The day 4 mean percent hatch ranged from 7.9% to 24.3%. The day 5 mean percent hatch ranged from 80.0% to 87.9%. Statistical analysis indicated that percent hatch was not significantly different from pooled controls in any test level on day 4 or day 5.
Alevin survival was analyzed for study day 5. Mean percent alevin survival ranged from 82.9% to 88.6%. Fry survival was analyzed at test termination on study day 35. Mean percent fry survival ranged from 93.6% to 100%. Statistical analysis indicated that both, alevin survival and fry survival, were not significantly different from pooled controls in any test level.
At test termination (study day 35), the fish were sacrificed and measured for standard length and dry weight. The mean lengths ranged from 20.2 to 20.8 mm. Mean dry weights for fish ranged from 72.4 to 80.8 mg. Statistical analysis indicated that standard length was not significantly different from pooled controls in any test level. For dry weight, the William's test showed a statistically significant difference at the highest test concentration (0.049 µg a.s./L) in comparison to the pooled control data.

Endpoint	**Control**	**Solvent control**	**0.003 µg a.s./L**	**0.006 µg a.s./L**	**0.012 µg a.s./L**	**0.024 µg a.s./L**	**0.049 µg a.s./L**
Day 4 - mean hatch	10.0%	7.9%	12.1%	24.3%	15.7%	15.0%	10.0%
Day 4 - mean hatch	83.6%	84.3%	87.9%	82.9%	85.7%	86.4%	80.0%
Day 5 – mean alevin survival	86.4%	85.7%	88.6%	84.3%	87.1%	87.1%	82.9%
Day 35 - mean fry survival	98.8%	97.5%	100.0%	98.8%	98.8%	96.3%	97.5%

Day 35 - mean standard length	20.8 mm	20.4 mm	20.8 mm	20.5 mm	20.4 mm	20.8 mm	20.2 mm
Day 35 - mean dry weight	78.0 mg	76.9 mg	78.8 mg	76.0 mg	77.4 mg	80.8 mg	72.4 mg

Endpoints for Sheepshead minnow exposed to deltamethrin in an Early Life Stage study based on mean measured concentrations

Test substance:	Deltamethrin
Test object:	Sheepshead minnow (*Cyprinodon variegatus*)
Exposure:	Early Life Stage (ELS) study, 35 d, flow-through test design (dose-response)
NOEC:	**0.024 µg a.s./L (mean measured)**
LOEC:	0.49 µg a.s./L (mean measured)

Conclusion:
The 35-day exposure of Sheepshead minnow to deltamethrin technical resulted in a NOEC of 0.024 µg a.s./L and a LOEC of 0.049 µg a.s./L based on dry weight.

CA 8.2.2.2 Fish full life cycle test

See point MCA 8.2.2. No additional studies were performed.

CA 8.2.2.3 Bioconcentration in fish

For studies already evaluated during the first EU review of deltamethrin, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

CA 8.2.3 Endocrine disrupting properties

As the aquatic profile of deltamethrin is characterized by fast dissipation from the water phase, no chronic exposure of fish is expected. Nevertheless, population relevant effects of deltamethrin on fish were studied in a flow-through early life stage test (ELS) and a fish full life cycle test (FFLC) with fathead minnow (*Pimephales promelas*). In the ELS a NOEC of 22 ng a.s./L (mean measured) based on growth was determined. In the FFLC the lowest NOEC of 17 ng a.s./L was found for growth (female weight) as well, and no effects on reproductive parameters were observed.
Based on the absence of relevant effects it can be concluded that deltamethrin is not a (potential) endocrine disrupter. This conclusion is supported by the results of a published Fish Screening Assay with *Danio rerio* conducted with deltamethrin ([redacted] 2006; M-460900-01-2).
No further testing is indicated to evaluate the endocrine disrupter potential of deltamethrin to fish.

Results from literature review

Report:	**KCA 8.2.3/01; De [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2006**
Title:	Reproductive aspects of zebrafish, Danio rerio, exposed to sublethal doses of deltamethrin. Aspectos reprodutivos do peixe-zebra, Danio rerio, exposto a doses

	subletais de deltametrina.
Source:	Archives of Veterinary Science, 11, 1, p. 48-53
DOI No: Document No:	- M-460900-01-2
Guidelines:	USEPA (ENVIRONMENTAL PROTECTION AGENCY). EPA/68-W-01-023: Fish screening assays for endocrine disruption, Ohio, 2002.
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY (Abstract from publication)

The deltamethrin is listed by the Environmental Protection Agency of the United States (US EPA) as a possible endocrine disruptor, being able to interfere in the reproductive system. It is a synthetic pyrethroid, with potent insecticide action, relatively low toxicity in mammals and limited persistence in the environment, but high toxic to aquatic organisms. It is also used in the human and veterinary medicines for prophylaxis and treatment of parasitic diseases. The aim of this study was to evaluate possible endocrine alterations in the reproduction of the zebrafish (*Danio rerio*) following the protocol of USEPA (2002). The fish were exposed to sublethal concentrations of deltamethrin (6 µg/L and 10 µg/L of the technical deltamethrin) and acetone, used as solvent, during 14 days. Parameters as number of eggs, hatching, size and histology of the gonads were evaluated. It was not observed any significant difference of the evaluated parameters among the groups. The deltamethrin didn't alter the reproduction of the zebrafish. In the present study the progeny was not evaluated, because was not included in the used protocol. However, endocrine disruptor substances can affect the neuroendocrine reproductive system and the alterations can appear in the progenitors or in the progeny in different stages of the development.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin tech. dissolved in acetone
Active substance(s):	Deltamethrin
Chemical state and description:	Not reported
Source of test item:	[redacted] Brasil Ltda.
Batch number:	Not reported
Purity:	98.8%
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Preparation of test solutions not described. Acetone used as solvent.

3. Test organism(s)

Species:	*Danio rerio*
Common name:	Zebrafish
Source of test species:	Not reported

4. Culture conditions of test organism(s)

No information provided on culture conditions of test organisms.

B. Study design and methods

1. Test procedure

Test system:	The test was conducted in accordance with the US EPA protocol for "Fish screening assays for endocrine disruption" Reproductive bioassay and histological procedures 16 L aquaria filled with dechlorinated filtered water. The aquaria had an interior net to protect the eggs which were laid on the bottom, from adults feeding on them. Egg were collected from the aquaria and counted using a magnifying glass, a light source and a pipette. The counted eggs were labelled with concentrations and days, and kept in separate containers in a bigger aquarium at a temperature of about 27°C. After 72 h, the hatched fries were counted. On the 14th day after the last egg collection and counting, one female and one male from each aquarium were sacrificed and weighed. The gonads were removed and weighed for the calculation of the gonadosomatic index (GSI), obtained using the formula: GSI = (gonad weight/body weight) x 100. Later, the gonads were fixed in an Alfac solution for 16 h, followed by routine histological procedures for embedding in paraffin. The slides were stained with haematoxylin and eosin and were analysed under a light microscope for morphological description of the different cellular development phases of the female and male gamete lineages as described by [redacted] (1996).
Test concentration(s):	6 and 10 µg/L
Control(s):	Negative control (dechlorinated filtered water only) and solvent control (acetone)
Number of replicates:	4 replicates, each with 6 adult fish (4 females, 2 males)
Test conditions:	Constant aeration, 25°C temperature, pH 6.5 ± 0.5, 16:8 hour day:night phase
Feeding:	Commercial fish food twice a day
Medium renewal:	Every day 4 litres of water were removed from each aquarium and replaced by 4 litres of fresh water containing deltamethrin at the corresponding concentration
Frequency of test item application:	Semi-static; test media partially renewed every 24 h
Test duration:	14 days
Endpoints:	Mortality, behavioural changes, external alterations (haemorrhages, discolouring), number of eggs, number of hatched eggs, gonadosomatic index of females and males
Statistics:	The variables fitted the normal distribution and were compared using analysis of variance (ANOVA), followed Bonferroni correction, for comparison between groups, with a level of significance $p < 0.05$. The results were expressed as mean □ standard error of the mean.

2. Measurements during the test

Water/medium parameters:	Sixteen 16-litre aquariums (four replicates for each group) with dechlorinated filtered water and constant aeration, 25 °C temperature, pH 6.5 ± 0.5 and 16/8 hour (day/night phase)

3. Chemical analysis

No chemical analysis

RESULTS

Neither external alterations such as haemorrhages or discolouring, nor general signs of intoxication, such as hyperventilation, changes in swimming patterns and feeding were noticed in the controls and any of the treatment levels. Analyses did not show significant differences between the control and the contaminated group regarding total number of eggs collected, number of hatched eggs and the GSI for the separate sexes during the study.

Mean and standard error for the number of eggs, number of eggs hatched, gonadosomatic index of females and males of *Danio rerio:*

Deltamethrin concentration [µg/L] (nominal)	Number of eggs	Number of hatched eggs	Gonadosomatic index of females (%)	Gonadosomatic index of males (%)
Control (water)	2,195 ± 381	1,077 ± 144	8.52 ± 1.01	1.63 ± 0.23
Control (acetone)	2,895 ± 189	783 ± 45	7.98 ± 0.73	1.87 ± 0.36
6	1,727 ± 214	827 ± 201	9.18 ± 0.32	1.56 ± 0.33
10	1,562 ± 306	840 ± 253	7.90 ± 0.99	1.60 ± 0.08

The histological analysis of the gonads of fish from the contaminated groups showed the same characteristics as those of the control group.

CONCLUSION

The results obtained in the present study indicate, that there was no effect on the parameters evaluated (mortality, behaviour, number of eggs, number of hatched eggs, gonadosomatic index, histopath of gonads) caused by deltamethrin at a nominal concentration of 10 µg/L.

Comment by the Notifier:

Reliabilty of the published data is limited due to missing analytical verification. However, test media were renewed every 24 hours, so a continuos exposure of fish can be assumed. The study is considered supplementary information only, as results from a screening assay are not suitable for TER calculations. Moreover, the available FFLC study with Fathead minnow delivers a more reliable and sensitive endpoints.
Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

For studies already evaluated during the first EU review of deltamethrin, please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

A new study with the active substance deltamethrin is available, resulting in a lower endpoint, compared to the one given in the EU list of endpoints. The study is summarized below, and the new endpoint will be considered in the risk assessment.

In order to complete the aquatic risk assessment for aquatic invertebrates, additional studies are provided for the metabolites of deltamethrin in this Supplemental Dossier. Acute *Daphnia* studies were conducted with the metabolites alpha-R-isomer and trans-isomer of deltamethrin, 4'OH-deltamethrin, Br_2CA, Serinyl-BrCA, mPBaldehyde and mPBacid. Respective study summaries are given below.

mPBaldehyde is not a major metabolite in the aquatic environment. The available study is summarized below for the sake of completeness, but no risk assessment will be provided for this minor metabolite.

Table 8.2.4.1- 1: Additional studies for acute toxicity of deltamethrin and its metabolites to *Daphnia magna*

Test substance	Test species	Endpoint	Reference
Deltamethrin	Invertebrate, acute *Daphnia magna*	EC_{50} 0.0131 µg/L (mm)	[redacted] **(2014) M-474111-01-1**
alpha-R-isomer of deltamethrin	Invertebrate, acute *Daphnia magna*	EC_{50} 0.0366 µg/L (mm)*	[redacted] **(2014) M-474118-01-1**
trans-isomer of deltamethrin	Invertebrate, acute *Daphnia magna*	EC_{50} 0.069 µg/L (mm)*	[redacted] **(2014) M-473835-01-1**
4'OH-deltamethrin (BCS-BY84407)	Invertebrate, acute *Daphnia magna*	EC_{50} 670 µg/L (mm)	[redacted] **(2013) M-465317-01-1**
Br_2CA (AE F108565)	Invertebrate, acute *Daphnia magna*	EC_{50} >100000 µg/L (nom)	[redacted] **&** [redacted] **(2001) M-199793-01-2**
Serinyl-BrCA (BCS-CW57835)	Invertebrate, acute *Daphnia magna*	EC_{50} 35300 µg/L (mm)	[redacted] **(2013) M-465372-01-1**
mPBaldehyde (AE F114152)	Invertebrate, acute *Daphnia magna*	EC_{50} 162 µg/L (mm)**	[redacted] **(2010) M-386854-01-1**
mPBacid (AE F109036)	Invertebrate, acute *Daphnia magna*	EC_{50} 85000 µg/L (mm)	[redacted] **(1983) RJO318B** [redacted] **number: CGA55186/0721 Letter of Access: M-479954-01-1**

mm – mean measured; nom = nominal

* Results from the studies with the alpha-R-isomer and the trans-isomer of deltamethrin <u>are not suitable for the use in aquatic risk assessments</u>. In both studies, the parent compound deltamethrin was also detected at concentrations, which are lethal to *Daphnia*, due to re-isomerization of the alpha-R-isomer and the trans-isomer into the parent compound deltamethrin under test conditions. Therefore, it is expected that deltamethrin contributed significantly to the toxic effects observed in these studies. In a conservative approach, endpoints were derived based on the mean measured concentrations of the respective metabolite alone. However, these endpoints overestimate the actual toxicity of the metabolites, as they do not consider the effects caused by the presence of deltamethrin. The available studies do not allow for a definite determination of the metabolite toxicity, and are therefore not considered adequate for a risk assessment. Nevertheless, these worst-case endpoints clearly demonstrate that neither the alpha-R-isomer nor the trans-isomer of deltamethrin is more toxic to aquatic organisms than the parent compound itself.

** mPBaldehyde is only a minor metabolite and will not be considered in the risk assessment.

Active substance deltamethrin

Report:	**KCA 8.2.4.1/03, [redacted]; 2014**
Title:	Acute toxicity of deltamethrin (tech.) to the waterflea Daphnia magna in a static renewal laboratory test system
Document No:	M-474111-01-1 (Rep. No: EBDAN150)
Guidelines:	OECD guideline 202,(2004); EC Council Regulation No 440/2008, Method C.2 (2008); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).
GLP:	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-renewal laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: Deltamethrin (tech.), batch AE F032640-01-15 (PMDN001265), specification No.: 102000001388-03, purity: 99.6% w/w (TOX 10229.00)
Daphnia magna (1st instars < 24 h old, 6 × 5 animals per concentration), exposed in a static-renewal test system for 48 (2 × 24) hours to nominal concentrations of 0, 10, 20, 40, 80 and 160 ng a.s./L (corresponding to mean-measured concentrations of 6.5, 12.9, 28.2, 47.7 and 99.5 ng a.s./L) without feeding.
The content of deltamethrin (AE F032640) in exposure media was measured for verification of the test item concentrations at the start and end of each renewal period. Additional analysis for identification of the metabolites AE F108569 (alpha-R-isomer of deltamethrin) and AE 0035073 (trans-isomer of deltamethrin) in exposure media was performed.

Findings:
Analytical results:
The accompanying chemical analysis of deltamethrin (AE F032640) in the freshly prepared test solutions revealed measured contents between 85% and 121% (mean: 95%) of nominal concentrations. The corresponding concentrations of the aged test solutions at the end of each 24-hour exposure period ranged between 31% and 39% (mean: 34%) of nominal. Therefore, the biological results were based on mean measured concentrations.

Biological results:

Toxicity of deltamethrin to *Daphnia magna*:

Mean measured test concentration [mg a.s./L]	**Exposed daphnids (=100%)**	**Immobilised daphnids**			
		24 h		**48 h**	
		n	**%**	**n**	**%**
Control	30	0	0	0	**0**
Solvent control *	30	0	0	0	**0**
6.5	30	0	0	5	**17**
12.9	30	0	0	19	**63**
28.2	30	0	0	22	**73**
47.7	30	3	10	27	**90**
99.5	30	8	27	24	**80**

* 0.1 mL acetone/L test media

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

EC_{50} values for *Daphnia magna* exposed to deltamethrin based on mean measured concentrations

Test substance:	Deltamethrin techn.
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):	155 (88.3-271) ng a.s./L (mean measured)
EC_{50} 48 h (95% C.I.):	**13.1 (10.1-17.0) ng a.s./L (mean measured)**

Conclusions:
Based on mean measured concentrations of deltamethrin (AE F032640), the 48-hour EC_{50} value for immobilisation was determined to be 13.1 ng a.s./L in a static-renewal system.

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Metabolite alpha-R-isomer of deltamethrin

Report:	**KCA 8.2.4.1/04, [redacted]; 2014**
Title:	Acute toxicity of alpha-R isomer of deltamethrin (tech.) to the waterflea *Daphnia magna* in a static-renewal laboratory test system
Document No:	M-474118-01-1 (Rep. No: EBDAL022)
Guidelines:	OECD guideline 202 (2004); EC Council Regulation No 440/2008, Method C.2 (2008) (formerly: EEC Directive 92/69/EEC, part C.2 (1992)
GLP:	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-renewal laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: Alpha-R-isomer of deltamethrin (tech.) (metabolite of deltamethrin), batch AE F108569-PU-02 (origin batch No. HSRM.6269-1-1), purity: 94.3% w/w (AZ 17287). The test item also contains 0.2% of deltamethrin as an impurity.
Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static-renewal test system for 48 (2 x 24) hours to nominal concentrations of 0, 12.5, 25, 50, 100, 200, and 400 ng pure metabolite (p.m.)/L without feeding.
The study was conducted using artificial fish-testing water.
The contents of alpha-R-isomer of deltamethrin (AE F108569) and its isomers deltamethrin (AE F032640) and trans-isomer of deltamethrin (AE 0035073) were measured in the freshly prepared and aged test media.

Findings:
Analytical results
The accompanying chemical analysis of AE F108569 (alpha-R-isomer of deltamethrin) in the freshly prepared test solutions at start of each renewal interval revealed single recoveries between 101% and 149% (mean: 139%) of the corresponding nominal concentrations. The corresponding concentrations of the aged test solutions at the end of each 24-hour exposure period ranged between 37% and 70% (mean: 62%) of nominal. This resulted in mean measured concentrations of 12.4, 21.3, 44.0, 86.1, 147.4 and 293.3 ng p.m./L. The detected concentrations of deltamethrin are within a range that is lethal to *Daphnia magna*. Therefore, it can be assumed, that deltamethrin contributed significantly to the acute toxicity observed in this study.
AE F108569 (deltamethrin) was formed in the exposure media via isomerisation. Mean measured concentrations of AE F108569 in the test media were < LoQ (6.33 ng/L), 4.08, 6.66, 9.91, 17.12 and 29.64 ng/L. Concentrations of AE F108569 were significantly higher in aged test media compared to freshly prepared media. The maximum measured concentration of AE F108569, i.e. 54.3 ng/L, was measured in aged media of the highest test concentration on day 2.
No amounts of AE 0035073 (trans-isomer of deltamethrin) were found in any of the measured water samples (LOQ: < 6.28 ng/L) and none of the measured compounds was detected in samples from untreated water and solvent control.

Biological results
The biological results are based on mean measured concentrations of AE F108569 (alpha-R-isomer of deltamethrin).
However, the reported endpoints for the alpha-R-isomer of deltamethrin are of <u>limited reliability</u>, as deltamethrin was also present in the test media at concentrations lethal to *Daphnia magna*, due to re-isomerization of the alpha-R-isomer into the parent compound deltamethrin under test conditions.

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Toxicity of alpha-R-isomer of deltamethrin (AE F108569) to Daphnia magna:

Mean measured test concentration [ng p.m./L]	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	%	n	%
Control	30	1	3	2	7
Solvent control *	30	2	7	2	7
12.4	30	5	17	8	**27**
21.3	30	5	17	14	**47**
44.0	30	7	23	16	**53**
86.1	30	15	50	21	**70**
147	30	10	33	26	**87**
293	30	17	57	30	**100**

* 0.1 mL acetone/L test media

An immobilisation of 6.7% was observed for untreated control animals as well as for the solvent control. Nevertheless, the immobilisation was <10%, which is the limit for acceptable control mortality according to the guidelines. For statistical evaluation of the dose-response pattern, this control mortality has been compensated using Abbott`s formula.

EC_{50} values for *Daphnia magna* exposed to BCS-BY84407 based on mean measured concentrations

Test substance:	alpha-R-isomer of deltamethrin (AE F108569)
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):*	121 (50.6-290) ng p.m./L (mean measured)
EC_{50} 48 h (95% C.I.):	**36.6 (26.4 - 50.7) ng p.m./L (mean measured)**

*) For the 24 h EC_{50} determination no clear dose-response relationship was observed.

Conclusions:

Based on mean measured concentrations of the alpha-R-isomer of deltamethrin (AE F108569), the 48-hour EC_{50} value for immobilisation was determined to be 36.6 ng pure metabolite/L in a static-renewal test system.

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Metabolite trans-isomer of deltamethrin

Report:	**KCA 8.2.4.1/05, [redacted]; 2014**
Title:	Acute toxicity of trans-isomer of deltamethrin (tech.) to the waterflea Daphnia magna in a static laboratory test system
Document No:	M-473835-01-1 (Rep. No: EBDAL028)
Guidelines:	OECD guideline 202,(2004); EC Council Regulation No 440/2008, Method C.2 (2008); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).
GLP:	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.

Material and methods:
Test item: Trans-isomer of deltamethrin (tech.) (metabolite of deltamethrin), batch AE 0035073 00 1B97 0001, purity: 95.1% w/w AE 0035073 (AZ16455). The test item also contains 1.4% of deltamethrin as an impurity.
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, 40, 80, 160, 320 and 640 ng pure metabolite (p.m.)/L without feeding.
The contents of the trans-isomer of deltamethrin (AE 0035073), and its isomers deltamethrin (AE F032640) and alpha-R-isomer of deltamethrin (AE F108569) were measured in the exposure media.

Findings:
Analytical results:
The contents of trans-deltamethrin (AE 0035073), and its isomers deltamethrin (AE F032640) and alpha-R isomer of deltamethrin (AE F108569) were measured in the exposure media.
The accompanying chemical analysis of the trans-isomer of deltamethrin (AE 0035073) in the freshly prepared test solutions revealed single recoveries between 93% and 98% of nominal values (mean: 96%) for the nominal concentration range of 40 – 320 ng p.m./L.
The initial measured recovery rate of 10% for the highest test concentration of 640 ng p.m./L does not reflect the recorded biological effects pattern, which showed a clear dose-response correlation up to 640 ng p.m./L. Moreover, the measured recovery for day 2 is within the expected range (6.78% of nominal). Due to the uncertainty regarding the actual test item concentration in this test level, it was excluded from the statistical evaluation and no mean measured concentration was calculated.
Geometric mean measured concentrations of AE F0035073 in the remaining test levels were: 0 (control and solvent control), 11.1, 23.1, 42.4 and 93.6 ng p.m./L.
The measured values for water samples from day 2 were below the LOQ of 25.1 ng/L. However, measured values were available in the raw data and were used for the calculation of geometric mean measured concentrations.
As analytical measurements at study initiation demonstrate, AE F032640 (deltamethrin) was quickly formed in the exposure media via isomerisation. The corresponding values for freshly prepared test solutions were 5.2, 8.4, 13.1, 20.9 and 22.1 ng/L in the nominal test concentrations of 80, 160, 320 and 640 ng AE F0035073/L.
The detected concentrations of deltamethrin were within a range that is lethal to *Daphnia magna*.

Therefore, it can be assumed, that deltamethrin contributed significantly to the toxicity observed in this study.
No amounts of the alpha-R isomer of deltamethrin (AE F108569) were found in any of the measured water samples and none of the measured compounds was detected in samples from untreated water and solvent control.

Biological results:

The biological results are based on mean measured concentrations of the trans-isomer of deltamethrin (AE 0035073).
However, the reported endpoints for the trans-isomer of deltamethrin are of limited reliability, as deltamethrin was also present in the test media at concentrations lethal to *Daphnia magna*, due to re-isomerization of the trans-isomer into the parent compound deltamethrin under test conditions.

Toxicity of trans-isomer of deltamethrin (AE 0035073) to Daphnia magna:

Mean measured test concentration [ng p.m./L]	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	%	n	%
Control	30	0	0	0	**0**
Solvent control *	30	0	0	0	**0**
11.1	30	0	0	0	**0**
23.1	30	0	0	0	**0**
42.4	30	1	3	9	**30**
93.6	30	1	3	19	**63**
Not calculated #	30	19	63	25	**83**

* 0.1 mL acetone/L test media
\# Highest test level (nominal concentration: 640 ng p.m./L) not considered for statistical evaluation, due to uncertainty regarding actual exposure concentration.

No immobility or other effects on behaviour were observed in the untreated control and the solvent control within 48 hours of exposure.

EC_{50} values for *Daphnia magna* exposed to trans-isomer of deltamethrin (AE 0035073):

Test substance:	trans-isomer of deltamethrin (AE 0035073)
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):	>93.6 (n.d.) ng p.m./L (mean measured)
EC_{50} 48 h (95% C.I.):	**69 (51-92) ng p.m./L (mean measured)**

Conclusions:
Based on mean measured concentrations of the trans-isomer of deltamethrin (AE 0035073), the 48-hour EC_{50} value for immobilisation was determined to be 69 ng pure metabolite/L in a static test system.

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Metabolite 4'OH-deltamethrin

Report:	**KCA 8.2.4.1/06, [redacted]; 2013**
Title:	Acute toxicity of BCS-BY84407 to the waterflea Daphnia magna in a static renewal laboratory test system
Document No:	M-465317-01-1 (Rep. No: EBDAL031)
Guidelines:	OECD guideline 202,(2004); EC Council Regulation No 440/2008, Method C.2 (2008); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).
GLP:	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 renewal laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: BCS-BY84407 (4'-OH-Deltamethrin), batch SES-12072-8-11, purity: 96.5% w/w (TOX09494-00)
Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) exposed in a static-renewal test system for 48 (2 x 24) hours to nominal concentrations of 0, 0.3, 0.6, 1.2, 2.4 and 4.8 µg pure metabolite/L (corresponding to mean-measured concentrations of 0, 0.18, 0.39, 0.73, 1.55 and 3.13 µg pure metabolite/L) and a solvent control (0.1 mL acetone/L) without feeding.
The content of BCS-BY84407 in exposure media was measured for verification of the test item concentrations at the start and end of each renewal 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 sub-lethal affected daphnids had to be recorded.

Findings:
Analytical results:
The analytically determined amounts of BCS-BY84407 in the freshly prepared test solutions at start of each renewal interval revealed recoveries between 93% and 108% (mean: 101%) of the nominal concentrations. The corresponding concentrations of the aged test solutions at the end of each 24-hour exposure period ranged between 20% and 35% (mean: 26 %) of nominal. Therefore, the biological results were based on mean measured concentrations.

No contaminations of BCS-BY84407 were detected in samples from the untreated water and solvent control.

Biological results:

Toxicity of BCS-BY84407 to *Daphnia magna*:

Mean measured test concentration [mg p.m./L]	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	%	n	%
Control	30	0	0	0	**0**
Solvent control *	30	0	0	0	**0**
0.18	30	0	0	2	**7**
0.39	30	1	3	13	**43**
0.73	30	2	7	16	**53**
1.55	30	9	30	22	**73**
3.13	30	17	57	27	**90**

* 0.1 mL acetone/L test media

No immobility or other effects on behaviour were observed in the untreated control and the solvent control within 48 hours of exposure.

EC_{50} values for *Daphnia magna* exposed to BCS-BY84407 based on mean measured concentrations

Test substance:	BCS-BY84407 (4'-OH-Deltamethrin)
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):	2.65 (1.90-3.68) mg p.m./L (mean measured)
EC_{50} 48 h (95% C.I.):	**0.67 (0.51-0.87) mg p.m./L (mean measured)**

Conclusion:
Based on mean measured concentrations of BCS-BY84407, the 48-hour EC_{50} value for immobilisation was determined to be 0.67 mg pure metabolite/L in a static-renewal system.

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Metabolite Br_2CA

Report:	**KCA 8.2.4.1/07, [redacted]; 2001**
Title:	Acute toxicity to *Daphnia magna* (Waterflea) AE F108565; substance, pure (Metabolite of Deltamethrin)
Document No:	M-199793-01-2 (Rep. No. C010889)
Guidelines:	OECD guideline 202 (2004); EC Council Regulation No 440/2008, Method C.2 (2008); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).
GLP:	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 renewal laboratory test system, expressed as EC_{50} for immobilisation.

Materials and methods:
Test item: AE F108565 (Br_2CA, metabolite of deltamethrin), purity: 98.8% w/w; specified by batch

code: AE F108565 00 1B99 0001 (AZ 08085).
Daphnia magna (1st instars < 24 h old, 2 x 10 animals per concentration), exposed in a static test system for 48 hours to nominal concentrations of 0, 1.0, 1.8, 3.2, 5.6, 10, 18, 56 and 100 mg test item/L without feeding. Chemical analysis of the freshly prepared and aged (48 hours old) test solutions was performed for the active ingredient AE F108565 using HPLC/UV.
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.

Results:
Analytical results:
Analyses of freshly prepared water for AE F108565 resulted in test item concentrations ranging from 81.1% to 114.1% of nominal values. Analyses of aged water (48 h) for AE F108565 at experimental termination resulted in test substance concentrations ranging from 68.6% to 136.9% of nominal values. The mean measured values over the time of exposure ranged from 75.1% to 115.7%. With the exception of the 48 h analyses from 1.0 and 1.8 mg/L all analysed concentrations of AE F108565 were within+ 20% of nominal at the start and the end of the study, all effect concentrations were based on nominal initial test concentrations.

Biological results:
No mortality and no intoxication symptoms were observed in any treatment level or the untreated control. The NOEC over 48 hours was 100 mg test item/L.

EC_{50} values for *Daphnia magna* exposed to AE F108565 (Br_2CA) based on nominal concentrations

Test substance:	AE F108565 (Br_2CA, metabolite of deltamethrin)
Test object:	*Daphnia magna*
Exposure:	48 hours, static test design (dose-response)
EC_{50} 24 h:	>100 mg test item/L (nominal)
EC_{50} 48 h:	**>100 mg test item/L (nominal)**

Conclusion:
Based on nominal concentrations of AE F108565 (Br_2CA), the 48-hour EC_{50} value for immobilisation was determined to be >100 mg test item/L in a static system.

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Metabolite Serinyl-BrCA

Report:	**KCA 8.2.4.1/08, [redacted]; 2013**
Title:	Acute toxicity of BCS-CW57835 to the waterflea Daphnia magna in a static renewal laboratory test system
Document No:	M-465372-01-1 (Rep. No: EBDAN001)
Guidelines:	None
GLP:	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 renewal laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: BCS-CW57835 (Serinyl-BrCA), batch BCS-CW57835-01-00, purity: 93.8% w/w (TOX09495-00)
Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration), exposed in a static renewal test system for 48 (2 x 24) hours to nominal concentrations of 0, 6.25, 12.5, 25, 50 and 100 mg pure metabolite (p.m.)/L (corresponding to mean-measured concentrations of 0, 6.52, 11.4, 26.0, 52.8 and 103 mg p.m./L) without feeding.
The content of BCS-CW57835 (Serinyl-BrCA) in exposure media was measured for verification of the test item concentrations at the start and end of each renewal 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 sub-lethal affected daphnids had to be recorded.

Results:
Analytical results
The analytically determined amounts of BCS-CW57835(Serinyl-BrCA) in the freshly prepared test solutions at the start of each renewal interval revealed recoveries between 76% and 105% (mean: 101%) of nominal concentrations. The corresponding concentrations of the aged test solutions at the end of each 24-hour exposure period ranged from 78% to 108% (mean: 103%) of nominal. Therefore, the biological results were based on mean measured concentrations.

No contaminations of BCS-CW57835 (Serinyl-BrCA) were detected in samples from the untreated water control.

Biological results

Toxicity of BCS-CW57835 (Serinyl-BrCA) to *Daphnia magna*:

Mean measured test concentration [mg p.m./L]	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	%	n	%
Control	30	0	0	0	**0**
6.52	30	0	0	1	**3.3**
11.4	30	0	0	5	**16.7**
26.0	30	4	13.3	12	**40.0**
52.8	30	7	23.3	17	**56.7**
103	30	17	56.7	27	**90.0**

No immobility or other effects on behaviour were observed in the untreated control within 48 hours of exposure.

EC_{50} values for *Daphnia magna* exposed to BCS-CW57835 (Serinyl-BrCA) based on mean measured concentrations

Test substance:	BCS-CW57835 (Serinyl-BrCA)
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):	90.2 (65.3-125) mg p.m./L (mean measured)
EC_{50} 48 h (95% C.I.):	**35.3 (27.6-45.0) mg p.m./L (mean measured)**

Conclusion:
Based on mean measured concentrations of BCS-CW57835 (Serinyl-BrCA), the 48-hour EC_{50} value for immobilisation was determined to be 35.3 mg pure metabolite/L in a static-renewal test system.

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Metabolite mPBaldehyde

Report:	**KCA 8.2.4.1/09, [redacted]; 2010**
Title:	Daphnia sp., Acute Immobilisation Test with Cyfluthrin-m-phenoxybenzaldehyde (AE F114152)
Document No:	M-386854-01-1 (Rep. No: 2010/0064/01)
Guidelines:	OECD TG 202 (2004) EU method C.2 (2008)
GLP:	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 renewal laboratory test system, expressed as EC_{50} for immobilisation.

Materials and methods:
Test item: Cyfluthrin-m-phenoxybenzaldehyde (AE F114152, mPBaldehyde), batch no. ABKBMPI125, purity: 99.5% w/w
Daphnia magna (1st instars < 24 h old, 2 x 10 animals per concentration), exposed in a static renewal test system for 48 (2 x 24) hours to nominal concentrations of 0, 0.09, 0.19, 0.41, 0.91 and 2 mg test item/L (corresponding to mean-measured concentrations of 0, 0.044, 0.077, 0.111, 0.170 and 0.865 mg pure metabolite (p.m.)/L after 24 h and 0.045, 0.078, 0.136, 0.237 and 1.116 mg p.m./L after 48 h, respectively) without feeding.
Chemical analysis of the freshly prepared and aged (24 hours old) test solutions was performed for the metabolite AE F114152 (mPBaldehyde) using HPLC/UV.
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.

Results:
Analytical results:
Analyses of freshly prepared water for AE F114152 resulted in test item concentrations ranging from 94.6% to 107.8% of nominal values. Analyses of aged water (24 h) for AE F114152 at experimental

termination resulted in test substance concentrations ranging from 12.7% to 51.5% of nominal values. Therefore, the biological endpoints were based on mean measured concentrations.

Biological results:

Toxicity of AE F114152 (mPBaldehyde) to *Daphnia magna*:

Nominal test concentration [mg test item/L]	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	%	n	%
Control	20	0	0	0	0
0.09	20	0	0	0	**0**
0.19	20	0	0	0	**0**
0.41	20	0	0	7	**35**
0.91	20	2	10	17	**85**
2.0	20	17	85	20	**100**

No immobility or other effects on behaviour were observed in the untreated control within 48 hours of exposure.

EC_{50} values for *Daphnia magna* exposed to AE F114152 (mPBaldehyde) based on mean measured concentrations

Test substance:	Cyfluthrin-m-phenoxybenzaldehyde (AE F114152)
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):	0.437 (0.321-0.628) mg p.m./L (mean measured)
EC_{50} 48 h (95% C.I.):	**0.162 (0.139-0.190) mg p.m./L (mean measured)**

Conclusion:
Based on mean measured concentrations of AE F114152 (mPBaldehyde), the 48-hour EC_{50} value for immobilisation was determined to be 0.162 mg pure metabolite/L in a static-renewal test system.

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Metabolite mPBacid

Report:	**KCA 8.2.4.1/10 [redacted]; 1983**
Title:	3-Phenoxybenzoic acid: Toxicity to first instar Daphnia magna (II)
Document No: Letter of Access:	RJ0318B M-479954-01-1
Guidelines:	Based on EPA (Reference 14)
GLP:	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.

Material and methods:
Test item: 3-phenoxybenzoic acid, purity: 99% w/w.
Daphnia magna (1st instars < 24 h old, 3 × 10 animals per concentration), exposed in a static test-

system for 48 hours in two test-systems (test I, test II) to nominal concentrations of 0, 25, 50, 100, 200 and 400 mg test item/L (corresponding to mean-measured concentrations of: Test I: <1.0, 24.0, 52.2, 104, 220 and 425 mg test item/L; Test II: <1.0, 25.2, 50.7, 104, 216 and 404 mg test item/L) without feeding.
The content of 3-phenoxybenzoic acid in exposure media was measured for verification of the test item concentrations at the start and end of the test by HPLC. To check if the 3-Phenoxybenzoic acid was in solution, samples of the higher concentrations (Test I: 400 and 200 mg/L, Test II: 400 and 100 mg/L) were passed sequentially through filters under vacuum.

Findings:

Analytical results:

Analyses of test item concentrations at test initiation showed recoveries between 95.6% to 108.0% of nominal values. Analyses of aged test solutions (48 h) at experimental termination resulted in test item concentrations ranging from 96.8% to 108.0% of nominal values. The biological results were based on mean measured concentrations.
Additional analysis of the series of filtered solutions of the two highest test concentrations in both tests indicated that > 85% of the 3-Phenoxybenzoic acid was in solution.

Biological results:

Toxicity of 3-Phenoxybenzoic acid to *Daphnia magna* in Test I:

Test I 6th April 1983						
Nominal test concentration [mg test item/L]	Mean measured test concentration [mg test item/L]	Exposed daphnids	Number of immobilised daphnids			
			24 h		48 h	
			n	%	n	%
Control	<1.0	30	0	0	0	0
25	24.0	30	0	0	0	0
50	52.2	30	0	0	0	0
100	104	30	4	13	21	70
200	220	30	25	83	30	100
400	425	30	30	100	30	100

Toxicity of 3-Phenoxybenzoic acid to *Daphnia magna* in Test II:

Test II 12th April 1983						
Nominal test concentration [mg test item/L]	Mean measured test concentration [mg test item/L]	Exposed daphnids	Number of immobilised daphnids			
			24 h		48 h	
			n	%	n	
Control	<1.0	30	0	0	0	0
25	25.2	30	0	0	0	0
50	50.7	30	0	0	0	0
100	104	30	5	17	21	70
200	216	30	30	100	30	100
400	404	30	30	100	30	100

No immobility or other effects on behaviourwere observed in the untreated control within 48 hours of exposure in Test I and Test II.

EC_{50} values for *Daphnia magna* exposed to 3-phenoxybenzoic acid based on mean measured concentrations

Test substance:	3-Phenoxybenzoic acid
Test object:	*Daphnia magna*
Exposure:	48 hours, static test design (dose-response)
EC_{50} 24 h (95% C.I.) – Test I:	155 (133-181) mg test item/L
EC_{50} 24 h (95% C.I.) – Test II:	139 (104-216) mg test item/L
EC_{50} 48 h (95% C.I.) – Test I:	85 (52-104) mg test item/L
EC_{50} 48 h (95% C.I.) – Test II:	85 (51-104) mg test item/L
EC_{50} 48 h (95% C.I.) – Mean of Test I & II:	**85 mg test item/L**

Conclusions:
Based on mean measured concentrations of 3-phenoxybenzoic acid, the 48-hour EC_{50} value for immobilisation was determined to be 85 mg test item/L in a static system.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Studies with additional aquatic invertebrate species were conducted for registrations outside the EU. The summary of an acute study with the freshwater amphipod *Hyalella azteca* in a water only system is provided below. An acute study with the marine species *Americamysis bahia* (formerly: *Mysidopsis bahia*, mysid shrimp) from 1991 was not evaluated during the first EU Review of deltamethrin, and is therefore summarized in this Supplemental Document.
In addition, studies on aquatic invertebrate species found in the public literature are summarized under this point. As none of the endpoints from these publications is considered relevant for the risk assessment, they are classified as supporting information only.

Table 8.2.4.2- 1: Additional studies for acute toxicity of deltamethrin to additional invertebrate species

Test substance	Test species	Endpoint	Reference
Deltamethrin	Invertebrate, acute *Hyalella azteca*	LC_{50} 0.17 ng a.s./L (mm)	[redacted] **(2013) M-461147-01-1**
Deltamethrin	Invertebrate, acute *Americamysis bahia*	LC_{50} 3.7 ng a.s./L (mm)	[redacted] **(1991) M-149478-01-1**

mm = mean measured

Report:	**KCA 8.2.4.2/01, [redacted]; 2013**
Title:	Deltamethrin – Acute Toxicity to Freshwater Amphipods (Hyalella Azteca) Under Flow-Through Conditions
Document No:	M-461147-01-1
Guidelines:	OCSPP Draft Guideline 850.1020
GLP:	yes

Objective:
A 96-hour flow-through test was conducted to determine the acute toxicity of deltamethrin to the freshwater amphipod, *Hyalella azteca*. The primary measure of acute toxicity was mortality. Results of the test are expressed as 96-hour median lethal concentration (LC_{50}) defined as the concentration of

deltamethrin estimated to be lethal to 50 percent of the test population at the specified time.

Material and methods:

Test item: Deltamethrin technical, batch no. EGDLTK113, purity: 99.52% w/w

Hyalella azteca, SMV Lot No. 040913, 9 day old at test initiation; source: Smithers Viscient cultures.

Organisms were exposed in a flow-through test system for 96 hours to nominal concentrations of 0, 0.1, 0.2, 0.4, 0.8 and 1.6 ng a.s./L (corresponding to mean-measured concentrations of 0, 0.061, 0.13, 0.28, 0.51, and 1.2 ng a.s./L) and a solvent control (0.05 mL acetone/L). The diluter delivered the control, solvent control and test solutions to the test vessels at a rate sufficient to provide approximately 10 test vessel volumes per 24-hour period, with a 90% replacement time of approximately 5 hours. Two replicate vessels (2 L glass beakers) per concentration and control contained 10 individuals each. The content of deltamethrin in exposure media was measured for verification of the test item concentrations at the start and end of the study.

The number of dead Hyalella was recorded at test initiation and after 24, 28, 72 and 96 hours of exposure. Death was determined by gently agitating the test solution around those amphipods that appeared to be immobile. If upon further inspection there was no observed movement, the Hyalella were considered dead. Biological observations and observations of the physical characteristics if each replicate test solution were also recorded.

Results:

Analytical results:

Measured concentrations at test initiation ranged from 34% to 69% of nominal values, and from 68-88% at test termination, respectively. Mean measured concentrations ranged from 61% to 75%. Biological endpoints are based on mean measured concentration.

No contaminations of deltamethrin were detected in samples from the untreated water and solvent control.

Biological results:

Toxicity of deltamethrin to *Hyalella azteca:*

Mean measured test concentration [ng a.s./L]	Exposed individuals (=100%)	Cumulative mortality							
		24 h		48 h		72 h		96 h	
		n	%	n	%	n	%	n	%
Control	20	0	0	0	0	0	0	0	0
Solvent control	20	0	0	0	0	0	0	0	0
0.061	20	0	0	0	0	0	0	0	0
0.13	20	3	15	4	20	4	20	7	35
0.28	20	5	25	9	45	12	60	16	80
0.51	20	4	20	5	25	12	60	19	95
1.2	20	3	15	8	40	11	55	17	85

No immobility or other effects on behaviour were observed in the untreated control and solvent control within 96 hours of exposure.

LC_{50} values for *Hyalella azteca* exposed to deltamethrin based on mean measured concentrations

Test substance:	Deltamethrin techn.
Test object:	*Hyalella azteca*
Exposure:	96 hours, flow-through test design (dose-response)
LC_{50} 96 h (95% C.I.):	**0.17 (0.13-0.22) ng a.s./L (mean measured)**

Conclusion:
Based on mean measured concentrations, the 96-hours LC_{50} value for *Hyalella azteca* exposed to deltamethrin was determined to be 0.17 ng a.s./L.

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Report:	**KCA 8.2.4.2/02, [redacted] 1991**
Title:	(Deltamethrin) – Acute toxicity to Mysid Shrimp (*Mysidopsis bahia*) under static renewal conditions
Document No:	M-149478-01-1 (Rep No: 91-7-3826)
Guidelines:	Ecological Effects Data Requirements 40 CFR 158.145, Guidelines Reference Number 72-3 (c)
GLP:	yes

Objective:
A 96-hour static-renewal test was conducted to determine the acute toxicity of deltamethrin to the saltwater mysid, *Americamysis bahia* (formerly: *Mysidopsis bahia*).

Materials and methods:
Test item: ^{14}C-Deltamethrin, Lot No. X595A (>95% radiopurity).
Juvenile *Americamysis bahia* (≤24 hours old) were exposed to nominal concentrations of 0, 0.78, 1.3, 2.2, 3.7, 6.0 and 10 ng/L for 96 hours in a static renewal test design. Natural filtered seawater (collected from [redacted] Massachusetts) was used as dilution water. Ten mysid shrimp were exposed in each replicate test vessel (20 per concentration and control). Test solutions were renewed following 24-, 48- and 72-hours of exposure. Mysids that were observed to be dead at the time of renewal were not transferred into the new test solutions. Mysids were fed with live brine shrimp nauplii once daily. All aquaria were examined after 24, 48, 72 and 96 hours of exposure as follows: mortalities were recorded, dead organisms were removed, and observations of the live mysid shrimp and the physical characteristics of the test solutions were recorded.

Results:
Analytical results:
Throughout the exposure period no visible sign of undissolved material was observed in any of the treatment levels.
The mean measured concentrations ranged from 32% to 60% of nominal values, with an average of 44% of nominal. The biological endpoints were based on the following mean measured concentrations: 0.25, 0.55, 0.78, 1.6, 3.6 and 4.9 ng a.s./L.

Biological results:

Toxicity of deltamethrin to *Americamysis bahia:*

Mean measured test concentration [ng a.s./L]	Exposed individuals (=100%)	Cumulative mortality							
		24 h		48 h		72 h		96 h	
		n	%	n	%	n	%	n	%
Control	20	0	0	0	0	1	5	1	5
Solvent control	20	2	10	2	10	2	10	2	10
0.25	20	1	5	2	10	2	10	2	10
0.55	20	0	0	0	0	0	0	0	0
0.78	20	2	10	4	20	5	25	5	25
1.6	20	2	10	4	20	4	20	4	20
3.6	20	1	5	4	15	7	35	9	45
4.9	20	3	15	10	50	20	100	20	100

The level of mortality in the control solutions (5-10%) was in accordance with the acceptability criterion outlined in the Standard Evaluation Procedured by EPA.
Sublethal effects (e.g., lethargy, erratic swimming behaviour) were observed among all of the surviving mysids, exposed to the 3.6, 1.6 and 0.78 ng/L test concentrations. No toxicant-related mortality or adverse effects were observed among mysids exposed to the two lowest test concentrations (0.25 and 0.55 ng a.s./L).

LC_{50} values for *Americamysis bahia* exposed to deltamethrin based on mean measured concentrations

Test substance:	Deltamethrin techn.
Test object:	*Americamysis bahia* (syn. *Mysidopsis bahia*)
Exposure:	96 hours, static renewal test design (dose-response)
LC_{50} 96 h (95% C.I.):	**3.7 (1.6-4.9) ng a.s./L (mean measured)**

Conclusion:
Based on the mean measured concentrations of deltamethrin, the 96-hour LC_{50} value for the saltwater mysid *Americamysis bahia* was estimated by nonlinear interpolation to be 3.7 ng a.s./L.

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Results from literature review

Report:	**KCA 8.2.4.2/03; [redacted]; 2012**
Title:	Acute toxic effects of deltamethrin on red swamp crayfish, Procambarus clarkii (Decapoda, Cambaridae)
Source:	Comparative Biochemistry and Physiology, Part C, 157 (2013) 280–286
DOI No:	10.1016/j.cbpc.2013.01.001
Document No:	M-462626-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY (Abstract from publication)

To investigate effects of deltamethrin on red swamp crayfish, *Procambarus clarkii*, an acute toxicity test was carried out. The results showed that the 24, 48 and 96 h LC_{50} values were 0.156, 0.099 and 0.056 µg/L,

respectively. The abnormal behavioral responses and toxic signs can be described as typical neurotoxic symptoms, characterized by ataxia, hyperexcitation, convulsion, and paralysis. The residue of deltamethrin in haemolymph, gill, muscle, and liver of the crayfish was under the limit of quantification of the GC-MS method after a 96-h exposure to 0.05 µg deltamethrin/L and a 24-h exposure to 0.1 µg deltamethrin/L. Besides that, the sublethal effects caused were assessed by using cytochrome c oxidase (CCO) activity, lactase dehydrogenase (LDH) activity, and lactic acid levels as sensitive biomarkers.
Results showed that 24 h exposure to 0.1 µg deltamethrin/L significantly inhibited the CCO activity (P <0.05), but increased LDH activity (P < 0.05) and the lactic acid level (P < 0.05) in gills, which further indicated that the aerobic metabolism was inhibited by deltamethrin in the gill during the anaerobic metabolism was stimulated.

The following summary is limited to the acute toxicity assessment of the publication. Evaluation of residues and enzyme analysis were not considered reliable or relevant for the aquatic risk assessment.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Technical grade deltamethrin
Active substance(s):	Deltamethrin
Chemical state and description:	Not specified
Source of test item:	Shanghai [redacted], Original Producer: India
Batch number:	Not specified
Purity:	98%
Storage conditions:	Not specified
Water solubility:	Not specified

2. Test organism(s)

Species:	*Procambarus clarkii*
Common name:	Red swamp crayfish
Gender:	Adult males
Mass:	Mean wet weight: 19.4 ± 2.0 g
Length:	Mean length: 8.20 ± 0.28 cm
Source of test species:	Collected from [redacted] Shanghai [redacted] Shanghai, P.R. China).

3. Breeding of test organism(s)

Housing conditions:	Acclimated to laboratory conditions for at least 1 week in plastic tanks (capacity 300 L) and fed with wild small fish every day. Water was changed daily. The mean concentration of water dissolved oxygen was kept at 7.3 ± 0.2 mg/L and mean pH was 8.6 ± 0.1. Feeding was discontinued for 24 h before the tests.
Temperature:	25°C
Photoperiod:	Natural light-dark cycle
Observations:	Mortality during this period was negligible.

B. Study design and methods

1. Test procedure

Test system:	96 h static-renewal exposure test in glass aquaria with 20 L test solution
Test concentrations:	0.1, 0.126, 0.158, 0.2, 0.25 µg/L (24 h); 0.1, 0.119, 0.141, 0.168, 0.2 µg/L (48 h); and 0.025, 0.040, 0.063, 0.100, 0.158 µg/L (96 h), all nominal values

Solvent:	Deltamethrin was dissolved in dimethylbenzene by using pesticide emulsifier 2201 (Jiangsu Zhongshan Chemical Co., P.R. China) for testing.
Control(s):	Water control group and solvent control group
Number of replicates:	Ten fish per tank, two replicate tanks per treatment
Test medium:	Not specified
Medium change intervals:	Every 24 h
Test conditions:	Culture conditions were consistent with the previous conditions. Crayfish were not fed during the experiments. Dead animals were removed immediately.
Measurements:	Number of dead animals (= absence of movement within 5 min after animals were gently probed with a glass rod)
Statistics:	LC_{50} values were calculated by the Linear Regression analysis method.

2. Chemical analysis

No chemical analysis conducted in exposure media. However, as the test media were renewed daily, reporting of endpoints based on nominal concentrations is considered acceptable.

RESULTS

During the acute toxic test, no mortality occurred in the water control group and the solvent control group.

The acute toxicity of deltamethrin to *P. clarkii* was time dependent and showed a clear dose-dependent response. The LC_{50} values are listed in the table below.

Regression equations, LC_{50} values and the 95% confidence limits of deltamethrin to *Procambarus clarkii* (Girard) at 24 h, 48 h and 96 h

Time (h)	Regression equation	R^2	LC_{50} (µg/L)	95% confidence limits (µg/L)
24	P = 11.085 + 7.55C	0.936	0.156	0.141-0.170
48	P = 11.420 + 6.4C	0.986	0.099	0.089-0.111
96	P = 9.710 + 3.765C	0.948	**0.056**	0.047-0.067

In the regression equation, the symbol P is the probability unit of mortality and C the logarithm of concentration of deltamethrin. R is the regression coefficient. The equations were determined using SPSS 13.0 software, and the LC_{50} value was calculated from each equation.

CONLCUSION

Exposure of the red swamp crayfish *Procambarus clarkii* to deltamethrin under static-renewal conditions resulted in a 96-h LC_{50} value of 0.056 µg/L based on nominal concentrations.

Comment by the Notifier:

The study is considered supplementary information only, as GLP studies are available to address this data point (acute toxicity to invertebrates). Reliability of the published data is limited due to missing analytical verification. However, test media were renewed every daily, so a continuos exposure of organisms can be assumed in this 96-hour study.

Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	**KCA 8.2.4.2/04; [redacted], M.; 2012**
Title:	Comparative study on the toxicity of pyrethroids, α-cypermethrin and deltamethrin to Ceriodaphnia dubia
Source:	Ecotoxicology andEnvironmentalSafety78 (2012) 9–13
DOI No: Document No:	10.1016/j.ecoenv.2011.07.018 M-462170-01-1
Guidelines:	Chronic toxicity test protocol was based on the USEPA Test Method 1002.0 for survival and reproduction using Ceriodaphnia dubia (USEPA, 2002b).
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY (Abstract from publication)

Two synthetic pyrethroids pesticides, α-cypermethrin and deltamethrinwere investigated as potential toxic contaminants. The acute and chronic bioassays were conducted using *Ceriodaphnia dubia*. The toxicity of α-cypermethrin an ddeltamethrin to *C. dubia* increased with increasing concentrations and exposure time. *C. dubia* was three times more sensitive to deltamethrin than to α –cypermethrin with 48-h EC_{50} of 0.06 µg/L and0.23 µg/L, respectively. The chronic EC_{50} values for α –cypermethrin and deltamethrin were 97.8 and 34.7 ng/L, respectively. Eight-day growth of *Ceriodaphnia* neonates during chronic exposures was the most sensitive endpoint measured in comparison to the endpoints of survival and number of neonates produced. To gain a better understanding of the link between acute and chronic toxicity,the acute-to chronic ratios (ACRs) were also calculated for survival, growth and reproduction endpoints. ACRs varied between11 and 224 for the two pyrethroids. These results suggest that at environmentally relevant low concentrations, α –cypermethrin and deltamethrin could have significant adverse effects on the survival, reproduction and growth of C. dubia.

The following summary is limited to the data reported for deltamethrin.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	deltamethrin
Active substance(s):	52918-63-5
Chemical state and description:	Not reported
Source of test item:	[redacted]
Batch number:	Not reported
Purity:	>99.8%
Storage conditions:	Not reported
Water solubility:	<0.0002 mg/L (25°C)

2. Test solutions

Vehicle/solvent:	acetone
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	The highest acetone concentration in the exposure vials was less than 0.05% in acute tests; 20 µL/L in chronic tests
Method of preparation:	Stock solutions at concentrations of 100 mg/L and 1000 mg/L in acetone (purity ≥99.5%) were sealed in 20mL scintillation vials and stored in the dark at 4 °C for preparing working stock solutions and test solutions, respectively.

3. Test organism(s)

Species:	*Ceriodaphnia dubia*
Common name:	cladoceran
Source of test species:	[redacted] laboratories

4. Culture conditions of test organism(s)

Culture medium:	Formulated water (hardness 80-100 mg/L as $CaCO_3$) according to the guidelines recommended by the US EPA. This moderately hard water was enriched with 2 µg/L selenium (as Na_2SeO_4). The culture water was replaced three times per week.
Temperature:	24 ± 1°C
Photoperiod:	16:8 light/dark
Light intensity:	Fluorescent lamp
pH:	Not reported
Oxygen saturation:	Not reported
Food and feeding regime:	The culture was fed a tri-algal mix consisting of *Ankistrodesmus* sp., *Chlamydomonas* sp. and *Pseudokirchneriella subcapitata* and a mixture of yeast, cereal leaves and trout chow (YCT). The culture was fed three times per week.
Acclimatisation prior to testing:	In-house cultures
Observations during acclimatisation:	none

B. Study design and methods

1. Test procedure – **Acute toxicity test**

Test system:	Laboratory test
Test concentration(s):	0.0.05,0.1,0.25,0.5,1,2.5 and 5µg/L (nominal)
Control(s):	Water control, solvent control (acetone, max. concentration less than 0.05%), and $CuSO_4$ as reference toxicant (tested in a range from 5 to 20 µg/L)
Number of replicates:	Four replicates, each with neonates
Test conditions:	Tests were conducted in 20 mL glass scintillation vials containing 18 mL of test solution. Each vial was sealed with a lid and shaken vigorously to obtain a homogeneous mixture.
Feeding:	no feed was provided
Medium renewal:	Static renewal (renewal after 24 h)
Frequency of test item application:	At test initiation and via renewed media after 24 h
Test duration:	48 hours
Endpoints:	Immobilization, defined as the failure to move within 15 s of the beaker being gently swirled
Statistics:	LC50 values with 95% confidence limits ($p<0.05$) were calculated by the Trimmed Spearman-Karber (TSK) analysis for lethal tests.

2. Measurements during the test

Water/medium parameters:	Water quality parameters were measured before and after the 24-hour renewal of the test solutions. Physico-chemical parameters including temperature (between 23 and 25 °C), electrical conductivity (between 248 and 260 mS/cm), dissolved oxygen (between 5.50 and 5.90 mg/L) and pH value (between 8.0 and 8.1) were recorded during the tests. Results of these water quality parameters are all acceptable with regard to

the US EPA guidelines.

3. Sampling

Sampling frequency: n.a.

Transport/storage of samples: n.a.

4. Chemical analysis

Guideline/protocol: Not reported

Method: Gas chromatograph (Agilent6890N), equipped with a split and splitless injector and µ-ECD detector. A 30 m x 0.25 mm x 0.25 µm HP-5MS fused silica capillary column was used.

Pre-treatment of samples: The spiked solution (20 mL) was extracted with 20 mL petroleum ether two times and rotary evaporated to 1 mL under a water bath at 45°C. After evaporation 1 µL of each sample was injected into a gas chromatograph.

Conduction: Chemical analysis was conducted in separate spiked samples (not exposure media) of 0.5 and 1.0 µg/L in Milli-Q water (pH 7) and test water (pH 8), respectively. The spiked solutions were analyzed by gas chromatography after 0, 4, 12 and 24 h

Reference item: Not reported

Recovery: approximately 50% of the nominal concentrations

Limit of detection: Not reported

Limit of quantification: Not reported

1. Test procedure – **Chronic toxicity test**

Test system: Laboratory test

The test was based on the US EPA Test Method 1002.0 for survival and reproduction using *Ceriodaphnia dubia*

Test concentration(s): 0, 2.5, 5, 10, 25, 50, 100, 200 ng/L (nominal)

Control(s): Water control, solvent control (acetone, 20 µg/L), and $CuSO_4$ as reference toxicant

Number of replicates: 10 replicates of individually kept new born *C. dubia*

Test conditions: 200 mL beaker with 100 mL Se-enriched moderately hard water, covered with plastic film.

Feeding: The organisms were fed with YCT 200µL and 500 µL Tri-algal mix (7.6x10^7 cells) after each water change

Medium renewal: daily

Frequency of test item application: Daily via media renewal

Test duration: 8 days

Endpoints: Survival and number of neonates produced were recorded daily. Test endpoints measured were survival, reproduction and growth of the adults.

Statistics: Data from the control and experimental groups were analysed by one-way ANOVA in conjunction with Tukey test. NOEC and LOEC values were determined by Tukey test for multiple comparisons with Toxstat software version 3.5 (Gulley and West Inc.). Statistical difference was accepted at $p<0.05$.

2. Measurements during the test

Water/medium parameters: Water quality parameters were measured in the beginning, middle and end of the test.

Results see above (acute test)

3. Sampling

Sampling frequency:	Survival and number of neonates produced were recorded daily. Adults were preserved in ethanol at study termination for length measurements
Transport/storage of samples:	preserved in 70% ethanol

4. Chemical analysis

See above (acute test)

RESULTS

1. Analytical findings:

The loss of deltamethrin form the test water at different time intervals is shown in Table 6 of the publication. After 24 h, the measured concentration of deltamethrin was approximately 50% of the nominal concentrations. The results showed that the degradation of deltamethrin in the test water (pH 8) was not significantly different from the spiked Milli-Q water samples (pH value of 7.0):

Table 6
Measured concentrations of deltamethrin spiked solutions at different time intervals.

Spiked solutions	0-h	4-h	12-h	24-h
A	0.43	0.42	0.37	0.23
B	0.85	0.86	0.70	0.45
C	0.44	0.41	0.33	0.21
D	0.86	0.81	0.67	0.4

A: spike 0.5 ng/L deltamethrin in the Mill-Q water (pH = 7.0).
B: spike 1.0 μg/L deltamethrin in the Mill-Q water (pH = 7.0).
C: spike 0.5 μg/L deltamethrin in the test water (pH = 8.0).
D: spike 1.0 μg/L deltamethrin in the test water (pH = 8.0).

2. Biological findings:

Acute toxicity test:

The calculated LC_{50} values incl. 95% confidence intervals are summarized in the table below. The mortality in the controls and the solvent controls was <10% and the 24-h LC_{50} for the $CuSO_4$ reference toxicant was between 3.5 and 5.8 µg/L which is within the normal range for this test.

LC_{50} values (µg/L) during acute exposure of the pyrethroid pesticide deltamethrin to *Ceriodaphnia dubia:*

24h LC_{50} (95% confidence interval)	0.84 (0.42-16.8) µg/L
48h LC_{50} (95% confidence interval)	**0.06 (0.04-0.10) µg/L**

Chronic toxicity test:

Survival: The mortality in the controls was <10%. The test organisms exposed to deltamethrin survived (>80%) up to a test concentration of 50 ng/L. Higher test concentrations induced higher mortality compared to control ($p<0.05$). Based upon these analyses, the NOEC and LOEC values for survival were 50 and 100 ng/L, respectively.

Reproduction: For the reproductive effect, the time to first brood and the number of young per female were examined. Deltamethrin had a significant effect on the time to the first brood at concentrations from 25 to 200 ng/L. The number of offspring was reduced in the higher concentrations. Based upon this analysis, the NOEC and LOEC values for reproduction were 10 and 25 ng/L, respectively.

Growth: For the growth effects, the length of adults was examined. The length of organisms was reduced at higher concentrations of deltamethrin.
Results from chronic testing are summarized in Table 4 of the publication:

Table 4
Survival, reproduction and growth of *Ceriodaphnia dubia* exposed to different concentrations of deltamethrin during 8-d chronic toxicity test. Each treatment comprised of ten replicates with one neonate per replicate.

Concentration (ng/L)	Adult survival (%)	Time to first brood (mean ± S.D., days)	No. of young/ adult[a] (mean ± S.D.)	Adult length (mean ± S.D., µm)
0	90	4.2 ± 0.5	17.0 ± 2.8	934 ± 20
2.5	100	4.1 ± 0.4	16.5 ± 3.5	921 ± 20
5	100	4.0 ± 0.0	14.8 ± 2.2	878 ± 28*
10	100	4.1 ± 0.3	16.4 ± 4.5	861 ± 28*
25	90	4.9 ± 0.6*	7.0 ± 2.4*	794 ± 9*
50	80	4.9 ± 0.7*	8.4 ± 4.5*	773 ± 23*
100	60*	5.5 ± 0.6*	3.8 ± 2.3*	683 ± 22*
200	20*	0	0*	539 ± 11*

[a] No. of young is based on 10 organisms, regardless of adult mortality.
* Indicates a significant difference compared to control ($p < 0.05$).

Summary of effect concentrations for deltamethrin based on 8-day chronic exposures to *Ceriodaphnia dubia*:

Survival	EC_{10} NOEC LOEC	41.9 ng/L 50 ng/L 100 ng/L
Reproduction	EC_{10} NOEC LOEC	5.4 ng/L 10 ng/L 25 ng/L

RESULTS SUMMARY

In an acute toxicity test with deltamethrin, the 48-hour EC_{50} for *C. dubia* was determined to be 0.06 µg/L based on nominal concentrations. In an 8-day chronic test, the NOEC was determined as 10 ng/L (nominal) based on effects observed on reproduction.

Comment by the Notifier:

Reliabilty of the published data is limited due to missing analytical verification of test concentrations. However, test media were renewed every 24 hours, so a continuos exposure of test organisms can be assumed. The results for both, acute and chronic testing, are considered supplementary information only, as GLP studies are available to address the data points (toxicity to additional aquatic invertebrate species).
Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	**KCA 8.2.4.2/05; [redacted]; 2013**
Title:	Comparative Toxicity of Pyrethroid Insecticides to Two Estuarine Crustacean Species, Americamysis bahia and Palaemonetes pugio
Source:	Environ Toxicol. 2013 Jan 30
DOI No:	10.1002/tox.21840
Document No:	M-462328-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2010;9(2):2092)

EXECUTIVE SUMMARY (Abstract from publication)

Pyrethroid insecticides are widely used on agricultural crops, as well as for nurseries, golf courses, urban structural and landscaping sites, residential home and garden pest control and mosquito abatement. Evaluation of sensitive marine and estuarine species is essential for the development of toxicity testing and risk-assessment protocols. Two estuarine crustacean species, *Americamysis bahia* (mysids) and *Palaemonetes pugio* (grass shrimp), were tested with the commonly used pyrethroid compounds, lambda-cyhalothrin, permethrin, cypermethrin, deltamethrin, and phenothrin. Sensitivities of adult and larval grass shrimp and 7-day-old mysids were compared using standard 96-h LC_{50} bioassay protocols. Adult and larval grass shrimp were more sensitive than the mysids to all the pyrethroids tested. Larval grass shrimp were approximately 18-fold more sensitive to lambda-cyhalothrin than the mysids. Larval grass shrimp were similar in sensitivity to adult grass shrimp for cypermethrin, deltamethrin, and phenothrin, but larvae were approximately twice as sensitive to lambda-cyhalothrin and permethrin as adult shrimp. Acute toxicity to estuarine crustaceans occurred at low nanogram per liter concentrations of some pyrethroids, illustrating the need for careful regulation of the use of pyrethroid compounds in the coastal zone.

The following summary is limited to the data reported for deltamethrin.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	deltamethrin tech.
Active substance(s):	deltamethrin
Chemical state and description:	not specified
Source of test item:	[redacted].
Batch number:	not specified
Purity:	≥ 97.7%
Storage conditions:	not specified
Water solubility:	not specified

2. Test solutions

Vehicle/solvent:	acetone
Source of vehicle/solvent:	not specified
Concentration of vehicle/solvent:	0.1%
Method of preparation:	not specified
Evidence of unsolved material:	not specified

A 3. Test organism(s)

Species:	*Palaemonetes pugio*
Common name:	Grass shrimp
Source of test species:	Adult grass shrimp were collected from [redacted] South Carolina, USA [redacted]
Stage of test organisms at study initiation:	Two life stages were used in the toxicity testing: Adults (approx. 15-25 mm in length) 24-h old larvae (approx. 2-3 mm in length)

A 4. Culture conditions of test organism(s)

Culture medium:	Seawater used in the experiments was supplied to the laboratory from [redacted] filtered (5 µm), UV-sterilized, activated carbon filtered, and diluted with deionized water to adjust salinity.
Temperature:	25 °C
Photoperiod:	16-h light, 8-h dark
Light intensity:	not specified
pH:	not specified
Oxygen saturation:	not specified
Salinity:	22 ppt
Food and feeding regime:	Adult shrimp were fed daily with Tetramin® fish flakes Larvae were fed *Artemia* nauplii (24 h old) daily.
Acclimatisation prior to testing:	7-14 days in 76 L tank Brooding traps were used to capture larvae released by ovigerous females.
Observations during acclimatisation:	not specified

B 3. Test organism(s)

Species:	*Americamysis bahia*
Common name:	Mysid shrimp
Source of test species:	Original stock obtained from [redacted] FL, USA
Stage of test organisms at study initiation:	7-day old "young adults", not sexually mature, approx. 4-6 mm in length

B 4. Culture conditions of test organism(s)

Culture medium:	See above
Temperature:	24.0–26.0°C
Photoperiod:	16-h light:8-h dark
Light intensity:	not specified
pH:	7.6–8.2
Oxygen saturation:	≥ 60%
Salinity:	18-22 ppt
Food and feeding regime:	Daily with *Artemia* nauplii (≤ 24-h old)
Acclimatisation prior to testing:	not specified
Observations during acclimatisation:	not specified

B. Study design and methods

1. Test procedure

Test system:	Static renewal (daily)
Test concentration(s):	1, 4, 11, 33, and 100 ng/L (nominal)
Control(s):	Solvent control (0.1% acetone)
Number of replicates:	Three replicates (10 animals per replicate)
Test conditions:	Adult grass shrimp were exposed in 4 L glass jars. Larval grass shrimp and mysid were exposed in 600 mL beakers with a test volume of 400 mL. Experiments were conducted in an environmental chamber set at 25°C, with a 16-h light:8-h dark photoperiod. Aeration was provided via a glass pipette.
Feeding:	Adult grass shrimp were not fed during exposure. Larval grass shrimp and 7-day-old mysids were fed three to four drops of newly hatched *Artemia* daily.
Medium renewal:	daily
Frequency of test item application:	Daily with medium renewal
Test duration:	96 h
Endpoints:	mortality
Statistics:	Median lethal concentrations (24 h and 96 h LC_{50} values) with 95% confidence intervals (CIs) were determined using nominal chemical concentrations (SAS Probit Analysis, PROC PROBIT, SAS V.9.1.3, Cary, NC).

2. Chemical analysis

No chemical analysis was conducted in the exposure media. However, as the test media were renewed daily, reporting of endpoints based on nominal concentrations is considered acceptable.

RESULTS

1. Validity criteria:

< 10% mortality in the controls; Water quality parameters within standard range: temperature 24.0–26.0°C, dissolved oxygen ≥60% saturation, pH 7.6– 8.2, salinity 18–22 ppt

2. Biological findings:

Mortality in grass shrimp and mysid showed a clear dose-related response. Median lethal concentrations after 96 hours based on nominal test concentrations are provided in the table below.

Median lethal concentrations, LC_{50} value (ng/L) and 95% confidence interval (CI) of deltamethrin determined for adult grass shrimp, larval grass shrimp, and juvenile mysids.

Test item	Time (h)	Larval grass shrimp LC_{50} ng/L (95% CI)	Adult grass shrimp LC_{50} ng/L (95% CI)	Juvenile mysid LC_{50} ng/L (95% CI)
Deltamethrin	24	20.80 (12.24–29.59)	23.20 (19.80–26.84)	113.3 (80.06–190.37)
	96	5.04 (4.11–6.18)	5.80 (4.70–7.15)	26.77 (20.91–34.27)

Deltamethrin caused approximately 20% mortality in grass shrimp at 4 ng/L, and approx. 100% mortality at 11 ng/L.

CONCLUSION

The 96 h LC50 values for deltamethrin determined for adult grass shrimp, larval grass shrimp and mysids in an acute static-renewal test, were 5.80 ng/L, 5.04 ng/L and 26.77 ng/L, respectively, based on nominal concentrations.

Comment by the Notifier:

The study is considered supplementary information only, as GLP studies are available to address this data point (acute toxicity to invertebrates). Reliability of the published data is limited due to missing analytical verification. However, test media were renewed every daily, so a continuos exposure of organisms can be assumed in this 96-hour studies.
Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

For studies already evaluated during the first EU review of this compound, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

The endpoint from the following table was evaluated during the first EU review (review report of Deltamethrin 6504/VI/99-final) and will be used in the risk assessment. No additional studies were performed.
However, a chronic study on *D. magna* was found in the public literature and is summarized under this point. As the endpoints from this publication are not considered relevant for the risk assessment, the publication is classified as supporting information only.

Table 8.2.5.1- 3: Chronic Daphnia toxicity of deltamethrin

Test substance	Test species	Endpoint	Reference
Deltamethrin	Invertebrate, chronic *Daphnia magna*	**NOEC 4.1 ng a.s./L (mm)**	[redacted] (1990) M-174975-01-1

mm = mean measured

Results from literature review

Report:	**KCA 8.2.5.1/02 [redacted]; [redacted]; [redacted]; [redacted], C.M.; [redacted]; [redacted]; Férard, J.F. ; 2013**
Title:	Effects of deltamethrin (pyrethroid insecticide) on growth, reproduction, embryonic development and sex differentiation in two strains of *Daphnia magna* (Crustacea, Cladocera)
Source:	Science of The Total Environment, Volumes 458-460, p. 47-53
DOI No: Document No:	10.1016/j.scitotenv.2013.03.085 M-462220-01-1
Guidelines:	
GLP:	no

EXECUTIVE SUMMARY

Acute and different chronic ecotoxic effects of deltamethrin have been investigated on two strains (coming from two different laboratories) of *Daphnia magna*. The effective concentrations immobilizing 50% of daphnids (EC_{50}s) after 24 h and 48 h were 9.40 and 0.32 µg/L, 8.86 and 0.63 µg/L for first strain (strain 1) and second strain (strain 2), respectively. Thus, there was an increase of deltamethrin ecotoxicity with time of exposure as confirmed by chronic studies. After 21 days of exposure to deltamethrin, daphnids have showed significant effects on survival at deltamethrin concentrations of 0.16 µg/L and 0.31 µg/L for strains 1 and 2, respectively. Eleven other endpointswere examined: body length, population growth rate and various reproductive parameters (days to first brood, number of broods, number of cumulative molts and number of neonates), embryotoxicity and appearance of males. IC_{50} values related to the number of juveniles per live adult were 11 and 46 ng/L for strains 1 and 2, respectively. Furthermore, an increase in embryo deformities was observed at the highest concentrations tested for both strains. Following deltamethrin exposure, undeveloped second antennae, curved or unextended shell spines, and curved post abdomen spines were observed in live neonates. The production of male juveniles was only registered with strain 1 at 0.16 µg/L.

The acute toxicity testing described in the publication is poorly documented and of low reliability. Therefore, only the information on chronic testing of D. magna is summarized in the following.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	DECIS EC25
Active substance(s):	deltamethrin
Chemical state and description:	not reported
Source of test item:	[redacted] (Germany)
Batch number:	not reported
Purity:	Deltamethrin, 25 g/L (nominal)
Storage conditions:	not reported
Water solubility:	not reported

2. Test solutions

Vehicle/solvent:	not reported
Source of vehicle/solvent:	not reported
Concentration of vehicle/solvent:	not reported
Method of preparation:	Stock solutions were prepared by dissolving the pesticide directly in water immediately before each experiment.
Evidence of unsolved material:	not reported

3. Test organism(s)

Species:	*Daphnia magna*
Common name:	Water flea
Source of test species:	Strain 1: from [redacted] France) and identified as clone A Strain 2: from the [redacted] Japan)

4. Culture conditions of test organism(s)

Culture medium:	LCV medium: a mixture (80:20) of Lefevre-Czarda (LC) medium and French mineral water called Volvic (V).
Temperature:	20°C
Photoperiod:	16:8 h light–dark cycle
Light intensity:	not reported
pH:	not reported
Oxygen saturation:	not reported
Food and feeding regime:	three algal species (5×10^6 *Pseudokirchneriella subcapitata*, 2.5×10^6 *Desmodesmus subspicatus*, and 2.5×10^6 *Chlorella vulgaris*/Daphnia/day).
Acclimatisation prior to testing:	Cultures kept at the testing facility for several years
Observations during acclimatisation:	not reported

B. Study design and methods

1. Test procedure

Test system:	Daphnid neonates (aged <24 h) were exposed individually in 60 mL glass beakers containing 50 mL test solution for 21 days.
Test concentration(s):	Control, 9, 20, 40, 80 and 160 ng/L (time-weighted mean) for strain 1, nominal concentrations ranged from 18 to 300 ng/L Control, 16, 37, 75, 150, 310 ng/L (time-weighted mean) for strain 2, nominal concentrations ranged from 30 to 580 ng/L
Control(s):	not specified, but included
Number of replicates:	10 replicates
Test conditions:	Temperature was controlled at 20 °C, photoperiod was maintained as culture conditions at 16:8 h light–dark cycle
Feeding:	Mixture of three algal species (5×10^6 *P. subcapitata*, 2.5×10^6 *D. subspicatus*, and 2.5×10^6 *C. vulgaris*/*Daphnia*/day) at test medium renewal
Medium renewal:	every 2 days
Frequency of test item application:	Via test media renewal, every two days
Test duration:	21 days
Endpoints:	The endpoints examined were longevity, body length, growth parameters (cumulative molts, growth rate), reproductive parameters (days to first brood, number of broods, total number of neonates per survival female), embryotoxicity (number of abnormal neonates, percentages of undeveloped antennules, curved or inextended shell spines, and curved post abdomen spines) and appearance of males.

Statistics:	All chronic data were tested for statistical significance by single factor one way analysis of variance followed by Dunnett's post hoc test. Significant differences were established at $p < 0.05$. All statistical analyses were performed with Statistica 6.0 software.

2. Measurements during the test

Water/medium parameters:	not reported

3. Sampling

Sampling frequency:	not reported
Transport/storage of samples:	not reported

4. Chemical analysis

Guideline/protocol:	not reported
Method:	GC-MS
Pre-treatment of samples:	Deltamethrin was extracted from the diverse test solutions with dichloromethane (CH_2Cl_2).
Conduction:	Deltamethrin was analyzed using a gas chromatograph equipped with an ion trap mass spectrometer (FOCUS-ITQ 700 Thermo Scientific Inc.) in electronic impact mode.
Reference item:	not reported
Recovery:	not reported
Limit of detection:	0.2 μL^{-1}
Limit of quantification:	0.5 μL^{-1}

RESULTS

1. Analytical findings:

After 24 and 48 h, nominal concentrations were decreased by 45% and 74%, respectively. Therefore, the tested concentrations were expressed as measured time-weighted means.

2. Biological findings:

Sublethal effects on survival, growth and different fecundity parameters of both strains registered at the end of exposure time are described in the table below. In terms of IC_{10}s and IC_{20}s number of neonates per live adult and the number of cumulative molts were the most sensitive indices of ecotoxicity for both strains, whereas number of broods, population growth rate (r), longevity and length (in this order) were less pertinent parameters. In terms of measured NOECs, the most sensitive parameters were also the number of neonates per live adult and the number of cumulative molts for strain 1 (NOEC: 9 ng a.s./L), whereas for stain 2 the length was the most sensitive parameter (NOEC: <16 ng a.s./L).

Mean (±S.D.), IC$_{20}$ and IC$_{10}$ values (with 95% confidence interval), and their ratios for longevity, size, molting, population growth rate, and reproduction parameters of the two *D. magna* strains exposed during 21 days to deltamethrin

Test concentration [ng a.s./L]#	Longevity [days]	Length [µm]	Number of cumulative molts	Population growth rate	Day to first brood	Number of broods	Number of neonates per surviving adult
Strain 1							
Control	21.0 ± 0.0	4.7 ± 0.3	10.4 ± 2.2	0.33 ± 0.01	8.2 ± 0.4	5.3 ± 1.2	114.9 ± 21.5
9	20.4 ± 1.3	4.7 ± 0.2	9.4 ± 1.3	0.31 ± 0.01	8.2 ± 0.4	5.8 ± 1.7	101.2 ± 29.1
20	20.4 ± 1.3	4.7 ± 0.4	8.2 ± 1.6*	0.30 ± 0.02	8.3 ± 0.5	4.4 ± 2.2	83.1 ± 34.1*
40	20.4 ± 1.3	4.6 ± 0.4	8.2 ± 1.1*	0.30 ± 0.01	9.3 ± 0.7*	4.4 ± 0.5	80.6 ± 22.5*
80	20.3 ± 1.2	4.3 ± 0.4*	7.9 ± 1.4*	0.28 ± 0.02*	9.8 ± 0.4*	4.0 ± 1.3	59.4 ± 1.0*
160	16.3 ± 5.1*	4.2 ± 0.3*	6.5 ± 2.1*	0.26 ± 0.01*	10.2 ± 0.6*	3.1 ± 3.8*	26.4 ± 34.2*
IC$_{10}$ [ng a.s./L]	130 (118-148)	120 (92-164)	7.4 (1.4-18.2)	36 (21-50)	n.c.	20 (2-60)	**11 (4-20)**
IC$_{20}$ [ng a.s./L]	160 (153-164)	> 160	34 (15-61)	152 (118-197)	n.c.	48 (15-148)	22 (12-34)
Strain 2							
Control	21.0 ± 0.0	4.4 ± 0.4	10.4 ± 1.2	0.33 ± 0.01	7.3 ± 0.5	4.9 ± 1.0	104.8 ± 11.1
16	21.0 ± 0.0	3.9 ± 0.1*	9.5 ± 1.0	0.32 ± 0.01	7.5 ± 0.5	4.9 ± 0.7	97.3 ± 13.7
37	21.0 ± 0.0	3.8 ± 0.2*	9.0 ± 1.3*	0.32 ± 0.02	7.6 ± 0.5	4.4 ± 1.9	93.3 ± 30.3
75	21.0 ± 0.0	3.8 ± 0.2*	8.2 ± 1.2*	0.32 ± 0.01	7.8 ± 0.8	4.2 ± 0.6	91.0 ± 17.2
150	19.5 ± 2.7	3.8 ± 0.2*	7.7 ± 0.8*	0.29 ± 0.10*	8.2 ± 1.2	4.2 ± 1.0	65.2 ± 16.7*
310	15.6 ± 5.8*	3.7 ± 0.2*	7.1 ± 0.9*	0.25 ± 0.02*	8.6 ± 1.5*	3.8 ± 3.3	48.1 ± 21.0*
IC$_{10}$ [ng a.s./L]	180 (169-197)	> 310	16 (9-25)	148 (116-181)	n.c.	58 (14-126)	**46 (24-70)**
IC$_{20}$ [ng a.s./L]	264 (254-273)	> 310	80 (61-105)	260 (235-295)	n.c.	231 (118-417)	87 (57-117)

Time-weighted measured concentrations
* Statistically significant difference compared to control (Dunnett's test, $p < 0.05$)
n.c. = not calculated

RESULTS SUMMARY

Based on time-weighted measured concentrations the following endpoints were derived in a 21-day chronic study with 2 strains of *Daphnia magna* under static-renewal conditions:

Strain 1: IC$_{10}$ = 11 ng/L (based on number of neonates per surviving adult)

Strain 2: IC$_{10}$ = 46 ng/L (based on number of neonates per surviving adult)

Comment by the Notifier:

The study is considered supplementary information only, as a GLP study is available to address this data point (chronic invertebrate toxicity) which results in a lower endpoint. The results for "number of cumulative molts" were not considered relevant, as this is not a standard endpoint according to OECD 211.
The information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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

A life-cycle study with the saltwater mysid *Americamysis bahia* was conducted to fulfill the data requirements for the registration of deltamethrin in the USA and is summarized below.

Table 8.2.5.2- 1: Additional studies for the reproductive and development toxicity of deltamethrin to additional invertebrate species

Test substance	Test species	Endpoint	Reference
Deltamethrin	Invertebrate, chronic *Americamysis bahia*	**NOEC 0.73 ng a.s./L (mm)**	[redacted] **(2012)** **M-437923-01-1**

mm = mean measured

Report:	**KCA 8.2.5.2/01 [redacted] 2012**
Title:	Deltamethrin: A flow-through life-cycle toxicity test with the saltwater mysid (*Americamysis bahia*)
Document No:	M-437923-01-1 (Rep. No: 149A-245A)
Guidelines:	U.S. EPA Series 850 – Ecological Effects Test Guidelines, OPPTS Number 850.1350: Mysid Chronic Toxicity Test and ASTM Standard E 1191-03 a: Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids.
GLP:	Yes

Objective:

The objective of this study was to evaluate the effects of deltamethrin on the survival, reproduction and growth of the saltwater mysid (*Americamysis bahia*) during chronic exposure under flow-through test conditions.

Materials and Methods:

Test item: [benzyl-^{14}C] Deltamethrin; Batch No.: 10562A; radiochemical purity: > 99%.

Saltwater mysids were exposed to a geometric series of five test concentrations, a negative (dilution water) and a solvent control (0.02 mL/L dimethylformamide) under flow-through conditions for 35 days. Target test concentrations were 0.33, 0.57, 1.1, 1.9 and 3.9 ng ^{14}C-deltamethrin/L (throughout the report referred to as deltamethrin). Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, approximately weekly during the test and at test termination.

Delivery of the test solutions to the test chambers was initiated two days prior to test initiation in order to achieve equilibrium of the test substance. Four replicate test chambers were maintained in each treatment and control group. At test initiation, each replicate contained one compartment with 15 neonate mysids, resulting in a total of 60 mysids in each treatment and control group. On Day 14 of the test, after mysids attained sexual maturity, male and female adults were paired in each treatment and control group, with a maximum of five reproductive pairs per replicate. Reproduction of the paired mysids was monitored through termination on Day 35. Observations for mortality and signs of toxicity were conducted daily throughout the test. At test termination, the total body lengths and dry weights of surviving first-generation mysids were measured.

Observations of the effects of deltamethrin on mortality, reproduction and growth were used to determine the no-observed-effect concentration (NOEC), the lowest-observed-effect concentration (LOEC), and the maximum acceptable toxicant concentration (MATC).

Results:

Analytical results:

The nominal concentrations selected for use in this study were 0.25, 0.50, 1.0, 2.0 and 4.0 ng a.s./L.

Once the delivery stocks were made and analyzed, it was determined with the sponsor to use target concentrations based on the results of the analysis. Target concentrations for this study were determined to be 0.33, 0.57, 1.1, 1.9 and 3.9 ng a.s./L.
Based on measurement of samples collected during the test, the mean measured test concentrations for this study were 0.26, 0.47, 0.73, 1.2 and 1.8 ng/L, representing 78.8%, 82.5%, 66.4%, 63.2% and 46.2% of target concentrations, respectively.

Biological results:

Mortality

After 14 days of exposure, mortality in the pooled control and in the 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 97.5%, 100%, 93.3%, 100% and 71.7%, respectively.

Reproduction

The mean percent of females producing young in the negative and solvent control groups was 88.9% and 100%, respectively. The mean percent of females producing young in the pooled control and in the 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 94.3%, 82.4%, 94.1%, 100%, 43.8% and 45.5%, respectively. Fisher's Exact test indicated there were statistically significant decreases in mean percent of females reproducing young in the 1.2 and 1.8 ng a.s./L treatment groups, in comparison to the pooled control ($p \leq 0.05$).
The mean number of young produced per female in the negative control and solvent control groups was 4.6 and 7.9, respectively. The mean number of young produced per female in the pooled control group and the 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 6.2, 2.8, 6.9, 8.0, 2.6 and 2.3 respectively. Dunnett's test indicated there were statistically significant decreases in the mean number of young produced per female in the 1.2 and 1.8 ng a.s./L treatment groups when compared to the pooled control ($p \leq 0.05$).
The mean number of young produced per reproductive day in the negative and solvent control groups was 0.236 and 0.388, respectively. The mean number of young produced per reproductive day in the pooled control and in the 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 0.312, 0.140, 0.333, 0.456, 0.185 and 0.117, respectively. Dunnett's test indicated there were statistically significant decreases in reproduction in the 1.8 ng a.s./L treatment group in comparison to the pooled control ($p \leq 0.05$).

Growth

Males: The mean total length and dry weight of male mysids in the negative control group was 7.31 mm and 1.06 mg, respectively. In the solvent control group, the mean total length and dry weight of males was 7.19 mm and 0.98 mg, respectively. The mean total length of male mysids in the pooled control and the 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 7.25, 7.16, 7.24, 7.10, 7.25 and 7.12 mm, respectively. The mean dry weight of males in the pooled control and the 0.26, 0.47, 0.73, 1.2 and 1.8 ng/L treatment groups was 1.02, 1.05, 1.00, 1.10, 1.11 and 1.08 mg, respectively. Dunnett's test indicated there were no statistically significant decreases in mean total length or mean dry weight in any of the treatment groups for males, in comparison to the pooled control ($p>0.05$).
Females: The mean total length and dry weight of female mysids in the negative control group was 7.59 mm and 1.38 mg, respectively. In the solvent control group, the mean total length and dry weight of females was 7.49 mm and 1.26 mg, respectively. The mean total length of female mysids in the pooled control and 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 7.54, 7.69, 7.47, 7.52, 7.41 and 7.44 mm, respectively. The mean dry weight of females in the pooled control and the 0.26, 0.47, 0.73, 1.2 and 1.8 ng a.s./L treatment groups was 1.32, 1.33, 1.42, 1.30, 1.34 and 1.24 mg, respectively. Dunnett's

test indicated there were no statistically significant decreases in mean total length or mean dry weight in any of the treatment groups for females, in comparison to the pooled control (p>0.05).

Chronic NOEC for *Americamysis bahia* exposed to deltamethrin based on mean measured concentrations

Test substance:	^{14}C-deltamethrin
Test object:	*Americamysis bahia*
Exposure:	35 days, flow-through test design (dose-response)
NOEC:	**0.73 ng a.s./L (mean measured)**
LOEC:	1.2 ng a.s./L (mean measured)

Conclusions:

Saltwater mysids (*Americamysis bahia*) were exposed to Deltamethrin at mean measured concentrations of 0.26 to 1.8 ng a.s./L under flow-through conditions for 35 days, and were evaluated for survival, reproduction and growth. Reproduction, measured as the percent of females that produced young and the mean number of young produced per female during the test, was the most sensitive biological endpoint measured. There was a statistically significant decrease in percent of reproductive females and number of young per female in the 1.2 and 1.8 ng a.s./L treatment groups. Consequently, the NOEC, based on reproduction, was 0.73 ng/L. The LOEC was 1.2 ng/L and the MATC was calculated to be 0.94 ng/L.

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Results from literature review

Report:	**KCA 8.2.5.2/02; [redacted] 2012**
Title:	Comparative study on the toxicity of pyrethroids, α-cypermethrin and deltamethrin to Ceriodaphnia dubia
Source:	Ecotoxicology andEnvironmentalSafety78(2012)9–13
DOI No: Document No:	10.1016/j.ecoenv.2011.07.018 M-462170-01-1
Guidelines:	Chronic toxicity test protocol was based on the USEPA Test Method 1002.0 for survival and reproduction using Ceriodaphnia dubia (USEPA, 2002b).
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

The summary of this publication is provided under point MCP 8.2.4.2.

Comment by the Notifier:

Reliabilty of the published data is limited due to missing analytical verification of test concentrations. However, test media were renewed every 24 hours, so a continuos exposure of test organisms can be assumed. The results are considered supplementary information only, as a GLP study is available to address this data point (chronic toxicity to additional aquatic invertebrate species).

Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CA 8.2.5.3 Development and emergence in Chironomus species

For studies already evaluated during the first EU review of this compound, please refer to corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.
The following endpoint, derived from a spiked water study evaluated during the first EU review (review report of Deltamethrin 6504/VI/99-final), is used in the risk assessment:

Table 8.2.5.3- 1: Long-term toxicity to *Chironomus riparius* exposed to deltamethrin

Test substance	Test species	Endpoint		Reference
Deltamethrin	Chironomid, chronic *Chironomus riparius*	NOEC	10 ng a.s./L	[redacted] (1998) M-152560-01-1

CA 8.2.5.4 Sediment dwelling organisms

A new chronic study with *Chironomus riparius* using a spiked sediment design was conducted and is summarized below:
In addition a spiked sediment study with *C. dilutus* was conducted for registration of deltamethrin in the USA. This study is considered supporting information only, as it was conducted according to specific US EPA Test Methods, and the data requirement according to Regulation (EC) No 1107/2009 was already addressed by the available study from [redacted] (2012; M-425202-01-1).

Table 8.2.5.4- 1: Additional studies for the toxicity of deltamethrin to sediment dwelling organisms

Test substance	Test species	Endpoint		Reference
Deltamethrin	Sediment dweller, chronic *Chironomus riparius*	EC_{10}	7.5 µg a.s./sed dw sed (nom)	**[redacted] (2012) M-425202-01-1**
Deltamethrin	Sediment dweller, chronic *Chironomus dilutus*	NOEC	1.5 µg a.s./kg sed (mm)	**[redacted] (2013) M-466314-01-1**

nom = nominal, mm = mean measured

Report:	**KCA 8.2.5.4/01; [redacted]; 2012**
Title:	*Chironomus riparius* 28-day chronic toxicity test with deltamethrin (tech.) in a water-sediment system using spiked sediment.
Document No:	M-425202-01-1(Rep. No: EBDAL036)
Guidelines:	OECD Guideline 218: “Sediment-Water Chironomid Toxicity Test Using Spiked Sediment” (adopted 13 April 2004)
GLP:	Yes (certified laboratory)

Objective:

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

Material and methods:

Test item: Deltamethrin (tech.), purity: 99.8%, batch-no.: ABJFDCO012, TOX09083-00 and specification No.: 102000001388.
First instar larvae of *Chironomus riparius*, 4 beakers per test concentration, control and solvent control (acetone) with 20 animals each were exposed in a static water sediment test system for 28 days to initial nominal concentrations of 2, 4, 8, 16, 32 and 64 µg a.s./kg dw sed (dry weight sediment). During the study the larvae were fed at least 3 times a week with a commercial ornamental fish food extract (trade name Tetra Phyll ®).
Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger. Additionally the temperature was measured once a week in the overlying water of additional test vessels of each test concentration incl. controls.
Dissolved oxygen was measured twice per week in the overlying water of the additional test vessels of each test concentration incl. controls and additionally in all test vessels at the end of the test (day 28).
The pH was measured once per week in the overlying water of the additional test vessels of each test concentration incl. controls and additionally in all test vessels at the end of the test (day 28).
The concentrations of deltamethrin (AE F032640), as well as of the metabolites trans-isomer of deltamethrin (AE 0035073) and α-R-isomer of deltamethrin (AE F108569) were analysed in the freshly prepared spiked sediments of all test concentrations and the controls on day -2. Their concentration in the overlying water, the pore water of the sediment and the sediment was analysed at day 0 (directly before inserting of the larvae), day 7 and day 28 in separate test vessels of all test concentrations and controls.
The test vessels were observed at least three times per week to make a visual assessment of any behavioural differences compared to the control. The sex, time point of emergence and number of emerged midges was recorded daily during the period of emergence. As only fully emerged adults are relevant for the endpoints of this study, larvae which did not yet mature were not taken into account for emergence rates and development time. To determine number and sex of emerged adults, the covering plates of each test container were carefully moved and the midges, which mostly stayed at the sides of the vessels, were enumerated; after identification of the sex (male midges have feathered antennae) midges were removed.

Dates of experimental work: 2010-10-28 to 2011-06-09

Results:

Measured temperature, pH and oxygen content in water did not deviate from defined guideline recommendations.

Analytical results:
Sediment analysis of deltamethrin on day -2 (directly after spiking) reflect high recoveries with 86% to 119% (mean: 109 %) of nominal concentrations in all test levels. Analyses in the sediment on day 0 showed stable recoveries of 101% to 116% (mean: 108 %) of nominal for all test concentrations. Thus all results are based on nominal concentrations of deltamethrin in the sediment, expressed in µg a.s./kg dw sed. On day 7, 98% to 115% (mean: 103 %) and on day 28, 76% to 110% (mean: 94%) of nominal were found, respectively.
Sediment analyses of the metabolites α-R-isomer of deltamethrin and trans-isomer of deltamethrin did not result in findings above the limit of quantification in any sample. Thus the results are based only on the findings of deltamethrin.

Chemical analyses of the overlying water and pore water for deltamethrin, and the metabolites α-R-isomer of deltamethrin and trans-isomer of deltamethrin over time did not result in findings above the limit of quantification, respectively.

Biological results:
Start of emergence was at day 14 and 15 for the controls and all test concentrations from 2 to 16 µg a.s./kg dw sed. The start of emergence was delayed for three days at a test concentration of 32 µg a.s./kg dw sed. and for four days at a test concentration of 64 µg a.s./kg dw sed.
87.5 % of the inserted (n= 160) larvae maturated to adults in the pooled controls after 28 days, fulfilling the guideline requirements.

Influence on emergence and development rate after 28 days (based on nominal concentrations of the test item in the sediment):

Nominal concentration [µg a.s./kg dw sed]	Number of introduced larvae	Number of emerged midges	Emergence of inserted larvae			Development rate (pooled sex)
			total [%]	male [%]	female [%]	(1/d)
Controls[1)]	160	140	87.5	48.1	39.4	0.059
2.00	80	71	88.8	46.3	42.5	0.058
4.00	80	67	83.8	48.8	35.0	0.059
8.00	80	60[2)]	75.0	40.0	35.0	0.058
16.0	80	53[2)]	66.3	31.3	35.0	0.056[2)]
32.0	80	40[2)]	50.0	27.5	22.5	0.054[2)]
64.0	80	18[2)]	22.5	12.5	10.0	0.051[2)]

1) Pooled control and solvent-control
2) Comparison of treatments with "pooled control" by the t-test procedure after Williams (Significance was α = 0.05, one-sided smaller)

The student-t-test for homogeneous variances indicates no statistically different distribution between sexes compared to the assumption of 50% females and 50% males. Therefore male and female results were pooled for further statistical analyses to increase the statistical power.
A statistically significant difference in emergence was estimated for the concentrations from 8 to 64 µg a.s./kg dw sed as compared to the pooled controls, resulting in a NOEC of 4 µg a.s./kg dw sed.
For the development rate (pooled sex) a statistically significant difference was estimated for the concentrations from 16 to 64 µg a.s./kg dw sed as compared to the pooled controls, resulting in a NOEC of 8 µg a.s./kg dw sed. The EC_{10} values for emergence and development rate were calculated to be 7.5 µg a.s./kg dw sed for emergence and 42.1 µg a.s./kg dw sed for development rate, respectively.

Conclusion:

Results are based on nominal concentrations of deltamethrin in µg a.s./ kg dw sed.

Endpoints	EC_{10}	EC_{20}	NOEC	LOEC
Emergence rate (pooled sex) (95 % confidence limits)	**7.5** (5.6-9.3)	12.6 (10.158-14.996)	4.0	8.0
Development rate (pooled sex)	42.1 (23.0-146.9)	383.5 (116.0-8938.5)	8.0	16.0

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Report:	**KCA 8.2.5.4/02; [redacted]; 2013**
Title:	Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to Deltamethrin Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods
Document No:	M-466314-01-1 (Rep. No: EBDAL086)
Guidelines:	EPA Test Methods, Smithers Viscient Protocol No.: 111910/EPA/Midge chronic
GLP:	Yes (certified laboratory)

Objective:
The purpose of this study was to determine the effects of deltamethrin, applied to sediment, on the life cycle of the midge (*Chironomus dilutus*).

Material and methods:
Test item: Deltamethrin technical, purity 99.52%, batch no. LGDL TK113, CAS No. 52918-63-5.
The dipteran midge *Chironomus dilutus* was exposed for 63 days in a spiked sediment study, with renewal of overlying water. Artificial sediment prepared according to OECD Guideline 218 was used in this study. Nominal sediment concentrations were 0.51, 0.77, 1.9, 4.8 and 12 µg a.s./kg, corresponding to mean measured concentrations of 0.22, 0.76, 1.5, 3.6 and 12 µg a.s./kg. The sediment was spiked and allowed to equilibrate for 14 days before test organisms were introduced. 300 mL glass vessels were filled with 100 mL sediment each (approx. 4 cm layer), before adding 175 mL of overlying water. At test initiation, one to two day old midges were added to the test vessels (12 midges/vessel, 12 replicates/concentration). During the study the test systems were kept between 22 to 24°C with a continuous illumination of 16 hours at 340 to 950 lux and 8 hours of darkness. The overlying water was laboratory well water: pH 6.4 to 7.6, conductivity 130 to 360 µS/cm, total hardness as $CaCO_3$ 28 to 52 mg/L, and total alkalinity as $CaCO_3$ 20 to 26 mg/L.

Results:
Based on the data obtained during this study, the following endpoints were generated based on mean measured sediment concentrations.

Endpoint	Mean Measured Sediment (µg/kg)	OC Normalized Sediment (µg/g OC)
Midge larval survival (Day 20)		
LOEC	9.1	> 0.35
NOEC	9.1	0.35
LC_{50} (95% confidence intervals)	> 9.1 (NA*)	> 0.35 (NA*)
Midge larval growth (Day 20)		
LOEC	3.6	0.14
NOEC	**1.5**	0.058
LC_{50} (95% confidence intervals)	8.3 (3.5-20)	0.32 (0.14-0.76)
Percent emergence (Day 63)		

Endpoint	Mean Measured Sediment (µg/kg)	OC Normalized Sediment (µg/g OC)
LOEC	3.6	0.14
NOEC	**1.5**	0.058
LC_{50} (95% confidence intervals)	> 9.1 (NA*)	> 0.35 (NA*)
Male emergence rate (Day 63)		
LOEC	3.6	0.14
NOEC	**1.5**	0.058
LC_{50} (95% confidence intervals)	> 9.1 (NA*)	> 0.35 (NA*)
Female emergence rate; Days to death for males; Days to death for females; Egg masses per mated female; Number of eggs per egg mass; Number of eggs per mated female; Percent hatch; Days to oviposition (Day 63)		
LOEC	> 9.1	> 0.35
NOEC	9.1	0.35
LC_{50} (95% confidence intervals)	> 9.1 (NA*)	> 0.35 (NA*)

* NA = Not Applicable EC_{50} value was empirically estimated. Therefore, corresponding 95% confidence intervals could not be calculated.
LOEC: Lowest-Observed-Effect Concentration
NOEC: No-Observed-Effect Concentration

Conclusion:
The lowest NOEC was determined to be 1.5 µg a.s./kg sed, based on the effects observed on larval growth and survival (day 20) and percent emergence (day 63) at the LOEC of 3.6 µg a.s./kg sed.

CA 8.2.6 Effects on algal growth

For studies already evaluated during the first EU review of this compound, please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.

For green algae, the endpoint from the following table was evaluated during the first EU review (review report of Deltamethrin 6504/VI/89-final), but was considered an "*uncertain value*" in the List of endpoints provided by EFSA.

Table 8.2.6-1: Toxicity to algal species exposed to deltamethrin

Test substance	Test species	Endpoint		Reference
Deltamethrin	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50}	>9100 µg a.s./L (im)	[redacted] (1990) M-149338-01-1
		E_rC_{50}	>9100 µg a.s./L (im)	

im = initial measured

Studies with additional algae species were conducted to fulfill the data requirements for the registration of deltamethrin in the USA and are summarized under MCA 8.2.6.2. All these studies were conducted at the practical solubility limit of deltamethrin. The lowest endpoint from the *Navicula* study will be used in the risk assessment as a conservative approach.

Table 8.2.6- 2: Additional studies for toxicity of deltamethrin to algae

Test substance	Test species	Endpoint		Reference
Deltamethrin	Algae, growth inhibition *Navicula pelliculosa*	E_rC_{50} (72 h) E_yC_{50} (72 h)	>3.1 µg a.s./L (im) >3.1 µg a.s./L (im)	[redacted] (2013) M-468384-01-1
Deltamethrin	Algae, growth inhibition *Anabaena flos-aquae*	E_rC_{50} (72 h) E_yC_{50} (72 h)	>3.6 µg a.s./L (im) >3.6 µg a.s./L (im)	[redacted] (2013) M-468386-01-1
Deltamethrin	Algae, growth inhibition *Skeletonema costatum*	E_rC_{50} (72 h) E_yC_{50} (72 h)	>3.4 µg a.s./L (im) >3.4 µg a.s./L (im)	[redacted] (2013) M-468465-01-1

im = initial measured

CA 8.2.6.1 Effects on growth of green algae

Please refer to point MCA 8.2.6.

CA 8.2.6.2 Effects on growth of an additional algal species

Report:	**KCA 8.2.6.2/01; [redacted] 2013**
Title:	Toxicity of deltamethrin technical to the freshwater diatom *Navicula pelliculosa* during a 96 hour exposure
Document No:	M-468384-01-1 (Rep. No: 7SRLS13C7)
Guidelines:	FIFRA Guideline123-2 (1982), OCSPP Guideline 850.4500 (2012), OECD Guideline 201 (2006)
GLP:	Yes (certified laboratory)

Objective:
The aim of the study was to determine the growth effects of deltamethrin technical to the freshwater diatom *Navicula pelliculosa* during a 96 hour exposure.

Material and methods:
Test item: Deltamethrin technical, purity 99.6%, batch no. PMDN001265, spec. no. 102000001388-03
The freshwater diatom *Navicula pelliculosa* were exposed under static conditions for 96-hours up to the functional limit of solubility. Nominal concentrations were control, solvent control (50 µL DMF/L), 0.25, 0.50, 1.0, 2.0, and 4.0 µg a.s./L. Deltamethrin was measured in the test solutions on Day 0 and Day 4.

Results:
Analytical results:
Measured deltamethrin concentrations ranged from 54% to 83% of nominal concentrations on day 0. At test termination (day 4), measured concentrations ranged from below the LOQ to 1.8% of nominal. The biological endpoints are reported based on the initial measured concentrations.

Initial measured concentrations were: <LOQ (for control and solvent control, 0.14, 0.27, 0.62, 1.7, and 3.1 µg a.s./L.

Biological results:
No effects of deltamehrin on the growth of *Navicula pelliculosa* were observed up to the highest concentration tested.

Toxicity to algae

Test substance	Deltamethrin Technical
Test object	*Navicula pelliculosa*
Exposure	96 hour, static
Growth rate 0-72 h E_rC_{50} (95% confidence interval)	> 3.1 µg a.s./L - functional limit of solubility (95% C.I. could not be calculated)
Yield 0-72 h E_yC_{50} (95% confidence interval)	> 3.1 µg a.s./L - functional limit of solubility (95% C.I. could not be calculated)

No cell abnormalities were observed in the control or treatment groups during the study.

Conclusions:
The 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be ≥ 3.6 µg a.s./L with LOEC and NOEC values of > 3.6 and ≥ 3.6 µg a.s./L, respectively, based on initial measured concentrations.

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Report:	**KCA 8.2.6.2/02; [redacted] 2013**
Title:	Toxicity of deltamethrin technical to the Cyanobacterium *Anabaena flos-aquae* during a 96 hour exposure
Document No:	M-468386-01-1 (Rep. No: EBDAL096)
Guidelines:	FIFRA Guideline 123-2 (1982), OCSPP Guideline 850.4500 (2012), OECD Guideline 201 (2006).
GLP:	Yes (certified laboratory)

Objective:
The aim of the study was to determine the growth effects of deltamethrin technical to the blue-green algae *Anabaena flos-aquae* during a 96 hour exposure.

Material and methods:
Test item: Deltamethrin technical, purity: 99.6%, batch no. PMDN001265, spec. no. 102000001388-03
The cyanobacterium, *Anabeana flos-aquae,* was exposed under static conditions for 96-hours up to the functional limit of solubility. Nominal concentrations were control, solvent control (50 µL DMF/L), 0.25, 0.50, 1.0, 2.0, and 4.0 µg a.s./L. Deltamethrin was measured in the test solutions on Day 0 and Day 4.

Results:
Analytical results:
Measured deltamethrin concentrations ranged from 88% to130% of nominal concentrations on day 0. At test termination (day 4), measured concentrations ranged from 7.9% to 28% of nominal. The biological endpoints are reported based on the initial measured concentrations.

Initial measured concentrations were: <LOQ (for control and solvent control, 0.32, 0.44, 0.95, 1.9, and 3.6 µg a.s./L.

Biological results:

No effects of deltamehrin on the growth of *Navicula pelliculosa* were observed up to the highest concentration tested.

Toxicity to algae

Test substance	Deltamethrin Technical
Test object	*Anabaena flos-aquae*
Exposure	96 hour, static
Growth rate 0-72 h E_rC_{50} (95% confidence interval)	>3.6 µg a.s/L - functional limit of solubility (95% C.I. could not be calculated)
Yield 0-72 h E_yC_{50} (95% confidence interval)	>3.6 µg a.s/L - functional limit of solubility (95% C.I. could not be calculated)

No cell abnormalities were observed in the control or treatment groups during the study.

Conclusions:
The 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be > 3.6 µg a.s./L with LOEC and NOEC values of > 3.6 and ≥ 3.6 µg a.s./L, respectively, based on initial measured concentrations.

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Report:	**KCA 8.2.6.2/03; [redacted] 2013**
Title:	Toxicity of deltamethrin technical to the saltwater diatom *Skeletonema costatum* during a 96 hour exposure
Document No:	M-468465-01-1 (Rep. No: EBDAL098)
Guidelines:	FIFRA Guideline 123-2 (1982), OCSPP Guideline 850.4500 (2012), OECD Guideline 201 (2006).
GLP:	Yes (certified laboratory)

Objective:
The aim of the study was to determine the growth effects of deltamethrin technical to the saltwater diatom *Skeletonema costatum* during a 96 hour exposure.

Material and methods:
Test item: Deltamethrin technical, purity: 99.6%, batch no. PMDN001265, spec. no. 102000001388-03
The saltwater diatom *Skeletonema costatum* was exposed under static conditions for 96-hours up to the functional limit of solubility. Nominal concentrations were control, solvent control (50 µL DMF/L), 0.25, 0.50, 1.0, 2.0, and 4.0 µg a.s./L. Deltamethrin was measured in the test solutions on Day 0 and Day 4.

Results:
Analytical findings:
Measured deltamethrin concentrations ranged from 51% to 85% of nominal concentrations on day 0. At test termination (day 4), measured concentrations ranged from 6% to 11% of nominal, with the

exception of the low level sample in which there was no recovery of deltamethrin. The biological endpoints are reported based on the initial measured concentrations.
Initial measured concentrations were: <LOQ (for control and solvent control, 0.14, 0.26, 0.73, 1.4, and 3.4 µg a.s./L.

Biological findings:
No effects of deltamehrin on the growth of *Skeletonema costatum* were observed up to the highest concentration tested.

Toxicity to algae

Test substance	Deltamethrin Technical
Test object	*Skeletonema costatum* (saltwater diatom)
Exposure	96 hour, static
Growth rate 0-72 hr E_rC_{50} (95% confidence interval)	3.4 µg a.s./L - functional limit of solubility (95% C.I. could not be calculated)
Yield 0-72 h E_yC_{50} (95% confidence interval)	3.4 µg a.s./L - functional limit of solubility (95% C.I. could not be calculated)

No cell abnormalities were observed in the control or treatment groups during the study.

Conclusions:
The 72-hour EC_{50} value for growth rate (E_rC_{50}) was determined to be > 3.4 µg a.s./L with LOEC and NOEC values of > 3.4 and ≥ 3.4 µg a.s./L, respectively, based on initial measured concentrations.

CA 8.2.7 Effects on aquatic macrophytes

According to the Commission Regulation (EU) No. 283/2013, studies on aquatic macrophytes are no data requirement for insecticides. However, a study with *Lemna gibba* was conducted to fulfill the data requirements for the registration of deltamethrin in the USA and is summarized below.

Table 8.2.7- 1: Additional studies for toxicity of deltamethrin to aquatic macrophytes

Test substance	Test species	Endpoint		Reference
Deltamethrin	Aquatic macrophytes, growth inhibition *Lemna gibba*	E_rC_{50} E_bC_{50}	>0.779 µg a.s./L (mm) >0.779 µg a.s./L (mm)	[redacted] **(2012) M-439085-01-1**

mm = mean measured

Report:	**KCA 8.2.7/01; [redacted] 2012**
Title:	Toxicity of deltamethrin technical to duckweed (Lemna gibba G3) under static-renewal conditions
Document No:	M-439085-01-1
Guidelines:	FIFRA Guideline,132-2 (1982), OPPTS-Guideline 850.4400 (1996), OECD Guideline 221 (2006)
GLP:	Yes (certified laboratory)

Objective: The aim of the study was to determine the growth effects of deltamethrin technical on *Lemna gibba* G3 to estimate the fifty percent effective concentration (EC_{50}) for deltamethrin technical.

Material and methods:
Test item: Deltamethrin techn., purity: 99.52%, batch no. FGDLTK113
The duckweed *Lemna gibba* G3 was exposed to the test item for 7 days under static-renewal (renewal on day 3) conditions. Testing was conducted up to the functional limit of solubility. Nominal concentrations were control, solvent control, 0.25, 0.5, 1.0, 2.0, and 4.0 µg a.s./L. Growth was determined by frond counts on Days 0, 3, 5 and 7 and frond dry weights from Day 0 and Day 7.

Results:

Analytical results:

Measured concentrations in the new test solutions ranged from 23% to 38% on nominal values. In the aged test solutions the maximum recovery was 7% of nominal, recovery for some test levels was below the LOQ (0.03 µg a.s./L). For samples with a recovery <LOQ, LOQ/2 was considered to calculated mean measured concentrations. The biological results are based on the following mean measured concentrations: control, solvent control, 0.047, 0.084, 0.166, 0.415 and 0.779 µg a.s./L.

Biological results:

Fronds in all test levels appeared normal relative to the control group during the course of the study.

Endpoints are summarized in the following table:

Test Substance	Deltamethrin technical
Test Object	*Lemna gibba* G3
Exposure	7-Day, static-renewal
7-day EC_{50} – frond count	> 0.779 µg a.s./L
7-day E_rC_{50} – growth rate for frond numbers	> 0.779 µg a.s./L
7-day E_bC_{50} – cumulative biomass for frond numbers	> 0.779 µg a.s./L
7-day EC_{50} – frond dry weight	> 0.779 µg a.s./L
7-day E_rC_{50} – growth rate for frond dry weight	> 0.779 µg a.s./L
Lowest Concentration With an Effect (LOEC)	> 0.779 µg a.s./L
Highest Concentration Without Toxic Effect (NOEC)	0.779 µg a.s./L

Conclusion:
The NOEC and LOEC in the 7-day exposure of *Lemna gibba* G3 to deltamethrin technical were 0.779 and > 0.779 µg a.s./L, respectively for the endpoints of 7 day frond counts, growth rate for frond numbers, and cumulative biomass for frond counts, and also dry weight and growth rate for dry weights. EC_{50} values for all endpoints could not be calculated within the range of tested concentrations and were determined to be greater than the highest test concentration (> 0.779 µg a.s./L).

CA 8.2.8 Further testing on aquatic organisms

No additional studies were performed.

Results from literature review

Report:	**KCA 8.2.8/04;** [redacted] **2007**
Title:	Influence of isolation on the recovery of pond mesocosms from the application of an insecticide. I. Study design and planktonic community responses
Source:	Environmental Toxicology and Chemistry, Vol. 26, No. 6, pp. 1265-1279
DOI No: Document No:	- M-294182-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The influence of relative isolation on the ecological recovery of freshwater outdoor mesocosm communities after an acute toxic stress was assessed in a 14-month-long study. A single concentration of deltamethrin was applied to 8 out of 16 outdoor 9 m^3 mesocosms to create a rapid decrease of the abundance of arthropods. To discriminate between external and internal recovery mechanisms, four treated and four untreated (control) mesocosms were covered with 1 mm mesh screen lids. The dynamics of planktonic communities were monitored in the four types of ponds. The abundance of many phytoplankton taxa increased after deltamethrin addition, but the magnitude of most increases was relatively small, probably due to low nutrient availability and the survival of rotifers. The greatest impact on zooplankton was seen in Daphniidae and, to a lesser extent, calanoid copepods. Recovery (defined as when statistical analysis failed to detect a difference in the abundance between the deltamethrin-treated ponds and corresponding control ponds for two consecutive sampling dates) of Daphniidae was observed in the water column 105 and 77 d after deltamethrin addition in open and covered mesocosms, respectively, and <42 d for both open and covered ponds at the surface of the sediments. Rotifers did not proliferate, probably because of the survival of predators (e.g.,cyclopoid copepods). These results confirm that the recovery of planktonic communities after exposure to a strong temporary chemical stress mostly depends upon internal mechanisms (except for larvae of the insect Chaoborus sp.) and that recovery dynamics are controlled by biotic factors, such as the presence of dormant forms and selective survival of predators.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin dissolved in an acetone-water mixture (5.8 mL acetone stock per 1 L water)
Active substance(s):	Deltamethrin
Chemical state and description:	Liquid; active substance dissolved in an acetone-water mixture
Source of test item:	[redacted], Germany
Batch number:	Not reported
Purity:	99%
Storage conditions:	Not reported
Water solubility:	> 0.2

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	5.8 mL acetone stock per 1 L water in "stock solution"
Method of preparation:	Not specified
Evidence of unsolved material:	Not reported

3. Test organism(s)

Species:	Plankton community of a freshwater pond
Common name:	Not applicable
Source of test species:	Artificial introduction of various species, as well as spontaneous development of plants, colonization by insects etc. during 1-year stabilization period (pre-treatment)

4. Culture conditions of test organism(s)

Not applicable

B. Study design and methods

1. Test procedure

Test system: Mesocosm study

Mesocosms were prepared in 2002 (filled with 7 m³ tap water and 400 L of sediment, artificial inoculation of aquatic organisms) and allowed to stabilize for about 1 year prior to treatment. The mesocosms are circular 9 m² outdoor tanks (3.2 m diameter x 0.9 m depth) located in Rennes, France.

Eight ponds were treated with deltamethrin and eight were retained as untreated controls

In the present study, half of the treated and control mesocosms were provided with fine mesh lids (1 mm mesh size) immediately after treatment. The lids were designed in order to minimize immigration of organisms from outside, thus creating relatively isolated ecosystems.

Phytoplankton assessment: Chlorophyll a levels were determined weekly in depth-integrated water samples. Detailed analysis of phytoplankton community structure was performed for 5 weeks after treatment to assess immediate changes in algae populations.

Zooplankton assessment: Zooplankton dynamics were assessed in both, water column and surface sediment samples. Samples were

	collected 1 and 2 days after treatment, and then weekly (with a break between December 2003 and March 2004). All the individuals found in the samples were identified to the lowest feasible level.
Test concentration(s):	Nominal concentration of 5.0 µg a.s./L, targeted to result in a final water concentration near deltamethrin solubility (~0.2 µg/L)
Control(s):	8 untreated controls (4 open ponds, 4 covered ponds)
Number of replicates:	4 replicates per treatment
Test conditions:	For measured physico-chemical parameters see results
Feeding:	Not applicable
Medium renewal:	Not applicable
Frequency of test item application:	Single application
Test duration:	April 2003 – May 2004
Endpoints:	Dynamics of phytoplankton and zooplankton
Statistics:	Analysis of variance (ANOVA) was used to compare data of different treatments. When repeated measures (RM) ANOVA indicated a significant effect of deltamethrin on a particular zooplankton group, the abundance data were analysed for each sampling date using one-way ANOVA. Recovery for single parameters was defined as when one-way ANOVA failed to detect a difference between treatment and control ponds for two consecutive sampling dates. Changes in the structure of planktonic communities were analysed by the principal response curve (PCR) method using the most practical level of taxonomic identification for each group. Monte Carlo permutation tests were performed at each sampling date to identify the dates when a significant difference occurred between the different treatments. Recovery of the community following deltamethrin treatment was considered achieved when Monte Carl permutation tests failed to detect a difference between treated and control ponds for two consecutive sampling dates. Significance was accepted at α = 0.05 for all statistical tests.

2. Measurements during the test

Water/medium parameters:	For measured physico-chemical parameters see results.

3. Sampling

Sampling frequency:	Depth-integrated water samples for deltamethrin analysis were collected using polyvinyl chloride tube samplers. 15 min, and 4, 24, 48, 96, and 168 h after treatment
Transport/storage of samples:	Water samples stored in amber glass bottles at -20°C

4. Chemical analysis

Guideline/protocol:	Not reported
Method:	Not reported
Pre-treatment of samples:	Extraction with dichloromethane
Conduction:	HP 5890 Series II gas chromatograph (Agilent Technologies France) equipped with an electron capture detector and a DB-1 column
Limit of detection:	Not reported
Limit of quantification:	0.025 µg/L

RESULTS

1. Analytical findings:

Maximum mean deltamethrin levels for the open and covered ponds were 0.102 and 0.125 µg a.s./L, respectively. As expected, deltamethrin levels decreased rapidly with time and were below the analytical detection limit (0.025 µg/L) before 168 h after treatment. Deltamethrin half-life in water of 35.7 and 57.3 h were calculated for open and covered ponds, respectively. Average exposure concentrations for 48 and 168 h are given in the table below.

Table A: Average exposure concentrations (AEC) of deltamethrin in pond water according to Van Wijngaarden et al. (1996); mean value ± standard error (n=4)

	Open mesocosms	**Covered mesocosms**
Maximum mean	0.102 (0.02) µg/L	0.125 (0.03) µg/L
AEC_{48h}	0.072 (0.018) µg/L	0.099 (0.028) µg/L
AEC_{168h}	0.031 (0.006) µg/L	0.045 (0.015) µg/L

2. Other measurements:

In general neither the lids, nor deltamethrin had broad impacts on water conditions in the mesocosms.

Table B: Mean (SE[a]) and range of variation of physical and chemical parameters measured in water for the four groups of mesocosms during the whole measurement period

Parameter	Open control		Covered control		Open deltamethrin		Covered deltamethrin	
	Mean (SE)	Min-max[b]	Mean (SE)	Min-max[b]	Mean (SE)	Min-max[b]	Mean (SE)	Min-max[b]
Water temperature [°C]	14.10 (0.40)	1.5-26.7	14.37 (0.39)	4.1-26.8	14.12 (0.40)	1.4-26.8	14.31 (0.39)	4.1-26.4
Dissolved oxygen [mg/L]	12.39 (0.16)	7.1-22.2	12.30 (0.18)	4.85-23.4	12.57 (0.15)	3.52-23.1	12.12 (0.18)	5.97-24
pH	9.56 (0.04)	7.83-10.91	9.53 (0.04)	6.51-11.15	9.7 (0.04)	7.8-11.18	9.42 (0.04)	7.94-10.75
Conductivity [µS/cm]	231.5 (2.8)	160-367	225.9 (2.7)	144-335	244.0 (2.8)	150-342	255.5 (2.8)	165-368
Total nitrogen [mg/L]	1.20 (0.14)	0.30-2.60	1.45 (0.18)	0.30-2.80	1.28 (0.16)	0.40-2.70	1.44 (0.16)	0.40-2.60
Total phosphorus [µg/L]	27.7 (2.52)	4.1-103.2	25.7 (2.52)	ND[c]-84.4	32.4 (2.78)	2.0-76.4	36.8 (4.25)	ND-176.0

[a] SE = standard error of the mean

[b] Range (minimum to maximum) for the whole measurement period

[c] ND = not detected

4. Biological findings:

Phytoplankton: Analysis of data from the whole study period indicated that mean chlorophyll a concentrations significantly varied with time ($p<0.001$), but no significant effect of deltamethrin or presence of lids was observed. Monthly analysis showed that deltamethrin did transiently affect chlorophyll a levels in May 2003. However, outside of this window neither deltamethrin, nor the lids

had any significant effect on chlorophyll a levels in the ponds.
A total of 79 different phytoplankton taxa were identified in the mesocosms. Chlorophyceae (green algae) was the most diverse class (27 taxa), followed by Bacillariophyceae (22 taxa) and Cyanobacteria (18 taxa). Principal response curve analysis of phytoplankton abundance data showed a significant effect of deltamethrin. However, this effect was only transient, and recovery of phytoplankton community structure was apparent within 4 weeks following the application.

Zooplankton: A total of 22 zooplankton taxa were identified.
Water column zooplankton: Copepod nauplii and rotifers of the genus *Keratella* were the most abundant zooplankton organisms in the water column in the open control ponds. The PRC analysis showed a highly significant effect of deltamethrin treatment. Monte Carlo permutation tests per sampling date indicated that the response of water column zooplankton to deltamethrin was immediate and statistically identical in the open and covered treated ponds. Recovery of the community occurred quite rapidly (within 28 days) in all ponds.
When water column data for the whole study period was considered, a negative effect of deltamethrin on the abundance of Daphniidae was detected, whereas the effect was positive for copepod nauplii in both open and covered ponds. Although PCR analysis indicated that recovery of the community occurred within 28 d, one-way ANOVA per sampling date showed that recovery of Daphniidae in the water column occurred more slowly (between 77 and 105 d after deltamethrin application). Deltamethrin also caused a reduction in the abundance of adults and copepodites of calanoids in the water column during the first 3 months post exposure. One-way ANOVA showed per sampling date showed, that the effects were larger in the open mesocosms and that the reduction in abundance of both life stages was significant for only 2 d after deltamethrin application. Recovery of these groups was noted as soon as 14 d after the treatment.

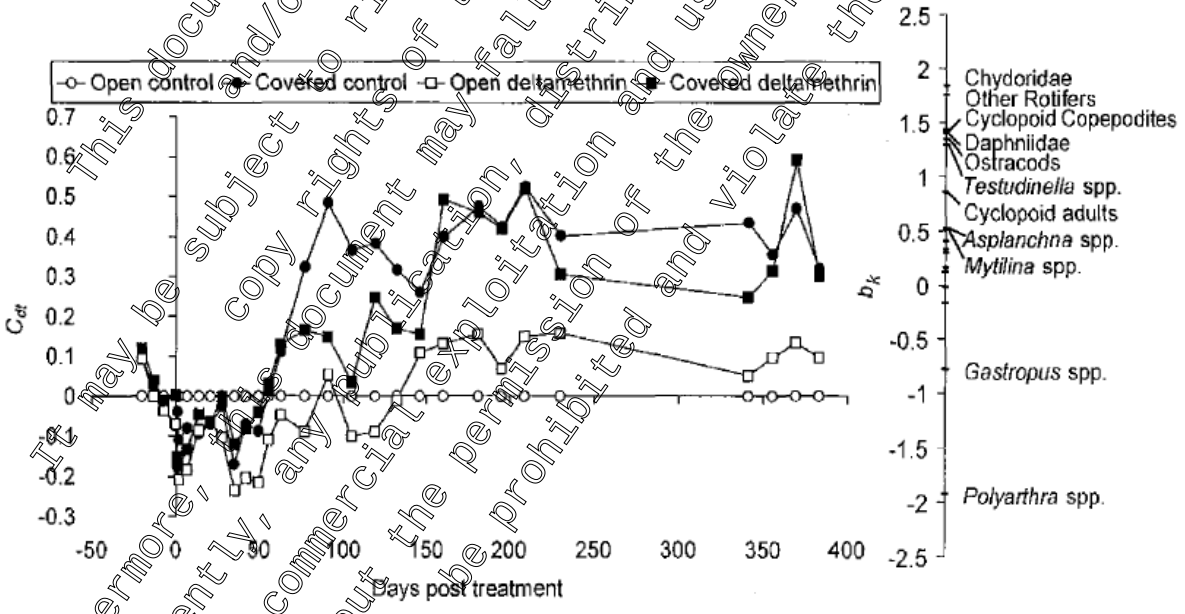

Fig. 5. Principal response curves (PRC) resulting from the analysis of water column zooplankton data set. The lines represent the course of the treatments in time. The vertical axis represents the difference in community structure between treatments and the controls expressed as regression coefficients (C_{dt}) of the PRC model. The species weight (b_k) can be interpreted as the affinity of the taxon to the principal response. Only species with a weight superior to 0.5 or inferior to −0.5 are shown.

Sediment surface zooplankton: The most abundant taxa in the sediment surface samples averaged over the whole experiment were Chydoridae, Ostracoda and other rotifers. As for the water column, PRC analysis of sediment zooplankton showed a significant effect of deltamethrin. Monte Carlo permutation

tests per sampling date indicated that deltamethrin affected the structure of the zooplankton community as soon as 2 d after application in both the open and covered mesocosms. The effects of the insecticide were seen over the first 2 weeks following treatment and PRC analysis indicated that recovery occurred within 42 d.

Effect of lids on zooplankton community: Monte Carlo permutation tests per sampling date indicated that the presence or absence of lids had an effect on water column zooplankton structure in control and treated ponds from 5 months after the application to the end of the study. Reduction in abundance due to the lids was noted in water column samples for *Polyarthra* spp., *Gastropus* sp., and *Chaoborus* larvae, whereas increases in abundance were seen for copepodites and adults of cylopoids, Daphniidae and Chydoridae, and various rotifers.

Monte Carlo permutation test per sampling date also indicated a small lid effect on sediment surface zooplankton, especially in spring 2004, 1 year after deltamethrin application. Increases in abundance due to the lids were seen for Chydoridae, whereas reductions were observed for *Polyarthra* spp. Further, with the exception of *Chaoborus* larvae in the sediment surface samples, effects of the lids were primarily seen later in the experiment with impacts being observed over the first 3 months.

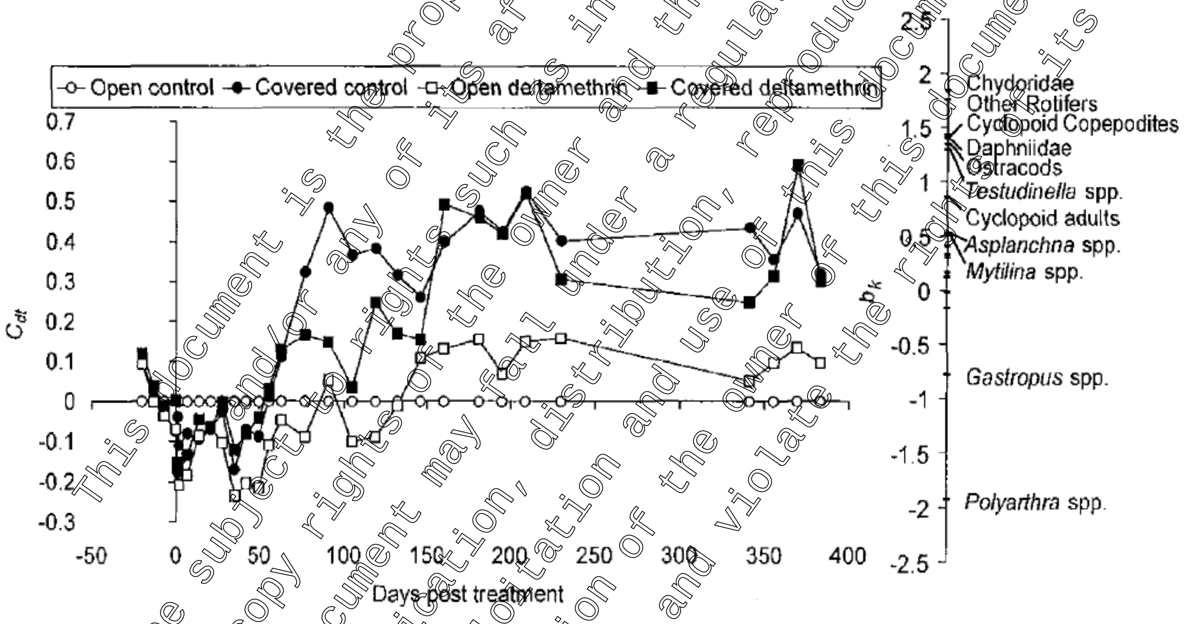

Fig. 5. Principal response curves (PRC) resulting from the analysis of water column zooplankton data set. The lines represent the course of the treatments in time. The vertical axis represents the difference in community structure between treatments and the controls expressed as regression coefficients (C_{dt}) of the PRC model. The species weight (b_k) can be interpreted as the affinity of the taxon to the principal response. Only species with a weight superior to 0.5 or inferior to −0.5 are shown.

CONCLUSION

Principal response curves analysis shows that immediate response of zooplankton to deltamethrin was functionally identical in open and covered ponds. Further, general patterns of survival among groups are in agreement with previous field studies on pyrethroids in freshwater systems that showed cladocerans as very sensitive, copepods less affected, and rotifers positively or neutrally affected by the insecticide.

Overall, the results presented here clearly indicate that the recovery of zooplankton following the addition of a rapidly disappearing insecticide to freshwater ponds primarily depends upon internal rather than on external recovery mechanisms. The dynamics of recovery, however, is influenced by many factors, including the availability of dormant forms (e.g. resting eggs of Daphniidae) and/or the presence and activity of predators (e.g. Chaoborus larvae).

Comment by the Notifier:

The intention of the study was to study recovery after a rapid and significant decrease in the abundance of arthropods in a series of freshwater outdoor lentic mesocosms.
Deltamethrin was specifically chosen because it degrades rapidly after release to aquatic systems and its degradation products are relatively non-toxic thus creating an instantaneous stress impact and allowing recovery to initiate almost immediately after the impact of the stressor with no residual suppression of populations due to the presence of the chemical.
The nominal test concentration of 5 µg a.s./L (maximum measured concentration: 0.125 µg a.s./L) is far above the suggested regulatory acceptable concentration, and therefore the results are only of limited value for the risk assessment. However, the results demonstrate that with time the systems recover even after this high application rate.
Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	**KCA 8.2.8/05; [redacted]; 2007**
Title:	Influence of isolation on the recovery of pond mesocosms from the application of an insecticide. II. Benthic macroinvertebrate responses
Source:	Environmental Toxicology and Chemistry, Vol. 26, No. 6, pp. 1280-1290
DOI No:	-
Document No:	M-294188-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The immediate response and recovery of the macrobenthic communities of non-isolated and isolated freshwater outdoor 9 m³ mesocosms following an acute stress caused by the addition of deltamethrin were studied over a 14-month period. To discriminate between internal and external recovery mechanisms, half of the treated ponds were covered by 1 mm mesh lids that restricted aerial recolonization. Both structural (abundance of the different taxonomic groups) and functional (litter breakdown) parameters were monitored. Insects were broadly reduced in numbers by deltamethrin addition. In general, non-insect groups were not affected or increased in abundance in deltamethrin-treated ponds, probably because of relative insensitivity to deltamethrin, reduced predation, and lower competition for food. No major change in litter breakdown rates was seen, probably because of functional redundancy among the macrobenthic community. Chironominae larvae recovered in open, treated mesocosms 42 d after deltamethrin addition and most insect groups recovered 84 d after the treatment date. However, the presence of lids significantly reduced insect recovery rate, suggesting that it largely depends on the immigration of winged forms (i.e., external recovery) from surrounding non- or less affected systems. These results indicate that the recovery time of macrobenthic communities in an affected natural pond would depend on spatial characteristics of the landscape and also the season that exposure occurs. Isolated ecosystems would display post-treatment insect recovery dynamics very different from highly connected ones, evolving toward alternate pseudo-equilibrium
states, possibly with lower biodiversity but with preserved functionality. Consequences for higher tier risk assessment of pesticides are discussed.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin dissolved in an acetone-water mixture (5.8 mL acetone stock per 1 L water)
Active substance(s):	Deltamethrin
Chemical state and description:	Liquid; active substance dissolved in an acetone-water mixture
Source of test item:	[redacted], Germany
Batch number:	Not reported
Purity:	99%
Storage conditions:	Not reported
Water solubility:	0.2

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	5.8 mL acetone stock per 1 L water in "stock solution"
Method of preparation:	Not specified
Evidence of unsolved material:	Not reported

3. Test organism(s)

Species:	Macrobenthic communities of a freshwater pond
Common name:	Not applicable
Source of test species:	Artificial introduction of various species, as well as spontaneous development of plants, colonization by insects etc. during 1-year stabilization period (pre-treatment)

4. Culture conditions of test organism(s)

Not applicable

B. Study design and methods

1. Test procedure

Test system: Mesocosm study

Mesocosms were prepared in 2002 (filled with 7 m³ tap water and 400 L of sediment, artificial inoculation of aquatic organisms) and allowed to stabilize for about 1 year prior to treatment. The mesocosms are circular 9 m² outdoor tanks (3.2 m diameter x 0.9 m depth) located in Rennes, France.

Eight ponds were treated with deltamethrin and eight were retained as untreated controls

In the present study, half of the treated and control mesocosms were provided with fine mesh lids (1 mm mesh size) immediately after treatment. The lids were designed in order to minimize immigration of organisms from outside, thus creating relatively isolated ecosystems.

Periphyton samples: Samples for the periphyton assessment were obtained with the use of glass microscope slides, which were put in suspended samplers located 20 cm below the water surface. Five slides per sampler, three samplers per mesocosm. The slides were maintained in the mesocosm for 3 weeks, then replace by new ones and stored at -20°C. One side of each slide was used for

chlorophyll a determination, whereas the other side was used to estimate periphyton ash-free dry weight.

Benthic invertebrates: A stratified sampling strategy based on the use of artificial substrates was implemented in each mesocosm. Sediment dwelling organisms were collected with triplicate multiplate samplers. Macrophyte-dwelling invertebrates were samples with the use of substrates made of four roughened polyethylene tubes, also in triplicate. Every three weeks, the two types of samplers were replaced. The collected organisms were identified at the lowest practical taxonomic level.

Emerging insects: Three 20 cm-diameter polyethylene funnels, topped by a transparent wasp trap filled with 50 mL of neutral aqueous formaldehyde, were suspended in each mesocosm, close to the macrophyte dwelling invertebrate samplers. The traps were emptied weekly, with individuals being identified at the most practical taxonomic level.

Litter breakdown: Three grams of air-dried alder (*Alnus glutinosa*) leaves, collected at abscission, were enclosed in two types of bags to assess litter breakdown. In coarse mesh bags (5 mm mesh size), litter degradation would depend on both the shredding of the leaves by invertebrates and microbial degradation, whereas in fine mesh bags (0.25 mm mesh size), only microbial degradation would occur. Fifteen bags of each type were introduced in the mesocosms. The bags were then collected in triplicate every 3 weeks. Contents were sorted, leaf residues oven-dried to a constant weight. Macroinvertebrates found in the bags were enumerated and identified. Change in leave weight over time was used to estimate the litter breakdown.

Test concentration(s):	Nominal concentration of 5.0 µg a.s./L, targeted to result in a final water concentration near deltamethrin solubility (~0.2 µg/L)
Control(s):	8 untreated controls (4 open ponds, 4 covered ponds)
Number of replicates:	4 replicates per treatment
Test conditions:	For measured physico-chemical parameters see results
Feeding:	Not applicable
Medium renewal:	Not applicable
Frequency of test item application:	Single application
Test duration:	April 2003 – May 2004
Endpoints:	Dynamics of benthic macroinvertebrate communities
Statistics:	Changes in the structure of invertebrate communities were analysed by the principle response curve (PRC) method, in which abundance values were ln (2x+1) transformed before analysis. Data for both types of substrate samplers were grouped before analysis. A Monte Carlo permutation test was used to identify dates for which a significant difference was apparent among treatments. This test was also used to determine community recovery, which was defined was the moment when the Monte Carlo permutation test failed to detect a difference between deltamethrin-treated and control mesocosms for two consecutive sampling dates. Two-way RM-ANOVA was used to assess the effect of treatment on the abundance of different taxa on the benthic substrates and the emergence traps, respectively. Different time periods were considered in the analysis: the whole study period to detect overall effects if the 4 treatments, and five separate time periods to

identify transient effects that might have occurred at specific times over the whole experiment. Abundance data were ln (x+1) transformed before analysis.

When repeated measures (RM)-ANOVA indicated a significant immediate effect of deltamethrin on the abundance of an invertebrate group, the abundance data were analysed for each sampling date using one-way ANOVA. Recovery was defined as when one-way ANOVA failed to detect a difference between treatment and control ponds for two consecutive sampling dates.

Data were analysed separately for the open and covered ponds to detect differences in recovery dynamics associated with isolation.

It should be noted that data on the abundance of emerging insects were not analysed with this approach, because emergence displays a clear seasonal pattern, which could lead to false positives.

Finally, the effects of deltamethrin and isolation on litter breakdown were analysed by two-way ANOVA for coarse and fine mesh bags, respectively.

2. Measurements during the test

Water/medium parameters: Dynamics of phytoplankton and zooplankton

3. Sampling

Sampling frequency: Depth-integrated water samples for deltamethrin analysis were collected using polyvinyl chloride tube samplers

15 min, and 4, 24, 48, 96, and 168 h after treatment

Transport/storage of samples: Water samples stored in amber glass bottles at -20°C

4. Chemical analysis

Guideline/protocol: Not reported

Method: Not reported

Pre-treatment of samples: Extraction with dichloromethane

Conduction: HP 5890 Series II gas chromatograph (Agilent Technologies France) equipped with an electron capture detector and a DB-1 column

Limit of detection: Not reported

Limit of quantification: 0.025 µg/L

RESULTS

1. Analytical findings:

The analytical results summarized for [redacted] (2007; M-294182-01-1) do also apply for this publication, which reports results from the very same mesocosm set-up.

3. Other measurements:

For result of measured physical and chemical parameters, reference is made to the summary of [redacted] (2007; M-294182-01-1).

4. Biological findings:

Periphyton. Chlorophyll a concentration and ash-free dry weight (AFDW) significantly varied with time, but did not show significant differences as a result of deltamethrin application or the presence of lids. A transient negative effect of deltamethrin was noted on the periphyton AFDW on two sampling dates. However, deltamethrin did not significantly affect periphyton chlorophyll a levels.

Benthic macroinvertebrates: Approx. 40 macroinvertebrate taxa were identified in the samples collected during the study period. Deltamethrin addition clearly killed many insects, as indicated by the numerous dead adults (e.g. backswimmers) seen on the water surface just after treatment, and PRC analysis of benthic macroinvertebrate data showed a highly significant effect of deltamethrin (p=0.002; Fig. 2). Furthermore, Monte Carlo permutation tests for each sampling date indicated that the response of benthic macroinvertebrates to deltamethrin was immediate and identical in both open and covered treated ponds, and that recovery occurred approx. 84 d after deltamethrin application in the open ponds. Figure 2 also shows that the lids had a significant effect on the structure of macrobenthic communities in both control and treated ponds. In fact, the lids had a greater effect on community structure than deltamethrin addition after the immediate effect of the insecticide in the treated ponds.

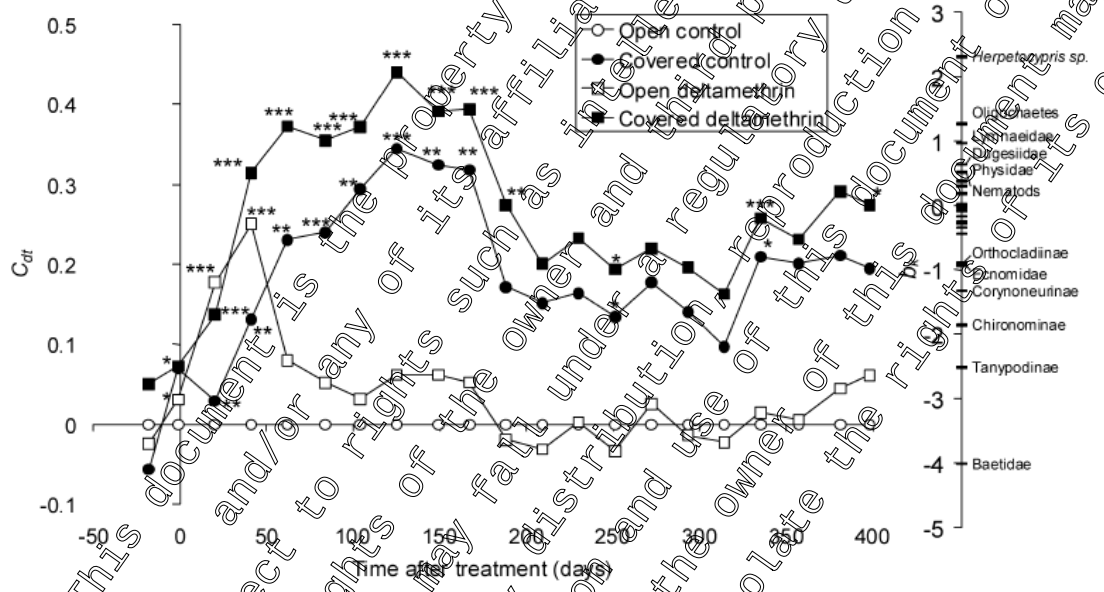

Fig. 2. Principal response curves resulting from the analysis of the benthic macroinvertebrate data set. The lines represent the course of the treatments in time. The vertical axis represents the difference in community structure between treatments and the controls expressed as regression coefficients (C_{dt}) of the principal response curves model. The species weight (b) can be interpreted as the affinity of the taxa to the principal response curves. Only species with a weight superior to 0.2 or inferior to −0.3 are shown. Results for Monte Carlo permutation test for each sampling date: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

Recovery patterns of benthic macroinvertebrates: The pattern of recovery among benthic macroinvertebrates was different in open and covered ponds. Larval Chironominae were the first insects to recover in the open, treated ponds (62 d after deltamethrin application), whereas most other groups (e.g. larvae of Baetidae, Corynoneurinae, and Ecnomidae and pupae of Chironomidae) and overall invertebrate biodiversity recovered 84 d after the application date. Larvae of Orthocladiinae and Caenidae took longer to recover: 104 and 109 d after deltamethrin application, respectively.

Recovery in the covered, treated mesocosms was very delayed relative to the open systems. For example, recovery took 149 d for overall community biodiversity and Chironominae larvae, 167 d for Chironomidae pupae, and 209 d for Coryneunorinae larvae. Recovery of Chironominae larvae was only transitory because no larvae were detected in either control or treated covered ponds after September 18, 2003. Furthermore, no recovery was observed for Ecnomidae larvae. Finally, Asselidae completely disappeared in all mesocosms, except in two covered control ponds, and Baetidae, Caenida, and Orthocladiinae larvae completely disappeared in the covered control ponds.

Emerging insects: The results observed for emerging insects are consistent with those reported for larvae. PRC patterns showed a highly significant effect of deltamethrin and covering on emergence. The PRC diagram clearly shows that deltamethrin application immediately changed the taxonomic structure of emerging insects. Recovery was observed approx. 84 d after deltamethrin application in the open ponds (it was slower in the covered ones), and covering significantly reduced the abundance of emerging insects in all treatments. Figure 3 shows that the effect of the lids on emerging insect abundances was less than that due to deltamethrin application. The apparent recovery in the covered ponds between 200 and 350 d after deltamethrin application is explained by the naturally sharp decrease of emergence during winter months (i.e. roughly between November and March).

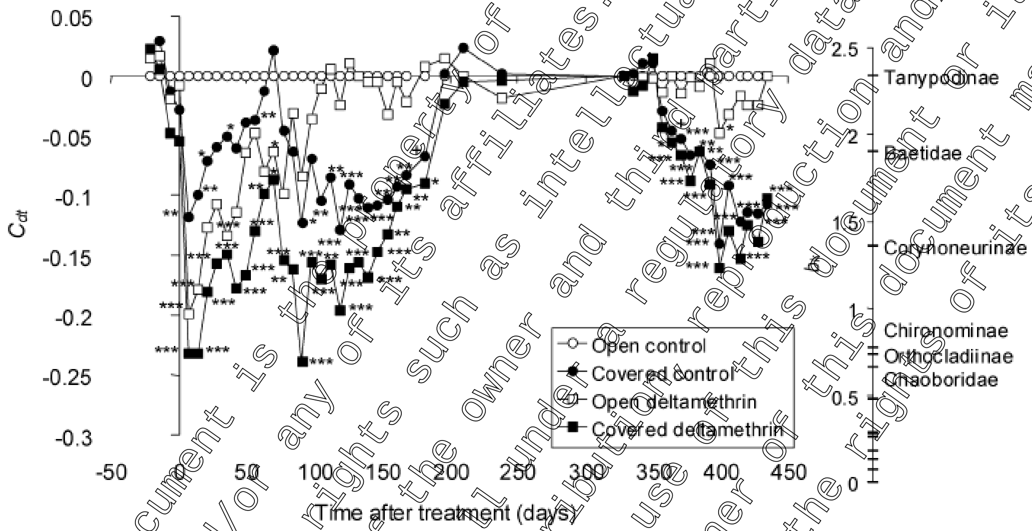

Fig. 3. Principal response curves resulting from the analysis of the emerging insect data set. The lines represent the course of the treatments in time. The vertical axis represents the difference in community structure between treatments and the controls expressed as regression coefficients (C_{dt}) of the principal response curves model. The species weight (b_k) can be interpreted as the affinity of the taxa to the principal response curves. Only species with a weight superior to 0.5 are shown. Results for Monte Carlo permutation test for each sampling date: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$; + $0.05 < p < 0.10$.

Litter breakdown: Deltamethrin application and isolation did not affect the litter breakdown. Snails of the families Physidae and Lymnaeidae were the most abundant invertebrates in the coarse mesh bags over the whole study period.

CONCLUSION

In contrast to the results obtained for zooplankton ([redacted] 2007; M-294182-01-1), results presented in this publication clearly indicate that the recovery of deltamethrin-sensitive species mostly depended on external rather than internal recovery.
The results obtained for emerging insects confirm that seasonality must be considered when assessing effects. Therefore, it is suggests that recovery assessment should primarily focus on benthic rather than on flying forms of insects, and studies should focus on the year periods when biodiversity and abundance of the communities are maximal.
In spite of their effects on the structure of the macroinvertebrate community, neither deltamethrin nor isolation had any effect on litter breakdown. This suggests that functional endpoints are less sensitive to deltamethrin than structural features of the macroinvertebrate community. Hence, an impoverishment of the macroinvertebrate community might not always be associated with a loss in ecological function because of the existence of ecological redundancy among species.

Comment by the Notifier:

The intention of the study was to study recovery after a rapid and significant decrease in the abundance of arthropods in a series of freshwater outdoor lentic mesocosms.
Deltamethrin was specifically chosen because it degrades rapidly after release to aquatic systems and its degradation products are relatively non-toxic thus creating an instantaneous stress impact and allowing recovery to initiate almost immediately after the impact of the stressor with no residual suppression of populations due to the presence of the chemical.
The nominal test concentration of 5 µg a.s./L (maximum measured concentration: 0.125 µg a.s./L) is far above the suggested regulatory acceptable concentration, and therefore the results are only of limited value for the risk assessment. However, the results demonstrate that with time the systems recover even after this high application rate.
Therefore, the information is classified as b) supplementary information (EFSA Journal 2011; 9(2): 2092).

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

New studies referring to the intrinsic toxicity of deltamethrin to bees, conducted since the first Annex I inclusion process are summarised in this document. Moreover, there is one old publication dating 1977, which investigated the oral and contact toxicity of deltamethrin. Two further studies with a technical concentrate of deltamethrin a summarised here in addition to complete the database. For all studies submitted during the frame of the first Annex I inclusion, please refer to the corresponding section in the Monograph and in the baseline dossier provided by Bayer CropScience.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	**KCA 8.3.1.1.1/01, Anonymous; 1977**
Title:	Acute Toxicity of DECAMETHRINE to Honey Bees
Document No:	M-150494-01-2
Guidelines:	No applicable at this time
GLP:	No

A range of compounds was investigated in the laboratory for their oral and contact toxicity to honey bees. The contact LD_{50} of deltamethrin was determined to be 0.047 µg a.s./bee. The oral LD_{50} of deltamethrin was determined to be 0.079 µg a.s./bee.

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Report:	**KCA 8.3.1.1.1/02, [redacted]; 2013**
Title:	Effects of deltamethrin tech. (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory
Document No:	M-444971-01-1 (Rep. No: 73581035)
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	Yes

Material and Methods:
Deltamethrin tech.: 99.9 % w/w (analytical), Origin Batch No.: ABKBDCK008; Batch Code: AE F032640-01-11; Customer Order No.: TOX-No: 09084-01; Specification No.: 102000001388; LIMS No.: 1212051.
Under laboratory conditions *Apis mellifera* 30 worker bees per treatment level were exposed for 72 hours to doses of 0.40, 0.20, 0.10, 0.05, 0.025 and 0.013 µg a.s. per bee by topical application (contact dose response test) and 30 worker bees per treatment level were exposed for 72 hours to doses of 0.70, 0.43, 0.26, 0.14, 0.091 and 0.063 µg a.s. per bee by feeding (oral dose response test, value based on the actual intake of the test item). The contact and oral tests were prolonged for further 24 hours up to 72 hours due to increasing mortality between 24/48 hours.

Findings
Toxicity to Honey Bees; laboratory tests

Test Item	Deltamethrin tech.	
Test object	*Apis mellifera*	
Exposure	oral (sugar syrup/acetone/water)	contact (solution in acetone)
Application rate µg a.s./bee	0.70, 0.43, 0.26, 0.14, 0.091 & 0.063	0.40, 0.20, 0.10, 0.050, 0.025 and 0.013
LD_{50} µg a.s./bee	24 hours: 0.22 48 hours: 0.20 72 hours: 0.19	24 hours: > 0.40 48 hours: 0.12 72 hours: 0.11
LD_{20} µg a.s./bee	24 hours: 0.11 48 hours: 0.11 72 hours: 0.11	24 hours: 0.083 48 hours: 0.061 72 hours: 0.062
LD_{10} µg a.s./bee	24 hours: 0.08 48 hours: 0.08 72 hours: 0.08	24 hours: 0.037 48 hours: 0.043 72 hours: 0.046
NOED µg a.s./bee*	24 hours: 0.091 48 hours: 0.091 72 hours: 0.091	24 hours: 0.025 48 hours: 0.025 72 hours: 0.025

Contact Test:
The contact toxicity test was prolonged for a further 24 hours up to 72 hours due to increasing mortality between 24/48 hours. Dose levels of 0.40, 0.20, 0.10 and 0.05 µg a.s./bee resulted in mortality of 96.7, 83.3, 33.3 and 20.0 % at test termination (72 hours). No mortality occurred in the 0.025 and 0.013 µg a.s./bee dose levels. There was 3.3 % mortality in the control group (water + 0.5 % Adhäsit) and 0.0 % in the solvent control group, respectively. Over the entire time of the test (72 hours) behavioural abnormalities (e.g. vomiting, movement coordination problems and/or apathy) were observed in the 0.40, 0.20 and 0.10 µg a.s./bee dose level groups. In the 0.050 µg a.s./bee dose group these behavioural abnormalities occurred during the 4 and 24-hrs assessment. In the 0.025 and 0.013 µg a.s./bee dose levels behavioural impairments only occurred 4-hrs following treatment.

Oral Test:
The oral toxicity test was also prolonged for a further 24 hours up to 72 hours due to increasing mortality between 24 and 48 hours. The maximum nominal dose levels of the test item (2.0, 1.0, 0.50, 0.25 and 0.13 µg a.s./bee) could not be achieved, because the bees did not ingest the full volume of treated sugar syrup solution even when offered over a period of 6 hours. Actual oral doses of 0.70, 0.43, 0.26, 0.14 and 0.091 µg a.s. per bee resulted in mortality ranging from 90.0 % to 10.0 % at the end of the test (after 72 hours). No mortality occurred in the 0.063 µg a.s./bee treatment as well as in

the water and solvent control groups, respectively. During the 4 hours and 24 hours assessments discoordinated movements and/or apathy were observed in all treatment groups (with exception of the 0.091 µg a.s./bee dose group). 24 hours following start of treatment a few bees were found apathetic in the 0.70, 0.43 and 0.26 µg a.s./bee dose levels. One bee was found apathetic during the 72 hours assessment in the 0.70 µg a.s./bee dose group. No more test item related behavioural abnormalities were found until the end of the test

Conclusion
The toxicity of deltamethrin tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} values (48 h and 72 h) of deltamethrin tech. were determined to be 0.12 and 0.11 µg a.s./bee, respectively. The oral LD_{50} values (24 h, 48 h + 72 h) were 0.22, 0.20 and 0.19 µg a.s./bee, respectively.

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Report:	**KCA 8.3.1.1.1/03, [redacted]; 1996**
Title:	Deltamethrin; Code: RU 22974 - Oral toxicity (LD 50) to honey bees (*Apis mellifera* L.)
Document No:	M-140579-01-1 (Rep. No: CW 94/084)
Guidelines:	EPPO 170
GLP:	Yes

Objective:
The objective of this study was to investigate the effects of deltamethrin as a stomach poison (LD_{50}) on adult honey bees by oral application of the test substance.

Materials and methods:
In an acute laboratory study the oral toxicity of deltamethrin on honey bees was tested. Adult worker bees were treated with 5 dose rates of deltamethrin in the diet (0.00001, 0.00005, 0.0001, 0.0005 and 0.0010% a.s.). The ingested dose rates per bee were 0.0010, 0.006, 0.0046, 0.0075 and 0.0104 µg/bee. Hoe 002960 00 EC40 C660 (ingested dose rates: 0.0768, 0.1164, 0.1459, 0.3313 and 0.5149 µg a.s./bee) was used as toxic reference substance. A 50% aqueous sucrose solution served as negative control. 5 replicates, each with 10 bees per cage were used. The mortality was assessed 24, 48 and 72 h after application.

Findings:
A summary of the acute oral toxicity of deltamethrin to honey bees is given in the table below.

Acute oral toxicity of deltamethrin to honey bees

Active substance in diet [%]	Ingested dose per bee [µg a.s./bee]	Mortality [%] After 24 h	After 48 h	After 72 h
Deltamethrin				
Control	--	0	0	2
0.00001	0.0010	2	4	6
0.00005	0.0006	0	2	2
0.0001	0.0046	2	2	2

		Mortality [%]		
Active substance in diet [%]	**Ingested dose per bee [µg a.s./bee]**	**After 24 h**	**After 48 h**	**After 72 h**
0.0005	0.0075	38	38	38
0.0010	0.0104	24	24	26
Reference substance: Hoe 002960 00 EC40 C660				
Control	--	0	0	2
0.0003	0.0768	6	6	6
0.0006	0.1164	18	20	22
0.0012	0.1459	32	32	32
0.0024	0.3313	60	60	60
0.0048	0.5149	100	100	100

The mortality was 38% in the treatment group ingested 0.0075 µg a.s./bee and 26% in the treatment group ingested 0.0104 µg a.s./bee. In the other treatment groups mortality was ≥ 6%.

Oral toxicity LD_{50} values of bees treated with deltamethrin

	LD_{50} oral [µg a.s./bee]		
	24 h	**48 h**	**72 h**
Test item	0.019	0.028	0.023
Toxic standard (Hoe 002960 00 EC40 C660)	0.213	0.211	0.215

Conclusion:
The LD_{50} was 0.028 µg deltamethrin/bee after 48 h and 0.023 µg deltamethrin/bee after 72 h.

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CA 8.3.1.1.2 Acute contact toxicity

In the study by [redacted] (2013) the acute oral and contact toxicity was assessed together.

Report:	**KCA 8.3.1.1.2/01, [redacted]; 2013**
Title:	Effects of deltamethrin tech. (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory
Document No:	M-444971-01-1 (Rep. No.: 73581035)
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	Yes

This study is presented under point KCA 8.3.1.1.1.

In the studies which are summarized below, only the contact toxicity was assessed.

Report:	**KCA 8.3.1.1.2/02, [redacted]; 1996**
Title:	Deltamethrin (Code: RU 22974): Contact toxicity (LD_{50}) to honey bees (*Apis mellifera* L.)
Document No:	M-149608-01-1 (Rep. No: CW 94/083)
Guidelines:	USEPA 141-1, EPPO 170
GLP:	Yes

Objective:
The objective of this study was to investigate the effects of deltamethrin as a contact poison (LD_{50}) on adult honey bees by topical application of the test substance.

Material and methods:
In an acute laboratory study the contact toxicity of deltamethrin on honey bees was tested. Adult worker bees were treated with 5 dose rates of Deltamethrin: 0.0001, 0.0005, 0.001, 0.005 and 0.010% a.s., corresponding to 0.001, 0.005, 0.01, 0.05 and 0.1 µg/bee. Hoe 002960 00 EC40 C660 was used as toxic reference substance at concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05% w/w product corresponding to 0.04, 0.08, 0.12, 0.16 and 0.2 µg a.s./bee. Acetone (dilutent for the test substance) and drinking water (dilutent for the reference substance) served as negative controls. 5 replicates, each with 10 bees per cage were used. The mortality was assessed 24, 48 and 72 h after application.

Findings:
A summary of the acute contact toxicity of deltamethrin to honey bees is given in the table below.

Acute contact toxicity of Deltamethrin to honey bees

Concentration [µg a.s./bee]	Mortality [%] After 24 h	After 48 h	After 72 h
Deltamethrin			
Control	0	01	1
0.001	0	2	3
0.005	16	16	16
0.01	19	20	20
0.05	38	40	41
0.1	45	49	50
Reference substance: Hoe 002960 00 EC40 C660			
Control	0	0	1
0.04	0	0	0
0.08	18	19	20
0.12	47	47	48
0.16	49	49	49
0.2	50	50	50

The mortality was 16, 20, 41 and 50% in the 0.005, 0.01, 0.05 and 0.1 µg a.s./bee treatment groups.

Contact toxicity LD_{50} values of bees treated with deltamethrin

	LD_{50}, contact [µg a.s./bee] (95% confidential limits)		
	24 h	**48 h**	**72 h**
Test item	0.015 (0.011 – 0.020)	0.012 (0.009 – 0.015)	0.012 (0.009 – 0.015)
Toxic standard (Hoe 002960 00 EC40 C660)	0.087 (0.080 – 0.093)	0.086 (0.079 – 0.092)	0.085 (0.077 – 0.091)

Conclusion:
The LD_{50} was 0.012 µg deltamethrin/bee after 48 h and after 72 h.

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Report:	**KCA 8.3.1.1.2/03, [redacted]; 2013**
Title:	Deltamethrin (tech.): Acute Contact Toxicity to the Bumble bee, *Bombus terrestris* L. under Laboratory Conditions
Document No:	M-477381-01-1 (Rep. No: S13-04467)
Guidelines:	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010), OECD Guideline 214 (1998), and on the review article of VAN DER STEEN (2001)
GLP:	Yes

Materials and Methods:

Test item:
- Name: Deltamethrin (tech.)
- TOX number: 09084-04
- Origin batch number: ABKBP9CK008
- Purity: 99.9 % w/w (analysed)

The contact toxicity of deltamethrin (tech.) to the bumble bee (*Bombus terrestris* L.) was determined in a dose-response test according to OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, bumble bees were exposed to 3.5, 7, 14, 28 and 56 µg a.s./bumble bee by topical application. Mortality and sub-lethal effects were assessed 24, 48, 72 and 96 hours after treatment. The control groups were exposed for the same period of time under identical exposure conditions to tap water and acetone, respectively.

Dates of work: 23 October 2013 – 10 November 2013

Findings:

In both control groups, treated either with tap water or acetone, no mortality was observed during the 96 h test period.

In the test item treatment group, a mortality of 73.3 % was observed at the highest dose level of 56 µg a.s./bumble bee at the final assessment after 96 hours.

In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

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

Deltamethrin (tech.)	Contact toxicity test [µg a.s./bumble bee]
LD_{50} (24 h)	> 56
LD_{50} (48 h)	36.8
LD_{50} (72 h)	37.4
LD_{50} (96 h)	36.0

In the test item treatment group, affected or moribund bumble bees were observed over all tested dose levels at the 24, 48 and 72 hour assessments. At the final assessment, 96 hours after start of the experimental phase, no remarkable sub-lethal effects were noticed.

The test item dose level of 14 µg deltamethrin a.s./bumble bee was determined to be the NOLD (No Observed Effect Dose) for mortality.

Conclusion:

The 96 hour contact LD_{50} value for deltamethrin (tech.) was determined to be 36 µg deltamethrin a.s./bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Deltamethrin EW 15, as technical deltamethrin is only very slightly soluble in water.

Report:	**KCA 8.3.1.2/01**
Title:	Deltamethrin EW 15B G - Assessment of Chronic Effects to the Honeybee, *Apis mellifera L.*, in a 10 Days Continuous Laboratory Feeding Test
Document No:	M-477250-01-1 (Rep. No.: S13-00151)
Guidelines:	No specific guidelines are available.
GLP:	Yes

Materials and Methods:

Test item:	Name:	Deltamethrin EW 15B G
	TOX-No.:	09629-00
	Batch-No.:	2012-000065
	Content of active substance (a.s.):	1.58 % w/w (analysed)

The chronic effects of the test item Deltamethrin EW 15B G on the honey bee, *Apis mellifera* L., were assessed in a 10 days continuous feeding test in the laboratory.

Over a period of 10 days, honey bees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing nominally 2, 6, 18, 54 and 162 mg a.s./kg of the test item Deltamethrin

EW 15B G 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 continuous 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 in an independent study by [redacted] Germany and the corresponding analytical report is attached as an integral part of this final report.

Dates of experimental work: 16 August 2013 – 11 September 2013

Findings

After 10 days of continuous exposure, mortality at all test item treatment levels of 2, 18, 54 and 162 mg a.s./kg of Deltamethrin EW 15B G were statistically significantly different when compared to the control group. Up to and including 6 mg a.s./kg, mortality after 10 days of continuous exposure was max. 10 %, and as such below the control mortality threshold level for study validity.

The cumulative control mortality was 1.0 %, as determined at the final evaluation after 10 days. The cumulative mortality at the treatment levels of 2, 6, 18, 54 and 162 mg a.s./kg Deltamethrin EW 15B G was 10.0, 0.0, 50.0, 100 and 100 %, (corrected 9.1, -1.0, 49.5, 100 and 100 %), respectively, at the final evaluation.

From the first assessment throughout the entire observation period of 10 days, at all treatment levels of Deltamethrin EW 15B G, sub-lethal effects or behavioural abnormalities were observed, showing a strong dose response dependency.

After 10 days of continuous exposure, by considering the actual food consumption of the honey bees, the accumulated nominal intake of the test item at the treatment levels of 2, 6, 18, 54 and 162 mg a.s./kg was 0.73, 1.77, 6.48, 12.48 and 8.10 µg a.s./bee, the corresponding average daily dose was therefore 0.07, 0.18, 0.65, 1.25 and 0.81 µg a.s./bee (nominal), respectively.

The overall mean daily consumption of the aqueous sucrose application (feeding) solution (i.e. the average value over 10 days per replicate) in the test item treatment groups of 6, 18 and 162 mg a.s./kg was statistically significantly different when compared to the untreated control group (29.3, 36.0 and 24.9 mg/bee at 6, 18 and 162 mg a.s./bee, respectively, compared to 44.4 mg/bee in the control group).

The mean daily consumption of the aqueous sucrose application (feeding) solution was often statistically significantly different (lower) between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison).

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 LDD_{50}

Treatment Level	Control[1]	Deltamethrin EW 15B G [mg a.s./kg][2]				
		2	6	18	54	162
Cumulative mortality after ten days of continuous exposure [%]	1.0	10.0*	0.0	50.0*	100*	100*
Corrected cumulative mortality after ten days of continuous exposure [%]	-	9.1	-1.0	49.5	100	100
Overall mean daily consumption of application (feeding) solution [mg/bee] [3]	44.4	36.9	29.3**	36.0**	41.5	24.9**
Mean nominal intake accumulated over ten test days [µg a.s./bee/10d]	-	0.73	1.77	6.48	12.48	8.10
Average daily dose (nominal) throughout ten days of continuous exposure [µg a.s./bee/d]	-	0.07	0.18	0.65	1.25	0.81
LC_{50} (95 % confidence limits)	15.1 mg a.s./kg (nominal) (11.9 to 19.3 mg a.s./kg)					
LDD_{50} (95 % confidence limits)	0.53 µg a.s./bee/day (nominal) (0.41 to 0.70 µg a.s./bee/day)					

1 Application (feeding) solution: 50 % (w/v) aqueous sucrose solution
2 Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing Deltamethrin EW 15B G
3 The mean values per replicate over the test period (non-rounded values) were used as basis for the calculation of the overall mean daily consumption of application (feeding) solution
* Statistically significantly different compared to the control; Fisher's Exact Test (Bonferroni-Holms corrected, right-sided, $p \leq 0.05$)
** Statistically significantly lower compared to the control group; Dunnett's t-Test (left sided, $p \leq 0.05$)
a.s. active substance; $LDD_{(50)}$: Lethal Dietary Dose$_{(50)}$

Analytical Results

The actual concentration of deltamethrin in the application (feeding) solutions, determined for each preparation day, was in the range from 63 to 94 % of the nominal concentration. The average actual concentration of deltamethrin over a period of 10 consecutive days per individual test item treatment level was within the range of 84 - 90 % of the nominal concentration, the overall average actual concentration of deltamethrin (over 10 consecutive days, over all treatment levels) accounted to 88 % of the nominal concentration. No residues of deltamethrin above the LOQ (10 µg/kg) were found in any of the control samples.

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 Deltamethrin EW 15B G at the treatment levels of 2, 6, 18, 54 and 162 mg a.s./kg resulted in dose-dependent effects on mortality, sub-lethal effects and behaviour.

The cumulative control mortality was 1.0 %, as determined at the final evaluation after 10 days. The cumulative mortality at the treatment levels of 2, 6, 18, 54 and 162 mg a.s./kg Deltamethrin EW 15B

G was 10.0, 0.0, 50.0, 100 and 100 %, (corrected 9.1, -1.0, 49.5, 100 and 100%) at the final evaluation, respectively. Up to and including 6 mg a.s./kg, mortality after 10 days of continuous exposure was max. 10 %, and as such below the control mortality threshold level for study validity. The overall mean daily consumption of the aqueous sucrose application (feeding) solution (i.e. the average value over 10 days) was lower at each test item treatment level when compared to the untreated control group, for some test item treatment levels the difference was statistically significant. The same holds true for the daily mean food consumption, which was in a day-by-day comparison often statistically significantly lower in the test item treatment groups when compared to control. This indicates that there was a repellent effect of the test item at all treatment levels.

The LC_{50} after 10 days of continuous exposure was determined to be 1.01 mg a.s./kg (nominal). The corresponding LDD_{50} (Lethal Dietary Dose), based on the actual consumption of the respective feeding solutions, was calculated to be 0.53 µg a.s./bee/day (nominal).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

A semi-filed honey bee brood study has been conducted with the formulated product (see KCP 10.3.1.3/01).

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

Deltamethrin EC 25 was the representative formulation of the last Annex I inclusion for deltamethrin. The current representative formulation of deltamethrin supported in this dossier, is Deltamethrin EW 15. Therefore new laboratory and extended laboratory studies on Ty*phlodromus pyri*, *Aphidius rhopalosiphi*, *Coccinella septempunctata* and *Chrysoperla carnea* have been conducted following the current guidelines. The tier 1 laboratory studies on Ty*phlodromus pyri* and *Aphidius rhopalosiphi* are summarized under point MCA 8.3.2.1 and MCA 8.3.2.2. The other studies are presented under point MCP 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Report:	**KCA 8.3.2.1 /01: [redacted]; 2000**
Title:	A laboratory dose-response study to evaluate the effects of AE F032640 00 EW01 B103 on survival and reproduction of the parasitoid wasp *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae)
Report No:	AE014ARL
Document No:	M-198587-01-1
Guidelines:	Barrett et al. (1994), Mead-Briggs et al. (2000), Mead-Briggs (1992) and Polgar (1988); Deviation: no major deviations occurred
GLP:	yes

Materials and Methods:

The test item AE F032640 00 EW01 B103 (Deltamethrin EW 15; purity: 1.48% w/w; oil in water emulsion; 15 g/1; Batch No.: TA124/99SG; Density: 1.022 g/mL; Certificate of Analysis Ref. Code: AZ 08183) was applied at concentrations of 0.150, 0.255, 0.510, 0.825, 1.725 and 3.000 g a.s./ha with a spray application volume of about 200 I/ha to glass plates. The control was treated with demineralized water. Dimethoate was used as toxic standard.
Aphidius rhopalosiphi was confined to test substance residues in ventilated cages. Four groups of 15 animals were exposed to each AE F032640 00 EW01 B103 test concentration and the water control.
Two groups of 15 animals were tested with the toxic standard. Mortality was assessed after a 2-day exposure period.
From the water control and two highest test rates of AE F032640 00 EW01 B103 causing less than 50% corrected mortality and which were below the expected LR_{50}, 20 impartially chosen females per treatment were each transferred to a cylinder containing untreated cereal plants infested with aphids (*Rhopalosiphum padi*) for a 1-day parasitisation period to provide a measure of reproductive success. The number of mummies produced was assessed 11 days later.

Findings:
Mortality in the toxic standard was 57% at 120 mg dimethoate/ha, which together with a control mortality of 5% and a control parasitisation rate of 19.1 mummies per female, fulfil the validity criteria for the study.

Summary of findings

Test item	AE F032640 00 EW01 B103			
Test organism	*Aphidius rhopalosiphi*			
Exposure on	dry spray deposit on glass plates (ventilated cages)			
Treatment	**Mortality after 2 d [%]**	**Corrected mortality after 2 d[a] [%]**	**Reproduction after 1 day (mean no. of mummies/female)**	**Reduction of reproduction relative to the control[b] [%]**
Control	5	-	19.1	-
0.150 g a.s./ha	3	-2 n.s.	-	-
0.255 g a.s./ha	8	3 n.s.	-	-
0.510 g a.s./ha	9	5 n.s.	11.7	39 *
0.825 g a.s./ha	31	28 *	4.1	79 *
1.725 g a.s./ha	65	63 *	-	-
3.000 g a.s./ha	63	61 *	-	-

LR_{50} = 1.726 g a.s./ha (confidence limits of 1.38 - 2.16 g a.s./ha)
[a] n.s. = not significant; * = significant; Fisher Exact Test, α = 0.05
[b] n.s. = not significant; one-way ANOVA/Fisher's LSD test, α = 0.05

Conclusions:

Exposure to residues of AE F032640 00 EW01 B103 (Deltamethrin EW 15) on artificial substrate, when applied at 6 different concentrations resulted in a LR_{50}, of 1.726 g a.s. /ha with confidence limits of 1.38 - 2.16 g a.s./ha. There was a reduction in reproductive success relative to the control of respectively 39% and 79% to the AE F032640 00 EW01 B103 concentrations 0.510 and 0.825 g a.s./ha that was

statistically different from the water control. Results of the toxic standard showed that the test set-up was sufficiently sensitive to detect potential adverse effects.

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Supplemental information from literature

Report:	**KCA 8.3.2.1 /02; [redacted]; 2005**
Title:	Increase of the Behavioral Response to Kairomones by the Parasitoid Wasp Leptopilina heterotoma Surviving Insecticides.
Source:	Arch. Environ. Contam. Toxicol., 49, 2, p. 186-191
DOI No:	DOI: 10.1007/s00244-004-0158-1
Document No:	M-460858-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

In this work, the authors have determined the sublethal effects of two insecticides, an organophosphorus (chlorpyrifos) and a pyrethroid (deltamethrin), on the arrestment, by host kairomones, of female parasitoids surviving an LD20 for 24 h. Material and methods as well as results are summarized here only for deltamethrin.

One-day-old female parasitoids were exposed in groups of ten in glass vials containing a piece of paper (length 5cm, width 8 mm) on which 12 µl of the insecticide diluted in acetone was deposited (pure acetone was used for controls). Pieces of paper were left 1 h on the bench for evaporation of the acetone before placing in vials. A small drop of honey was placed on the side of the vial to feed the parasitoids and vials were put at 20°C at 12:12 light: dark. The mortality of *L. heterotoma* was assessed after 24 h of contact with the treated piece of paper. For determining the regression line of mortality of the strain, 5 increasing concentrations of insecticide were used, and for each concentration 30 adults were exposed to the insecticide. Then, the lethal dose 20% (LD20) used for the experiments was estimated (log-probit program).

For the kairomon tests, the effects of deltamethrin on the behavior of female parasitoids toward their host kairomones were determined. For this, females exposed to the LD 20 of deltamethrin (treated females, i.e., exposed to the insecticide with the method used for the determination of lethal doses) and non-treated females (exposed to residues of acetone) were used. Then, mated *L. heterotoma* female (no signs of intoxication) was placed on a glass plate where two patches of agar (one control and one with kairomones) were deposited and covered with a Petri dish. For each test, the position of the two patches of agar was saved and the behaviour of the female was recorded during 8 min with a computerized video tracking device.

For deltamethrin, the LD20 was 2817.7 ng per piece of paper [2817.7 ng /8 cm² equivalent to 35.2 g/ha].

For both treated and non-treated insects, mean values are always higher than the indifference area indicating females were arrested by the kairomone patch. The values obtained for the females exposed to deltamethrin are always higher than the values obtained for the females unexposed.

This increase was not followed up by a modification of the kinetics of the behavior. In both control and exposed conditions, parasitoids regularly increased their residence time on the kairomone patch indicating that no saturation to kairomones had occurred. In a field situation where hosts could be scarce, this increase in arrestment could be advantageous for parasitoids by increasing their host finding.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	98%
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*E. heteroloma* Thomson
Cultivar:	
Source of test species:	[redacted] France
Age of test organisms at study initiation /	1 to 6 days old
Crop growth stage at treatment:	
Holding conditions prior to test:	Parasitoids were reared at 25C and 12:12 light:dark on D. melanogaster Meigen
Acclimatisation:	After emergence, parasitoid adults were stored from 1 to 6 days at 20C in vials with sweet agar-agar (2% v:w) at 12:12 light:- dark until the experiments were conducted.

B. Study design and methods

1. Test procedure

Test system (study type):	Lethal Doses Test and Kairomone Test
Duration of study:	*Lethal Doses Test:* 24 h and *Kairomone Test:* 8 min
Treatments:	*Lethal Doses Test:* 24 h and *Kairomone Test:* 8 min
Test concentrations	*Lethal Doses Test:* 5 increasing concentration; *Kairomone Test:* LD20
Number of replicates:	*Lethal Doses Test:*3 replicates; *Kairomone Test:*-
Individuals per replicate:	*Lethal Doses Test:*10 individuals; *Kairomone Test:*individuals which did not show any apparent sign of intoxication after exposure to LD20 of deltamethrin
Test units (type and size):	*Lethal Doses Test:* glass vials containing a piece of paper (length 5 cm, width 8 mm) and *Kairomone Test:* glass plates with patches of agar (control and kairomones)
Application / device, nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	*Lethal Doses Test:* glass vials containing a piece of paper (length 5 cm, width 8 mm) and *Kairomone Test:* glass plates with patches

	of agar (control and kairomones)
Temperature / relative humidity:	*Lethal Doses Test:*20°C *Kairomone Test:* 20°C
Photoperiod:	*Lethal Doses Test:*12:12 ligth:dark *Kairomone Test:* -
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg -soil]:	-
Fertilization:	-
3. Observations and measurements:	
Analytical parameters measured:	
Biological parameters measured:	LD20, residence time, walked distance, linear speed, angular speed
Measurement frequency:	*Lethal Doses Test:* after 24 h; *Kairomone Test:* during 8 min
Statistical analyses:	Logit-probit analysis (Raymond 1985) based on Finney (1971), Student's t-tests after arcsine square root transformation

RESULTS

1. Validity criteria:

No validity criteria were mentioned.

3. Biological findings:

For deltamethrin, the LD20 was 2817.7 ng per piece of paper (95% confidence interval: 2105.2–3430.1 ng) [2817.7 ng /8 cm² equivalent to 35.2 g/ha].

For both treated and non-treated insects, mean values are always higher than the indifference area indicating females were arrested by the kairomone patch. The values obtained for the females exposed to deltamethrin are always higher than the values obtained for the females unexposed.

Values for control females are near or inside the indifference area indicating they have no particular interest for the control patch. For females exposed to deltamethrin, the time spent on the control patch is linearly decreasing during time until almost reaching the indifference area (linearity test: $F_{14,496}$ = 0.45, ns; slope (-1.55) significantly different from 0. Furthermore, parasitoid females exposed to deltamethrin presented a not significant decrease of the walked distance, a significant decrease of the linear speed and a not significant increase of the angular speed

Table 1: Locomotor activity during the 8 min of recording for control females (Non-treated) and females exposed to an LD20 of deltamethrin during 24 hours before experiment (Treated)*

	Mean (SE)		t	
	Non-treated (n = 35)	**Treated (n = 32)**		
Walked distance (cm)	89.0 (2.84)	82.3 (2.97)	1.43	ns
Linear speed (cm/s)	0.573 (0.004)	0.560 (0.003)	2.66	$p < 0.01$
Angular speed (degree/s)	643 (11.5)	670 (9.44)	1.80	ns

* ns, not significant

For both treated and non-treated insects, mean values are always higher than the indifference area indicating females were arrested by the kairomone patch. The values obtained for the females exposed to deltamethrin are always higher than the values obtained for the females unexposed.
Values for control females are near or inside the indifference area indicating they have no particular interest for the control patch. For females exposed to deltamethrin, the time spent on the control patch is linearly decreasing during time until almost reaching the indifference area (linearity test: $F_{14,496}$ = 0.45, ns; slope (-1.55) significantly different from 0. Furthermore, parasitoid females exposed to deltamethrin presented a not significant decrease of the walked distance, a significant decrease of the linear speed and a not significant increase of the angular speed. In a field situation where hosts could be scarce, this increase in arrestment could be advantageous for parasitoids by increasing their host finding.

Comment by the Notifier

The publication indicated a low toxicity of deltamethrin to the tested parasitoid with an LC20 of 35.2 g a.s./ha. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	**KCA 8.3.2.1 /03; [redacted]; 2013**
Title:	The sublethal effects of deltamethrin on *Trichogramma* behaviors during the exploitation of host patches
Source:	Science of The Total Environment, Volume No. 447, p. 274-279
DOI No.: Document No:	10.1016/j.scitotenv.2012.12.096 M-462302-01-1
Guidelines:	No
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

This study identified the effects of an LD_{20} of deltamethrin on the behavior of *Trichogramma brassicae* females infesting a patch of host eggs. The study found that females that survived exposure to the insecticide infested fewer host eggs; spent more time on unsuitable, previously infested host eggs; and infested more previously infested host eggs than controls. The insecticide also induced an increase in antennal and ovipositor rejection of previously infested host eggs. These results are discussed in the light of the mode of action of pyrethroid insecticides.

MATERIAL AND METHODS

A. Material

1. <u>Test material</u>

Test item:	deltamethrin
Active substance(s):	deltamethrin
Adjuvant/ Surfactant:	not reported
Source of test item:	[redacted], France
Lot/Batch number:	n/a
Purity:	99 %
Storage conditions:	not reported

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	not reported
Concentration of vehicle/solvent:	not reported

3. Test organism(s)

Species:	*Trichogramma brassicae*
Cultivar:	not reported
Source of test species:	not reported
Age of test organisms at study initiation / Crop growth stage at treatment:	not reported
Rearing conditions:	This strain was reared on *Ephestia kuehniella* eggs (Lepidoptera, Pyralidae) killed by UV radiation to prevent non-parasitized eggs from emerging. Host eggs were supplied in excess. Therefore only one *T. brassicae* egg was laid per *E. kuehniella* egg. The rearing and experiments were conducted at 21 °C under a 12 L:12 D photoperiod (light phase from 7:30 am to 7:30 pm).
Acclimatisation:	not reported

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory study on glass plates
Duration of study:	24 h
Treatments:	not reported
Test concentrations	The theoretical dose inducing 20% of mortality (LD_{20}) was 40.60 ng (active ingredient) per piece of paper (95% confidence interval: 28.83–50.00 ng). This dose was used for testing the effects of deltamethrin on the behavior of *Trichogramma*.
Number of replicates:	5 groups of 50 individuals were exposed to a control solution and 4 solutions of increasing concentrations of insecticide.
Individuals per replicate:	50 individuals
Test units (type and size):	glass vials (3 cm in length, 5 mm in diameter)
Application / device / nozzles:	3 µl of deltamethrin diluted in acetone was deposited on pieces of paper (2.2 cm×4 mm), which were left for 1 h on the lab bench to allow the total evaporation of acetone to occur. The pieces of paper were then introduced into each vial containing a tested female. Papers on which pure acetone was deposited were used as controls.
Water volume:	not reported
Calibration of sprayer:	not reported

2. Environmental conditions

Test medium:	glass vials
Temperature / relative humidity:	21 °C
Photoperiod:	12:12
Lighting	not reported

Fertilization:	Two days prior to emergence, *E. kuehniella* eggs infested by *Trichogramma* were individually isolated in glass vials (3 cm in length, 5 mm in diameter) with a minute drop of honey. The males and females were sexed 48 h after the first emergences, and each female was then placed with one male for fertilization. After at least 1.5 h, the males were removed.
3. Observations and measurements:	
Biological parameters measured:	observation of the behavior of insects on host patches (Two days prior to emergence, *E. kuehniella* eggs infested by Trichogramma were individually isolated in glass vials (3 cm in length, 5 mm in diameter) with a minute drop of honey. The males and females were sexed 48 h after the first emergences, and each female was then placed with one male for fertilization. After at least 1.5 h, the males were removed, and the females were exposed to an LD_{20} of deltamethrin as described in "Determination of lethal doses" section. The females were left in their exposure vial until their behavior on a patch of host eggs was observed. The following behaviors were counted and their duration recorded with JWatcher software (Blumstein et al., 2006)[3]: – Entry into the group of healthy (not infested) eggs – Entry into the group of infested eggs – Climbing onto an egg – Drumming on an egg – Drilling an egg – Antennal rejection (the egg is left after antennal drumming) – Ovipositor rejection (the egg is left after drilling without infesting it) – Egg rejection (sum of the last two behaviors) – Oviposition – Moving intra-patch (the parasitoid walks between eggs) – Exit from the group of healthy eggs – Exit from the group of infested eggs – Moving extra-patch (the parasitoid walks on the square sheet of paper at a distance of at least 2 mm from the eggs) – Resting by the parasitoid (immobility or feeding on the egg) – Revisiting the patch (the parasitoid comes back to the patch after having left it).
Measurement frequency:	n/a
Statistical analyses:	The variability of the duration of each behavior as a function of the rank of the repetition and treatment (insecticide or control) was analyzed with generalized estimating equations (GEE). GEE, a regression method described by Liang and

[3] Blumstein DT, Daniel JC, Evans CS. JWatcher. URL http://www.jwatcher.ucla.edu 2006 [accessed 8 October 2012].

Zeger (1986)[4], allows us to test the influence of different factors on a variable that is non-normally distributed with repeated measures for the same individual (Ballinger, 2004[5]). The GEE method is derived from the generalized linear model, which allows the incorporation of correlations between measures. The data were compared using a Wald test. The statistical software R (Ihaka and Gentleman, 1996[6] http://www.r-project.org/) with the package geepack (Halekoh et al., 2006[7]) was used for the analysis.

RESULTS

The theoretical dose inducing 20% of mortality (LD_{20}) was 40.60 ng (active ingredient) per piece of paper (95% confidence interval: 28.83–50.00 ng) [40.60 ng/1.72 cm^2 equivalent to 2.31 g/ha].

A total of 85.2% (SE 1.70) of the host eggs identified as infested became black during their development. Infested eggs become black when the parasitoid has reached the 3rd larval stage (Voegelé, 1978[8]). Therefore, the emergence rate would be, at most, 85.2%. This result corresponds to the observations of [redacted] (2009)[9], which estimated the emergence rate of *T. brassicae* reared at 25 °C in *E. kuehniella* eggs killed by UV radiation to range between 75 and 95%. Based on these findings, observations of infestation appear to be reliable.

A total of 78.8% (SE 1.45) of the eggs used for the group of infested eggs became black. The 6.4% difference between this result and the 85.2% blackening of the infested eggs in the group of healthy eggs is statistically significant (Wilcoxon rank sum test: W=173, pb0.05). Therefore, it is possible that the group of infested eggs was infested at an approximate percentage of only 94%, even though the value of 78.8% falls within the 75–95% interval of emergences estimated by [redacted] (2009).

At the beginning of each observation, females were deposited on the group of infested eggs. The majority of them rapidly left this group and approached the healthy group. However, 28% of the females exposed to the insecticide stayed on the infested group and infested their first host egg in that group, whereas only 11% of the control females did so (χ^2=1.60, df=1, NS). Both the females exposed to the insecticide and the controls superparasitized the first egg, which was infested at least twice, but they rarely superparasitized the subsequent eggs. No significant differences between the exposed females and the controls were observed. The females exposed to deltamethrin spent significantly more time on the group of infested eggs than the controls. They laid significantly fewer eggs in the healthy group and

4 Liang KY, Zeger SL. Longitudinal data analysis using generalized linearmodels. Biometrika 1986;73:13–22.

5 Ballinger GA. Using generalized estimating equations for longitudinal data analysis. Organ Res Methods 2004;7:127–50.

6 Ihaka R, Gentleman R. R: a language for data analysis and graphics. J Comput Graph Stat 1996;5:299–314.

7 Halekoh U, Hojsgaard S, [redacted] J. The R package geepack for generalized estimating equations. J Stat Softw 2006;15:1–9.

8 Voegelé J. Utilisation des trichogrammes. Bull Tech Inf Minist Agric 1978;332–333: 447–52.

9 [redacted] La lutte biologique et les Trichogrammes. Application au contrôle de la pyrale du maïs. Paris: Le Manuscrit; 2009

more in the infested group than the controls (Table 1). They also infested fewer host eggs in the healthy group than the controls (74% and 94%, respectively, Table 2).

Contingency table for the total numbers of eggs laid by *T. brassicae* females according to the group of host eggs in which they were laid (healthy or infested eggs) and the type of treatment received by parasitoid females (exposed or not exposed to the insecticide).

	Group		total
	healthy	infested	
Control	181	2	183
Treated	139	10	149
total	320	12	332

Treated insects were exposed to an LD_{20} of deltamethrin. Effect of the insecticide on the choice of the group of host egg: χ2=7.44, df=1, pb0.01.

Contingency table for the numbers of host eggs infested and not infested in the group of healthy eggs according to the type of treatment received by parasitoid females (exposed or not exposed to the insecticide).

	Healthy group		total
	Infested eggs	Non-infested eggs	
Control	152	10	162
Treated	120	42	162
total	272	52	324

Treated insects were exposed to an LD_{20} of deltamethrin. Effect of the insecticide on the number of hosts infested: χ2=23.46, df=1, p < 0.001.

The female parasitoids exposed to deltamethrin rejected more host eggs than the controls. This increase in the rejection rate was due to an increase in both antennal and ovipositor rejections, whereas there was no significant difference between the exposed and the control females in the number of climbing behaviors. The exposure to the insecticide had no effect on the behaviors of drumming, drilling, oviposition and intra-patch moving (results not shown).

The females exposed to deltamethrin spent significantly more time on the group of infested eggs than the controls. They laid significantly fewer eggs in the healthy group and more in the infested group than the controls. They also infested fewer host eggs in the healthy group than the controls. The female parasitoids exposed to deltamethrin rejected more host eggs than the controls.

Comment by the Notifier

The publication indicated a sensitivity of the tested parasitoid with an LC20 of 2.31 g a.s./ha. Reduced reproduction performance was also observed for Aphidius rhopalosiphi at a test rate of 0.825 g a.s./ha sprayed onto grass plates. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	**KCA 8.3.2.1 /04; [redacted]; 2012**
Title:	The effects of deltamethrin applied at sublethal concentrations on the adults of *Anagrus nilaparvatae* (Hymenoptera: Mymaridae).
Source:	ARPN Journal of Agricultural and Biological Science VOL. 7, NO. 12, pp. 1032-1037
DOI No.: Document No:	- M-462184-01-1
ISSN No.:	1990-6145
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Anagrus nilaparvatae is one of major parasitoids for *Nilaparvata lugens* eggs. This research aimed to investigate the effects of deltamethrin on the longevity, development time, emergence rate of progeny, actual and potential fecundity of *A. nilaparvatae*. The insecticide was applied to the parasitoid adults at sublethal concentrations using the contact method in a test tube. The tested concentration was 0.023 ppm (LC_{10}) and 2.235 ppm (LC_{40}), and the control was treated with acetone. Each parasitoid surviving from deltamethrin treatment was exposed into *N. lugens* eggs in the rice seedlings for 24 hours. The seedling was then removed and substituted with new seedling until the parasitoid died. Each treatment was repeated 10 times. The application of deltamethrin at sublethal concentrations decreased the longevity of adults, increased the development time of progeny, decreased the actual and potential fecundity, but no effect on the emergence rate. These findings suggest that the application of deltamethrin to rice plants could reduce the potency of *A. nilaparvatae* as a biological control agent of *N. lugens*.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: deltamethrin (technical grade) containing 97% active ingredient
Active substance(s): deltamethrin
Adjuvant / Surfactant: not reported
Source of test item: [redacted], Indonesia)
Lot/Batch number: not reported
Purity: not reported
Storage conditions: not reported

2. Test solutions

Vehicle/solvent: acetone
Source of vehicle/solvent: not reported
Concentration of vehicle/solvent: not reported

3. Test organism(s)

Species: *Nilaparvata lugens*
Anagrus nilaparvatae
Cultivar: not reported

Source of test species:	*Nilaparvata lugens*: initial population was obtained from the [redacted] (Yogyakarta, Indonesia). *Anagrus nilaparvatae*: initial parasitoid population was obtained from [redacted] (Yogyakarta, Indonesia) by trapping (Trisyono, 1991; Maryana, 1994; Yaherwandi and Syam, 2007).
Age of test organisms at study initiation / Crop growth stage at treatment:	not reported
Holding conditions prior to test:	not reported
Acclimatisation:	not reported

B. Study design and methods

1. Test procedure

	Toxicity of deltamethrin to *Anagrus nilaparvatae*
Test system (study type):	contact method
Guideline deviation:	not reported
Duration of study:	1 h exposed to the test item
Treatments:	each treatment was repeated four times
Test concentrations	0.015625-16 ppm
Number of replicates:	not reported
Individuals per replicate:	10 females
Test conduction:	The test tube was wetted with 0.1 ml solution of each concentration. The test tube was rolled so that the whole inner surface was covered with deltamethrin solution and then was allowed to evaporate for one hour. Ten female parasitoids were put into the test tube and allowed to be exposed to the insecticide for one hour. The surviving parasitoids were then moved to the clean test tube containing a rice seedling which had been infested with N. lugens eggs aged two days and had 10% honey solution on the aluminium foil (0.5x4 cm).
Test units (type and size):	tube (1.3 cm in diameter, 10 cm in length)
Application / device / nozzles:	not reported
Water volume:	not reported
Calibration of sprayer:	not reported
	The effects of deltamethrin applied at sublethal concentrations on the adults of *Anagrus nilaparvatae* and its subsequent life
Test system (study type):	contact method
Guideline deviation:	not reported
Duration of study:	not reported
Treatments:	Each treatment was repeated 10 times.
Test concentrations	not reported
Number of replicates:	not reported
Individuals per replicate:	not reported
Test conduction:	Rice seedling was enclosed with tissue paper measuring 1x3 cm (4 layers) at the root and then wrapped in an aluminium

foil of the same size. The tissue paper was immersed in water to keep the plant fresh. The edge of the seedling was cut to make its length the same as the length of the test tube (10 cm). One seedling was put into the test tube with its root at the bottom of the tube. The seedlings were prepared according to the number of treatments. Three female adults of *N. lugens* that were about to lay eggs were put into the test tube that contained plants, and then it was capped with gauze cloth stuck with duck tape. The *N. lugens* adults were allowed to lay eggs on the rice plants for two days and then taken out. *A. nilaparvatae* that was still alive after treatment of LC_{10} and LC_{40} was put into each test tube. The rice seedlings were replaced daily by new seedlings that also contained *N. lugens* eggs.

Test units (type and size): not reported

Application / device / nozzles: not reported

Water volume: not reported

Calibration of sprayer: not reported

2. Observations and measurements:

Analytical parameters measured: no analytics performed

Biological parameters measured: Mortality of parasitoid. Observation was made on the parasitoid's longevity, developmental time, emergence rate of new progeny, and fecundity.

Measurement frequency: not reported

Statistical analyses: A probit analysis (Finney, 1971) was performed using Software SAS 9.3.1 Portable.
Analysis of Variance (ANOVA) was performed using Completely Random Design employing SAS 9.1.3. Portable. Analysis was continued with LSD test when significant differences existed (Gomez and Gomez, 1995). The data were analyzed using software SAS 9.1.3. Portable.

RESULTS

LC_{10} and LC_{40} of deltamethrin with the contact method on *A. nilaparvatae* were 0.023 ppm and 2.235 ppm (Table-1).

Table-1. The toxicity of deltamethrin to newly emerged adults of *Anagrus nilaparvatae* employing the contact method*.

Parameter	Value
Number of test insects	360
LC_{10} (95 % FL) (ppm)	0.023 (0.002 - 0.064)
LC_{40} (95 % FL) (ppm)	2.235 (0.962 -12.172)
LC_{50} (95 % FL) (ppm)	6.935 (2.331 - 81.901)
Solpe +/- SE	0.51 ± 0.05

* Parasitoids were released into the deltamethrin-treated test tube for an hour

The longevity and developmental time of *A. nilaparvatae* were not significantly different between those of the control and the treatment of deltamethrin with sublethal concentrations. However, the longevity of parasitoid tended to shorten and the developmental time tended to increase with the increasing concentrations of deltamethrin (Table-2). The actual fecundity of *A. nilaparvatae* in the control was significantly higher compared with those treated with deltamethrin at LC_{10} and LC_{40}. Increasing the concentration of deltamthrin tended to decrease in the actual fecundity of *A. nilaparvatae*. In the same way, the potential fecundity of the control was higher compared with those treated with deltamethrin. The higher the sublethal concentration applied, the lower the potential fecundity of *A. nilaparvatae*. The percentage of the emerging female parasitoids was extremely high (> 95%) and the application of insecticide did not affect the emergence of female parasitoids. The actual fecundity of *A. nilaparvatae* decreased up to 50% as a result of the sublethal effect (LC_{40}) of deltamethrin. Deltamethrin applied at high concentrations also reduced potential fecundity >50%.

Table-2. The effects of deltamethrin applied at sublethal concentrations on newly emerged adults of *Anagrus nilaparvatae* and its subsequent life.

Concentration	Longevity	Developmental	Fecundity	
			Actual (offspring/female)	Potential (eggs/female)
Control	2.0 a	10.0 a	26.8 a	40.4 a
LC_{10} (0.023 ppm)	1.8 a	10.1 a	19.3 b	23.7 b
LC_{40} (2.235 ppm)	1.5 a	10.2 a	13.7 b	15.7 c

Note: Parasitoid adults were treated individually using the contact method. Each treatment was repeated 10 times. The averages followed by the same letters in the columns were not significantly different at 5% level with the LSD test.

LC_{10} and LC_{40} of deltamethrin with the contact method on *A. nilaparvatae* were 0.023 ppm and 2.235 ppm. The longevity and developmental time of *A. nilaparvatae* were not significantly different between those of the control and the treatment of deltamethrin with sublethal concentrations. Increasing the concentration of deltamthrin tended to decrease in the actual fecundity of *A. nilaparvatae*. In the same way, the potential fecundity of the control was higher compared with those treated with deltamethrin.

Comment by the Notifier

The publication indicated a sensitivity of the tested parasitoid with an LC50 of 6.935 ppm. The 95% confidence limit ranges from 2.331 – 81.901 ppm. The basic mortality data that were used for this LCx calculation are not presented but the very wide range of the confidence limits do indicate a high variability. It is known form the available regulatory data on *Aphidius rhopalosiphi* that deltamethrin can have lethal and sublethal effects on parasitoids under tier 1 laboratory conditions. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	KCA 8.3.2.1 /05; [redacted]; 2006
Title:	A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp Aphidius ervi.
Source:	Chemosphere, 65, 10, p. 1697-1706
DOI No: Document No:	10.1016/j.chemosphere.2006.04.082 M-460882-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The aim of this study was to assess the effects of deltamethrin treatments on the parasitoid Aphidius ervi using a multi-step bioassay. The authors evaluated its effects on parasitoid emergence, adult survival and longevity, and host searching. Two exposure methods were used, topical and spray, to evaluate impact of the deltamethrin field rate (6.25 g a.i./ha) and also to compare the methods of exposure.

Experiment 1 - lethal concentration 50 (LC50): To establish the concentration–mortality relationship, insects were exposed to seven concentrations increasing with a geometrical ratio of 2 (from 0.29 to 18.75 ng/cm^2 of exposed surface area). Deltamethrin (tech.) was introduced with acetone on the internal surface of the tubes (200 µl). As control was used pure acetone. After complete evaporation of acetone at room temperature, ten females were placed per tube, with two drops of honey on a small plastic strip (4 replicates of at least 30 wasp (3 tubes of 10 females)). Exposure was performed at 15 ± 1°C, 65% ± 5% relative humidity, and under a 12L:12D photoperiod.

Experiment 2 - effects of deltamethrin on emergence and longevity: A spray exposure method and a topical exposure method for controlled individual dosage ([redacted] 1985 [redacted] 2001) were used. *Spray application:* Mummified S. avenae aphids containing A. ervi were attached on rectangular glass plates and then treated with deltamethrin (Decis micro®) using a Burgerjon-type Potter-tower device. Test concentrations were 6.25 g a.i./ha and 62.5 g a.i./ha. Water treatment was used as control. Two hours after the insecticide exposure, treated mummies were removed from the glass plates, placed individually in glass tube and kept at 15 ± 1°C, 55% ± 5% relative humidity until emergence. For each concentration and control, 42-133 mummies were exposed. The mummies were observed twice a day and the percentage of emergence was calculated for each group. Then, emerging adults were placed individually in glass tubes with access to food (dilute honey solution). The parasitoids were observed twice a day and the longevity in days was calculated for each group. *Topical application:* Mummified aphids were attached on rectangular glass and were treated by receiving a topical application of 0.3 µl deltamethrin (Decis micro®) using a 2-µl syringe provided with bevel point. 37-136 mummies were exposed to test concentrations of 6.25 g a.i./ha and 62.6 g a.i./ha. Water was used as control. Two hours after the topical treatment, mummies followed the same procedure as described in case of spray exposition.

Experiment 3 – toxicity of deltamethrin on leaves: *A. ervi* were exposed to deltamethrin (Decis micro®) on canola leaves. Insecticide was applied using the Burgerjon-type Potter tower. Test concentrations were 6.25 g a.i./ha and 0.5 g a.i./ha. Water-sprayed leaves served as controls. As exposure chamber was used a modified version from [redacted] (1996). Ten parasitoids were introduced

per unit (10 replicates for each treatment). After 24 h, the dead parasitoids were counted. Exposure conditions were 20 ± 1°C, 65% ± 5% relative humidity and 12L:12D photoperiod.

Experiment 4 – effects of deltamethrin on orientation behaviour: A. ervi females were exposed to four concentrations from 0.20 to 2.34 ng/cm² (by a geometrical ratio of 2). After 24 h exposure period, the number of dead parasitoids was counted and the survivors were collected and placed individually in Petri dishes for use in behaviour experiments within 2 h following the end of exposure.

Oriented responses towards aphid-infested plant odour were investigated in a four-armed olfactometer. The odour source was constituted by canola stems, kept in water, with a total of seven to eight leaves infested by *Myzus persicae* (400-500 aphids after 7 days of infestation). Experimental conditions were 25°C, fluorescent light (800 lux), and 70% relative humidity. Only one of four fields was odorized. A female parasitoid was introduced into a vial connected to the four-armed olfactometer. Observations started when, the female entered the chamber, and lasted for 1 min. The position of the female was recorded on a computer using event recorder software ("the Observer").

The concentration–mortality relationship for *A. ervi* exposed to deltamethrin on glass estimated the LC50 at 3.36 ± 0.53 ng/cm². The percentage of emergence of *A. Ervi* was not significantly decreased after exposure to 6.25 g a.i./ha with both application methods. However, when exposed to a topical application of 62.5 g a.i./ha, a 30% reduction of emergence was found when compared to the control group. In the case of spray application at this concentration, no significant effect appeared. During the first 48 h after emergence, there was no significant mortality in individuals coming from mummies treated topically or by spray at the field rate. In the groups exposed to 62.5 g a.i./ha, significantly more individuals died during the first 48 h than in the control. Longevity was significantly reduced by ca. 21% when exposed by spray at 6.25 g a.i./ha, but not in the case of a topical application. 62.5 g a.i./ha decreased significantly the longevity of emergent parasitoids in case of spray application as well as topical application. Mortality of *A.ervi* on treated leaves increased significantly for concentrations of 0.5 g a.i./ha and 6.25 g a.i./ha when compared to control. These concentrations induced 8.0 ± 3.3% and 71.0 ± 5.3% of mortality , respectively. Furthermore, the number of eggs potentially laid by a female was 185.10 (±2.90), 157.43 (±4.74) and 105.14 (±7.85), respectively, for the control group, 6.25 and 62.5 g a.i./ha when exposed to sprayed deltamethrin at the mummy instar. In the case of topical exposure of deltamethrin, the number of eggs was 185.21 (±4.80), 188.78 (± 2.12) and 131.19 (±9.51), respectively, for he control group and those exposed to 6.25 and 62.5 g a.i./ha. Furthermore, the orientation behaviour test exhibited for all groups of parasitoids a significant attraction towards the aphid-infested plant odour. A significant effect between treatments and control could not be observed.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin (tech.) and Decis micro®
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	Deltamethrin (tech.): [redacted], France); Decis micro®: [redacted] France, Lyon)
Lot/Batch number:	-
Purity:	98% (Deltamethrin (tech.) and 25 g a.i./l (Decis micro®)

Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Aphidius ervi* (Mummified *Sitobion avenae* aphids containing *A.ervi*)
Cultivar:	-
Source of test species:	[redacted] (Belgium)
Age of test organisms at study initiation / Crop growth stage at treatment:	The mummies used were 24 h old. When adults were used, the female parasitoids were 24-48h old.
Holding conditions prior to test:	They were stored in plastic Petri dishes and placed in an environmental chamber at 20 ± 1 °C, 65 ± 5% relative humidity, and light regime 18L: 6D.
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Lethal concentration test, Longevity and emergence test, Toxicity of deltamethrin on leaves, orientation behaviour test
Duration of study:	*Lethal concentration test*, *exposure to treated leaves* and *orientation behaviour* had exposure duration of 24 h. The longevity and emergence test had an exposure time of 2 h.
Treatments:	*Lethal concentration test*: glass tubes; *Longevity and emergence test*: realistic spray exposure and topical exposure; *toxicity of deltamethrin on leaves*: sprayed residues on canola leaves; orientation behaviour test: glass tubes
Test concentrations	4 concentrations, increasing by a geometrical ratio of 2 from 0.29 to 2.34 ng/cm² for *lethal concentration test* and *orientation behaviour test*; 6.25 and 62.5 g a.i./ha for *acute emergence and longevity test*; 0.5 and 6.25 g a.i./ha for *exposure to treated leaves*
Number of replicates:	4 (*lethal concentration test*), 0 (*longevity and emergence test*) , 10 (*toxicity of deltamethrin on leaves*) , 4 (*orientation behaviour test*)
Individuals per replicate:	10 females (*lethal concentration test*), 42-133 mummies for spray application and 37-136 mummies for topical application (*longevity and emergence test*) , 10 females (*toxicity of deltamethrin on leaves*) , 10 females (*orientation behaviour test*)
Test units (type and size):	*Lethal concentration test:* Glass tubes (length: 9.3 cm; diameter: 2.3 cm; internal surface: 67.4 cm²), *Longevity and emergence test Spray application:* rectangular glass plates, *topical application*: -, *toxicity of deltamehtrin on leaves:* The exposure chambers were slightly modified from Jansen(1996)[10] (diameter: 5 cm; height: 2 cm). *Orientation behaviour:* Glass tubes (length: 9.3 cm; diameter: 2.3 cm; internal surface: 67.4 cm²) and four-armed

[10] Jansen, J.P., 1996. Side effects of insecticides on *Aphidius rhopalosiphi* (Hym. Aphiddidae) in laboratory. Entomophaga 41, 37–43

	olfactometer
Application / device / nozzles:	*Longevity and emergence test: Spray application*: Burgerjon-type Potter-tower; *Topical exposure*: 2-µl syringe provided with bevel point
Water volume:	Sprayed volume with Burgerjon-type Potter-tower: 4 ml
Calibration of sprayer:	-
2. Environmental conditions	
Test medium:	*Lethal concentration test:* glass tubes; *Longevity and emergence test*: realistic spray exposure and topical exposure; toxicity of deltamethrin on leaves: sprayed residues on canola leaves; orientation behaviour test: glass tubes
Temperature / relative humidity:	15 ± 1°C (lethal concentration test and longevity and emergency test), 20±1°C (acute exposure to residues on leaves), 15±1°C during deltamethrin exposure for 2 h and 25°C in the olfactometer (orientation behavior test)
Photoperiod:	12L:12D (*lethal concentration test*) – (*longevity and emergency test*), 12L:12D (*acute exposure to residues on leaves*), 12L:12D (*orientation behavior test*)
Lighting:	- (*lethal concentration test*) – (*longevity and emergency test*), - (*acute exposure to residues on leaves*), fluorescent light with 800 lux in the olfactometer(*orientation behavior test*)
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-
3. Observations and measurements:	
Analytical parameters measured:	-
Biological parameters measured:	-
Measurement frequency:	-
Statistical analyses:	Probit analyses for calculation of LC50 values; χ^2 test; Mann-Whitney test with Bonferroni adjustment method; log-likelihood ratio test (G test); logistic regression; Kolmogorov-Smirnov test

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

Experiment 1 - lethal concentration 50 (LC50): The concentration–mortality relationship for *A. ervi* exposed to deltamethrin on glass estimated the LC50 at 3.36 ± 0.53 ng/cm² after 24 h.

Experiment 2 – effects of deltamethrin on emergence and longevity: The percentage of emergence of *A. Ervi* was not significantly decreased after exposure to 6.25 g.a.i./ha with both application methods.

However, when exposed to a topical application of 62.5 g a.i./ha, a 30% reduction of emergence was found when compared to the control group. In the case of spray application at this concentration, no significant effect appeared. During the first 48 h after emergence, there was no significant mortality in individuals coming from mummies treated topically or by spray at the field rate. In the groups exposed to 62.5 g a.i./ha, significantly more individuals died during the first 48 h than in the control. Longevity was significantly reduced by ca. 21% when exposed by spray at 6.25 g a.i./ha, but not in the case of a topical application. 62.5 g a.i./ha decreased significantly the longevity of emergent parasitoids in case of spray application as well as topical application. Furthermore, the number of eggs potentially laid by a female was 185.10 (±2.90), 157.43 (±4.74) and 105.14 (±7.85), respectively, for the control group, 6.25 and 62.5 g a.i./ha when exposed to sprayed deltamethrin at the mummy instar. In the case of topical exposure of deltamethrin, the number of eggs was 185.21 (±4.80), 188.78 (± 2.12) and 131.19 (±9.51), respectively, for he control group and those exposed to 6.25 and 62.5 g a.i./ha.

<u>*Experiment 3 – toxicity of deltamethrin on leaves*</u>: Mortality of *A. ervi* on treated leaves increased significantly by 8.0 ± 3.3% for concentrations of 0.5 and by 71.0 ± 5.3% for a concentration of 6.25 g a.i./ha when compared to control.

<u>Experiment 4 – effects of deltamethrin on orientation behaviour</u>: Furthermore, the orientation behaviour test exhibited for all groups of parasitoids a significant attraction towards the aphid-infested plant odour. A significant treatment effect (compared to the control) was not observed.

Comment by the Notifier

The publication indicated a sensitivity of the tested parasitoid with an LR50 of 0.336 g a.s./ha on artificial substrate (glass). This LR50 rate indicates a significant lower sensitivity compared to T. pyri with an LR50 value of 0.00439 g a.s./ha on glass plates. Reduced reproduction performance was also observed for Aphidius rhopalosiphi. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	KCA 8.3.2.1 /06; [redacted]; 2006
Title:	Multistep bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment.
Source:	Environ. Toxicol. Chem., 25, 10, p. 2675-2682,
DOI No: Document No:	10.1897/05-562R.1 M-460881-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Under laboratory conditions, the lethal and sublethal effects of deltamethrin on the aphid parasitoid Diaeretiella rapae M'Intosh (Hymenoptera: Braconidae) were studied at the mummy stage and in emerging adults. Following a multistep bioassay, analyses were aimed at evaluating the effects of

deltamethrin at various crucial steps in the recolonization process following a deltamethrin treatment:

Experiment 1 – Effects of deltamethrin on parasitoid emergence on longevity: Oilseed rape leaves bearing mummified aphids parasitized by *D. rapae* (2-3 days old) were attached on glass plates and treated with formulated deltamethrin (Decis micro®) by using a Burgerjon-type Potter tower. Test concentrations were 0.5, 5, 6.25 and 50 g a.s./ha. Water sprayed leaves were used as controls. After two hours, treated mummies were removed from the leaves and placed individually in gelatin capsules to check rapidly for emergence. Mummies were kept at 20 ± 1°C and 65% ± 5% relative humidity until emergence. For each test concentration (and water control), seven replicates of one leaf bearing 10 ± 1 mummies were made. They were observed twice a day. Endpoint was emergence. The, emerging adults were transferred individually in petri dishes with access to food (diluted honey solution, 80%) and were observed twice a day to investigate longevity.
Experiment 2 – Toxicity of deltamethrin on leaves: Ten parasitoids (*D. rapae*) were introduced per unit and were exposed to deltamehtrin (Decis micro®) on residues on leaves. Test concentration were 0.5, 5, 6.25 and 50 g a.i./ha and were applied by using a Burgerjon-type Potter tower. The used exposure units were slightly modified from those developed by Jansen (1996)[11] and recommended by Mead.Briggs et al.(1998)[12]. Dead parasitoids were counted 24 h after exposure. Pesticide exposure was performed at 20 ± 1°C and 65% ± 5% relative humidity under a 12:12-h light:dark photoperiod.
Experiment 3 – Effects of deltamethrin on orientation behaviour: Parasitoid exposure to pesticide: Ten parasitoid females (*D. rapae* and *Aphidius matricariae*) were exposed to four concentrations, increasing by a factor of two (acetone solutions of a.i. deltamethrin, range: 0.29-2.34 ng/cm²). As test unit was used a glass tube. Exposure was performed at 15 ± 1°C and 65% ± 5% relative humidity under a 12:12-h light:dark photoperiod. After 24-h exposure period, the number of dead parasitoids was counted, and the survivors were used for the behavioural tests. *Behavioral tests:* Oriented responses toward aphid-infested plant odor were investigated in a four-armed olfactometer. The odor source constituted of oilseed rape stems, with a total of seven to eight leaves infested by *M. persicae*. The relative humidity was 70% and the temperature was 25°C. Female paraistoids were introduced individually into the four armed olfactometer. The position of the female was recorded continuously on a computer using Observer event-recorder software to compute the overall time spent in each field.

Deltamethrin reduced the percentage of emergence from mummies, but only when exposed to the 50 g a.i./ha concentration. Mortality during the first 48 h after emergence was 17.3, 24.0, 8.0, 8.6 and 2.9% for 50, 6.25, 5.0, 0.5 g a.s./ha and control. However, for all concentrations tested, the insecticide induced a decrease in longevity after emergence from sprayed mummies and significant adult mortality when parasitoids walked on fresh residues on leaves. Indices were defined and predicted a high mortality and, thus, reduction of recolonization capacities. However, deltamethrin had no effect on orientation behavior toward aphid-infested plants for adults that survived a residual exposure to the insecticide.

MATERIAL AND METHODS

[11] Jansen JP. 1996. Side effects of insecticides on Aphidius rhopalosiphi (Hym. Aphiddidae) in laboratory. Entomophaga 41: 37–43.
[12] Mead-Briggs M, Brown K, Candolfi MP, Coulson M, Klepka S, Kühner C, Longley M, Maise S, McIndoe E, Miles M, [redacted]C, Ufer A. 1998. Development and ring-testing of a standardized laboratory test for parasitic wasps, using the aphid-specific parasitoid *Aphidius rhopalosiphi*. In Haskell PT, McEwen P, eds, Ecotoxicology. Kluwer Academic, London, UK, pp 80–88.

A. Material

1. Test material

Test item:	Decis micro® and pure deltamethrin
Active substance(s):	Deltamethrin (emulsifiable formulation 25000 mg a.i./L) and deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted] (Lyon, France) and [redacted], France)
Lot/Batch number:	-
Purity:	- and 98%
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Acetone for pure deltamethrin
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Diaeretiella rapae* M'Intosh; For experiment 3, *Aphidius matricariae* were also tested to confirm the results obtained with D.rapae
Cultivar:	-
Source of test species:	Laboratory strain
Age of test organisms at study initiation /	24 to 48 h
Crop growth stage at treatment:	-
Holding conditions prior to test:	All insects were reared in environmental chambers at 23 ± 1°C under a 18:6-h light/dark photoperiod. *Myzus persicae* was reared on *Vicia fabae* L.; *D. rapae* was reared on *M. persicae* transferred on *Brassicae napus* leaves. At the mummy stage, parasitized aphids were kept individually in plastic Petri dishes until emergency of adults. Adult females were mated at emergence and then stored in groups of five in glass tubes for 24 h. During this time, they were supplied with a dilute honey solution in water (80%).
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Experiment 1: Effects of deltamethrin on parasitioid emergence and longevity; Experiment 2: Toxicity of deltamethrin on leaves; Experiment 3: Effects of deltamethrin on orientation behavior
Duration of study:	*Experiment 1:* - (Endpoint: longevity); *Experiment 2:* 24 h; *Experiment 3:* parasitoid exposure to pesticide: 24 h and behavioral test: - (Endpoint: orverall time spent in each field of the olfactometer
Treatments:	*Experiment 1*: Oilseed rape leaves bearing mummified aphids parasitized by *D. rapae* (treated with Decis micro® and control); *Experiment 2: D. rapae* females were exposed to Decis micro® residues in leaves (and control); Experiment 3: *D. rapae* females were exposed to acetone solutions of deltamethrin which were

	applied to the inner surface of glass tubes (and control)
Test concentrations	*Experiments 1 and 2*: 0.5, 5, 6.25 and 50 g a.i./ha; *Experiment 3:* four concentrations, increasing by a factor of two (concentration range: 0.29-2.34 ng/cm²)
Number of replicates:	*Experiment 1*:7 replicates ; *Experiment 2: 5 to 7 replicates* *Experiment 3:* not mentionied
Individuals per replicate:	*Experiment 1:* 10 ± 1 mummies; *Experiment 2:* 10 parasitoid females; *Experiment 3:*10 parasitoid females
Test units (type and size):	*Experiment 1:* Mummies were kept in gelatin capsules and emerging adults were placed in Petri dishes (diameter, 5.3 cm); *Experiment 2:* slightly modified exposure units from those developed by Jansen (1996)[11] and recommended by Mead-Briggs et al. (1998)[12]; *Experiment 3:*glass tubes (length, 9.3 cm; diameter, 2.3 cm; internal surface, 67.4 cm²) and olfactometer
Application / device / nozzles:	*Experiment 1:* Burgerjon-type Potter tower; *Experiment 2:* Burgerjon-type Potter tower; *Experiment 3:* Microman® pipette
Water volume:	-
Calibration of sprayer:	-
<u>2. Environmental conditions</u>	
Test medium:	*Experiment 1:*Oilseed rape leaves on a glass plate; *Experiment 2:* leaves; *Experiment 3:* glass tubes
Temperature / relative humidity:	*Experiment 1:* 20 ± 1°C / 65% ± 5% RH; *Experiment 2:* 20 ± 1°C / 65% ± 5% RH; *Experiment 3:*parasitoid exposure to pesticide: 15 ± 1°C / 65% ± 5% RH; behavioural test: 25°C / 70% RH
Photoperiod:	*Experiment 1:* -; *Experiment 2:*12:12-h light:dark; *Experiment 3:* parasitoid exposure to pesticide: 12:12-h light:dark
Lighting	:*Experiment 3:* behavioural test: 800 lux
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-
<u>3. Observations and measurements:</u>	
Analytical parameters measured:	-
Biological parameters measured:	*Experiment 1:* Emergence, Longevity; *Experiment 2:* Mortality; *Experiment 3:*Mortality and orientation response
Measurement frequency:	*Experiment 1:*twice a day; *Experiment 2:* only after test end (24 h); *Experiment 3:*parasitoid exposure to pesticide: every hour for the first 10 h and after 24 h; *behavioral tests:* no interval
Statistical analyses:	Mann-Whitney test with Bonferroni adjustment; chi-square test; Friedman analysis of variance on ranks; logistic regression of the percentage of time spent in the odor as a function of detlamethrin concentration (linear model); Kolmogorov-Smirnov; Calculation of a reproductive potential index based on a study by Hag Ahmed

(1989)[13]; Wilcoxon sign-rank test; Calculation of a population survival index;

RESULTS

Validity criteria:

For experiments 1 and 2, no validity criteria were mentioned. For experiment 3, control mortality remained less than 10% (Hassan 1998)[14].

Biological findings:

Deltamethrin reduced the percentage of emergence from mummies, but only when exposed to the 50 g a.s./ha concentration. Mortality during the first 48 h after emergence was 17.3, 24.0, 8.0, 8.6 and 2.9% for 50, 6.25, 5.0, 0.5 g a.s./L and control. However, for all concentrations tested, the insecticide induced a decrease in longevity after emergence from sprayed mummies and significant adult mortality when parasitoids walked on fresh residues on leaves. However, deltamethrin had no effect on orientation behavior toward aphid-infested plants for adults that survived a residual exposure to the insecticide.

The reproductive potential of D. rapae was significantly reduced for parasitoid populations exposed to deltamethrin at the mummy instar. The number of eggs potentially laid by a female during her entire life was approximately 209, 170, 178, 153, and 122 for the control group and those exposed to 0.5, 5.0, 6.25, and 50 g a.s./ha, respectively.

Comment by the Notifier

It is known form the available regulatory data on *Aphidius rhopalosiphi* that deltamethrin can have lethal and sublethal effects on parasitoids under laboratory conditions (LR50 = 1.726 g a.s./ha on glass plates). Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report:	**KCA 8.3.2.1 /07;** [redacted] **; 2004**
Title:	Oviposition behaviour and patch-time allocation in two aphid parasitoids exposed to deltamethrin residues
Source:	Entomol. Exp. Appl., 112, 3, p. 227-235
DOI No: Document No:	10.1111/j.0013-8703.2004.00198.x M-460857-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Under laboratory conditions, this study investigated the impact of deltamethrin on the oviposition behaviour of two hymenopterous parasitoids of aphids, *Aphidius matricariae* (Haliday) and

[13] Hag Ahmed SEMK. 1989. Biological control of glasshouse *Myzus persicae* (Sulzer) using *Aphidius matricariae* Haliday. PhD thesis. University of London, London, UK.

[14] Hassan S. 1998. Guideline for the evaluation of side effects of plant protection products on *Trichogramma cacoeciae*. IOBC/ WPRS Bulletin 21:118–128.

Diaeretiella rapae (McIntosh) (Hymenoptera: Braconidae).

Determination of deltamethrin doses.

First, adult female parasitoids were exposed to six (*A. matricariae*) or seven (*D. rapae*) doses of dry residues of deltamethrin on glass increasing by a factor of two for 24 h (30 insects per dose in each species) in order to establish a regression line of mortality. Three doses were then estimated from the regression line: a LD_{50}, a LD_{20} and a $LD_{0.1}$.

Parasitoid exposure to pesticide (for behavioural test).

Acetone solutions of deltamethrin were applied to the inner surface of glass tubes (pure acetone was used as control). The tubes were left for 1 h on the bench to allow complete evaporation of the acetone before introducing the parasitoids. Ten female parasitoids were placed in each tube and two drops of honey were deposited deposited on a small plastic strip so that they would not be contaminated by the insecticide. Pesticide exposure was achieved at 15 ± 1 °C, 65 ± 5% r.h., and under a L12:D12 photoperiod. After a 24-h exposure period, the number of dead parasitoids was counted and the survivors collected and placed individually in Petri dishes. The behavioural tests were performed within 2 h of the end of exposure.

Behavioural tests.

Parasitoid females were placed individually on an aphid patch (*M. perisoae* on *oilseed rape*). Observations were carried out through a binocular microscope. The observations were carried out at a temperature of 20 ± 1 °C. The behaviours recorded were: 'antennal contact' (brief contact between an antenna of the parasitoid and an aphid body), 'antennal examination' (contact between both antennae of the parasitoid and an aphid body), 'sting attempt' (when the ovipositor was extruded next to an aphid or into an aphid exuvia), and 'sting' (ovipositor insertion into an aphid). Because these behaviours were very brief, only their frequency was recorded. The frequency and duration of four other behaviours were also recorded: 'walking onto aphid patch', 'walking out of aphid patch', 'grooming', and 'time spent immobile'. The observation period lasted until the parasitoid flew away or left the patch for more than 60 s.

For *D. rapae* and *A. matricariae*, respectively, the doses that induced 50% mortality (LD_{50}) were 1.36 ng cm^{-2} and 1.01 ng cm^{-2}, LD_{20} was 0.68 ng cm^{-2} and 0.34 ng cm^{-2}, and $LD_{0.1}$ was 0.10 ng cm^{-2} and 0.02 ng cm^{-2}.

When using these doses to expose females before behavioural testing, corrected mortalities were equal to 6.20 ±3.16% for $LD_{0.1}$, 17.54 ± 6.40% for LD_{20}, and 48.35 ± 3.85% for LD_{50} in the case of *D. rapae*, and 5.49 ± 3.37% for $LD_{0.1}$, 17.75 ± 5.67% for LD_{20}, and 46.58 ± 6.66% for LD_{50} in the case of *A. matricariae*.

In both parasitoid species, there was no significant effect of the three deltamethrin doses on the frequencies of 'antennal contact', 'antennal examination', 'sting', 'sting attempt', and of the two behavioural sequences considered. There was no significant difference between the species when we considered the number of behavioural items per minute. However, there was a significant difference between the two species regarding the 'initiation of host handling' sequence, but not the 'host acceptance' sequence. The three deltamethrin doses did not significantly modify patch residence time.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted]
Lot/Batch number:	-
Purity:	98%
Storage conditions:	-
Form:	crystals

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Diaeretiella rapae* and *Aphidius matricariae*
Cultivar:	-
Source of test species:	Field-collected parasitoids were yearly incorporated in the laboratory strain
Age of test organisms at study initiation:	24–48 h old
Crop growth stage at treatment:	
Holding conditions prior to test:	23 ± 1 °C, under a L16:D8 photoperiod
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Determination of LD values and behavioural tests
Duration of study:	*Determination of LD values: 24 h*; *Behavioural tests:* Exposition for 24 h; behavioural tests were performed within 2 h of the end of exposure and lasted until the parasitoid flew away.
Treatments:	Deltamethrin and Control (Acetone)
Test concentrations:	*Determination of LD values:* six (*A. matricariae*) or seven (*D. rapae*) doses increasing by a factor of two; *Behavioural test:* LD_{50}, LD_{20} and $LD_{0.1}$
Number of replicates:	-
Individuals per replicate:	*Determination of LD values:*30 females per doses; *Behavioural test:*10 females per doses
Test units (type and size):	*Determination of LD values:* exposure on glass; *Behavioural test:* During exposure to deltamethrin a glass surface was used as test unit (length: 9.3 cm; diameter: 2.3 cm; internal surface: 67.4 cm^2) During behavioural test, two-leaf stage oilseed rape leafs were used.
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	*Determination of LD values:* glass plates; behavioural test: glass plates (Exposure time) and oilseed rape leafs
Temperature / relative humidity:	*Determination of LD values:* not given; *Behavioural test:* 15 ± 1 °C / 65 ± 5% r.h. (Exposure time), 20 ± 1 °C (Behavioural test).
Photoperiod:	*Determination of LD values:* not given; *Behavioural test:* L12:D12(Exposure time),
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality; ‘antennal contact’ (brief contact between an antenna of the parasitoid and an aphid body), ‘antennal examination’ (contact between both antennae of the parasitoid and an aphid body), ‘sting attempt’ (when the ovipositor was extruded next to an aphid, or into an aphid exuvia), and ‘sting’ (ovipositor insertion into an aphid). Because these behaviours were very brief, only their frequency was recorded. The frequency and duration of four other behaviours were also recorded: ‘walking onto aphid patch’, ‘walking out of aphid patch’, ‘grooming’, and ‘time spent immobile
Measurement frequency:	*Determination of LD values*: after 24 h; *Behavioral test*: after 24 h (Exposure time),
Statistical analyses:	Logarithmic transformation of doses and a probit transformation of mortalities. Abbott corrected mortalities, generalized linear model based on a Gamma distribution and log-link function

RESULTS

Validity criteria:

No validity criteria were mentioned

Biological findings:

For *D. rapae* and *A. matricariae*, respectively, the doses that induced 50% mortality (LD_{50}) were 1.36 ng cm^{-2} and 1.01 ng cm^{-2}, LD_{20} was 0.68 ng cm^{-2} and 0.34 ng cm^{-2}, and $LD_{0.1}$ was 0.10 ng cm^{-2} and 0.02 ng cm^{-2}.

When using these doses to expose females before behavioural testing, corrected mortalities were equal to 6.20 ± 3.16% for $LD_{0.1}$, 17.54 ± 6.40% for LD_{20}, and 48.35 ± 3.85% for LD_{50} in the case of *D. rapae*, and 5.49 ± 3.37% for $LD_{0.1}$, 17.75 ± 5.67% for LD_{20}, and 46.58 ± 6.66% for LD_{50} in the case of *A. matricariae*.

In both parasitoid species, there was no significant effect of the three deltamethrin doses on the

frequencies of ‘antennal contact’, ‘antennal examination’, ‘sting’, ‘sting attempt’, and of the two behavioural sequences considered. There was no significant difference between the species when we considered the number of behavioural items per minute. However, there was a significant difference between the two species regarding the ‘initiation of host handling’ sequence, but not the ‘host acceptance’ sequence. The three deltamethrin doses did not significantly modify patch residence time.

Table 1: Mean number (± SE) of behaviours related to host handling per minute and sequences per minute for *Diaeretiella rapae* and *Aphidius matricariae* females previously exposed to three doses of deltamethrin ($LD_{0.1}$, LD_{20}, or LD_{50}) for 24 h or a control, on *Myzus persicae* patches. The statistical comparison between control and the three doses for each species is provided

	Average frequencies per min of behaviours and sequences					
	Antennal contact	Antennal examination	Sting attempt	Sting	Initiation of host-handling	Host acceptance
Aphidius matricariae						
Control n= 34	0.57 ± 0.08	0.37 ± 0.08	0.56 ± 0.11	0.30 ± 0.06	0.09 ± 0.04	0.18 ± 0.05
$LD_{0.1}$ n= 33	0.45 ± 0.06	0.65 ± 0.15	0.83 ± 0.19	1.11 ± 0.71	0.14 ± 0.05	0.08 ± 0.02
LD_{20} n= 34	0.58 ± 0.11	0.42 ± 0.10	0.75 ± 0.29	0.43 ± 0.15	0.14 ± 0.05	0.23 ± 0.08
LD_{50} n= 36	0.83 ± 0.22	0.67 ± 0.25	0.60 ± 0.16	0.39 ± 0.12	0.10 ± 0.06	0.18 ± 0.31
Diaeretiella rapae						
Control n = 35	0.78 ± 0.10	0.70 ± 0.15	0.63 ± 0.17	0.42 ± 0.11	0.11 ± 0.03	0.34 ± 0.06
$LD_{0.1}$ n= 34	0.75 ± 0.11	0.75 ± 0.14	0.81 ± 0.16	0.30 ± 0.06	0.14 ± 0.03	0.30 ± 0.06
LD_{20} n= 35	0.93 ± 0.16	0.93 ± 0.19	0.81 ± 0.16	0.38 ± 0.11	0.25 ± 0.08	0.28 ± 0.08
LD_{50} n= 35	0.67 ± 0.09	0.62 ± 0.13	1.03 ± 0.29	0.40 ± 0.10	0.08 ± 0.03	0.28 ± 0.05

Comment by the Notifier

The publication indicated a sensitivity of the tested parasitoid with an LR50 of 0.101 g a.s./ha and 1.36 g a.s./ha on artificial substrate (glass). This LR50 rates indicate a significant lower sensitivity compared to T. pyri with an LR50 value of 0.00439 g a.s./ha on glass plates. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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CA 8.3.2.2 Effects on Typhlodromus pyri

Report:	**KCA 8.3.2.2/01, [redacted] 2010**
Title:	A laboratory dose-response study to evaluate the effects of Deltamethrin EW 15 g/L on survival of the predaceous mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) on glass
Document No:	M-387027-01-1 (Rep. No: B156TPL)
Guidelines:	Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products (Blümel et al., 2000).
GLP	GLP study

Material and Methods:

The insecticide Deltamethrin EW 15 g/L (active ingredient: deltamethrin, content: 15.35 g/L, sample description: TOX08992-00, batch no.: 2010-002975) was applied to all inner parts of ventilated glass cages at 5 nominal rates, ranging from 1.66 mg a.s./ha to 27.04 mg a.s./ha, at a spray application volume of approximately 200 L/ha. The control was treated with deionised water. Dimethoate at a rate of 8 mL product/ha (nominal 400 g/L a.i., 0.08% of the highest recommended field rate) was used as a toxic reference.

Typhlodromus pyri Scheuten was exposed in groups of 10 per unit to dry residues within 1.5 hours after application. There were 8 units for the water control, 7 units for each Deltamethrin EW 15 g/L treatment and 5 units for the toxic reference. Mortality was assessed after a 7-day exposure period.

Findings:

Low mortality in the control treatment indicated that test animals were in good condition. Mortality in the toxic reference treatment showed that test animals were sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected with the set-up used in this experiment.

Summary of findings

Test Organims	*Typhlodromus pyri*		
Test Item	Deltamethrin EW 15 g/L		
Exposure	7 days in ventilated glass cages		
Nominal application volume	200 L/ha		
	Mortality after 7 days		
Water control	10%		(Standard deviation 11%)
Application rates of Deltamethrin EW 15 g/L	Corrected mortality after 7 days (reference 3 days)		Standard deviation
1.66 mg a.s./ha	36%	P = 0.001*	19%
3.32 mg a.s./ha	48%	P < 0.001*	15%
6.68 mg a.s./ha	58%	P < 0.001*	17%
13.44 mg a.s./ha	80%	P < 0.001*	19%
27.04 mg a.s./ha	97%	P < 0.001*	8%
Toxic reference	100%	P < 0.001*	0%
LR_{50}	4.39 mg a.s./ha (CL 3.42 and 5.65 mg a.s./ha)		
Other observations	A dose related effect was observed on development.		

* Statistically significantly different from deionised water control. Statistical analysis: Fisher's exact test

Conclusion:

After 7 days of exposure to Deltamethrin EW 15 g/L at rates equivalent to 1.66 mg a.s./ha or higher, survival of *Typhlodromus pyri* was statistically significantly reduced compared to the water control. The LR_{50} was calculated as 4.39 mg a.s./ha with 95% confidence limits of 3.42 and 5.65 mg a.s./ha.

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CA 8.4 Effects on non-target soil meso and macrofauna

For all studies submitted during the frame of the first Annex I inclusion please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience. A summary is provided in the table below:

Table 8.4 - 1: EU-agreed endpoints on effects on non-target soil meso and macrofauna

Test item	EU agreed endpoints	Endpoints used in risk assessment
Earthworm, acute		
Deltamethrin (tech.)	LC_{50} >1290 mg a.s./kg dws	not required

dws = dry weight soil; a.s. = active substance

In order to complete the risk assessment for deltamethrin limit tests on reproduction of *Hypoaspis aculeifer*, *Folsomia candida* and *Eisenia fetida* were conducted with the representative formulation and the soil metabolites Br_2CA and mPBacid.

Table 8.4 - 2: Reproduction tests on non-target soil meso and macrofauna with deltamethrin EW15 and soil metabolites

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Earthworm, reproduction				
Deltamethrin EW 15A G	*Eisenia fetida* reproduction 56 d, mixed	NOEC	281 mg prod./kg dws 4.22 mg a.s./kg dws	[redacted] **(2012)** **M-426439-01-1**
		$NOEC_{corr.}$	140.5 mg prod./kg dws[A] **2.11 mg a.s./kg dws[A]**	
Br_2CA	*Eisenia fetida* reproduction 56 d, mixed	NOEC	10 mg/kg dws	[redacted] **(2011)** **M-403733-01-1**
		$NOEC_{corr.}$	**5 mg/kg dws[A]**	
mPBacid	*Eisenia fetida* reproduction 56 d, mixed	NOEC	10 mg/kg dws	[redacted] **(2011)** **M-402952-01-1**
		$NOEC_{corr.}$	**5 mg/kg dws[A]**	
Collembola, reproduction				
Deltamethrin EW 15A G	*Folsomia candida* reproduction 28 d, mixed	NOEC	178 mg prod./kg dws 2.67 mg a.s./kg dws	[redacted] **(2010)** **M-397993-01-1**
		$NOEC_{corr.}$	89 mg prod./kg dws[A] **1.34 mg a.s./kg dws[A]**	
Br_2CA	*Folsomia candida* reproduction 28 d, mixed	NOEC	≥100 mg/kg dws	[redacted] **(2010)** **M-398826-01-1**
		$NOEC_{corr.}$	**≥50 mg/kg dws[A]**	
mPBacid	*Folsomia candida* reproduction 28 d, mixed	NOEC	≥100 mg/kg dws	[redacted] **(2010)** **M-398820-01-1**
		$NOEC_{corr.}$	**≥50 mg/kg dws[A]**	
Soil mites, reproduction				
Deltamethrin EW 15A G	*Hypoaspis aculeifer*	NOEC	32 mg prod./kg dws 0.48 mg a.s./kg dws	[redacted] **(2010)** **M-393654-01-1**

Test item	Test species, test design	Ecotoxicological endpoint		Reference
	reproduction 14 d, mixed	**NOEC$_{corr.}$**	16 mg prod./kg dws[A] **0.24 mg a.s./kg dws[A]**	
Br_2CA	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	≥100 mg/kg dws	[redacted] (2011) M-400275-01-1
		NOEC$_{corr.}$	**≥50 mg/kg dws[A]**	
mPBacid	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	≥100 mg/kg dws	[redacted] (2011) M-400270-01-1
		NOEC$_{corr.}$	**≥50 mg/kg dws[A]**	

dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected

Bold values: endpoints used for risk assessment

[A] corrected by factor of 2 due to lipophilic substance (i.e. log P_{ow} > 2)

CA 8.4.1 Earthworm, sub-lethal effects

Report:	**KCA 8.4.1/01, [redacted]; 2011**
Title:	Br_2CA (Metabolite of deltamethrin, AE F108565): Sublethal toxicity to the earthworm *Eisenia fetida* in artificial soil with 5% peat
Document No:	M-403733-01-1 (1010481025)
Guidelines:	OECD 222 (2004), ISO 11268-2
GLP:	yes

Objective:

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.

Material and methods:

Test item Br_2CA (Metabolite of deltamethrin, AE F108565), Product code: AE F108565 00 1B99 0001, Origin Batch No.: 2N6185C, CAS No.: 53179-78-5, Chemical name: (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid, analysed purity: 98.8 % w/w.

1[st] test run: Adult earthworms (*Eisenia fetida andrei*, about 3 months old) were exposed to 100 mg test item/kg soil dry weight (dws) containing 73.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 18 – 22 °C and a photoperiod: light : dark = 16 h : 8 h (610 lx) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

2[nd] test run: Adult earthworms (*Eisenia fetida andrei*, about 3 months old) were exposed to 10 – 18 – 32 –56 - 100 mg test item/kg soil dry weight (dws) containing 73.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 18.0 – 21.7 °C and a photoperiod: light : dark = 16 h : 8 h (750 lx) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

Findings:

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	Br_2CA (Metabolite of deltamethrin, AE F108565) *Eisenia fetida* Artficial soil		
	Mortality	**Biomass change**	**Reproduction**
	[mg test item/kg dws]		
LOEC	> 100	32	18
LC_{50}/EC_{50}	> 100	> 100	25
95% confidence limit	-	-	22 (lower cl) 28 (upper cl)
NOEC	≥ 100	18	10

Observations:

1st test run

Br_2CA (Metabolite of deltamethrin, AE F108565) [mg test item/kg w.w.]		
	Control	**100**
Mortality of adult worms after 4 weeks		
Mortality (%)	0	6.3**
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)		
Mean (mg)	72.3	-102.2*
Mean (%)	20.8	-28.6
Number of juveniles per surviving adult worm after 8 weeks		
Mean	6.9	0.0
Number of juveniles per replicate after 8 weeks		
Mean	68.8	0.0*
Reduction of reproduction per treatment (%)		
% to control		-100

* statistically significantly different compared to control (Student-t-test, Welch-t test, p≤ 0.05, one sided smaller)

** statistically significantly different compared to control (Fisher's Exact Binomial Test, p ≤ 0.05, one-sided greater

Validity criteria (1st test run)

- Adult mortality: ≤ 10% (being 0% after 4 weeks)
- Number of juveniles per replicate ≥ 30 (being 56, 96, 69, 82, 79, 41, 55 and 72)
- Coefficient ov variation of reproduction: ≤ 30% (being 25.5%)

2nd test run

Br$_2$CA (Metabolite of deltamethrin, AE F108565) [mg test item/kg dws]						
	Control	**10**	**18**	**32**	**56**	**100**
Mortality of adult worms after 4 weeks						
Mortality (%)	1.3	0	0	2.5	2.5	0
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)						
Mean (mg)	102.6	104.5	100.3	77.4*	54.0*	-52.8
Mean (%)	28.9	29.6	28.3	22.0	15.5	-14.9
Number of juveniles per surviving adult worm after 8 weeks						
Mean	7.9	7.4	5.8	2.3	0.9	0.0
Number of juveniles per replicate after 8 weeks						
Mean	78.3	74.3	58.3*	22.8*	9.0*	0.3*
Reduction of reproduction per treatment (%)						
% to control	-	-5.1	-25.6	-70.9	-88.5	-99.7

Validity criteria (2nd test run)
- Adult mortality: ≤ 10% (being 1.3% after 4 weeks)
- Number of juveniles per replicate ≥ 30 (being 59, 98, 65, 78, 84, 74, 89 and 79)
- Coefficient ov variation of reproduction: ≤ 30% (being 16.0%)

In a reference test, the number of juveniles was reduced by 73.7 and 99.7 % 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.

Conclusion:

Br$_2$CA (metabolite of deltamethrin, AE F108565) showed no statistically significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to 100 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 32, 56 and 100 mg test item/kg soil dws.

The test item showed statistically significantly adverse effects on reproduction at 18, 32, 56 and 100 mg test item/kg soil dws. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 10 mg test item/ kg soil dws, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 18 mg test item/kg soil dws. The EC$_{50}$ for number of juveniles was calculated to be 25 mg test item/kg soil dws with 95 % confidence limits ranging from 22 to 28 mg test item/kg soil dws.

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Report:	**KCA 8.4.1/02, [redacted], 2011**
Title:	mPBacid (Metabolite of deltamethrin, AE F109036): Sublethal toxicity to the earthworm *Eisenia fetida* in artificial soil with 5% peat
Document No:	M-402952-01-1 (11 10 48 099 S)
Guidelines:	OECD-Guideline No. 222 (2004), ISO 11268-2 (1998)
GLP:	Yes (certified laboratory)

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.

The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998).

Materials and Methods:

Test item mPBacid (Metabolite of deltamethrin, AE F109036), Batch code: AE F109036 00 1B99 0001, Origin Batch No.: 400976/1, CAS No.: 3739-38-6, Chemical name: 3-phenoxybenzoic acid, analysed purity: 98.6 % w/w.

1st test run: Adult earthworms (*Eisenia fetida andrei*, about 3 months old) were exposed to 100 mg test item/kg soil dry weight (d.w.) containing 73.7 % quartz sand, 20 % kaolin clay, 5% sphagnum peat and 0.3 % CaCO3, at 18 – 22 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

2nd test run: Adult earthworms (*Eisenia fetida andrei*, about 3 months old) were exposed to 10 – 18 – 32 – 56 - 100 mg test item/kg soil dry weight (d.w.) containing 73.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 18.0 – 21.7 °C and a photoperiod: light : dark = 16 h : 8 h (720 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: quartz sand, solvent control: none.

Dates of work: 1st test run: September 28, 2010 – November 23, 2010
2nd test run: December 07, 2010 – February 01, 2011

Results:

Validity Criteria	Recommended	Obtained 1st run	Obtained 2nd run
Adult mortality	≤ 10%	0% after 4 weeks	
Number of juveniles per replicate	≥ 30	73, 49, 85, 95, 68, 86, 65 and 81	88, 108, 93, 98, 74, 81, 66 and 92
Coefficient of variation of reproduction	≤ 30%	19.4%	15.4%

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) is routinely tested at concentrations of 5 and 10 mg product/kg soil dry weight.

In the most recent study with Nutdazim 50 FLOW (BioChem project No. R 10 10 48 007 S, dated August 05, 2010), the number of juveniles was reduced by 73.7 and 99.7% at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 22.8 and 0.3) after 8 weeks of test duration when compared to control (mean number of juveniles = 86.6).

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	mPBacid (Metabolite of deltamethrin, AE F109036) *Eisenia fetida* Artificial soil		
	Mortality	Biomass change	Reproduction
	[mg test item/kg soil d.w.]		
LOEC	> 100	18	18
LC_{50}/EC_{50}	> 100	> 100	31
95 % confidence limit	-		29 (lower cl) 32 (upper cl)
NOEC	≥ 100	10	10

Observations:

1st test run

mPBacid (Metabolite of deltamethrin, AE F109036) [mg test item/kg soil d.w.]		
	Control	100
Mortality of adult worms after 4 weeks		
Mortality (%)	0 0 2.5	0
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)		
Mean (mg)	82.3	-179.5*
Mean (%)	21.7	-47.2
Number of juveniles per surviving adult worm after 8 weeks		
Mean	7.5	0.0
Number of juveniles per replicate after 8 weeks		
Mean	75.3	0.0*
Reduction of reproduction per treatment (%)		
% to control	-	-100

* Statistically significantly different compared to control (Student-t-test, $p \leq 0.05$, one-sided smaller)

2nd test run

mPBacid (Metabolite of deltamethrin, AE F109036) [mg test item/kg soil d.w.]						
	Control	10	18	32	56	100
Mortality of adult worms after 4 weeks						
Mortality (%)	0	0	2.5	0	2.5	0
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)						
Mean (mg)	139.9	130.1	114.6*	94.6*	70.4*	-33.9*
Mean (%)	39.3	37.1	32.7	27.0	19.8	-9.7
Number of juveniles per surviving adult worm after 8 weeks						
Mean	8.8	9.1	7.2	4.1	1.4	0.1
Number of juveniles per replicate after 8 weeks						
Mean	87.5	91.0	71.5*	41.0*	13.3*	1.0*
Reduction of reproduction per treatment (%)						
% to control	-	4.0	-18.3	-53.1	-84.9	-98.9

* Statistically significantly different compared to control
(Williams Multiple Sequential t-test, p ≤ 0.05, one-sided smaller)

Conclusion:

mPBacid (Metabolite of deltamethrin, AE F109036) showed no statistically significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to 100 mg test item/kg soil dry weight, i.e. the highest concentration tested.
The test item showed statistically significantly adverse effects on growth and reproduction at 18, 32, 56 and 100 mg test item/kg soil d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 10 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 18 mg test item/kg soil d.w. The EC_{50} for number of juveniles was calculated to be 31 mg test item/ kg soil d.w. with 95 % confidence limits ranging from 29 to 32 mg test item/kg soil d.w.

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Report:	**KCA 8.4.1/03; [redacted]; 2012**
Title:	Deltamethrin EW 15A G: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5 % peat
Document No:	M-426439-01-1
Guidelines:	ISO/DIS 11268 -2 (1998); OECD 222: April 13, 2004
GLP:	yes

Material and methods:

Deltamethrin EW 15A G (Sample description: TOX08992-00; Batch ID: 2010-002975; Material No. 05759284; Specification No. 102000013165 - 05; content: 15.35 g deltamethrin/L; density: 1.023 g/mL).
Adult *Eisenia fetida* (approx. 5 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 5 % peat content) to

the nominal test concentrations of 50, 89, 158, 281 and 500 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings: Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table (values in this table are rounded values).

Test object	***Eisenia fetida***					
Test item	Control	DLP EW 15A G				
mg test item/kg dry weight artificial soil	---	50	89	158	281	500
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	87.60	88.55	78.78	79.01	87.08	80.58
Standard Deviation	10.48	13.71	8.90	11.97	5.47	7.40
Mean number of offspring per test vessel after 56 days **	190.8	206.3	199.8	188.3	177.3	153.0 **
Standard Deviation	25.4	29.3	25.7	22.8	26.9	9.4
Coefficient of variance (%)	13.3	14.2	12.9	12.1	15.2	6.2
% of control	---	108.1	104.7	98.7	92.9	80.2

* no statistical significance compared to the control (Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$)
** statistical significance compared to the control (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$)

Observations
Mortality
After 28 days of exposure no worms died in the control group and no mortality was observed at any test item concentration.

Effects on growth
Statistically significant different values for the growth relative to the control were not observed.
Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 500 mg test item/kg dry weight artificial soil
LOEC related to growth: > 500 mg test item/kg dry weight artificial soil

Effects on reproduction
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 50, 89, 158 and 281 mg test item/kg dry weight artificial soil. Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the highest test concentration of 500 mg test item/kg dry weight artificial soil.
Therefore, based on biological and statistical significance:
NOEC related to reproduction: 281 mg test item/kg dry weight artificial soil

LOEC related to reproduction: 500 mg test item/kg dry weight artificial soil

Conclusions

Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 281 mg test item/kg dry weight artificial soil.

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

Report:	**KCA 8.4.2/01 [redacted]; 2011**
Title:	Br_2CA (Metabolite of deltamethrin, AE F108565): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Document No:	M-400275-01-1 (Rep. No: 101048104S)
Guidelines:	OECD 226 (2008)
GLP:	Yes

Material and methods:

Test item Br_2CA (Metabolite of deltamethrin, AE F108565), Product code: AE F108565 00 1B99 0001, Origin Batch No.: 2N6185C, CAS No.: 53179-78-5, chemical name: (1R,3R)-3-(2,2-dibromovinyl)-2,2-
dimethyl-cyclopropanecarboxylic acid, analysed purity: 98.8 % w/w. 10 adult soil mites (females) were exposed to 100 mg test item/kg dry weight (dws) of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 18.9 - 20.3 °C and a photoperiod light : dark = 16 h : 8 h (500 lx) and were fed every 2 days with Tyrophagus putrescentiae ([redacted]). Mortality and reproduction were determined after 14 days. Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg soil dws; control: deionised water, solvent control: none.

Findings:

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Br_2CA *Hypoaspis aculeifer* Artificial Soil	
	Adult mortality	Reproduction
	mg test item/kg soil dws	
LOEC	>100	100
NOEC	100	100

Observations:

Endpoint	Br_2CA (mg test item/ kg soil dws)	
	Control	100
Mortality of soil mites after 14 days (%)	7.5	6.3
Mean number of juveniles after 14 days	235.5	226.0
CV (%)	13.0	14.3
Reduction of reproduction	-	4.0

Endpoint	Br_2CA (mg test item/ kg soil dws)	
	Control	100
(% to control)		

No statistically significant difference compared to control (Fisher's Exact Binomial Test for mortality, $p \leq 0.05$; Student-t-test for reproduction; $p \leq 0.05$). Calculations were done using non-rounded values
Percent reduction: $(1-R_t/R_C) * 100\%$
R_t = the reproduction observed in the treated group(s)
R_c = the reproduction observed in the control group

Conclusion:
In this test all validity criteria have been fulfilled.
The test item Br_2CA (Metabolite of deltamethrin, AE F108565) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg dws.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dws and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg dws.

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Report:	**KCA 8.4.2/02, [redacted]; 2011**
Title:	mPBacid (Metabolite of deltamethrin, AE F109036): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Document No:	M-400270-01-1 (Rep. No: 10104810S)
Guidelines:	OECD 226 (2008): Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*
GLP:	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. The test was performed as limit test according to the OECD guideline 226 (2008).

Material and methods:
Test item mPBacid (Metabolite of deltamethrin, AE F109036), Batch code: AE F109036 00 1B99 0001, Origin Batch No.: 400976T, CAS No.: 3739-38-6, Chemical name: 3-phenoxybenzoic acid, analysed purity: 98.6 % w/w.

10 adult soil mites (females) were exposed to 100 mg test item/kg dry weight (d.w.) of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % CaCO3, at 18.9 - 20.3 °C and a photoperiod: light : dark = 16 h : 8 h (490 lx) and were fed every 2 days with *Tyrophagus* putrescentiae ([redacted]). Mortality and reproduction were determined after 14 days.
Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.i./kg soil d.w.; control: deionised water, solvent control: none.

Findings:

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item **Test object** **Exposure**	**mPBacid (Metabolite of deltamethrin, AE F109036)** ***Hypoasis aculeifer*** **Artificial soil**	
	Adult mortality	**Reproduction**
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥ 100	≥ 100

Observations:

Endpoint	**mPBacid (Metabolite of deltamethrin, AE F109036)** **(mg test item/kg soil d.w.)**	
	Control	**100**
Mortality of soil mites after 14 days (%)	8.8	6.3
Mean number of juveniles after 14 days	280.5	289.3
CV (%)	9.3	6.4
Reduction of reproduction (% to control)	-	-3.1

No statistically significant difference compared to control
(Fisher's Exact Binomial Test for mortality, $p \leq 0.05$; Student-t-test for reproduction; $p \leq 0.05$)
Calculations were done using non-rounded values
Percent reduction: $(1 - R_t / R_c) * 100$ %
R_t = the reproduction observed in the treated group(s)
R_c = the reproduction observed in the control group

In a separate study (BioChem project No. R 10 10 48 003 S, dated March 24, 2010), the EC50 (reproduction) of the reference item Dimethoate EC 400 was calculated to be 6.6 mg a.i./kg soil d.w. Th results of the reference test demonstrate the sensitivity of the test system.

Validity criteria (for the control group)

	Recommended	**Obtained**
Mean mortality of adult females	≤ 20 %	8.8 %
Mean number of juvenile per replicate	≥ 50	**280.5**
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	6.4 %

Conclusion:

The test item mPBacid (Metabolite of deltamethrin, AE F109036) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg d.w.

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

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Report:	**KCA 8.4.2/03, [redacted]; 2010**
Title:	Br_2CA (Metabolite of deltamethrin, AE F108565) - Effects on the reproduction of the collembolans *Folsomia candida*
Document No:	M-398826-01-1 (Rep. No: 101048103S)
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes

Material and methods:
Test item Br_2CA (Metabolites of deltamethrin, AE F108565), Product code: AE F108565 00 1B99 0001, Batch No.: 2N6185C, CAS No.: 53179-78-5, Chemical name: OR, 3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid, analysed purity: 98.8% w/w. 10 Collembola (9-12 days old) were exposed to 100 mg test item/kg soil dry weight of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$, at 18.4 - 21.1°C and a photoperiod, light:dark = 16h : 8h (750 lux) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days. Toxic standard 44-67-100-150-225 mg boric acid/kg dws; control: deionised water, solvent control: none.

Findings:

Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	Br_2CA *Folsomia candida* Artificial Soil	
	Adult mortality	Reproduction
	mg test item/kg soil dws	
LOEC	> 100	> 100
NOEC	= 100	= 100

Observations:

Endpoint	Br_2CA (mg test item/ kg soil dws)	
	Control	100
Mortality of parental collembolans after 4 weeks (%)	3.8	2.5
Mean number of juveniles after 4 weeks CV %	714.9 15.4	651.4 9.7
% Reduction of reproduction compared to control	-	8.9

CV: coefficient of variation
Percent reduction: $(1-R_t/R_c) * 100\%$
R_t = the reproduction observed in the treated groups
R_c = the reproduction observed in the control group

Conclusion:
In this test all validity criteria have been fulfilled.
The test item Br_2CA (Metabolite of deltamethrin, AE F108565) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans Folsomia candida in artificial soil at 100 mg test item/kg dws.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dws and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg dws.

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Report:	**KCA 8.4.2/04, [redacted], 2010**
Title:	mPBacid (Metabolite of deltamethrin, AE F109036): Effects on the reproduction of the collembolan Folsomia candida
Document No:	M-398820-01-1 (Rep. No: 101048100S)
Guidelines:	OECD 232 (2009), OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009); Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants
GLP:	Yes

Objective:
The purpose of this study was to determine potential effects 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. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted. The test was performed as limit test according to the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).

Material and methods:
Test item mPBacid (Metabolite of deltamethrin, AE F109036), Batch code: AE F109036 00 1B99 0001, Batch No.:400976/1,CASNo.:3739-38-6,Chemicalname:3-phenoxybenzoicacid, analysed purity: 98.6 % w/w.

10 Collembola (9-12 days old) were exposed to 100 mg test item/kg soil dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % CaCO3, at 18.4 – 21.1 °C and a photoperiod: light : dark = 16 h : 8 h (750 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

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

Findings:

Effects on mortality and reproduction of *Folsomia candida*

Test item **Test object** **Exposure**	**mPBacid (Metabolite of deltamethrin, AE F109036)** ***Folsomia candida*** **Artificial soil**	
	Adult mortality	**Reproduction**
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥ 100	≥ 100

Observations:

Endpoint	**mPBacid (Metabolite of deltamethrin, AE F109036)** **(mg test item/kg soil d.w.)**	
	control	**100**
Mortality of parental collembolans after 4 weeks (%)	2.5	1.3
Mean number of juveniles after 4 weeks	751.4	723.8
CV %	15.1	13.1
% Reduction of reproduction compared to control	-	3.7

CV: coefficient of variation
Percent reduction: $(1-R_t/R_c) * 100$ %
R_t = the reproduction observed in the treated groups
R_c = the reproduction observed in the control group

Validity criteria
- Mean adult mortality: ≤ 20 % (observed: 2.5 %)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 751.4/vessel)
- Coefficient of variation for the mean number of juveniles: ≤ 30 % (observed: 15.1 %)

In a separate study, the EC_{50} (reproduction) of the reference item boric acid was calculated to be 108.6 mg product/kg soil dry weight. Therefore, the observed effects assure a high sensitivity of the test system.

Conclusion:
The test item mPBacid (Metabolite of deltamethrin, AE F109036) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil at 100 mg test item/kg d.w.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg d.w.

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Report:	**KCA 8.4.2/05, [redacted] 2010**
Title:	Deltamethrin EW 15A G: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil with 5% peat.
Document No:	M-393654-01-1 (Rep. No: KRA-HR-39/10)
Guidelines:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
GLP:	Yes

Material and methods:
Test item: Deltamethrin EW 15A G; (Bach ID: 2010-002975; Material No.: 05759284; Specification No.: 102000013165 - 5; Master recipe ID: 0108025-001; Sample description: TOX08992-00; content: 15.35 g deltamethrin/L; density: 1.023 g/mL).
Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 18, 32, 56, 100, 178 mg test item/kg dry weight artificial soil were 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 (29 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast and with nematodes bred on watered oat flakes. 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.

Findings:

Test item	**Deltamethrin EW 15A G**		
Test organism			
Test substrate	**Artificial soil**		
mg test item/kg soil dry weight	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	7.5	288.1 ± 55.3	-
18	12.5	260.3 ± 29.2	90.3
32	0.0	249.0 ± 27.7	86.4
56	15.0	195.8* ± 37.4	67.9
100	25.0	175.8* ± 25.9	61.0
178	22.5	196.3* ± 37.5	68.1
NOEC (mg test item/kg dry weight artificial soil)			**32**
LOEC (mg test item/kg dry weight artificial soil)			**56**

* statistical significance (Williams test one-sided smaller, α = 0.05)

Observations:

Mortality:
In the control group 7.5 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} can not be calculated and is considered to be > 178 mg test item/kg dry weight artificial soil.
More than 10 adults were obtained in one replicate because together with the adult females juveniles were transferred to the test vessels and became adult during the test run.

Reproduction:
Concerning the number of juveniles statistical analysis (Williams test, one-sided smaller, α = 0.05) revealed significant difference between control and the three highest concentrations tested (56, 100 and 178 mg test item/kg dry weight artificial soil).
The numbers of juveniles indicate that inadvertently inserted juveniles didn't reproduce during the test run. Therefore all counted juveniles were considered as reproduction of the initially inserted adults.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 32 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 56 mg test item/kg dry weight artificial soil. The EC_{50} was 380 mg test item/kg dry weight artificial soil.

Conclusions:
In this test all validity criteria have been fulfilled.
NOEC: 32 mg test item/kg dry weight artificial soil.
LOEC: 56 mg test item/kg dry weight artificial soil.

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Report:	**KCA 8.4.2/06, [redacted]; 2010**
Title:	Deltamethrin EW 15 A G: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Document No:	M-397993-01-1 (Rep.No: FRM-COLL-102/10)
Guidelines:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals – Collembolan Reproduction Test in Soil
GLP:	Yes

Material and methods:

Deltamethrin EW 15A G (analytical findings: 15.35 g/L corresponding to 1.50 % w/w, batch ID: 2010-002975, master recipe ID: 0108025-001, specification no.: 102000013165-05, sample description: TOX08992-00. Since the first test run on the test item did not provide a final result, a second test run was performed studying higher concentrations. 10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 4 replicates for the treatment group) were exposed to control (water treated), 18, 32, 56, 100 and 178 mg test item/kg artificial soil dry weight in the 1st test run and 316, 562 and 1000 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.

Findings:

Test item	**Deltamethrin EW 15 A G**		
Test organism	***Folsomia candida***		
Test substrate	**Artificial soil**		
mg test item/kg soil dry weight	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
1st test run			
Control	3.8	1450 ± 76	-
18	2.5	1442 ± 76	**99** n.s.
32	7.5	1528 ± 200	**105** n.s.
56	2.5	1340 ± 76	**92** n.s.
100	10.0	1341 ± 125	**93** n.s.
178	12.5	1556 ± 108	**107** n.s.
2nd test run			
control	6.3	1421 ± 138	-
316	2.5	1294 ± 152	**91***
562	77.5	443 ± 5.7	**3.1***
1000	75.0	46.8 ± 7.6	**3.3***
$NOEC_{reproductioon}$ (mg test item/kg soil (dry weight)			**178**
$LOEC_{reproduction}$ (mg test item/kg soil (dry weight)			**316**

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

Observations:

Mortality:

In the control group 3.8% (1st run) and 6.3% (2nd run) of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20% mortality. The highest mortality rate of 77.5% was observed in the treatment group with 562 test item/kg artificial soil dry weight.

Reproduction:

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no statistically significant differences between the control and any treatment group in the 1st test run. In the 2nd test run statistical analysis revealed statistically significant differences up to the highest treatment group with 1000 mg test item/kg artificial soil dry weight. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 178 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 316 mg test item/kg artificial soil dry weight.

Conclusions:

In this test all validity criteria have been fulfilled.

$NOEC_{reproduction}$: 178 mg test item/kg artificial soil dry weight

$LOEC_{reproduction}$: 316 mg test item/kg artificial soil dry weight

Supplemental information from literature research

Report:	**KCA 8.4.2/07; [redacted]; 2006**
Title:	Soil microbial and faunal community responses to Bt maize and insecticide in two soils.
Source:	J. Environ. Qual., Volume 35, Issue 3, Page 734-741
DOI No: Document No:	10.2134/jeq2005.0344 M-460894-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The effects of maize (Zea mays L.), genetically modified to express the Cry1Ab protein (Bt), and an insecticide (deltamethrin) on soil microbial and faunal communities were assessed in a glasshouse experiment. Material and methods as well as results are summarized only for deltamethrin and non-*Bt* maize.

Three plant growth stages (five-leaf, flowering, and maturity) on two soils ([redacted]) with and without insecticide treatment, with five replicate pots per treatment were tested. Pots were watered to constant weight three times per week with tap water. A topdressing of 100 mL liquid N–P–K fertilizer (16–5–32) (equivalent to 80 kg N/ha) was added to all pots assigned to the final sample (at maturity) after 83 d growth. After 39 d growth, when the plants had five leaves, and 87 d growth, during flowering, half the pots were treated with insecticide (Decis 2.5% w/w deltamethrin). The

recommended application rate of 200 mL/ha equates to 2.5 mL/plant given a typical sowing of 80 000 seeds/ha . An aqueous solution of Decis was prepared at 0.45 mL L21 and 5.5 mL (2.5 mL Decis) sprayed directly onto the soil surface. Pots not receiving Decis were sprayed with the same volume of water. Spraying took place 24 h before sampling.
Afterwards, the plant was carefully removed from the pot and soil shaken from the roots. Plants were separated into leaves, stems, cob (at maturity only), and roots, dried, weighed and milled (0.2 mm mesh). Then, carbon and N content were measured following combusting using a Europa Scientific 20-20 mass spectrometer.
Soil was mixed carefully and used for analysis with subsamples being frozen at -80°C, for phospholipid fatty acid analysis or at-20°C for the later determination of residual insecticidal activity toward non-target soil insects.
Gravimetric water content was determined at 105°C.
Nematodes were extracted from ca. 20 g fresh soil from each sample using a modified Whitehead and Hemming tray technique[15].
Total numbers of protozoa (i.e., active and encysted forms) were estimated by a most probable number technique[16]. The presence of flagellates, ciliates, and amoebae were recorded after 7, 14, and 21 d. Numbers were calculated according to Hurley and Roscoe (1983)[17] and biomass calculated using approximate weights (Griffiths and [redacted], 1993)[18].
Micro-arthropods were extracted from 100 g soil, over a 5-d period, using a Tullgren funnel apparatus (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) and preserved in 70% ethanol. Total micro-arthropod numbers were counted under low-power microscopy.
Soil–saline suspension remaining from the protozoan measurement was used to determine the community-level physiological profile[19]. Sterile NMAS were given into the suspension and absorbance of each well at 595 nm was read initially and after incubation of 3, 4 and 5 d at 15°C.

Soil temperature was the same regardless of treatment with average daily fluctuations between 12 and 24°C Volumetric soil water content varied between watering intervals, from 30 to 13% as maximum and minimum during the experiment.
Results indicated that there were no effects of insecticide on plant weight, carbon content or nitrogen content within soil type.
When the nematode community at the mature stage was analyzed, there were differences in the proportions of bacterial-feeders, with fewer in soil treated with insecticide than without insecticide ($p < 0.01$); and plant feeders, with more in soil treated with insecticide than without insecticide ($p < 0.05$). The nematode community was altered by the application of insecticide (indicated by Principal component plot). Differences due to the application of insecticide to Monumental were largely due to reduced proportions of *Pratylenchus* and Rhabditidae and increased proportions of Helicotylenchidae. Protozoa, Mites and microarthropods indicated no significant effects of insecticide application.

[15] Whitehead, A.G., and J.R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55:25–38.
[16] Darbyshire, J.F., R.E. Wheatley, M.P. Greaves, and R.H.E. Inkson. 1974. A rapid method for estimating bacterial and protozoan populations in soil. Rev. Ecol. Biol. Sol 11:465–474.
[17] Hurley, M.A., and M.E. Roscoe. 1983. Automated statistical analysis of microbial enumeration by dilution series. J. Appl. Bacteriol. 55:159–164.
[18] [redacted] B.S., and S. [redacted] 1993. Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. Biol. Fertil. Soils 15:201–207.
[19] Garland, J.L., and A.L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilisation. Appl. Environ. Microbiol. 57:2351–2359.

Furthermore, tests with the cabbage root fly in soil sampled at the mature stage of maize growth indicated no effect of Decis on development of the larvae.
Additionally, there were no significant effects of insecticide on the amount of phospholipid fatty acid in soils. Finally, the community level physiological profile was not significantly influenced by the insecticide treatment.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 2.5% w/w deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted] Cambridge, UK
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test organism(s)

Species:	Nematodes, Protozoa and Micro-arthropods
Source of test species:	Species were already in the collected soils ([redacted] Denmark and [redacted] France)

B. Study design and methods

1. Test procedure

Test system (study type):	Glasshouse experiment (Soil Microbial and Faunal Community Responses)
Duration of study:	Up to 123 days
Treatments:	Two different soils ([redacted]), Decis, control and three growth stages of monumental maize (five leaf stage (after 39 d of growth), at flowering (87 d of growth) and at maturity (122 d of growth))
Application rate:	Decis: 0.45 ml/L
Number of replicates:	5 replicates per treatment
Application / device / nozzles:	Decis was sprayed directly onto the soil surface
Water volume:	5.3 ml (2.5 µl Decis)
Verification of dispersion:	-

2. Environmental conditions

Test medium:	Soil was collected from the field sites at [redacted] Denmark and [redacted] France where Bt maize (MON810, Cry1Ab) was being grown. Soil was collected separately from the top 5 cm and the lower 5 to 20 cm-
Soil type:	[redacted]: sandy loam (62.2% sand, 23.2% silt and 8.3% clay); [redacted]: clay loam (20% sand, 41% silt and 30% clay)
Gravimetric water content:	[redacted]: 29.9%; [redacted]: 26.2%
Temperature / relative humidity	Light period: 20°C; dark period: 15°C
Photoperiod	16:8-h light:dark
Lighting	< 100 W/m²

pH_{CaCl2}:	[redacted]: 5.6; [redacted]: 7.1
Organic matter (C_{org}):	[redacted]: 6.4%; [redacted]: 4.8%
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	Gravimetric water content and soil temperature
Biological parameters measured:	Leave, stem, cob (at maturity only) and root weight; Carbon and nitrogen content in plant; Phospholipid fatty acid content in soil Insecticidal activity toward non-target soil insects (using cabbage root fly (*Delia radicum* L.), Number of Nematodes, Number and biomass of protozoa (Flagellates, ciliates and amoebae), Number of micro-arthropods, Community-level physiological profile
Measurement frequency:	Test were determined 24 h after exposure and soil aliquots were sampled; For insecticidal activity toward non-target soil insects, larvae and pupae of *Delia radicum* L. were collected after 3 weeks of incubation; For nematodes, the total number were counted 48 hours after aliquot collection; For protozoa, the number were recorded 7, 14 and 21 d after aliquot incubation; Micro-arthropods were counted after a 5 day extraction of an aliquot; The community-level physiological profile were determined after incubation for 3, 4 and 5 d.
Statistical analyses:	Standard analysis of variance (ANOVA), LSD-Test, Principal component analysis

RESULTS

1. Validity criteria:

No validity criteria were mentioned.

2. Analytical measurements:

Soil temperature was the same regardless of treatment with average daily fluctuations between 12 and 24°C Volumetric soil water content varied between watering intervals, from 30 to 13% as maximum and minimum during the experiment.

4. Biological findings:

Results indicated that there were no effects of insecticide on plant weight, carbon content or nitrogen content within soil type.

When the nematode community at the mature stage was analyzed, there were differences in the proportions of bacterial-feeders, with fewer in soil treated with insecticide than without insecticide ($p < 0.01$); and plant feeders, with more in soil treated with insecticide than without insecticide ($p < 0.05$). The nematode community was altered by the application of insecticide (indicated by Principal component plot). Differences due to the application of insecticide to Monumental were largely due to reduced proportions of *Pratylenchus* and Rhabditidae and increased proportions of Helicotylenchidae. Protozoa, Mites and microarthropods indicated no significant effects of insecticide application.

Furthermore, tests with the cabbage root fly in soil sampled at the mature stage of maize growth indicated no effect of Decis on development of the larvae.
Additionally, there were no significant effects of insecticide on the amount of phospholipid fatty acid in soils. Finally, the community level physiological profile was not significantly influenced by the insecticide treatment.

Table 1: Mean abundance (g^{-1} dry soil) and percentage composition of bacterial- (BF), fungal- (FF), omnivore (OM), and plant-feeding (PF) nematodes under mature maize (Monumental), with or without insecticide, growing in [redacted] or [redacted] soil under glasshouse conditions. Data are means of five replicates.

Soil	Treatment	Abundance g^{-1}	Composition %			
			BF	FF	OM	PF
[redacted]	Control	32.2	40.2	18.6	6.4	32.3
	Decis	37.8	36.9	18.1	12.6	31.2
[redacted]	Control	22.4	52.0	16.1	14.3	12.9
	Decis	22.9	41.9	16.1	15.3	21.4

RESULTS SUMMARY
Results indicated that there were no effects of insecticide on plant weight, carbon content or nitrogen content within soil type.
When the nematode community at the mature stage was analyzed, there were differences in the proportions of bacterial-feeders, with fewer in soil treated with insecticide than without insecticide ($p < 0.01$); and plant feeders, with more in soil treated with insecticide than without insecticide ($p < 0.05$). The nematode community was altered by the application of insecticide (indicated by Principal component plot). Differences due to the application of insecticide to Monumental were largely due to reduced proportions of *Pratylenchus* and Rhabditidae and increased proportions of Helicotylenchidae. Protozoa, mites and microarthropods indicated no significant effects of insecticide application.
Furthermore, tests with the cabbage root fly in soil sampled at the mature stage of maize growth indicated no effect of Decis on development of the larvae.
Additionally, there were no significant effects of insecticide on the amount of phospholipid fatty acid in soils. Finally, the community level physiological profile was not significantly influenced by the insecticide treatment.

Comments by the Notifier: Summarized under MCA 8.4.2/08 below**.**

Report:	**KCA 8.4.2/08; [redacted] 2011**
Title:	Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) with registered insecticides for Spodoptera frugiperda ([redacted], 1797) (Lepidoptera: Noctuidae) under laboratory conditions.
Source:	Crop protection,29, 6 p. 545-549
DOI No:	10.1016/j.cropro.2009.12.012
Document No:	M-461809-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The fall armyworm, *Spodoptera frugiperda* ([redacted], 1797) (Lepidoptera: Noctuidae) is considered the main key pest of corn crops in Brazil. Entomopathogenic nematodes (EPNs) may be used to control this pest, applied together with other different entomopathogen agents or phytosanity products in the spraying mixture. Thus, the objective of work was to evaluate the compatibility of EPNs with different insecticides used of *S. frugiperda* control in laboratory conditions. Three species of EPNs (*Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri*) and 18 insecticides registered to control of *S. frugiperda* in corn crops were tested. Compatibility of the insecticides with EPNs was evaluated by observing mortality and infectivity of infecting juveniles (IJs) 48 h after immersion in solution of the insecticide formulations. Material and methods as well as results are summarized only for deltamethrin.

Therefore, a methodology suggested by Negrisoli Jr. et al. (2008)[20] was adopted. To prepare the stock solution, one litre of Decis 25 EC (concentration: 0.2 L/ha; Spray volume: 10-20 L/ha) was prepared proportionally to the double doses that would be normally applied in 1 ha. From this solution, 1 mL aliquots of each product were placed in five test tubes and later 2500 IJs were added with 1 ml of distilled water to each treatment. Each tube consisted in one replicate. The bioassay was performed in environmental controlled chamber at 22 ± 1 °C; RH of 70 ± 10% with 12 h photoperiod. Nematode mortality was evaluated 48 h after their exposure to the product. Therefore, one aliquot of 0.1 ml was collected and 100 IJs were assessed under the stereo microscope.

Afterwards, the remaining treatments were rinsed three times with 3 ml of distilled water. Then, a volume of 0.2 ml (containing approx. 100 IJs) from the bottom of each tube were distributed in five Petri dish plates containing filter paper previously wetted with 1.8 ml distilled water. Each plate received ten last instar *G. mellonella* larvae. After this, they were incubated for 5 days at 22 ± 1 °C; RH of 70 ± 10% with 12 h photoperiod. Then, dead larvae were collected and stored into darkness for three more days. Finally, they were dissected in order to verify nematode's presence. The bioassays were performed two times.

Mortality of *H. indica*, S. carpocapsae and S. glaseri IJs was 10, 17.2 and 11.6%, respectively. Infectivity, that is, capacity of *H. indica*, *S. carpocapsae* and *S. glaseri* to cause *G. mellonella* death was 74.8, 80.6 and 78.6, respectively.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 25 EC
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

[20] [redacted] Jr., A.S., [redacted] C.R.C., Moino Jr., A., 2008. Avaliaçao da compatibilidade de produtos fitossanitarios com nematoides entomopatogenicos (Rhabditida: Steinernematidae, Heterorhabditidae) utilizando o protocolo modificado da IOBC/WPRS. Nematologia Brasileira 32 (2), 111–116.

Vehicle/solvent:	Distilled water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-
<u>3. Test organism(s)</u>	
Species:	*Heterorhabditis indica* IBCB-n5, *Steinernema carpocapsae* Sta Rosa and *Steinernema glaseri* Sta Rosa
Cultivar:	-
Source of test species:	*Heterorhabditis indica* (isolated [redacted] Sao Paulo, Brazil), *Steinernema carpocapsae* Sta Rosa and *Steinernema glaseri* Sta Rosa (isolated in [redacted] Sao Paulo, Brazil)
Age of test organisms at study initiation / Crop growth stage at treatment:	Infecting juveniles
Holding conditions prior to test:	Nematodes were multiplied on at instar *G. mellonella* larvae, according to Kaya and Stock (1997)[21]. Infecting juveniles were stored in aqueous suspension at 12°C in darkness.
Acclimatisation:	-

B. Study design and methods

<u>1. Test procedure</u>	
Test system (study type):	Effect of insecticide on viability and infectivity
Duration of study:	Mortality assessment: 48 h; Infectivity assessment: 10 days (48 h exposition to deltamethrin during mortality assessment + 5 days contact to *G. mellonella* larvae + 3 days storage of dead *G. melloella* larvae)
Treatments:	Control and Decis EC 50
Test concentrations	To prepare the stock solution, one litre of Decis 25 EC (concentration: 0.2 L/ha; Spray volume: 10-20 L/ha) was prepared proportionally to the double doses that would be normally applied in 1 ha. From this solution, 1 mL aliquots of each product were placed in five test tubes and were diluted with 1 ml distilled water.
Number of replicates:	5 (Bioassays were conducted two times)
Individuals per replicate:	Mortality assessment: 2500 IJs (only 100 IJS used for assessment); Infectivity assessment: 100 IJs (from mortality assessment and 10 last instar *G. mellonella* larvae
Test units (type and size):	Mortality assessment: Glass test tube; Infectivity assessment: Petri Dish plates (9 cm diameter)
<u>2. Environmental conditions</u>	
Test medium:	Mortality assessment: treated distilled water; Infectivity assessment: wetted filter paper
Temperature / relative humidity:	22 ±1 °C; RH of 70 ± 10%
Photoperiod:	12 h
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-

[21] Kaya, H.K., Stock, S.P., 1997. Techniques in insect nematology. In: Lacey, L.A. (Ed.), Manual of Tecniques in Insect Pathology. Academic Press, San Diego, California, pp. 281–324.

Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality, Infectivity (described the capacity of tested nematodes to cause *G. mellonella* larval death)
Measurement frequency:	After 48 h (Mortality assessment), 5 days later (Infectivity assessment), 3 days later (verifying of nematode presence)
Statistical analyses:	Tukey's HDS test

RESULTS

1. Validity criteria:

No validity criteria were stated.

3. Biological findings:

Mortality of *H. indica*, S. carpocapsae and S. glaseri IJs was 10, 17.2 and 11.6%, respectively. Infectivity, that is, capacity of *H. indica*, *S. carpocapsae* and *S. glaseri* to cause *G. mellonella* death was 74.8, 80.6 and 78.6, respectively.

Table 1: Effect of Decis 25 EC on the mortality (average ± SE) and infectivity[a] (average ± SE) of *Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri* (temperature 22 ± 1 °C, relative humidity of 70 ± 10% and photophase of 12 h),

	Heterorhabditis indica	***Steinernema carpocapsae***	***Steinernema glaseri***
Mortality			
Control (%)	4.6 ± 0.4	4.0 ± 1.2	4.8 ± 1.6
Decis (%)	10.0 ± 1.6	17.2 ± 0.7*	11.6 ± 2.1
Infectivity (%)			
Control (%)	91.4 ± 0.9	94 ± 0.6	94.6 ± 1.4
Decis (%)	74.8 ± 1.6*	80.6 ± 0.7*	78.6 ± 1.5*

[a] measured by *Galleria mellonella* larvae mortality.

* Statistically different by Tukey test at P = 0.05.

RESULTS SUMMARY

Mortality of *H. indica*, *S. carpocapsae* and *S. glaseri* IJs was 10, 17.2 and 11.6%, respectively. Infectivity, that is, capacity of *H. indica*, *S. carpocapsae* and *S. glaseri* to cause *G. mellonella* death was 74.8, 80.6 and 78.6, respectively.

Comments by the Notifier: Risk of Deltamethrin EW 15 for soil nematodes

[redacted] 2006 (MCA 8.4.2/07, M-460894-01-1) found an impact of Decis (2.5% Deltamethrin) on soil nematode community at 200 ml Decis/ha. Densities of bacterial feeding nematodes were reduced by up to 19.4 %, whereas the abundances of omnivore and plant feeding nematodes were up

to 2-times and 1.7-times higher in samples treated with Decis, respectively. However, a negative long-term impact of the representative formulation Deltamethrin EW15 on nematode community cannot be concluded from this study since the study was performed with Decis EC 2.5% (different formulation type) and the last observation took place 84 days after application, so only a mid-term observation was performed. A reduction in abundances was only observed for bacterial feeding nematodes (19.4%). Fungal-, omnivore, and plant-feeding nematodes were not negatively impacted by Decis. Thus, no negative long-term impact on the structure and functioning of the soil nematode community can be concluded.
In addition, [redacted] 2010 (MCA 8.4.2/08, M-461809-01-1) demonstrated that Decis EC 50 g/L is compatible (class 1) with the three Entomopathogenic nematodes *Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri* (biocontrol agents for the fall armyworm *Spodoptera frugiperda*), tested under laboratory conditions.
Thus, no unacceptable risk of Deltamethrin EW 15 on soil nematode community and functioning can be considered from the use of up to 2 x 7.5 g Deltamethrin/ha in cauliflower.

Report:	**KCA 8.4.2/09; [redacted] 2005**
Title:	Action of pesticides to Metarhizium anisopliae in soil.
Source:	Neotrop. Entomol., 34, 6, p. 961-971
DOI No: Document No:	http://dx.doi.org/10.1590/S1519-566X2005000600013 M-460907-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The objective of the present study was to analyze a possible toxic action of some active ingredients present in acaricides, fungicides, insecticides and herbicides, used at the doses recommended by the manufacturers, on the entomopathogenic fungus M. *anisopliae* in soil based on the measurement of respiratory activity.
2 ml of conidial suspension (1×10^8 conidia/ml; Isolate E9 of M. anisopliae (Metsch.) Sorkin from the spittlebug Deois flavopicta (Stal)) were incubated in 100 g portions of autoclaved soil (yellow red podzol of a sandy/medium texture collected January 2003; 0-20 cm depth; 21°21'02" S and 48°31'17" W; 65% of its saturation capacity). After 48 h at 27°C ± 0.5°C, the respiratory activity was measured using an adaption of the method described by Jenkinson & Powlson (1976)[22].
Next, 2.5 ml pesticide solution were spread over the whole soil surface with a pipette at the amount and concentration calculated to obtain the dose (per mm² soil surface) recommended by the manufacturer (50 ml/100L). The second measurement was made 48h after application of the pesticide, followed by an additional eight measurements every 48h and five measurements every four days, for a total of 40 days of incubation. The assay consisted of the treatment with two controls, one consisting of soil only and the other of soil inoculated with the fungus. The assay was performed in five replicates.
For the toxicity of deltametrin to M. *anisopliae* in soil, no significant difference in fungal respiratory activity was observed between this treatment and the control.

MATERIAL AND METHODS

[22] Jenkinson, D.S. & D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil - V. A method for measuring soil biomass. Soil Biol. Biochem. 8: 209- 213.

A. Material

1. Test material

Test item:	Decis 50 SC
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Metarhizium anisopliae*
Cultivar:	-
Source of test species:	Spittlebug *Deois flavopicta* ([redacted])
Age of test organisms at study initiation /	-
Crop growth stage at treatment:	
Holding conditions prior to test:	27°C
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Chronic toxicity assay
Duration of study:	40 days
Treatments:	Deltamethrin, pur soil control and soil fungus control
Test concentrations:	50 ml/100 L
Number of replicates:	5
Individuals per replicate:	2 ml conidial suspension (1.8 x 10^8 conidia/ml)
Test units (type and size):	1700 ml glass pots
Application / device / nozzles:	Pipette
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Soil
Temperature / relative humidity:	27 ± 0.5°C
Photoperiod:	-
Lighting	-
pH:	5.5
Organic matter (C_{org}):	26 g/dm ³
Ca	38 $mmol_c/dm^3$
Cation exchange capacity:	76.5 mmol/dm³
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Respiratory activity
Measurement frequency:	48 h after incubation of the fungus; 48 h after application of the pesticide, followed by additional eight measurments every 48 h and five measurements every four days for a total of 40 days of incubation
Statistical analyses:	F-test, Tukey test

RESULTS

1. Validity criteria:

No validity criteria were stated.

2. Biological findings:

For the toxicity of deltametrin to M. *anisopliae* in soil, no significant difference in fungal respiratory activity was observed between this treatment and the control.

Table 1: Respiratory activity (mg CO2100g-1 soil) of M. anisopliae in autoclaved soil submitted to the action of deltamethrin

Period analysed (days)	Control	Deltamethrin
0-2	22.23	22.0
2-4	12.7	12.4
4-6	7.9	8.5
6-8	7.6	8.1
8-10	7.1	7.4
10-12	5.5	5.0
12-14	4.6	5.0
14-16	3.8	3.6
16-18	3.5	3.4
18-20	3.6	4.0
20-24	25.	2.3
24-28	2.0	2.1
28-32	2.3	2.5
32-36	1.8	1.8
36-40	2.0	1.9
Means	6.0	6.0

Tukey test indicated no significant differences between deltamethrin treatment and the control.

RESULTS SUMMARY

For the toxicity of deltametrin to M. *anisopliae* in soil, no significant difference in fungal respiratory activity was observed between this treatment and the control.

Comments by the Notifier

Concentrations which were tested were very high and not in a relevant range for the intended use of Deltamethrin EW15. In addition, no effects were seen, so the study has no impact on the risk assessment and will not be further considered.

Report:	**KCA 8.4.2/10; [redacted]; 2007**
Title:	Comparative effects of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms (Eisenia fetida).
Source:	Pestic. Biochem. Physiol., 89, 1, p. 31-38
DOI No: Document No:	10.1016/j.pestbp.2007.02.005 M-460908-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Laboratory tests were conducted to compare the effects of various concentrations of lindane and deltamethrin on mature earthworms (*Eisenia fetida*) cultured in artificial soil during typical acute (14d) and subchronic (42d) exposure periods. The effects of the two pesticides on earthworm mortality, growth inhibition, and cellulase activity were determined for different exposure durations. Material and methods as well as results are summarized only for deltamethrin.

Acute toxicological test: 10 adult worms in 750 g of wet artificial soil were exposed to 5, 25, 50 100, 150, 500 and 1000 mg(deltamethrin)/kg dry soil for 14 days. Additionally a positive control was carried out using 2 ml of acetone. Three replicates were used for each dose. The containers were kept in an incubation chamber (20 ± 1 °C, 70-90% relative humidity with continuous illumination at 400-800 lx) throughout the test period. During the test period earthworms were separated from the test substrate, counted, cleaned with deionized water, and weighed on days 3, 7, 10, and 14. Two earthworms were selected from each container to determine cellulose activity at the end of the acute test period using a carboxymethlycellulose assay. The mortality was determined by counting the number of dead earthworms (lack of movement, no responds to a definite tactile stimulus or missing).

Subchronic toxicological test: The nominal test concentrations were 5, 25 and 50 mg/kg dry soil. During the first 14 days, the earthworms were cultured using the same procedure as for the acute toxicity test. From day 15 to 42, additional food (finely ground and dried cattle dung) was added once a week to every replicate (treatment plus control). Earthworms were fed by applying about 0.5 g cow dung per worm to the soil surface once a week. The same procedure but with acetone was used as control solvent. After the incubation periods of 28 and 42 days, the earthworms were removed from the substrate, counted, and weighed. Cellulase activity was determined only at the end of the subchronic test period (day 42) using a carboxymethlycellulose assay. The mortality was determined by counting the number of dead earthworms (lack of movement, no responds to a definite tactile stimulus or missing).

Earthworms exposed to deltamethrin showed dose-dependent toxic effects on growth and cellulose activity only from the acute exposures. The LC50 was 432.9 mg/kg in the acute toxicological test. Mortality levels were lower than 12% after exposure to deltamethrin for 28 and 42 days. During the 14-day exposure period, the growth inhibition for all of the earthworms cultured in the deltamethrin-treated soil were positive and significantly different ($P < 0.001$) from those for the controls. In the subchronic test, deltamethrin was found capable of inhibiting earth worm growth, with effects ranging from 16.8% to 26% after 28 days of exposure and from19.8% to 36.3% after 42 days. Cellulase activity in earthworms was inhibited in groups exposed to deltamethrin for 14 or 42 days. Maximum inhibitions of 24.9% and 23.6% were observed in the 5 and 50 mg/kg groups, respectively, with a minimum inhibition of 6.9% in the 100 mg/kg group.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted], China
Lot/Batch number:	-
Purity:	99%
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	2 ml containing 5, 25, 50, 100, 150, 500 and 1000 mg/kg dry soil (acute) and 5, 25 and 50 mg/kg dry soil (subacute)

3. Test organism(s)

Species:	*Eisenia fetida*
Cultivar:	
Source of test species:	China [redacted]
Age of test organisms at study initiation / Crop growth stage at treatment:	3 month old
Holding conditions prior to test:	In the dark at 14± 1°C
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Acute and sub-chronic test design
Duration of study:	Acute: 14 d; subchronic: 42 d
Treatments:	Deltamethrin and acetone control
Test concentrations	5, 25, 50, 100, 150, 500 and 1000 mg/kg dry soil (acute) and 5, 25 and 50 mg/kg dry soil (sub-chronic)
Number of replicates:	3
Individuals per replicate:	10
Test conditions:	20 ± 1°C, 70–90% relative humidity with continuous illumination at 400–800 lx
Test units (type and size):	-
Application / device / nozzles:	-
Water volume:	Soil moisture contents were adjusted to 35% of the basic substrate's dry weight by adding deionized water.
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Artificial soil mixture of sand, sphagnum peat, kaolinite clay, and calcium carbonate.
Temperature / relative humidity:	20 ± 1°C
Photoperiod:	-

Lighting	400–800 lx
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable	-
micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-
3. Observations and measurements:	
Analytical parameters measured:	-
Biological parameters measured:	Mortality, growth inhibition, weight and cellulose activity
Measurement frequency:	Mortality: after 3, 7, 10 and 14 days (acute), after 28 and 42 days (sub-chronic); Growth inhibition: 3, 7, 10 and 14 days (acute), after 28 and 42 days (sub-chronic) *Cellulase activity*: after 14 (acute) and 42 days (chronic)
Statistical analyses:	Probit regression, ANOVA using Student-Newman-Keuls post hoc pairwise multiple comparison procedure; Shapiro-Wilk test; Levene's test; Kruskal-Wallis

RESULTS

1. Validity criteria:

No validity criteria were mentioned.

3. Biological findings:

Mortality: The median lethal concentrations (LC50) of deltamethrin, was 432.9 mg/kg (14 days). Mortality levels were lower than 12% after exposure to deltamethrin for 28 and 42 days.

Growth Inhibition: During the 14-day exposure period, the growth inhibition for all of the earthworms cultured in the deltamethrin- treated soil were positive and significantly different ($P < 0.001$) from those for the controls. The decreases in weight after the 7- and 10-day exposures to deltamethrin were found statistically to be dose-dependent (ANOVA: $P < 0.01$ for the 7 days of exposure; $P < 0.05$ for the 10 days of exposure), with a significant difference between the lower (5 and 25 mg/kg) and higher dosage groups (100 and 150 mg/kg). In contrast, no significant difference was observed among the various concentrations for days 3 and 14 (ANOVA: $P > 0.05$ for 3 days of exposure; Kruskal–Wallis H test: $P > 0.05$ for 14 days of exposure).
In addition, deltamethrin was found capable of inhibiting earthworm growth, with effects ranging from 16.8% to 26.6% after 28 days of exposure and from 19.8% to 36.3% after 42 days. However, the inhibition showed no sign of dose dependency (ANOVA: $p \geq 0.05$ for 28 days and 42 days).

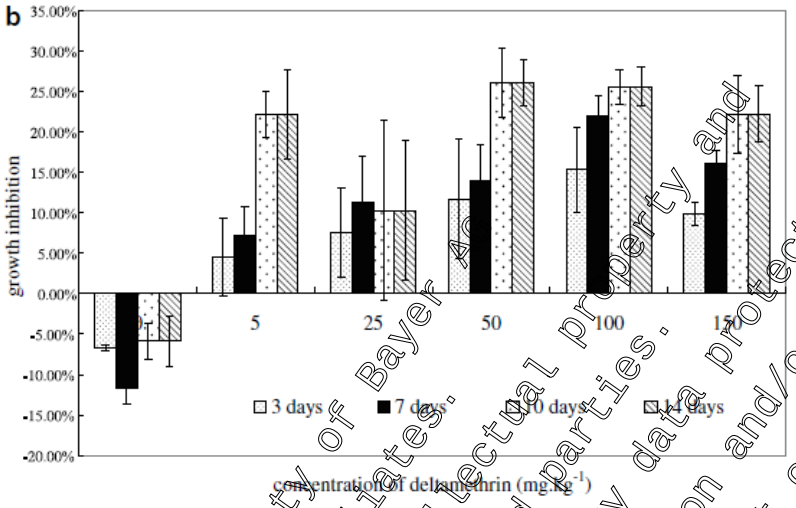

Fig. 1. (a) Growth inhibition in earthworms from acute exposure to lindane. (b) Growth inhibition in earthworms from acute exposure to deltamethrin.

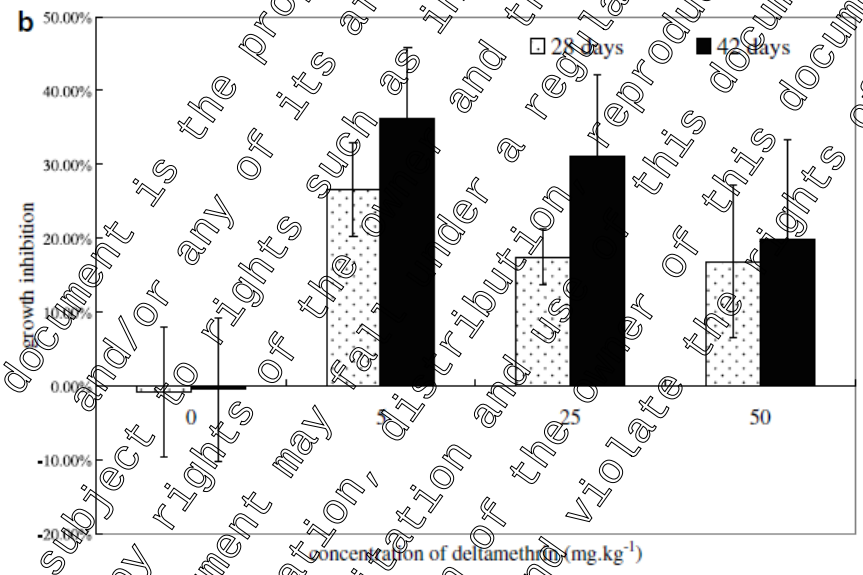

Fig. 2. (a) Growth inhibition in earthworms from subchronic exposure to lindane. (b) Growth inhibition in earthworms from subchronic exposure to deltamethrin.

Cellulase activity: Cellulase activity in earthworms was inhibited in groups exposed to deltamethrin for 14 or 42 days. Maximum inhibitions of 24.9% and 23.6% were observed in the 5 and 50 mg/kg groups, respectively, with a minimum inhibition of 6.9% in the 100 mg/kg group. One-way ANOVA (using the S–N–K test) showed a significant difference between the cellulase activity in the controls and those in earthworms exposed to deltamethrin at various concentrations for 14 days ($P < 0.05$).

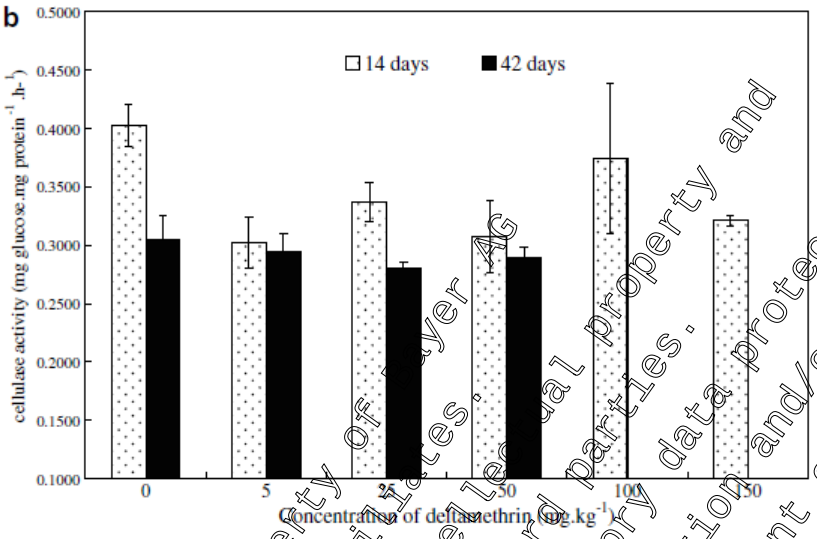

Fig. 3. (a) Cellulase activity in earthworms from acute and subchronic exposures to lindane. (b) Cellulase activity in earthworms from acute and subchronic exposures to deltamethrin.

RESULTS SUMMARY

Earthworms exposed to deltamethrin showed dose-dependent toxic effects on growth and cellulose activity only from the acute exposures. The LC50 was 432.9 mg/kg in the acute toxicological test (14 d). In addition, deltamethrin was found capable of inhibiting earthworm growth, with effects ranging from 16.8% to 26.6% after 28 days of exposure and from 19.8% to 36.3% after 42 days. Cellulase activity in earthworms was inhibited in groups exposed to deltamethrin for 14 or 42 days. Maximum inhibitions of 24.9% and 23.6% were observed in the 5 and 50 mg/kg.

Comments by the Notifier

Effect on growth of *E. fetida* was observed at 5 mg deltamethrin/kg. However, the concentrations tested are not in a relevant range for the intended use of Deltamethrin EW15. Therefore this study is not considered further in the risk assessment.

Report:	**KCA 8.4.2/11; [redacted] 2013**
Title:	Avoidance and reproduction tests with the predatory mite *Hypoaspis aculeifer*: effects of different chemical substances
Source:	Environmental Toxicology and Chemistry, Vol. 33, No. 1, 2014
DOI No.: Document No.:	10.1002/etc.2421 M-469671-01-1
Guidelines:	Organisation for Economic Co-operation and Development. 2008. Test No. 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. OECD Guidelines for the Testing of Chemicals. Paris, France.
GLP:	No. Published study (peer-reviewed article).
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The behaviour of deltamethrin on the avoidance and reproduction on mite was investigated. The LC_{50} was 16.30 mg/kg. The EC_{50} based on reproduction was 9.88 mg/kg and the EC_{50} based on avoidance was > 32 mg/kg.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis flussig, 2.8% active substance
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	Not reported
Source of test item:	[redacted]
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test solutions

Vehicle/solvent:	Acetone

3. Test organism(s)

Species:	*Hypoaspis aculeifer*
Source of test species:	[redacted], Germany)
Holding conditions prior to test:	Specimens were cultured in the laboratory, kept at 20°C with a photoperiod of 16:8 h, light:dark cycle in plastic containers lined with an 8:1 ratio of plaster of Paris and activated charcoal substrate. The substrate was moistened once a week with dejonized water, and cheese mites (*Tyrophagus putrescentiae*) were added ad libitum as a food source.

B. Study design and methods

1. Test procedure

Test system (study type):	Avoidance behavior of mites. Survival and reproduction of mites.
Duration of study:	Avoidance behaviour of mites: 48 h Survival and reproduction of mites: 14 d
Number of replicates:	Avoidance behaviour of mites: five Survival and reproduction of mites: Four replicates were used in all treatments, except in tests in which a solvent control was not needed where 6 replicates were used for the control treatments. For each treatment, 1 extra replicate for moisture and pH measurements.
Individuals per replicate:	Avoidance behaviour of mites: ten Survival and reproduction of mites: ten
Test units (type and size):	Avoidance behaviour of mites: cylindrical 150-mL glass beakers used divided into 2 sections (treated and control soil)

2. Environmental conditions

Test medium:	
Temperature / relative humidity:	Each soil treatment used in the experiment was moistened so as to make 50% of the maximum water holding capacity.

Lighting	Avoidance behaviour of mites: light:dark cycle of 16 h:8 h Survival and reproduction of mites: light:dark cycle of 16 h:8 h
pH:	The pH was adjusted to 6.0 +/- 0.5 by adding 0.2% $CaCO_3$.

3. Observations and measurements:

Biological parameters measured:	Avoidance behavior, survival and reproduction Avoidance behaviour of mites: NR = (C-T)/Nx100 where NR is the net response, C is the number of mites observed in the control soil, T is the number of mites observed in test soil, and N is the total number of mites per replicate. Dual-control test was done with pairwise comparison with 1-tailed Student's t test. The avoidance median effective concentration (EC_{50}) values were estimated using the trimmed Spearman Karber method. One-way analysis of variance was used to test the effects of exposure on mite avoidance response. When differences were observed, Tukey post-hoc comparison was used to ascertain where the differences lie.
Statistical analyses:	Survival and reproduction of mites: Differences in survival and reproduction of mites between water control and acetone control treatments were checked with a Student's t test. One-way analysis of variance was used to test the effects of increasing exposure concentrations on survival and reproduction of the mites. When differences were observed, Tukey post hoc comparison was used to ascertain where the differences lie. The median lethal concentration (LC_{50}) and reproduction EC_{50} values were calculated using trimmed Spearman Karber and nonlinear regression methods (logistic-3 parameter), respectively[23]. Significant differences between the avoidance EC_{50} values for each compound and the reproduction EC_{50} values and the LC_{50} values were ascertained when confidence limits do not overlap.

RESULTS

1. Validity criteria:

Avoidance behaviour of mites:

The validity criterion requiring no significant difference in avoidance response in dual control was achieved in all tests. Also, the validity criterion of the collembolan avoidance test, requiring at least 80% recovery, was adopted in the present study.

Survival and reproduction of mites:

No significant changes occurred in soil pH because of chemical spiking or test duration. The mean adult survival in all control soils was 80% or greater, and mean juvenile production was 100 or more, with a coefficient of variation of 30% or less. Therefore, all validity criteria were met in these tests.

2. Biological findings:

[23] Environment Canada. 2005. Guidance Document. Statistical Methods for Environmental Toxicity Tests. ESP 1/RM/46. Environmental Protection Series, Ottawa, ON.

Significant attraction ($p < 0.05$) was seen at 32 mg/kg (Figure 1).

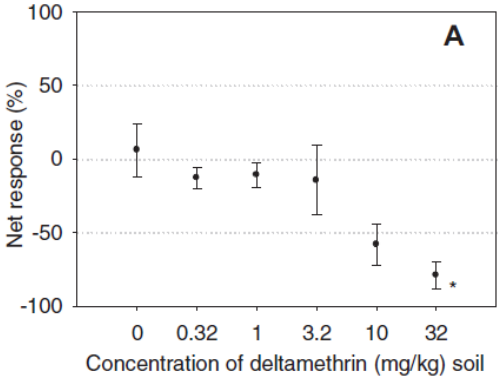

Figure 1. Mean (+/- standard error, n=5) net response of *Hypoaspis aculeifer* after its exposure for 48 h in 2-chamber avoidance tests combining untreated soil with deltamethrin

Reproduction NOEC was found at 3.2 mg/kg shown in figure 2.

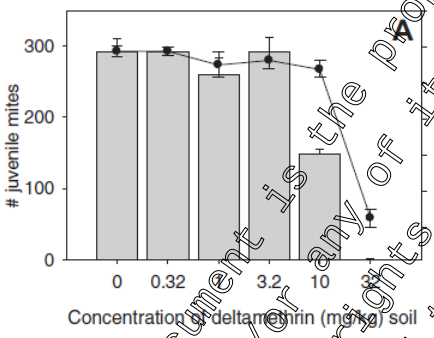

Figure 2. Mean (+/- standard error, n=4) survival and reproduction of the predatory mites (*Hypoaspis aculeifer*) after exposure for 14 d in Organisation for Economic Cooperation and Development soil with 5% organic matter content and spiked with deltamethrin

Table 1. The median lethal concentration (LC_{50}), reproduction and avoidance median effective concentration (EC_{50}) values (with corresponding 95% confidence intervals) for the effects on the survival, reproduction, and avoidance behavior of the predatory mite *Hypoaspis aculeifer* exposed to deltamethrin

Test substance	LC_{50} (mg/kg)	Reproduction EC_{50} (mg/kg)	Avoidance EC_{50} (mg/kg)
Deltamethrin	16.30 (13.59–19.55)	9.88 (8.74–12.04)	>32

RESULTS SUMMARY

The LC_{50} was 16.30 mg/kg. The EC_{50} based on reproduction was 9.88 mg/kg and the EC_{50} based on avoidance was > 32 mg/kg.

Comments by the Notifier

Avoidance behavior is not a relevant endpoint in the risk assessment for plant protection products. The entity which is to be protected is the population. So, relevant endpoints are mortality and reproduction. No mortality was observed at 10 mg Deltamethrin/kg and no effects on reproduction were observed at 3.2 mg Deltamethrin/kg. The endpoints determined in the OECD reproduction test with Deltamethrin

EW 15 (NOEC = 0.48 mg deltamethrin/kg ($NOEC_{corr.}$ = 0.24 mg/kg; KCA 8.4; KCA 8.4.2/05), on which the risk assessment is based, are clearly lower than the endpoints observed in this study. Thus, this study is not further considered in the risk assessment.

CA 8.4.2.1 Species level testing

See point MCA 8.4.2 above.

CA 8.5 Effects on soil nitrogen transformation

For studies already evaluated during the first EU review of this compound, please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer CropScience. In order to complete the risk assessment for soil micro-organisms additional tests on nitrogen mineralization with the soil metabolites Br_2CA and mPBacid were conducted. A summary of the endpoints is provided below:

Table 8.5 - 1: Effects of deltamethrin and soil metabolites on soil nitrogen transformation

Test item	Test design	Ecotoxicological endpoint		Reference
N-transformation				
Deltamethrin (tech.)	Study duration 28 d	**no unacceptable effects**	≥0.375 kg a.s./ha **≥0.5 mg a.s./kg dws**	[redacted] (1994) M-133031-01-2
Br_2CA	Study duration 28 d	**no unacceptable effects**	≥0.177 kg/ha **≥0.24 mg/kg dws**	[redacted] **(2011)** **M-400292-01-1**
mPBacid	Study duration 28 d	**no unacceptable effects**	≥0.177 kg/ha **≥0.24 mg/kg dws**	[redacted] **(2011)** **M-400287-01-1**
C-transformation				
Deltamethrin (tech.)	Study duration 56 d	no unacceptable effects	≥0.375 kg a.s./ha ≥0.5 mg a.s./kg dws	[redacted] (1994) M-133032-01-2

dws = dry weight soil; a.s. = active substance
Bold values: endpoints used for risk assessment

Report:	**KCA 8.5/02, [redacted]; 2011**
Title:	Br_2CA (Metabolite of deltamethrin, AE F108565): Effects on the activity of soil microflora (Nitrogen transformation test)
Document No.:	M-400292-01-1 (Rep. No: 101048077N)
Guidelines:	OECD 216 – Nitrogen Transformation Test
GLP	GLP study

Materials and Methods:

Br_2CA (Metabolite of deltamethrin, AE F108565), (analytical findings: 98.8 % w/w (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (AE F108565), Product code: AE F108565 00 1B99 0001, Batch ID: 2N6185C), was used in the test. A loamy sand soil (DIN 4220) was exposed for 28 days to 0.24 mg test item/kg soil dry weight. Application rate was equivalent to 0.177 kg test item/ha. Determination of the nitrogen transformation (NO3-nitrogen production) in soil enriched with Lucerne meal (concentration in soil 0.5 %). NH4-nitrogen, NO3- and NO2-nitrogen were determined using the Autoanalyzer II (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment). The coefficients of variation in the control (NO3-N) were maximum 6.4 % and thus fulfilled the demanded range (≤ 15 %).

Findings:

Effects on nitrogen transformation in soil after treatment with Br_2CA (Metabolite of deltamethrin, AE F108565)

Time Interval (days)	Control	0.24 mg Br_2CA/kg dry weight soil	
	Nitrate-N[1)]	Nitrate-N[1)]	% difference to control
0-7	1.57 ± 0.12	1.78 ± 0.24	+ 13 [n.s.]
7-14	1.37 ± 0.17	0.69 ± 0.06	- 49.5*
14-28	0.80 ± 0.12	0.79 ± 0.13	- 1.5 [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 $p \leq 0.05$)

* = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +37.6 %, +51.4 % and +27.1 % at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application (Appendix 4: Reference test 5. Results of the reference test, page 27).

Observations:

At time interval 7-14 days after application Br_2CA (Metabolite of deltamethrin, AE F108565) caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.24 mg/kg dry soil. However, no adverse effects of Br_2CA (Metabolite of deltamethrin, AE F108565) on nitrogen transformation in soil could be observed at the test concentration of 0.24 mg/kg dry soil, 28 days after application. Only a negligible difference to control of -1.5 % (test concentration 0.24 mg/kg dry soil) was measured at the end of the 28-day incubation period (time interval 14-28).

Conclusion:

In this test the validity criteria have been fulfilled.

Br_2CA (Metabolite of deltamethrin, AE F108565) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO3-N production) at the end of the 28-day incubation period (time interval 14-28). The study was performed in a field soil at a

concentration of 0.24 mg test item/kg soil, which is equivalent to an application rate of 0.177 kg test item/ha.

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Report:	**KCA 8.5/03; [redacted], 2011**
Title:	mPBacid (Metabolite of deltamethrin, AE F109036): Effects on the activity of soil microflora (Nitrogen transformation test)
Document No:	M-400287-01-1 (Rep. No: 101048076N)
Guidelines:	OECD-Guideline No. 216 (2000)
GLP:	Yes (certified laboratory)

Objectives:
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 mPBacid (Metabolite of deltamethrin, AE F109036), (analytical findings: 98.6 % w/w 3-phenoxybenzoic acid (AE F109036), Batch ID: AE F109036 00 1B99 0001, Origin Batch No.:400976/1), was used in the test. A loamy sand soil (DIN 4220) was exposed for 28 days to 0.24 mg test item/kg soil dry weight. Application rate was equivalent to 0.177 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 and 28 days after treatment).

In the most recent test, dated 07.01. - 18.02.2010, the toxic standard dinoterb caused an effect of +37.6%, 51.4 % and 27.1 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Dates of work: November 04, 2010 – December 02, 2010

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤15%	6.4%

Effects on nitrogen transformation in soil after treatment with mPBacid (Metabolite of deltamethrin, AE F109036)

Time Interval (days)	**Application rate**						
	Control			**[mPBacid (Metabolite of deltamethrin, AE F109036)]**			
				0.24 mg/kg dry weight soil			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control
0-7	1.57	±	0.12	1.64	±	0.51	+4.5 n.s
7-14	1.37	±	0.17	0.80	±	0.75	-41.5 n.s
14-28	0.80	±	0.12	0.73	±	0.12	-9.8 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)

Observations

At time interval 7-14 days after application, mPBacid (Metabolite of deltamethrin, AE F109036) caused a temporary inhibition of the daily nitrate rate at the tested concentration of 0.24 mg/kg dry soil. However, no adverse effects of mPBacid (Metabolite of deltamethrin, AE F109036) on nitrogen transformation in soil could be observed at the test concentration of 0.24 mg/kg dry soil, 28 days after application. Only a negligible difference to control of -9.8 % (test concentration 0.24 mg/kg dry soil) was measured at the end of the 28-day incubation period (time interval 14-28).

Conclusion:

mPBacid (Metabolite of deltamethrin, AE F109036) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO3-N production) at the end of the 28-day incubation period (time interval 14-28). The study was performed in a field soil at a concentration of 0.24 mg test item/kg soil, which is equivalent to an application rate of 0.177 kg test item/ha.

Supplemental information from literature research

Report:	**KCA 8.5/04; [redacted]; 2010**
Title:	Effect on the Biological Activity of Ordinary Chermozem from Contamination with Modern Pesticides
Source:	Agrochemistry, 2010, No. 11, pp. 339-44
DOI No: Document No:	Not given M-462161-01-2
Guidelines:	None
GLP:	No. Published study (peer-reviewed article).
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The aim of the present study is to determine the extent of the effect of modern pesticides on the population of soil microorganisms and the fermentation activity of a carbonate chernozem soil. Also the phytotoxicity of the pesticides was recorded.

No change in catalase activity was observed but dehydrogenase activity was reduced by 18% at concentrations of 1000 mg Decis/kg. Maximum application rates of 100,000 mg/kg resulted in a reduction of garden radish germination of 68%. At 50 mg/kg root and shoot length was reduced.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis (2.5% EC)
Active substance(s):	Deltamethrin
Chemical state and description:	Not specified
Source of test item:	Not specified
Batch number:	Not specified
Purity:	Not specified
Storage conditions:	Not specified
Water solubility:	Not specified

2. Soil:

Name / Classification	Carbonate Chernozem
Source, sampling date	[redacted] (Oktyabr'skii region, Rostov oblast)
Soil type:	Not specified
pH:	Not specified
Organic carbon content:	Not specified
Other information:	Not specified

B. Study design and methods

1. Sampling

Sampling technique:	Not specified
Sampling frequency:	Not specified
Sampling depth:	Top 30 cm
Number of samples per site/soil type:	Not specified
Sample treatment:	Soil was incubated at the optimum humidity and temperature

2. Test procedure:

Method:	The population of microorganisms was determined by the method of planting a soil suspension in solid agarized media. Beef-extract agar was used to isolate the ammonifying bacteria. The population of fungi was counted in an acidified Czapek's medium. The abundance of nitrogen-fixing bacteria of genus *Azotobacter* was determined by the method of aggregate growth in Ashby's nitrogen-free medium. The phytotoxicity of the soil was assessed from the change in parameters in the germination of seeds of garden radish of the red variety with a white tip and the degree of initial growth of the seedlings (length of roots, length of shoots). The fermentation activity of the soil was determined from the activity of the enzymes catalase and dehydrogenase.
Test concentrations:	Experiment 1: 0.1 – 10 mg/kg soil Experiment 2: 50 – 100000 mg/kg soil

Test duration	Experiment 1: 14 days Experiment 2: not stated
Statistics:	Data obtained were processed by statistical variance and dispersion analysis methods.

3. Chemical analysis

Guideline/protocol:	Not specified
Method:	Not specified
Pre-treatment of samples:	Not specified
Conditions::	Not specified
Recovery:	Not specified
Limit of detection:	Not specified
Limit of quantification:	Not specified

RESULTS

No regular change in catalase activity was observed after introduction of deltamethrin. The maximum deviation from the control did not exceed 15%, but showed slightly reduced activity of catalase. High doses of pesticides had quite a weak effect on the activity of soil enzymes. Only maximum doses of deltamethrin had a strong inhibitory effect on them. While low doses did not produce any reliable effect, doses of 1000 mg/kg Decis and more reduced dehydrogenase activity by a factor of 18%.

The introduction of Decis into the soil in doses up to 10,000 mg/kg had virtually no effect on the degree of germination of the garden radish seeds. The maximum dose of pesticides introduced (100,000 mg/kg) resulted in a reduction in this factor by 68%. Five days after planting the seeds, even the minimum dose of pesticides reduced the length of the roots and shoots of garden radish. A further increase in pesticide dose to the super-high level did not lead to a reduction in root length. The maximum pesticide dose led to a considerable suppression in the growth of the radish shoots. The length of the shoots was a more sensitive indicator of contamination, since on increasing the dose it decreased to 10% of control.

RESULTS SUMMARY

No change in catalase activity was observed after introduction of deltamethrin. Dehydrogenase activity was reduced by 18% at concentrations of 1000 mg Decis/kg. Maximum application rates of 100,000 mg/kg resulted in a reduction of garden radish germination of 68%. Even the minimum doses (50 mg/kg) reduced root and shoot length.

Comments by the notifier

No effects on biological activity were observed even at high concentrations. Concentration levels tested are very high compared to PEC in soil, thus, this study is considered not relevant for the risk assessment for the intended use of Deltamethrin EW15.

Report:	KCA 8.5/05; [redacted] 2009
Title:	Deltamethrin Degradation and Soil Microbial Activity in a Riparian Wetland Soil.
Source:	Soil Sci., 174, 4, p. 220-228
DOI No: Document No:	10.1097/SS.0b013e3181a09ea8 M-460927-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The effects of deltamethrin, in the presence and absence of nitrate, on soil microbial activity (as reflected by the rates of soil microbial basal respiration, denitrification, and methanogenesis) were studied in a riparian wetland soil under both aerobic and anaerobic conditions. Material and methods as well as results are summarized for deltamethrin only.

A microcosm study was carried out with soil collected from vicinity of a wetland. The soil was then amended with 50, 125, and 250 mg deltamethrin/ kg dry weight soil. Each deltamehtrin concentration was tested under aerobic and anaerobic conditions (6 replicates). Control nonamended soils were also included in the experiment. Samples were incubated in the dark at 20°C. CO_2, N_2O, N_2, and CH_4 content in the gas phase was measured after 2, 15, 28 and 58 days of incubation using a gas chromatograph with thermal conductivity detector. Deltamethrin concentrations were assessed after 15, 28 and 58 days of incubation, immediately after anaylsis of gas-phase. Consequently, data on gas-phase composition after 28 and 58 days of incubation correspond to mean values from four and two replicates. Recovery of deltamethrin was 78%, 75%, and 93% for the 50, 125, and 250 mg deltamethrin/kg amended soils.

Half-life values for deltamethrin degradation ranged from 27 to 49.3 days, depending on experimental conditions. Deltamethrin, under aerobiosis, had a significantly increasing effect on soil respiration in all concentration compared to the control. Whereas, under anaerobiosis, deltamethrin had an inhibitory effect on soil respiration. An antagonistic effect between deltamethrin degradation and denitrification activity was observed. Furtermore, deltematrhin also influenced the rate of methanogenesis. It was concluded that deltamethrin, designed to affect specific functions of its target organisms, also has an effect on nontarget organisms, that is, the soil microbial community.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted], Spain
Lot/Batch number:	-
Purity:	≥99%
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	hexane
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	Soil microorganisms
Cultivar:	-
Source of test species:	[redacted]
Age of test organisms at study initiation / Crop growth stage at treatment:	-
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Microcosm study
Duration of study:	58 days
Treatments:	Anarob and aerob Deltamethrin and anaerob and aerob nonamended control
Test concentrations:	50, 125, and 250 mg/kg dry weight (dw) soil
Number of replicates:	6 replicates
Individuals per replicate:	-
Test units (type and size):	Aerob: 500 ml airtight glass jars; Anaerob: 120 ml airtight glass jars
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Soil (0-20 cm top layer, brownish black color, and clay-sandy texture with abundant fine-size roots, 2 mm sieved)
Temperature / relative humidity:	20°C
Photoperiod:	Darkness
Lighting:	-
pH:	8.3
Organic matter (C_{org}):	1.7 g/kg
$CaCO_3$	
Ca^{2+}:	6162 mg/kg
Cation exchange capacity:	-
Electrical conductivity:	0.18 dS/m
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	Sand: 29.8%; Clay: 38.7%; Silt 31.5%
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	Deltamethrin, CO_2, N_2O, N_2, and CH_4 concentration
Biological parameters measured:	-
Measurement frequency:	2, 15, 28 and 58 after incubation
Statistical analyses:	Fisher`s protected least significant difference (PLSD) test

RESULTS

1. Validity criteria:
No validity criteria were mentioned.

2. Biological findings:
Half-life values for deltamethrin degradation ranged from 27 to 49.3 days, depending on experimental conditions. Deltamethrin, under aerobiosis, had a significantly increasing effect on soil respiration in all concentration compared to the control. Whereas, under anaerobiosis, deltamethrin had an inhibitory effect on soil respiration. An antagonistic effect between deltamethrin degradation and denitrification activity was observed. Furtermore, deltematrhin also influenced the rate of methanogenesis. It was concluded that deltamethrin, designed to affect specific functions of its target organisms, also has an effect on nontarget organisms, that is, the soil microbial community.

Kinetic Parameters of Deltamethrin Degradation under Both Aerobic and Anaerobic Conditions

Deltamethrin Concentration (mg/kg dw soil	$t_{1/2}$, Days (aerob)	$t_{1/2}$, Days (anaerob)
50	27.4	37.5
125	47.8	49.3
250	44.4	21.8

Effect of Deltamethrin Concentration on the Rates of Soil Microbial Basal Respiration (Units, mg CO_2 /(kg DW Soil * Day)) under Aerobic and Anaerobic Conditions

Incubation, Days	Control	50 mg/kg dw soil	125 mg/kg dw soil	250 mg/kg dw soil
Aerobic				
2	652.67 ± 5.66^{aA}	846.50 ± 61.98^{aB}	730.70 ± 26.24^{aC}	741.20 ± 14.25^{aC}
15	143.08 ± 6.78^{bA}	212.92 ± 7.98^{bB}	234.37 ± 4.48^{bC}	226.60 ± 20.27^{bC}
28	135.45 ± 2.69^{bA}	163.35 ± 11.55^{cB}	163.67 ± 4.74^{cB}	161.76 ± 7.72^{cB}
58	63.80 ± 1.74^{cA}	127.93 ± 1.52^{dB}	87.99 ± 2.40^{dC}	93.22 ± 3.14^{dC}
Anaerobic				
2	75.29 ± 2.65^{aA}	74.40 ± 4.63^{aA}	65.41 ± 4.88^{aA}	68.21 ± 3.53^{aA}
15	45.78 ± 3.89^{bA}	38.89 ± 5.18^{bB}	29.61 ± 1.15^{bC}	28.41 ± 1.21^{bC}
28	31.97 ± 1.64^{cA}	33.31 ± 6.83^{bA}	22.83 ± 0.97^{cB}	20.51 ± 1.01^{cB}
58	17.70 ± 2.34^{dA}	23.98 ± 3.17^{bA}	18.38 ± 7.62^{cA}	12.72 ± 0.42^{dA}

Numbers followed with different letters or asterisks are significantly different (P G < 0.05 or lower) according to Fisher PLSD test (lower-case letters: among sampling times; upper-case letters: among treatments)

Effect of Deltamethrin Concentration on the Rates of Denitrification (Units, mg N_2 /(kg DW Soil * Day)) under Anerobic Conditions

Incubation, Days	Control	50 mg/kg dw soil	125 mg/kg dw soil	250 mg/kg dw soil
2	53.24 ± 6.87^{aA}	39.79 ± 2.48^{aB}	84.66 ± 10.26^{aC}	128.36 ± 44.68^{aC}
15	168.93 ± 7.46^{bA}	81.34 ± 4.69^{bB}	16.17 ± 4.52^{bC}	16.12 ± 4.05^{bC}
28	124.90 ± 5.88^{cA}	72.63 ± 6.05^{bB}	9.15 ± 1.76^{bC}	8.77 ± 0.75^{c}
58	71.10 ± 3.79^{dA}	46.93 ± 4.11^{cB}	9.78 ± 1.41^{bC}	4.82 ± 0.04^{cD}

Numbers followed with different letters or asterisks are significantly different (P G < 0.05 or lower) according to Fisher PLSD test (lower-case letters: among sampling times; upper-case letters: among treatments)

Effect of Deltamethrin Concentration on the Rates of Methanogenesis (Units, mg CH_4 /(kg DW Soil * Day)) under Anerobic Conditions

Incubation, Days	Control	50 mg/kg dw soil	125 mg/kg dw soil	250 mg/kg dw soil
2	-	-	-	-
15	$0.24 \pm 0.05aA$	$0.25 \pm 0.12aA$	$0.34 \pm 0.06aA$	$0.13 \pm 0.03aB$
28	$2.80 \pm 0.67bA$	$3.42 \pm 0.66bA$	$5.53 \pm 0.14bB$	$2.72 \pm 0.28bA$
58	$3.13 \pm 0.27cA$	$2.71 \pm 0.56bA$	$3.83 \pm 0.92cB$	$5.39 \pm 0.20cC$

Numbers followed with different letters or asterisks are significantly different (P G < 0.05 or lower) according to Fisher PLSD test (lower-case letters: among sampling times; upper-case letters: among treatments)

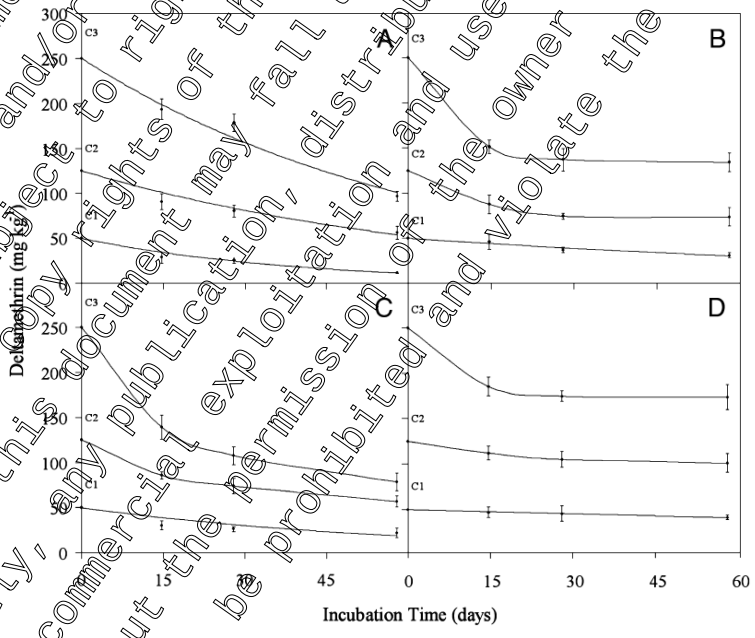

FIG. 1. Deltamethrin degradation curves: (A) aerobic without nitrate, (B) aerobic with nitrate, (C) anaerobic without nitrate, and (D) anaerobic with nitrate.

RESULTS SUMMARY

Half-life values for deltamethrin degradation ranged from 27 to 49.3 days, depending on experimental conditions. Deltamethrin, under aerobiosis, had a significantly increasing effect on soil respiration in all concentration compared to the control. Whereas, under anaerobiosis, deltamethrin had an inhibitory

effect on soil respiration. An antagonistic effect between deltamethrin degradation and denitrification activity was observed. Furtermore, deltamethrin also influenced the rate of methanogenesis. It was concluded that deltamethrin, designed to affect specific functions of its target organisms, also has an effect on nontarget organisms, that is, the soil microbial community.

Comments by the Notifier

Impacts were seen at 50 and 250 mg Deltamethrin/kg, and thus, the concentrations tested were not in a relevant range for the intended use of Deltamethrin EW15. So, this study was not further considered in the risk assessment.

Report:	**KCA 8.5/06; [redacted]; 2009**
Title:	Effect of pesticides and insecticide combinations on Azospirillum sp. in groundnut soils.
Source:	Pollut. Res., 28, 1, p. 105-109
DOI No: Document No:	- M-461209-01-1
Guidelines:	no
GLP:	no
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Effect of selected pesticides and insecticide (thiram, difeneconazole, deltamethrin, cypermethrin, endosulfan and profenofos) combinations on *Azospirillum* sp. in groundnut soils were determined. However, material and methods as well as results are summarized only for deltamethrin.
Samples of black clay soil and red sandy clay soils collected from groundnut cultivated fields of [redacted] India). Five gram portions of non-flooded groundnut soils in 15 x 150 mm test tubes were treated with 10, 25, 50, 75 and 100 µg/g deltamethrin (equivalent to 1, 2.5, 5, 7.5 and 10 kg/ha deltamethrin). The samples were incubated at 28±4°C and the moisture content was maintained at 60% WHC throughout the experimental period. Seven and 14 days after pesticide treatment, *Azospirillum* population was estimated in duplicates using N_2-free media and the numbers were calculated by most probable number (MPN) technique using probability tables[24].
Soil application of deltamtehrin up to 5.0 kg/ha enhanced the population of *Azospirilum* sp., at 7 and 14 days incubation in black soil; whereas in case of red soil, soil application of deltamethrin up to 2.5 kg/ha improved the population of *Azospirillum* sp., at 7 and 14 days.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-

[24] [redacted], M. 1965. Most probable Number Method for microbial populations. In: Methods of Soil Analysis'. (Ed. CA. Black). Part2, pp. 1467-1472.Am. Soc.Agr. Madison, Wisconsin, U.S.A.

Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Azospirillum* sp.
Cultivar:	-
Source of test species:	Black soil and red sandy soil from [redacted] India)
Age of test organisms at study initiation / Crop growth stage at treatment:	-
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	-
Duration of study:	14 days
Treatments:	Deltamethrin and Control (untreated)
Test concentrations:	10, 25, 50, 75 and 100 µg/g deltamethrin (equivalent to 1, 2.5, 5, 7.5 and 10 kg/ha deltamethrin)
Number of replicates:	-
Individuals per replicate:	2.1 x 10^3 g^{-1} soil
Test units (type and size):	15 x 150 mm
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Black soil and red sandy soil from Anantapur (Andhrapradesh, India)
Temperature / relative humidity:	28°C ± 4°C
Photoperiod:	-
Lighting:	-
pH:	Black soil: 7.7; Red soil: 6.6
Organic matter (C_{org}):	Black soil: 1.44% ; Red soil: 0.72%
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	Black soil: 71.3% sand, 20.7% silt, 8.0 % clay; Red soil: 67.3% sand, 26.1% silt, 6.6 % clay
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Population of *Azospirillum* sp.
Measurement frequency:	7 and 14 days after soil application
Statistical analyses:	Duncan's Multiple Range (DMR) test

RESULTS

1. Validity criteria:
No validity criteria were stated.

2. Biological findigs:
Soil application of deltamethrin up to 7.5 kg/ha enhanced the population of *Azospirilum* sp., at 7 and 14 days incubation in black soil and red soil in comparison to the control.

Population (MPN x 10^4 g^{-1} soil) of *Azospirillum* sp. as affected by application of deltamethrin in black soil

Initial 0-day population	Soil incubation, in days, after insecticide application											
	7 days						14 days					
	0	1.0	2.5	5.0	7.5	10.0	0	1.0	2.5	5.0	7.5	10.0
2.1	5.6a (100)	11.0b (196)	15.0c (267)	21.0d (375)	30.0e (232)	8.5f (151)	4.3a (100)	7.5b (174)	10.0c (232)	13.0d (302)	11.0e (253)	6.9f (160)

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (PS0.05) from each other according to DMR test.

Population (MPN x 10^4 g^{-1} soil) of *Azospirillum* sp. as affected by application of deltamethrin in red soil

Initial 0-day population	Soil incubation, in days, after insecticide application											
	7 days						14 days					
	0	1.0	2.5	5.0	7.5	10.0	0	1.0	2.5	5.0	7.5	10.0
2.1	4.5a (100)	8.3b (184)	18.0c (400)	13.0d (288)	11.0e (244)	3.1f (68)	3.3a (100)	6.3b (190)	13.0c (393)	10.0d (303)	5.8e (175)	2.7f (81)

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (PS0.05) from each other according to DMR test.

RESULTS SUMMARY
Soil application of deltamethrin up to 7.5 kg/ha enhanced the population of *Azospirilum* sp., at 7 and 14 days incubation in black soil and red soil in comparison to the control.

Comments by the Notifier
A stimulatory effects of Deltamethrin on Azospirillum sp were seen between 10 and 100 mg Deltamentrin/kg, which is by order of magnitude higher than the relevant concentrations in the risk assessment for the intended use of Deltamethrin EW15. Thus, the study was not further considered in the risk assessment.

Report:	**KCA 8.5/07; [redacted]; 2013**
Title:	Deltamethrin degradation and effects on soil microbial activity
Source:	Journal of Environmental Science and Health, Volume 48, Issue 7, Page 575-581
DOI No: ISSN No: Document No:	10.1080/03601234.2013.774900 0360-1234 (Print); 1532-4109 (Online) M-462470-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Deltamethrin [(S)-cyano-3-phenoxybenzyl-cis-(1R,3R)-2,2-dimethyl) cyclo–propane carboxylate],1] labelled at gem-dimethyl groups of the cyclopropane ring was applied on two Egyptian soils at a level of 10 mg/kg soil for a laboratory incubation experiment under aerobic and anaerobic conditions. A steady decrease of soil extractable ^{14}C-residues, accompanied by a corresponding increase of nonextractable bound ^{14}C-residues was observed over a 90-day incubation period. The percentage of evolved $^{14}CO_2$ increased with time under aerobic and anaerobic conditions in both soils. The effect of deltamethrin on soil microorganisms as well as the counter effect of microorganisms on the insecticide was also investigated. As the incubation period increased, the inhibitory effect of the insecticide on the microorganisms decreased and the evolution of carbon dioxide depended on the applied dose. The nature of soil methanol soluble residues was determined by chromatographic analysis which revealed the presence of the parent insecticide as the main product in addition to four metabolites: 3-(2′,2′-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (**II**); 3-phenoxybenzaldehyde (**III**); 3-phenoxybenzoic acid (**IV**); 3-phenoxybenzyl alcohol (**V**).

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	14C-Deltamethrin[(S)-cyano-3-phenoxybenzyl-cis-(1R,3R)-2,2-dimethyl) cyclopropane-carboxylate; 1] labeled at gem-dimethyl groups of the cyclopropane ring
Chemical state and description:	not reported
Source of test item:	[redacted], France
Batch number	not reported
Purity:	98 %
Storage conditions:	not reported
Water solubility:	not reported
Other specifications if stated (e.g. log Pow):	not reported

2. Soil:

Name / Classification	Information provided in table 1
Source, sampling date and storage conditions	The soils were taken from the north and south [redacted] Egypt, air- dried and passed through a 2mm screen before use. The soils were stored below 0◦C until used. At the start of the

experiment, soil was thawed and air dried overnight.

B. Study design and methods

1. Test procedure

Test conduction: To determine the fate of ^{14}C-deltamethrin in soil under anaerobic and aerobic conditions, the moist soils were spiked with 10 mg deltamethrin/Kg soil containing 0.5 µCi of the ^{14}C-chemical and flasks were incubated at about 25°C in the darkness for 90 days.
To determine the effect of deltamethrin on soil microbial activity, 3.7 × 104Bq of U-14C-glucose in water (1mL) was added to soil of the biometer flask that was spiked with three different doses of the insecticide 1, 3 and 10 mg deltamethrin/Kg soil. The insecticide and glucose solutions were applied to the soil surface using a micropipette, and the flasks were closed and incubated in triplicate at 25°C for 14 days. The evolved $^{14}CO_2$ was monitored by periodically determining ^{14}C- activity in 1 mL aliquots of the alkaline solutions removed from the side arm with a syringe. Cumulative $^{14}CO_2$ evolution was expressed as percent of the applied dose.

2. Sampling

Sampling frequency: At certain time intervals (1, 15, 30, 45, 60 and 90 days) samples of the alkaline solution were directly determined for their radioactivity and the soil in the biometer flask was analyzed for its extractable and bound residues.

Number of samples per site/soil type: triplicate

3. Chemical analysis

Method: HPLC and TLC

Conduction: The HPLC analysis was performed on a Waters-Association Model 510 equipped with a Waters-Association Model U6K Loop-injector and an UV Tunable Absorbance Detector. The TLC analysis of methanolic extract was determined using precoated silica gel plates 20×20 F254 Merck (Germany).

Radioactivity measurement: Radioactivity in extractable solutions was determined by Liquid ScintillationCounting (LSC) using a dioxane-based scintillation cocktail.

Table 1: Characteristics of the used soils

Soil Texture	pH	Organic matter	sand	silt	clay	W.H.C* (g.100g^{-1})
Clay loamy	7.70	1.85	21.15	20.19	58.66	41.4
Silt clay	7.78	0.95	11.25	55.15	33.60	35.7

*Water holding capacity.

RESULTS

1. Validity criteria:

No validity criteria defined.

2. Analytical findings:

Degradation of ^{14}C-deltamethrin in clay loamy and silt clay soils showed that a considerable amount of this insecticide was mineralized during 90 days with liberation of $^{14}CO_2$. As shown in Tables 2–5, the percentage of mineralization increases with time under aerobic and anaerobic conditions.

A significant increase of evolved $^{14}CO_2$ was observed after two weeks until the end of the experiment. Its maximum value for clay loamy soil reached about 9.3 and 10.3% (Table 2) in case of anaerobic conditions and 10.3% and 11.3% (Table 4) under aerobic conditions after two and three months, respectively (Fig. 1). The maximum value of $^{14}CO_2$ evolution for silt clay soil was 7.6 and 9.6% (Table 3) in case of anaerobic conditions and 6.8% and 9.2% (Table 5) under aerobic conditions after 60 and 90 days, respectively (Fig. 2).

A gradual decrease in percentage of extractable ^{14}C-residues of treated soils with time was observed (Tables 2–5).

On the other hand, there was a gradual increase in unextractable ^{14}C-residues "bound" in the two soils during the 90-day incubation period probably due to the strong pesticide adsorption to the solid phase. In both soils, it is found that the percentage of recovery ranged from 78–92% of the applied radiocarbon in case of aerobic and anaerobic conditions (Tables 2–5). The obtained results from thin layer chromatographic analysis of soil extractables indicated that the parent insecticide represents the main product (75–80%) in addition to four metabolites II, III, IV and V (20–25%). These results are in accordance with those obtained from high performance liquid chromatographic analysis (Table 6).

Incubation of clay loamy and silt clay soils with three different concentrations of non-labelled deltamethrin (1 and 3 and 10 mg/Kg soil) and U-14C -glucose resulted in liberation of an appreciable amount of $^{14}CO_2$ which increased during the 14 days of incubation (Table 7; Figs. 4 and 5).

Table 2. Fate of ^{14}C-deltamethrin in clay loamy soil under anaerobic conditions for 90 days.

Sampling time (day)	*Soil*	*^{14}C-Residues in soil% applied dose*		*$^{14}CO_2$%*	*Total ^{14}C-recovered (%)*
		^{14}C-Extract	*^{14}C-Bound*		
1	C	91.0 ± 0.90	3.2 ± 0.10	1.2 ± 0.03	95.40
	T	88.0 ± 0.80	2.1 ± 0.20	1.15 ± 0.01	91.25
15	C	86.0 ± 0.70	3.8 ± 0.20	2.8 ± 0.02	92.60
	T	83.0 ± 0.60	2.4 ± 0.30	2.4 ± 0.02	87.80
30	C	83.0 ± 0.60	4.6 ± 0.20	4.6 ± 0.01	91.20
	T	79.0 ± 0.40	3.5 ± 0.10	4.0 ± 0.03	86.50
45	C	76.0 ± 0.40	7.2 ± 0.30	7.4 ± 0.03	90.60
	T	72.0 ± 0.50	7.0 ± 0.20	7.1 ± 0.02	86.10
60	C	68.9 ± 0.30	11.5 ± 0.10	9.7 ± 0.01	89.20
	T	65.0 ± 0.20	10.2 ± 0.20	9.3 ± 0.02	84.50
90	C	64.15 ± 0.40	14.7 ± 0.30	10.8 ± 0.02	89.80
	T	59.0 ± 0.50	13.9 ± 0.20	10.3 ± 0.03	83.20

C = control; T = treated. *Mean ± standard deviation.

Table 3. Fate of ^{14}C-deltamethrin in silt clay soil under anaerobic conditions for 90 days.

Sampling time (day)	*Soil*	*^{14}C-Residues in soil% applied dose*		*$^{14}CO_2$%*	*Total ^{14}C-recovered (%)*
		^{14}C-Extract	*^{14}C-Bound*		
1	C	*93.42 ± 0.90	1.42 ± 0.10	0.20 ± 0.03	95.04
	T	87.92 ± 0.66	1.21 ± 0.09	0.067 ± 0.02	89.20
15	C	88.56 ± 0.70	3.68 ± 0.05	1.52 ± 0.02	93.76
	T	83.22 ± 0.80	3.08 ± 0.06	1.24 ± 0.01	87.54
30	C	80.12 ± 0.90	7.89 ± 0.04	3.66 ± 0.02	91.67
	T	78.10 ± 0.70	7.43 ± 0.07	3.44 ± 0.03	88.97
45	C	71.63 ± 0.45	10.12 ± 0.03	5.69 ± 0.02	87.44
	T	67.71 ± 0.90	9.66 ± 0.05	5.26 ± 0.02	82.63
60	C	68.85 ± 0.65	11.88 ± 0.04	8.45 ± 0.04	89.18
	T	60.51 ± 0.75	11.19 ± 0.08	7.64 ± 0.01	79.34
90	C	62.39 ± 0.55	13.56 ± 0.06	10.05 ± 0.05	86.00
	T	58.21 ± 0.60	12.38 ± 0.07	9.59 ± 0.03	80.18

C = control; T = treated. *Mean ± standard deviation.

Table 4. Fate of ^{14}C-deltamethrin in clay loamy soil under aerobic conditions for 90 days.

Sampling time (day)	*Soil*	*^{14}C-Residues in soil% applied dose*		*$^{14}CO_2$%*	*Total ^{14}C-recovered (%)*
		^{14}C-Extract	*^{14}C-bound*		
1	C	*93.21 ± 1.20	2.01 ± 0.04	0.24 ± 0.02	95.46
	T	90.46 ± 1.00	1.29 ± 0.03	0.13 ± 0.03	91.88
15	C	88.46 ± 0.40	2.93 ± 0.03	3.12 ± 0.02	94.00
	T	85.34 ± 0.90	2.02 ± 0.04	2.64 ± 0.03	90.00
30	C	81.6 ± 0.35	6.10 ± 0.02	7.88 ± 0.01	95.58
	T	76.67 ± 0.66	5.64 ± 0.06	6.39 ± 0.02	88.70
45	C	76.8 ± 0.60	8.11 ± 0.06	9.12 ± 0.01	94.03
	T	70.23 ± 0.48	7.72 ± 0.05	8.83 ± 0.01	86.78
60	C	67.20 ± 0.41	9.35 ± 0.04	11.31 ± 0.05	87.86
	T	63.25 ± 0.50	8.92 ± 0.02	10.26 ± 0.03	82.43
90	C	61.89 ± 0.23	11.06 ± 0.03	12.69 ± 0.02	85.64
	T	58.69 ± 0.82	10.23 ± 0.01	11.34 ± 0.04	80.26

C = control; T = treated. *Mean ± standard deviation.

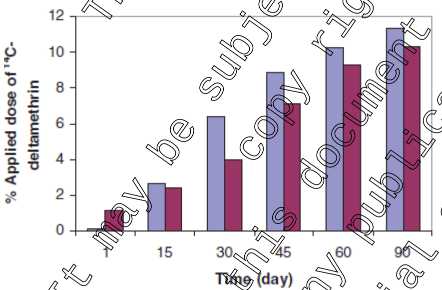

Fig. 1. Cumulative $^{14}CO_2$ for clay loamy soil spiked with ^{14}Cdeltamethrin under anaerobic and aerobic conditions during 90 days (color figure available online).

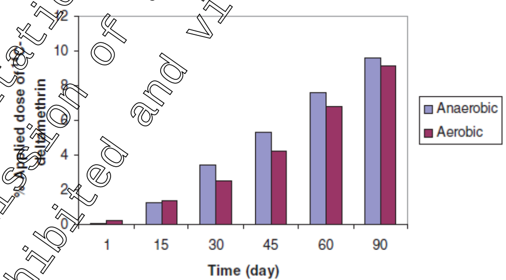

Fig. 2. Cumulative $^{14}CO_2$ for silt clay soil spiked with ^{14}Cdeltamethrin under anaerobic and aerobic conditions during 90 days color figure available online).

Table 5. Fate of ^{14}C-deltamethrin in silt clay soil under aerobic conditions for 90 days.

Sampling time (day)	Soil	^{14}C-Residues in soil% applied dose		$^{14}CO_2$%	Total ^{14}C-recovered (%)
		^{14}C-Extract	^{14}C-bound		
1	C	*93.33 ± 1.01	0.65 ± 0.08	0.24 ± 0.04	93.89
	T	91.52 ± 0.90	0.61 ± 0.09	0.21 ± 0.03	92.34
15	C	87.56 ± 0.88	1.25 ± 0.06	1.48 ± 0.02	90.29
	T	84.32 ± 0.80	1.04 ± 0.08	1.33 ± 0.05	86.69
30	C	81.21 ± 0.90	3.39 ± 0.07	3.05 ± 0.03	87.65
	T	78.20 ± 0.60	3.16 ± 0.09	2.49 ± 0.01	83.85
45	C	79.31 ± 0.75	6.20 ± 0.06	4.65 ± 0.02	90.16
	T	76.54 ± 0.70	5.89 ± 0.05	4.20 ± 0.05	86.63
60	C	65.30 ± 0.91	10.12 ± 0.08	7.36 ± 0.03	82.78
	T	61.56 ± 0.65	9.74 ± 0.07	6.8 ± 0.04	78.10
90	C	60.89 ± 0.85	13.21 ± 0.09	10.21 ± 0.01	84.31
	T	58.52 ± 0.45	12.78 ± 0.06	9.19 ± 0.02	80.19

C = control; T = treated. *Mean ± standard deviation.

Table 6. R_f and R_t values of deltamethrin and its metabolites.

Compounds	R_f Values			Retention time (R_t)	
	Sys. A	Sys. B	Sys. C	Sys. D	%
I	0.64	0.84	0.78	8.55	75–80
II	0.27	0.12	0.38	5.11	8–10
III	0.60	0.75	0.72	4.62	5–7
IV	0.04	0.05	0.2	2.02	3–5
V	0.01	0.03	0.10	4.05	2–3

I: Deltamethrin;
II: 3-(2′,2′-dibromovinyl)-2, 2-dimethylcyclopropane carboxylic acid;
III: 3-phenoxybenzaldehyde;
IV: 3-phenoxybenzoic acid;
V: 3-phenoxybenzyl alcohol.

Table 7. Percentage of $^{14}CO_2$ evolved after incubating ^{14}C-glucose with silt clay and clay loamy soil in the presence and absence of deltamethrin for 14 days.

Sampling time (day)	Soil type	$^{14}CO_2$ evolved (% of applied dose)			
		Control ± S.D.	1 mg/ Kg ± S.D.	3mg/ Kg ± S.D.	10 mg/Kg ± S.D.
1	Silt clay	1.73 ± 0.01	0.65 ± 0.01	0.35 ± 0.02	0.035 ± 0.03
	Clay loamy	2.56 ± 0.02	0.89 ± 0.01	0.46 ± 0.01	0.052 ± 0.02
2	Silt clay	2.45 ± 0.02	1.18 ± 0.02	1.05 ± 0.01	0.076 ± 0.01
	Clay loamy	2.76 ± 0.03	2.18 ± 0.03	1.65 ± 0.01	0.176 ± 0.04
4	Silt clay	4.83 ± 0.01	3.54 ± 0.06	2.10 ± 0.03	0.192 ± 0.05
	Clay loamy	5.03 ± 0.03	4.58 ± 0.02	2.95 ± 0.02	0.227 ± 0.05
8	Silt clay	6.69 ± 0.05	5.03 ± 0.02	3.79 ± 0.03	0.316 ± 0.04
	Clay loamy	9.76 ± 0.04	6.00 ± 0.03	4.61 ± 0.03	0.377 ± 0.02
10	Silt clay	8.32 ± 0.03	7.16 ± 0.04	6.13 ± 0.03	0.463 ± 0.01
	Clay loamy	13.11 ± 0.04	9.06 ± 0.04	6.15 ± 0.05	0.550 ± 0.05
14	Silt clay	13.61 ± 0.04	12.08 ± 0.05	9.28 ± 0.04	0.758 ± 0.02
	Clay loamy	20.15 ± 0.07	16.50 ± 0.05	11.82 ± 0.07	1.20 ± 0.02

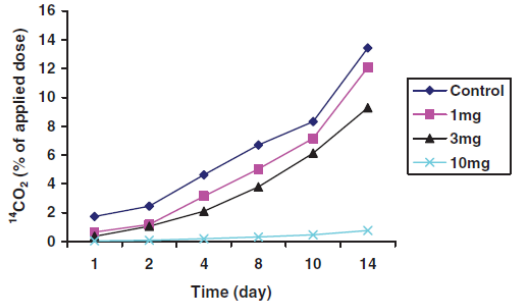

Fig. 4. Cumulative $^{14}CO_2$ after incubation of ^{14}C-glucose with silt clay soil in presence and absence of deltamethrin for 14 days (color figure available online).

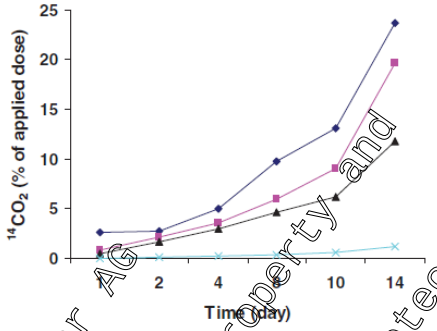

Fig. 5.Cumulative $^{14}CO_2$ after incubation of ^{14}C-glucose with clay loamy soil in presence and absence of deltamethrin for 14 days (color figure available online).

RESULTS SUMMARY

The mineralization of ^{14}C-deltamethrin in clay loamy soil was higher than in silt clay soil under anaerobic and aerobic conditions. The soil binding was increased with time whereby the extractable ^{14}C-residues simultaneously decreased during 90 days. The percentage of recovery ranged from 80–92% of the applied radiocarbon in both soils under anaerobic and aerobic conditions. Chromatographic analyses of methanol extractable ^{14}C-residues of the used soils revealed the presence of deltamethrin in addition to four compounds. Incubation of both soils with three different concentrations of non-labelled deltamethrin and U-^{14}C-glucose resulted in liberation $^{14}CO_2$ which increased during 14 days. As incubation period increased, the inhibitory effect of the insecticide decreased and evolution of $^{14}CO_2$ depended on applied dose.

Comments by the Notifier

The concentrations tested are by orders of magnitude higher than the relevant exposure in the risk assessment for the intended use of Deltamethrin EW15. Thus, the study is not further considered in the risk assessment.

Report:	**KCA 8.5.08; [redacted] 2011**
Title:	Effect of pesticides on microbial diversity and urease in groundnut (*Arachis hypogaea* L.) soil.
Source:	Dynamic soil, dynamic plant; 5 / Special Issue 1; pp. 75-82
ISBN:	1749-6590
Document No.:	M-476820-01-1
Guidelines:	none
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The influence of deltamethrin at 0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 kg/ha was assessed for their effects on the activity of urease (measured terms of hydrolysis of urea by sodium hypochlorite method) and microbial populations like bacteria, fungi and actinomycetes in two different agricultural soils, collected from a fallow groundnut fields in Anantapur district. The effects were dose dependent.

Higher doses (7.5, 10.0 kg/ha) were either toxic or innocuous to the urease activity and microbial population. The significant stimulation in the activity of urease was associated with 2.5 kg/ha deltamethrin.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis (2.8% EC)
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	Not reported
Source of test item:	[redacted] India Ltd
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test organism(s)

Species:	Urease; Microbial population (fungi, bacteria, actinomycetes)
Cultivar:	Natural colony
Source of test species:	From a soil collected in a semi-arid zone [redacted], India, to a depth of 12cm
Holding conditions prior to test:	Not reported
Acclimatisation:	Not reported

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory study
Duration of study:	Urase activity: 40 days of soil incubation 7 d after incubation: bacterial population Further 3 d in the dark: fungal population
Treatments:	6 treatments
Test concentrations:	0.0, 1.0, 2.5, 5.0, 7.5, 10.0 kg/ha
Number of replicates:	Duplicates
Test units (type and size):	Test tubes (25x150mm)
Application / device / nozzles:	Mixing into the soil and homogenised

2. Environmental conditions

Test medium:	Black clay soil and red sandy soil
Temperature / relative humidity:	Incubation: urease activity: 37°C; bacterial population: 30°C; fungi: 28+/-4°C; actinomycetes: 30°C
Photoperiod:	Not reported
Lighting:	Not reported
pH:	Not reported
Organic matter (C_{org}):	Not reported
$CaCO_3$	Not reported
Cation exchange capacity:	Not reported

3. Observations and measurements:

Analytical parameters measured:	None
Biological parameters measured:	Urease activity, population growth of fungi, actinomycetes and bacteria

Measurement frequency: At the end of each study type; in total 40 d
Statistical analyses: Duncan´s multiple range test

RESULTS AND DISCUSSION

1. Biological findings:

Fungal populations in both soils increased with increasing concentrations (up to 5 kg/ha) of deltamethrin (table 1). Concentrations of deltamethrin up to 7.5 kg/ha increased the population of actinomycetes, too (table 2). In addition, Bacterial populations were significantly higher in black soil treated with deltamethrin and was enhanced with increasing concentrations (up to 7.5 kg/ha) (table 3).

Higher doses (7.5, 10.0 kg/ha) of deltamethrin were either toxic or innocuous to the urease activity (table 4). The enzyme activity was continued up to 20 d of incubation and then decline in urease activity was observed in both black and red soil and at all concentration (table 5).

Table 1: Effect of deltamethrin, at varying concentrations, on population of fungi (CFU x 10^5 g/dry soil

	Black soil	Red soil
0.0	16 +/- 1.154 b	12 +/- 1.154 c
1.0	19. +/- 0.577 a	14 +/- 1.154 b
2.5	26 +/- 1.154 a	19 +/- 0.577 a
5.0	20 +/- 1.154 a	16 +/- 1.154 b
7.5	15 +/- 0.577 b	12 +/- 1.154 c
10.0	11 +/- 0.577 c	6 +/- 1.154 d

Table 2: Effect of deltamethrin, at varying concentrations, on population of actinomycetes (CFU x 10^5 g/dry soil

	Black soil	Red soil
0.0	115 +/- 2.886 d	96 +/- 1.154 e
1.0	120 +/- 2.886 c	121 +/- 0.577 c
2.5	140 +/- 2.886 b	135 +/- 2.886 b
5.0	164 +/- 2.309 a	151 +/- 0.577 a
7.5	120 +/- 2.886 c	110 +/- 2.886 d
10.0	95 +/- 2.886 e	86 +/- 1.154 f

Table 3: Effect of deltamethrin, at varying concentrations, on population of bacteria (CFU x 10^5 g/dry soil)

	Black soil	Red soil
0.0	155 +/- 2.886 e	135 +/- 2.886 e
1.0	178 +/- 1.154 d	152 +/- 1.154 d
2.5	250 +/- 2.886 a	172 +/- 1.154 b
5.0	207 +/- 1.155 b	195 +/- 2.886 a
7.5	191 +/- 0.577 c	164 +/- 1.154 c
10.0	134 +/- 1.155 f	120 +/- 2.886 f

Table 4: Effect of different concentrations of deltamethrin on urease activity in black and red soil after 10d

	Black soil	Red soil
0.0	134 +/- 2.309 c	84 +/- 2.309 c
1.0	138 +/- 1.155 c	88 +/- 1.154 c
2.5	160 +/- 2.887 a	114 +/- 1.154 a
5.0	150 +/- 2.887 b	102 +/- 1.154 b
7.5	126 +/- 2.309 d	78 +/- 1.154 d
10.0	112 +/- 1.155 e	62 +/- 1.154 e

Table 5: Influence of deltamethrin (2.5 kg/ha) on urease activity in black and red soil

		Soil incubation (days)			
		10 d	**20 d**	**30 d**	**40 d**
Black soil	Deltamethrin	160 +/- 1.154 a	170 +/- 2.886 a	160 +/- 5.733 a	140 +/- 2.886 a
	Control	134 +/- 1.154 d	144 +/- 2.309 c	136 +/- 1.732 d	120 +/- 2.886 c
Red soil	Deltamethrin	114 +/- 1.154 b	128 +/- 1.154 b	116 +/- 1.154 b	82 +/- 1.154 b
	Control	84 +/- 1.154 d	100 +/- 1.154 c	88 +/- 1.154 d	62 +/- 1.154 c

CONCLUSION
Higher doses (7.5, 10.0 kg/ha) of deltamethrin were either toxic or innocuous to the urease activity and microbial population. The significant stimulation in the activity of urease was associated with 2.5 kg/ha deltamethrin.

Comments by the Notifier
The concentrations tested are by orders of magnitude higher than the relevant exposure in the risk assessment for the intended use of deltamethrin EW15. Thus, this study is not considered further in the risk assessment.

Report:	**KCA 8.5/09 [redacted]; 2012**
Title:	Fertilization can modify the non-target effects of pesticides on soil microbial communities
Source:	Soil Biology & Biochemistry, Vol. 48, p. 125-134
DOI No.:	10.1016//j.soilbio.2012.01.021
Document No.:	M-458656-01-1
Guidelines:	ISO 16072, 2002. Soil Quality e Laboratory Methods for Determination of Microbial Soil Respiration. ISO 17155, 2002. Soil Quality e Determination of Abundance and Activity of Soil

	Microflora Using Respiration Curves.
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

A three-month mesocosm experiment was performed to unravel interactions between pesticides (difenoconazole: fungicide, deltamethrin: insecticide, ethofumesate: herbicide) and fertilizers (NPK synthetic fertilizer, compost) regarding the potential non-target effects of pesticides on soil microbial communities. To this aim, pesticides and fertilizers were applied to soil at a rate of 5 mg active ingredient kg^{-1} DW soil and 185 mg N kg^{-1} DW soil, respectively. Soil sampling was done after 0, 7, 30, 60 and 90 days of incubation in order to determine pesticide degradation rates and microbial properties: enzyme activities, basal respiration, substrate-induced respiration, potentially mineralizable N, nitrification rate and denitrification potential. Deltamethrin caused a short-term inhibitory effect on microbial activity in non-fertilized soils, but not in fertilized soils. A short-term antagonistic effect between NPK fertilization and deltamethrin presence was found regarding their inhibitory effect on potentially mineralizable N. In compost fertilized soils, pesticides counteracted the stimulatory effect of compost on denitrification potential. By the end of the incubation, deltamethrin in non-fertilized soils was degraded by 85%, with half-life (t1/2 = time required for a 50% dissipation of initial concentration) values of 35.9 days.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Audace® EC
Active substance(s):	Deltamethrin 2.5%
Source of test item:	Not stated
Batch number/CAS number:	Not stated
Purity:	Not stated
Stability of test compound:	Not given
Water solubility:	Not given

2. Test organism(s)

Species:	Soil microbial community
Source of test species:	Collected with test medium from riparian zone of the [redacted] located in the [redacted] (northern Spain).
Holding conditions prior to test:	Soil samples with microbial community were transferred to laboratory in dark plastic bags, manually homogenized, air-dried at 25 °C during 48 h and sieved to <2 mm.

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory: soil microbial activity (respiration and nitrification)
Deviation from guidelines:	Not reported.
Duration of study:	90 days
Treatments:	Total of 6 treatments in a 2 x 3 factorial scheme with first factor deltamethrin (added or not added) and second factor fertilization (non-fertilized or NPK synthetic fertilizer or organic compost

	fertilizer). – 6 further treatments are with respect to non-deltamthrin pesticides (fungicide and insecticide).
Test concentrations:	5 mg deltamethrin/kg dws (corresponding to recommended field application rate assuming soil bulk density of 1 g/cm^3 and soil depth of 1 cm)
Number of replicates:	3
Test units (type and size):	10 L plastic pots filled with 4 kg dry weight soil (dws) resulting in a soil layer of appr. 10 cm.
Application / device:	Mixed into soil with a rotary mixer

2. Environmental conditions

Test medium:	Chernozem calcic with clay sandy texture; natural soil (top 25-cm)
Source of test medium	Riparian zone of [redacted] located in [redacted] (northern Spain).
Temperature:	22 ± 1 °C
Photoperiod:	24 h dark
Soil moisture	Weekly adjusted to 60% water holding capacity throughout the incubation period
pH:	8.3
Organic matter (C_{org}):	17.0 g/kg dws
Soil characteristics:	2.3 g total N/kg dw soil, C/N ratio of 7.8 and electrical conductivity lf0 0.18 ds/m.
Soil history/fertilization:	No known pesticide or fertilizer application for the last 15 years.

3. Observations and measurements:

Analytical parameters measured:	Active substance concentration per kg dw soil (by GC-MS)
Biological parameters measured:	Soil microbial respiration, enzyme activities and N-transformation
Measurement frequency:	At 0, 7, 30, 60 and 90 days after treatment.
Statistical analyses:	ANOVA for analyzing microbial properties, Pearson's correlation coefficients and Principal Component analysis to establish relationships among soil properties, and for enzyme activity the treated-soil quality index (T-SQI) was calculated for 7 and 90 days of incubation (= after treatment).

RESULTS

1. Deltamethrin degradation in soil:

Table 1 - Kinetic parameters of deltamethrin degradation in non-fertilized (NF), NPK-fertilized (NPK) and compost-fertilized (Compost) soils.

Fertilizer	A	k1 (d^{-1})	B	k2 (d^{-1})	$t_{1/2}$ (d)	r^2
NF	3.594	0.022	1.498	0.014	35.9	0.997
NPK	3.211	0.020	1.874	0.020	35.6	0.999
Compost	4.383	0.029	0.617	4.243	19.4	0.996

Pesticide degradation in soil was described by a bi-exponential model [PC (t) = $A*e^{(-k1*t)} + B*e^{(-k2*t)}$], where [PC (t) = pesticide concentration at t time; A and B = constants; k1 and k2 = degradation kinetic constants for the first and second component of the curve; t = time]. $t_{½}$ = half-life or time required for a 50% dissipation of initial pesticide concentration.

Pesticide degradation fitted more accurately to a bi-exponential kinetic model than to classical first-order models (Figure 1).

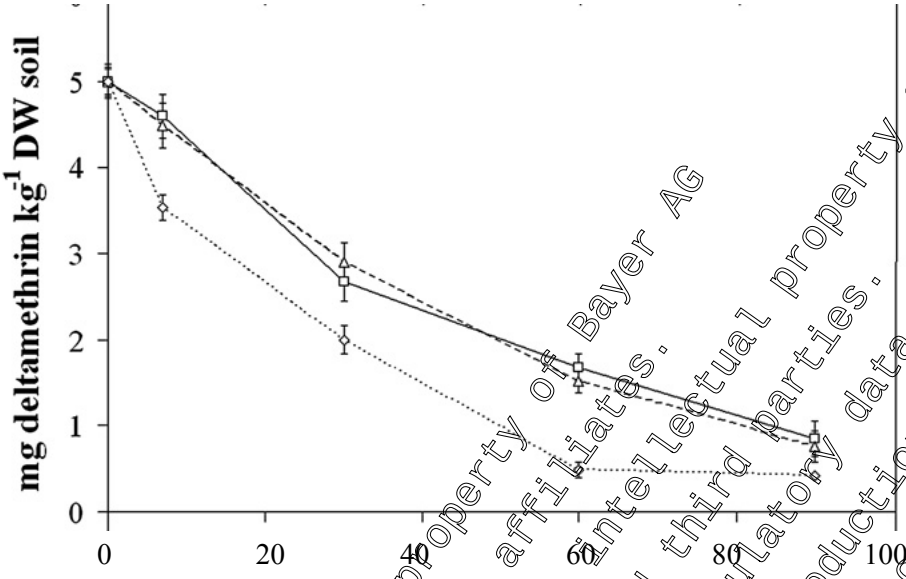

Figure 1 - Deltamethrin concentration in non-fertilized (NF), NPK-fertilized (NPK) and compost-fertilized (Compost) soils. Mean values (n = 3) ± S.D.

2. Biological findings:

Soil microbial respiration:
- Deltamethrin led to lower values of dehydrogenase activitiy compared to pesticide-free controls
- deltamethrin resulted in higher values of the respiratory quotient (Q_R) at day 7 (33% higher compared to pesticide-free controls)
- In pesticide-free controls, no effect of NPK or compost fertilization was found regarding Q_R values
- NPK-fertilized soils treated with deltamethrin showed lower values of Q_R than pesticide-treated non-fertilized soils. By contrast, compost-fertilized soils treated with pesticides showed similar values of Q_R than pesticide-treated non-fertilized soils

N-transformation:
- Deltamethrin had slightly inhibitory effect on mineralizable nitrogen (N_{min}) at day 7
  In NPK fertilized soils, the addition of deltamethrin led to lower values of N_{min} at day 7
- Compostfertilized soils treated with deltamethrin showed similar N_{min} values to those of non-fertilized pesticide-free controls
- No effect of deltamethrin on values of $N\text{-}NH_4^+$ compared to pesticide-free controls

Enzyme activity:
- Deltamethrin led to lower T-SQI value at day 90
  In pesticide-free controls, NPK- and compost-fertilized soils showed lower and higher T-SQI values, respectively, than non-fertilized soils
- Deltamethrin counteracted the stimulatory effect of compost on T-SQI values
- At day 7 and 90, in NPK-fertilized soils, the presence of deltamethrin counteracted the inhibitory effect of NPK fertilizer on T-SQI values

RESULTS SUMMARY

Deltamethrin caused a short-term inhibitory effect on microbial activity in non-fertilized soils, but not in fertilized soils. A short-term antagonistic effect between NPK fertilization and deltamethrin presence was found regarding their inhibitory effect on potentially mineralizable N. In compost fertilized soils, pesticides counteracted the stimulatory effect of compost on denitrification potential. By the end of the incubation, deltamethrin in non-fertilized soils was degraded by 85%, with half-life (t1/2 = time required for a 50% dissipation of initial concentration) values of 35.9 days.

Comments by the Notifier
The concentrations tested are very high and are not in a relevant range for the intended use of Deltamethrin EW15. Thus, this study is not further considered in the risk assessment.

Report:	**KCA 8.5/10; [redacted]; 2011**
Title:	Influence of pesticides, alone and in combination, on phosphatase activity in soils of groundnut (Arachis hypogaea L.) fields
Source:	Dynamic soil, dynamic plant; 5, Special Issue 1; pp. 70 – 74
ISBN: Document No.:	1749-6500 M-463427-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY
The influence of deltamethrin at 0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 kg/ha was assessed for test its non-target effect towards the activity of phosphatase in two different agricultural soils, collected from a fallow groundnut fields in Anantapur district. The effects were dose dependent. Phosphatase was more pronounced in soil samples treated with 2.5 kg/ha of deltamethrin. As a conclusion, the pesticide enhances the activity of phosphatase at field rate.

MATERIAL AND METHODS
A. Material
1. Test material

Test item:	Decis (2.8% EC)
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	Not reported
Source of test item:	[redacted] India Ltd
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test organism(s)

Species:	Phosphatase activity
Cultivar:	Natural colony
Source of test species:	From a soil collected in a semi-arid zone [redacted] India, to a depth of 12cm

Holding conditions prior to test: Not reported
Acclimatisation: Not reported

B. Study design and methods

1. Test procedure

Test system (study type): Laboratory study
Duration of study: 40 d
Treatments: 6 treatments
Test concentrations 0.0, 1.0, 2.5, 5.0, 7.5, 10.0 kg/ha
Number of replicates: Duplicates
Test units (type and size): Test tubes (25x150mm)
Application / device / nozzles: Mixing into the soil and homogenised

2. Environmental conditions

Test medium: Black clay soil and red sandy soil
Temperature / relative humidity: Incubation: 28+/-4°C. After 10 d: incubation at 30°C for 30 min
Photoperiod: Not reported
Lighting Not reported
pH: Not reported
Organic matter (C_{org}): Not reported
$CaCO_3$ Not reported
Cation exchange capacity: Not reported

3. Observations and measurements:

Analytical parameters measured: None
Biological parameters measured: Phosphatase activity
Measurement frequency: 10, 20, 30 and 40 d
Statistical analyses: Duncan´s multiple range test

RESULTS AND DISCUSSION

1. Biological findings:

The phosphatase activity was enhanced in both soils with/without deltamethrin upon further incubation for another 10 d (table 1). The application of deltamethrin increased the enzyme activity up to 5 kg/ha and decreased the activity with increasing pesticide concentration in both soils (table 2).

Table 1: Influence of deltamethrin (2.5 kg/ha) on phosphatase activity in black and red soil

		Soil incubation (days)			
		10 d	**20 d**	**30 d**	**40 d**
Black soil	Deltamethrin	148 +/- 1.154 b	177 +/- 1.732 a	140 +/- 5.773 b	125 +/- 5.773 b
	Control	98 +/- 1.154 d	120 +/- 5.773 d	110 +/- 5.773 c	80 +/- 5.773 d
Red soil	Deltamethrin	115 +/- 8.660 a	135 +/- 2.886 a	120 +/- 11.547 a	98 +/- 1.154 a
	Control	62 +/- 1.154 c	89 +/- 0.577 c	50 +/- 5.773 d	42 +/- 1.154 d

Table 2: Effect of different concentrations of deltamethrin on phosphatase activity in black and red soil after 10d

	Black soil	Red soil
0.0	98 +/- 2.309 c	62 +/- 1.154 e
1.0	112 +/- 1.154 b	80 +/- 5.773 c
2.5	148 +/- 1.154 a	115 +/- 8.660 a
5.0	110 +/- 5.773	92 +/- 1.154 b
7.5	80 +/- 2.886 d	70 +/- 5.773 d
10.0	75 +/- 2.886 d	52 +/- 1.154

CONCLUSION

Phosphatase was more pronounced in soil samples treated with 2.5 kg/ha on deltamethrin. In addition, the application of deltamethrin increased the enzyme activity up to 5 kg/ha and decreased the activity with increasing pesticide concentration in both soils. Therefore, deltamethrin enhance the activity of phosphatase at field rate.

Comments by the Notifier

The concentrations tested are by orders of magnitude higher than the relevant exposure in the risk assessment for the intended use of Deltamethrin FW15. Thus, this study was not further considered in the risk assessment.

Report:	**KCA 8.5/11; [redacted]; 2011**
Title:	Laboratory study of biological interaction between entomopathogenic fungi Beativeria bassiana (Bals.) Vuill. and some pesticides used in integrated plant protection systems.
Source:	Analele Universităţii din Craiova, seria Agricultură – Montanologie – Cadastru Vol. XLI 2011/2
DOI No:	Not given
Document No.:	M-462287-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

This paper aimed to assess the effect of deltamethrin of an entomopathogenic fungi *Beauveria bassiana* - active ingredient of fungal bio-insecticides. The formulations was tested at three different concentrations (mean concentration (MC), half and twice the MC). The results indicated that the formulation with deltamethrin is not compatible with *B. bassiana* and it caused a strong or complete inhibition in its development.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	deltamethrin
Active substance(s):	EC 2.5
Adjuvant / Surfactant:	not reported
Source of test item:	not reported
Lot/Batch number:	not reported
Purity:	not reported
Storage conditions:	not reported

2. Test organism(s)

Species:	*Beauveria bassiana* (Balsamo)
Source of test species:	isolated from natural outbreaks of infection (*Leptinotarsa decemlineata*), preserved by successive passages on test insects (*Tenebrio molitor*) and reisolated on PDA medium (25 ± 1°C dark).
Acclimatisation:	not reported

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory study
Duration of study:	*Germination Assessment*: 24 h of incubation *Assessment of effect on vegetative growth and sporulation:* eight days of incubation in the thermostat
Treatments:	not reported
Application rate:	5 MC (=mean concentration of commercial product diluted in 1000l water) 1 / 1 x FR, 1/ 2 x FR and 2 / 1 x FR (field recommendation).
Number of replicates:	not reported
Individuals per replicate:	not reported
Test conduction:	*Germination Assessments* Spore suspensions were prepared from pure cultures, obtained on PDA medium and their concentration was determined by Burker chamber. Spore suspension was calibrated at a concentration of 106 conidia/ml diluted in sterile distilled water to which was added Tween 80 (0.01%). The test pesticides were diluted with 9 ml sterile distilled water each / repetition / treatment and after homogenization were inoculated with 1 ml conidia suspension and left in contact for an hour. Same amount of spores in sterile distilled water and Tween 80 (0.01%) was considered as control. Tests were performed using microscope slides disinfected with ethanol. After demarcation of three areas on the bottom of the slides, they were placed in Petridishes, their moisture been kept inside by a filter paper wetted with distilled water. Slides were supported on two match sticks to prevent reaching the Petridish. Surface of each strip was covered with ~ 4 ml of

culture medium PDA (Sigma. Fluka) and each demarcated area were dropped ~ 0.05 ml conidian suspension -pesticide mixture.

Assessment of effect on vegetative growth and sporulation

Inoculum was produced by *B. bassiana* growing on PDA medium for 20 days at 24°C.

Test pesticides were included in the sterile and cooled PDA culture medium, at the final concentrations mentioned before. After homogenization the mixtures obtained were distributed in Petri dishes (30ml/treatment) and inoculated with fungal inoculum in three points per Petri dish, using microbiological loop (three plates / treatment). Same amount of medium but without pesticide was used as control. to (,) and fungicidal action / fungistatic was assessed by measuring mycelium growth (colony diameter) of 10 randomly chosen colonies of each treatment. Average diameters for the three repetitions were compared with those of the control, thereby calculating the percentage mycelium increase inhibition as in previous test. From 10 colonies randomly taken were cut with a glass tube (d = 7mm) disks, necessary for quantification of spores production. Each disc was individually distributed in tubes with 10 ml of sterile distilled water and Tween 80 (0.01%) and was homogenized until the spores were completely detached from the surface of culture media. The obtained suspension was suitably diluted for counting in Burker chamber. For each colour / each repetition have been two readings (24 squares) and their average was used for statistical calculations.

2. Environmental conditions

Temperature: *Germination Assessments:* 26 +/- 1°C
Assessment of effect on vegetative growth and sporulation: 24° ± 1°C in the dark

2. Observations and measurements:

Conditional (eg weather) parameters: not reported

Biological parameters measured: none

Measurement frequency: not reported

Statistical analyses: Inhibition of germination (%) = (G%C - G% var) / G% C x 100, where:
G% C - the germination percentage of the Control
G% var - the germination percentage / treatment
Data were analysed using ANOVA and comparisons between test environments have been using ANOVA test (ONE WAY) ($p < 0.05$) using BioStat 2008.

RESULTS

1. Validity criteria:

No validity criteria defined.

2. Biological findings:

Deltamethrin treatments completely inhibited germination of *Beauveria bassiana.* The formulation of deltametrhin at 1 / 1 and 1 / 2 concentrations, almost completely inhibited vegetative growth (> 95%). Sporulation of vegetative mycelium was completely inhibited (1 / 1 and 2 / 1 FR), but in low concentration (1/2) enhanced the sporulation.

Table 1. Effect of deltametrin on some biological parameters of *Beauveria bassiana*

concentration	Spore germination (%) N = 3 blades		Vegetative growth reduction (%) N = 10 colonies		Sporulation reduction (%) (x108/mL) N = 3 colonies /treatment	
	Mean ± ES (%)	(%)	Mean ± ES (cm)	(%)	Mean ± ES (increase number x 108)	(%)
½	0	100	0,94±0,05	30	31,8±1,7	<6
1/1	0	100	0,045±0,01	97	0	100
2/1	0	100	0	100	0	100

Table 2: T Factor and compatibility of deltametrin on fungi-toxic effect on strain BbS 1.07 (*Beauveria bassiana*)

concentration	T factor	Classification
½	98,8	Toxic
1/1	0,6	Toxic
2/1	0	Toxic

RESULTS SUMMARY

Deltamethrin treatments completely inhibited germination of *Beauveria bassiana.* The formulation of deltametrhin at 1 / 1 and 1 / 2 concentrations, almost completely inhibited vegetative growth (> 95%). Sporulation of vegetative mycelium was completely inhibited (1 / 1 and 2 / 1 FR), but in low concentration (1/2) enhanced the sporulation.

Comments by the Notifier

The tested formulation differs from the representative use formulation, so the study is considered not suitable for assessing the risk for Decis EW15 and is therefore not further considered in the risk assessment.

Report:	**KCA 8.5/12; [redacted]; 2013**
Title:	Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil
Source:	Science of the Total Environment 449 (2013) 345–354
DOI No.: Document No.:	10.1016/j.scitotenv.2013.01.079 M-462303-01-1
Guidelines:	ISO 16072, 2002. Soil Quality e Laboratory Methods for Determination of Microbial Soil Respiration. ISO 17155, 2002. Soil Quality e Determination of Abundance and Activity of Soil Microflora Using Respiration Curves.
GLP:	No (published paper).
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

An experiment was performed to study non-target effects of deltamethrin (insecticide) on microbial parameters in a clay-loam soil. Pesticides were applied as commercial formulations to soil samples at different concentrations (5, 50 and 500 mg kg^{-1} dws) and then incubated under laboratory conditions for 3 months. Throughout the incubation period, microbial parameters were determined at days 7, 30, 60 and 90. At 5 mg kg^{-1} dws, deltamethrin did not cause significant changes in soil microbial parameters. In contrast, at 500 mg kg^{-1} dws, pesticide application decreased overall soil microbial activity, negatively affecting the activity of soil enzymes.
Similarly, at 500 mg kg^{-1} dws, deltamethrin did not cause a pesticide-induced stress on soil microbial communities, as reflected by the respiratory quotient. Besides, deltamethrin at 50 and 500 mg kg^{-1} dws resulted in lower values of denitrification potential. It was concluded that, although pesticide concentration had a somewhat inconsistent and erratic effect on soil microbial parameters, pesticide application at 500 mg kg^{-1} dws did have an impact on many of the microbial parameters studied here.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Audace® (2.5% deltamethrin)
Active substance(s):	Deltamethrin((S)-cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate)
Source of test item:	Not stated
Batch number/CAS number:	Not stated
Purity:	Nominal 2.5% active substance
Stability of test compound:	Not given
Water solubility:	Not given

2. Test organism(s)

Species:	Soil microbial community
Source of test species:	Collected with test medium from riparian zone of [redacted] located in [redacted] (northern Spain).
Holding conditions prior to test:	Soil samples with microbial community were transferred to laboratory in dark plastic bags, manually homogenized, air-dried at 25°C during 48 h and sieved to <2 mm.

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory: soil microbial activity (soil microbial respiration, enzyme activities and N-transformation)
Deviation from guidelines:	Not reported.
Duration of study:	90 days
Treatments:	Control and pesticide treatment groups
Test concentrations:	5, 50 and 500 mg deltamethrin kg^{-1} dry weight soil (dws)
Number of replicates:	4 replicated mesocosms
Test units (type and size):	10 L plastic pots filled with 4 kg dws, soil layer of appr. 10 cm
Application / device:	Mixed into soil with a rotary mixer

2. Environmental conditions

Test medium:	Chernozem calcic with clay-loam texture; natural soil (0-25 cm)
Source of test medium	Riparian zone of [redacted] located in [redacted] (northern Spain).
Temperature:	22 ± 1 °C
Photoperiod:	24 h dark
Soil moisture	60% water holding capacity
pH:	8.3
Organic matter (C_{org}):	17.0 g kg^{-1} dws
Soil characteristics:	2.3 g total N kg^{-1} dw soil, C/N ratio of 7.8 and electrical conductivity of 0.18 ds m^{-1}.
Soil history/fertilization:	No known pesticide or fertilizer application for the last 15 years (no deltamethrin residues as confirmed by GC-MS).

3. Observations and measurements:

Analytical parameters measured:	Active substance concentration per kg dw soil
Biological parameters measured:	Soil basal respiration, substrate-induced respiration, potentially mineralizable nitrogen (N_{min}), nitrification rate, diversity of ammonium-oxidizing bacteria, denitrification potential and enzyme activities: dehydrogenase, β-glucosidase, urease, arylsulfatase, alkaline phosphatase
Measurement frequency:	At 0, 7, 30, 60 and 90 days after treatment.
Statistical analyses:	ANOVA for analyzing microbial properties, Pearson's correlation coefficients and Principal Component analysis to establish relationships among soil properties, and for enzyme activity the treated-soil quality index (T-SQI) was calculated for 7 and 90 days of incubation (= after treatment).

4. Chemical analysis

Guideline/protocol:	As described by [redacted] et al (2012) [25]
Method:	GC-MS
Pre-treatment:	From each mesocosm, 250 g fresh weight soil were sieved (< 2 mm) and stored at 4 °C until analysis
Conduction:	See above
Recovery:	> 99%
LOD:	Not specified
LOQ:	Not specified

RESULTS

1. Deltamethrin degradation in soil:

[25] Muñoz-Leoz B, [redacted] C, Antigüedad I, [redacted] E. Fertilization can modify the non-target effects of pesticides on soil microbial communities. Soil Biol Biochem 2012;48:125–34.

Table 2 - Kinetic parameters of deltamethrin dissipation in soil

Deltamethrin concentration	A	k1 (d^{-1})	B	k2 (d^{-1})	$t_{1/2}$ (d)	r^2
5 mg kg^{-1} dw	2.3	0.0783	2.7	0.0061	29.0	1.000
50 mg kg^{-1} dw	31.9	0.0031	18.0	0.0804	78.0	0.999
500 mg kg^{-1} dw	372.4	0.0002	127.6	0.4032	1381	1.000

Pesticide degradation in soil was described by a bi-exponential model [$PC(t) = A*e^{(-k1*t)} + B*e^{(-k2*t)}$], where PC (t) = pesticide concentration at t time; A and B = constants; k1 and k2 = degradation kinetic constants for the first and second component of the curve; t = time]. $t_{½}$ = half-life or time required for a 50% dissipation of initial pesticide concentration.

Pesticide degradation fitted more accurately to a bi-exponential kinetic model than to classical first-order models (Figure 1).

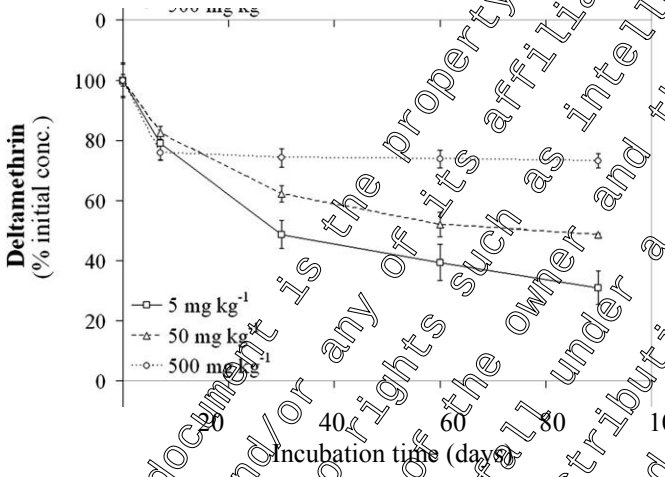

Figure 2 - Deltamethrin concentration in soil throughout the experiment. Pesticide concentration at a given incubation time is expressed as % of the initial pesticide concentration. Mean values (n = 4) ± S.D.

2. Biological findings:

- Deltamethrin had no effect on dehydrogenase activity at 5 and 50 mg kg^{-1}, while at 500 mg kg^{-1} dehydrogenase activity was reduced at day 90
- Higher values of Q_R (respiratory quotient: ratio of basal respiration to substrate-induced respiration) were not found in any treated samples with deltamethrin.
- N_{min} values (an indicator of biologically active soil N) were not affected at 5 mg deltamethrin kg^{-1}, while they were reduced at day 7 at 50 mg kg^{-1} and on days 7, 60 and 90 at 500 mg kg^{-1}
- NH_4^+ values of Deltamethrin were significantly higher at 50 and 500 mg kg^{-1} on day 7 only
- 50 and 500 mg deltamethrin kg^{-1} dws resulted in similar values of denitrification potential
- Deltamethrin did not increase the diversity of ammonium-oxidizing bacteria
- The T-SQI was not affected at 5 mg deltamethrin kg^{-1}, while significantly lower values were observed at 50 and 500 mg kg^{-1} at several sampling moments.

RESULTS SUMMARY

Pesticide degradation rates were dependent upon concentration: higher values of half-life time were observed at increasing pesticide concentrations. At 5 mg kg^{-1} dws, no pesticide-related relevant changes in soil microbial communities occurred. At higher concentrations, some adverse impacts on soil microbial communities were detected. In particular, at 500 mg kg^{-1} dw soil, deltamethrin application decreased overall soil microbial activity, negatively affecting the activity of soil enzymes (it was found that the higher the concentration, the lower the T-SQI value). Even at high concentrations, deltamethrin caused a pesticide-induced stress on soil microbial communities, as reflected by the respiratory quotient and a lower of N_{min} at the end of the incubation.

Comments by the Notifier

No significant impact was seen at concentrations of 5 mg Deltamethrin/kg, which is clearly above the relevant exposure in the risk assessment. However, this study demonstrates that the soil microbial community is not impacted at concentration of 5 mg Deltamethrin/kg.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

According to the EU data requirements, screening data shall establish whether test substances exhibit herbicidal or plant growth regulatory activity. The data shall include testing from at least six plant species from six different families including both mono- and dicotyledons. To cover this data requirement limit tests on vegitative vigor and seedling emergence were conducted with the representative formulation Deltamethrin EW15 with eleven plant species, eight dicotyledonous and three monocotyledonous species, at tested rates 6.5 times higher than the maximum single representative use rate of 7.5 g a.s./ha. Since effects were less than 50% for all eleven tested plant species at exaggerated rates in both studies it can be concluded that Deltamethrin does not exhibit herbicidal or plant growth regulatory activity.

The studies on non target plants (seedling emergence and vegetative vigour) conducted with the representative formulation Deltamethrin EW 15 are presented under point KCA 8.6.2.

CA 8.6.2 Testing on non-target plants

Report:	**KCA 8.6.2/01, [redacted]; 2011**
Title:	Deltamethrin EW 15A G: Vegetative Vigour Limit Test for Non Target Plants on Eleven Plant Species
Document No:	M-402931-01-1 (Rep. No: S10-02921)
Guidelines:	OECD 227 (2006)
GLP:	Yes

Material and methods

Test item: Deltamethrin EW 15 A G, Batch No.: 2010-002975, Active ingredients: Deltametrhin (AE F032640), Content of a.s. (analysed): 15.35 g/L

Study Objective: This vegetative vigour limit test was designed to evaluate the potential effects of the test item after application on the above-ground portions of plants under defined conditions in a green house. The effects on vigour and growth in relation to the control cultures were determined over a test period of 21 days following application of test item

Plant species: *Allium cepa, Avena sativa, Beta vulgaris, Brassica napus, Cucumis sativus, Fagopyrum esculentum, Glycine max, Helianthus annuus, Linum usitatissium, Solanum lycopersicum, Zea mays*
Test design: The experimental phase was performed in a controlled environment greenhouse in E-46820 Anna, Valencia, Spain. Eight dicotyledonous and three monocotyledonous species were cultivated in soil, to which Deltamethrin EW 15A G was applied at one rate of 48.5 g a.s./ha to young plants at the BBCH 12-14. Results were compared to a water treated control. Each treatment group consisted of a total of 30 plants. The test duration was 21 days after application. During this period plants were assessed for phytotoxicity symptomps on day 7, 14, 21. The effects on plant dry weight were determined at test termination.
Exposusre time: 21 days after application
Endpoints: Phytotoxicity, dry weight of shoots
Test rates: 0 (control), 48.5 g a.s./ha
Test conditions: Air temperature (min/max) [°C]: 5.0/31.0
Relative humidity (min/max [%]: 25.5/81.0
Photoperiod (light/dark) [h]: 16/8
Light intensity (min/max) [1000 lux]: 11.5/<.30

Findings
Effects of Deltamethrin EW 15A G applied at 48.5 g a.s./ha relative to control plants, for phytotoxicity and dry biomass are summarised in the following table.

Phytotoxicity effects (mean values) and inhibition of biomass after 21 days relative to controlplants

Plant species	Mean Phytotoxicity (%)	Inhibition of Dry Biomass (%)
Beta vulgaris	0.0	6.5
Brassica napus	0.0	14.1
Cucumis sativus	0.0	17.6
Fagopyrum esculentum	0.0	5.8
Glycine max	0.0	4.3
Helianthus annuus	0.0	3.7
Linum usitatissimum	0.0	17.4*
Solanum lycopersicum	0.0	-4.2
Allium cepa	0.0	-4.9
Avena sativa	0.0	-1.9
Zea mays	0.0	-12.3

*significantly different compared to the control
[1)] Calculated with the highest %-value per replicate

Conclusion
Validity criteria were fulfilled for all eleven species tested. Considering dry biomass data Linum usitatissimum was the most sensitive plant species tested with a statistically significant reduction of 17.4% when exposed to Deltamethrin EW 15A G at 48.5 g a.s./ha. Since effects on dry biomass were less than 50% and no phytotoxic symptoms occurred for all eleven tested plant species a low potential risk to terrestrial plants is concluded from Deltamethrin EW 15A G at 48.5 g a.s./ha.

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Report:	**KCA 8.6.2/02, [redacted]; 2011**
Title:	Deltamethrin EW 15A G: Seedling Emergence Test for Non Target Plants on Eleven Plant Species
Document No:	M-403202-01-1 (Rep. No: S10-02920)
Guidelines:	OECD 208 (2006)
GLP:	Yes

Material and methods

Test item: Deltamethrin EW 15A G, Batch No: 2010-002975, Active ingredients: Deltamethrin (AE F032640), content of a.s. (analysed): 15.35 g/L.

Study objective: This seedling emergence limit test was designed to evaluate the potential effects of the test item on seedling emergence and seedling early growth, by observation of the germination and growth of plant seeds after application of the test item to soil, under defined conditions in a greenhouse. The inhibition of plant emergence and early growth in relation to control cultures was determined over a test period of 21 days following 50 % emergence in the control.

Plant species: *Allium cepa, Avena sativa, Beta vulgaris, Brassica napus, Cucumis sativus, Fagopyrum esculentum, Glycine max, Helianthus annuus, Linum usitatissimum, Solanum lycopersicum, Zea mays*

Test design: The experimental phase was performed in a controlled environment greenhouse in [redacted] Valencia, Spain.

Eight dicotyledonous and three monocotyledonous species were cultivated in soil, to which Deltamethrin EW 15A G was applied at one rate of 48.5 g a.s./ha. Results were compared to a water treated control. Each treatment group consisted of a total of 30 plants. The test duration was 21 days after 50 % of the seeds in the control had emerged. During this period plants were assessed for seedling emergence and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant dry weight were determined at test termination.

Exposure time: 21 days after 50% in the control had emerged

Endpoints: Emergence, phytotoxicity, dry weight of shoots

Test rates: 0 (control), 48.5 g a.s./ha

Test conditions: Air temperature (min/max) [°C]: 11.0, 32.5, Relative humidity (min/max) [%]: 28.0/81.5, Photoperiod (light/dark) [h]: 16/8, Light intensity (min/max) [1000 lux] 11.5/< 30

Findings

Effects of Deltamethrin EW 15 applied at 48.5 g a.s./ha relative to control plants for seedling emergence, phytotoxicity and dry biomass are summarised in the following table.

Phytotoxicity effects (mean values) and inhibition of emergence and biomass after 21 days relative to control plants.

Plant species	Inhibition of Emergence (%)	Mean phytotoxicity (%)	Inhibition of Dry Biomass (%)
Beta vulgaris	3.3	5.0	17.1
Brassica napus	41.4	9.0	21.2
Cucumis sativus	3.3	0.0	31.1
Fagopyrum esculentum	6.9	0.0	18.9
Glycine max	29.6	0.0	19.1
Helianthus annuus	0.0	0.0	0.0
Linum usitatissimum	7.1	1.0	8.3
Solanum lycopersicum	0.0	0.0	-1.3
Allium cepa	15.6	6.7	-12.5
Avena sativa	8.5	0.0	2.9
Zea mays	-5.9	1.3	-3.9

* significantly different compared to the control
[1)] Calculated with the highest %-value per replicate

Conclusion

Validity criteria were fulfilled for ten species tested. Phytotoxic effects occurred in the control group of the species *Helianthus annuus*.

Considering seedling emergence, phytotoxicity and dry biomass, these data showed that only the seedling emergence of *Brassica napus*, *Glycine max* and *Allium cepa* was reduced on one or two assessment dates and the dry biomass of *Cucumis sativus* (31.1% reduction) was significantly reduced following exposure to Deltamethrin EW 15 at an application rate of 48.5 g a.s./ha. The other tested species showed no or only slight effects on the observation parameters.

Slight symtomps of phytotoxicity were observed in the tested species *Beta vulgaris*, *Brassica napus*, *Linum usitatissimum*, *Allium cepa and Zea mays*.

Since effects on emergence, phytotoxicity and biomass were all less than 50% at the rate of 48.5 g a.s./ha for all eleven tested plant species a low potential risk to terrestrial plants is concluded.

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

No studies on other terrestrial organisms are necessary.

CA 8.8 Effects on biological methods for sewage treatment

For studies already evaluated during the first EU review of this compound, please refer to the corresponding section in the Monograph and to the studies in the baseline dossier provided by Bayer Crop Science.

CA 8.9 Monitoring data

No ecological monitoring studies were conducted. For monitoring of deltamethrin in the environement please refer to MCA 7.5.