Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Prothioconazole

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

[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

Indroduction

A dossier on prothioconazole (CAS No. 178928-70-6) was submitted February 2002 by Bayer CropScience to the EU RMS United Kingdom for agricultural use as a fungicide. Prothioconazole was included into Annex I of the Council Directive 91/414/EEC by the Commission Directive 2008/44/EC published 4 April 2008, with an entry into force by 1 August 2008.

This Supplemental Dossier contains only detailed summaries of studies, which were not part of the dossier during the first Annex I inclusion of prothioconazole and were, therefore, not evaluated during the first EU review of this compound. In order to facilitate discrimination between new and old information, the new information is written in black letters whereas *grey letters describe the old information.*

All studies, which have been already submitted by Bayer CropScience for the first Annex I inclusion, are contained in the Monograph and its Addenda and are included in the Baseline dossier provided by Bayer CropScience.

A synonymous name for prothioconazole used at several locations in this Supplemental Dossier is JAU 6476.

Due to changes in trigger for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments since the first Annex I inclusion of prothioconazole, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table CA 8-1). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.

Table CA 8-1: Definition of the residue for risk assessment*

Compartment	Residue definition for risk assessment
Soil	Prothioconazole, JAU 6476-S-methyl *(M01)* and JAU 6476-desthio *(M04)*
Groundwater	Prothioconazole, JAU 6476-S-methyl *(M01)* and JAU 6476-desthio *(M04)*
Surface water	Prothioconazole, JAU 6476-S-methyl *(M01)*, JAU 6476-desthio *(M04)*, JAU 6476-thiazocine *(M12)*, 1,2,4-triazole *(M13)* and JAU 6476-triazolylketone *(M42)*
Sediment	Prothioconazole, JAU 6476-S-methyl *(M01)*, JAU 6476-desthio *(M04)*, JAU 6476-thiazocine *(M12)*, 1,2,4-triazole *(M13)* and JAU 6476-triazolylketone *(M42)*
Air	Prothioconazole and JAU 6476-desthio *(M04)*

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

Plant metabolites

In addition to the active substance, its metabolite JAU 6476-desthio is assessed in the dietary exposure and risk assessment of terrestrial vertebrates (birds and mammals).

A list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound prothioconazole, is presented in Document N3 of this dossier.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

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

Table CA 8.1.1- 1: Endpoints used in risk assessment and additional studies for prothioconazole

Test substance	Test species	Ecotoxicological endpoint		Reference
Prothioconazole	acute, oral *Colinus virginianus* (Bobwhite quail)	**LD_{50}**	**> 2000 mg a.s./kg bw**	[redacted] (1999), M-013030-01-1 KCA 8.1.1.1/01
	acute, oral *Serinus canaria* (Canary)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] & [redacted] (2010) M-364387-01-1 KCA 8.1.1.1/03
	Short-term, 5-day feeding *Colinus virginianus* (Bobwhite quail)	LC_{50} LDD_{50}	> 5000 mg a.s./kg diet > 1413 mg a.s./kg bw/d	[redacted] (2001) M-054770-01-1 KCA 8.1.1.2/01
	Short-term, 5-day feeding *Anas platyrhynchos* (Mallard duck)	LC_{50} LDD_{50}	> 5000 mg a.s./kg diet 2457 mg a.s./kg bw/d	[redacted] (1998) M-055523-01-1 KCA 8.1.1.2/03
	Reprod. 21 w dietary *Anas platyrhynchos* (Mallard duck)	**NOEC** **NOEL**	**700 mg a.s./kg diet** **78 mg a.s./kg bw/d**	[redacted] (2000), M-035123-01-1 KCA 8.1.1.3/02
	Reprod. 20 w dietary *Colinus virginianus* (Bobwhite quail)	NOEC NOEL	≥ 1000 mg a.s./kg diet ≥ 86 mg a.s./kg diet	[redacted] (2000), M-042334-01-1 KCA 8.1.1.3/01
JAU 6476-desthio	acute, oral *Colinus virginianus* (Bobwhite quail)	**LD_{50}**	**> 2000 mg p.m./kg bw**	[redacted] (1990) M-013315-01-1 KCA 8.1.1.1/02
	Short-term, 5-day feeding *Colinus virginianus* (Bobwhite quail)	LC_{50} LDD_{50}	4090 mg p.m./kg diet 603[a)] mg p.m./kg bw/d	[redacted] (1998), M-056229-02-1 KCA 8.1.1.2/02 and [redacted] (2006) M-268832-02-1 KCA 8.1.1.2/04
	Reprod. 22 w dietary *Colinus virginianus* (Bobwhite quail)	**NOEC** **NOEL**	**173 mg p.m./kg diet** **14.8 mg p.m./kg bw/d**	[redacted] (2002), M-090509-01-1 KCA 8.1.1.3/03
	Reprod. 20 w dietary *Anas platyrhynchos* (Mallard duck)	NOEC NOEL	≥ 500 mg p.m./kg diet ≥ 63 mg pm/kg bw/d	[redacted] et al. (2001), M-079949-01-1 KCA 8.1.1.3/04

a) [redacted].; 2006; M-268832-02-1, KCA 8.1.1.2/04: For JAU 6476-desthio the short term LC_{50} for bobwhite quail was determined to be 4090 mg pm/kg diet by probit analysis. As for the parent compound the short-term risk assessment for this metabolite will be based on the daily dietary dose that caused 50% mortality (LDD_{50}). To calculate the LDD_{50} value for this study the mortality data have been re-evaluated by probit analysis on the base of the daily dose data from the report. The probit analysis has been conducted with the computer program "ToxRatPro", Version 2.09. A LDD_{50} of 603 mg pm/kg bw/d has been calculated. For further details, please refer to CA 8.1.1.2.

CA 8.1.1.1 Acute oral toxicity to birds

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

Report:	KCA 8.1.1.1/03 [redacted],;[redacted]; 2010; M-364387-01-1
Title:	Toxicity of JAU 6476 technical (prothioconazole) during an actue oral LD50 with the canary (Serinus canaria)
Report No.:	EBJAL065
Document No.:	M-364387-01-1
Guideline(s):	OPPTS 850.2100 OECD 223
Guideline deviation(s):	- Canary bird feed (Living World Premium Canary Food) contaminant screening analysis was not conducted for this study however the nutrient analysis was presented from the supplier. These data were not collected in accordance with Good Laboratory Practice procedures (no protocol, study director, or in-life inspections). [40CFR160.90(g)] - Public water analysis was conducted by the Kansas City Missouri Water Services Laboratory. These data were not collected in accordance with Good Laboratory Practice procedures (no protocol, study director, or in-life inspections). [40CFR160.90(g)] - Corn oil screening analyses for pesticides, chlorinated hydrocarbons, and toxic metals were conducted by Covance Laboratories, Madison, WI. These data were not collected in accordance with Good Laboratory Practice procedures (no protocol, study director, or in-life inspections). [40CFR160.90(g)]
GLP/GEP:	**yes**

Objective:

The purpose of this study was to estimate the acute oral toxicity of JAU 6476 technical (Prothioconazole) to the Canary (*Serinus canaria*). Test methods were in agreement with OECD 223 and US Environmental Protection Agency test guidelines.

Material and methods:

Test item: JAU 6476 technical (Prothioconazole), purity: 98.3% w/w, Batch No. AE 1344248-02-1, TOX07816-01, Origin batch No. PFV0672333

Adult canaries (*Serinus canaria*) were orally dosed based on body weight with the JAU 6476 technical (Prothioconazole) at a limit dose level of 2000 mg active substance (a.s.)/kg body weight. Five males and five females were tested per treatment level and observed daily for clinical symptoms for 14 days post-dose administration.

During the whole experiment, birds were individually housed indoors in stainless stell breeder type cages. Average temperature was 22°C and average humidity was 54%. The photpheriod was 10 h light and 14 h dark with a light intensity of 304 lux. The birds were provided food *ad libitum* during acclimation and study duration. However, birds were fasted for approximately 16 h prior to dose administration.

Study endpoints of bird body weight and daily feed consumption were also monitored during the study period.

Findings:

No dose-related effects were seen in adult canaries dosed with 2000 mg a.s./kg body weight. There were no statistically significant reductions in body weight or growth at the 2000 mg a.s./kg body weight dose level. There were also no dose related reductions in feed consumption at the 2000 mg a.s./kg body weight dose level. No mortalities were noted during this study. Post-mortem examinations were not conducted for this study.

Table CA 8.1.1.1- 1: Acute oral toxicity of JAU 6476 technical to *Serinus canaria*

	Adult mortality (mg a.s./kg body weight)
LD_{50}	> 2000
Lowest Observed Adverse Effect Level (LOAEL)	> 2000
No Observed Adverse Effect Level (NOAEL)	2000

Conclusion:

The acute oral LD_{50} of JAU 6476 technical (Prothioconazole) to the canary was >2000 mg a.s./kg body weight based on a limit dose test. The NOAEL was 2000 mg a.s./kg body weight and the LOAEL was >2000 mg a.s./kg body weight based on all investigated parameters.

CA 8.1.1.2 Short-term dietary toxicity to birds

In the 5 day dietary LC_{50} study with JAU 6476-desthio in Bobwhite quail ([redacted],; 2006; M-056229-02-1, KCA 8.1.1.2/02), late mortalities were observed at the two top dose level after several days of reduced food consumption and body weight effects.

Results from this study are presented below.

Findings:

Table CA 8.1.1.2- 1: Effect on mortality, bodyweight and food consumption after 5 days of exposure to JAU 6476-desthio

Test level	Mortality [# dead / all]	Bodyweight [2] [g/bird]			Bodyweight [2] change [%]		Food consumption [2] [g/bird/d]	
conc. [1] (dose)	(day of mortality)	day1	day 5	day 8	day 1 ⇔ day 5	day 5 ⇔ day8	(d1-d5)	(d5-12)
0 (0)	0/20 -	26.0	35.2	49.1	+35.0	+39.6	6.3	10.2
313 (101)	0/10 -	24.1	37.8	51.5	+ 57.1	+ 36.1	9.8	9.7
625 (166)	0/10 -	26.8	36.8	51.8	+ 37.1	+ 40.9	8.3	13.4
1250 (297)	0/10 -	23.3	30.2	43.5	+ 29.7	+44.2	6.4	8.5
2500 (408)	1/10 (5)	23.9	28.1	40.2	+17.7	+43.0	4.1	7.9
5000 (705)	7/10 (3,4,5,5,5,5,6)	22.6	20.6	34.1	- 9.1	+65.9	2.9	11.8

(1 conc.: nominal concentration in [mg/kg food];
dose: daily dietary dose in [mg/kg bw/d] based on measured concentrations
(2: for birds alive at the respective time point

At the two top test levels mortalities occurred after several days of reduced food consumption leading to severe body weight loss. The seven chicks dying around day 5 at 5000 ppm had a mean bodyweight of 16.3 g/bird (see Table CA 8.1.1.2- 3); i.e less than 50% of the control bird weight of 35.2 g at day 5. All birds found dead were extremely emaciated. Since no other severe clinical symptoms were observed, it has to be assumed that they died on starvation.
During the post-exposure period the food consumption and bodyweight of the surviving birds started to recover.

Figures copied from the original report

Figure CA 8.1.1.2- 1: Average Body Weights

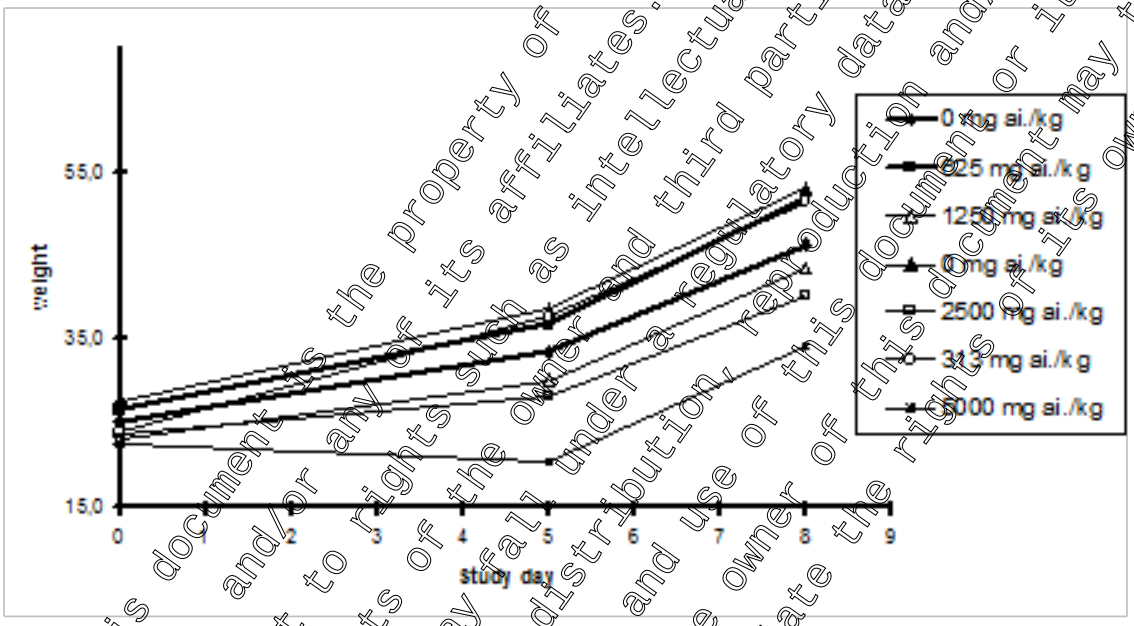

Figure CA 8.1.1.2- 2: **Average Food Consumption / Bird / 24h.**

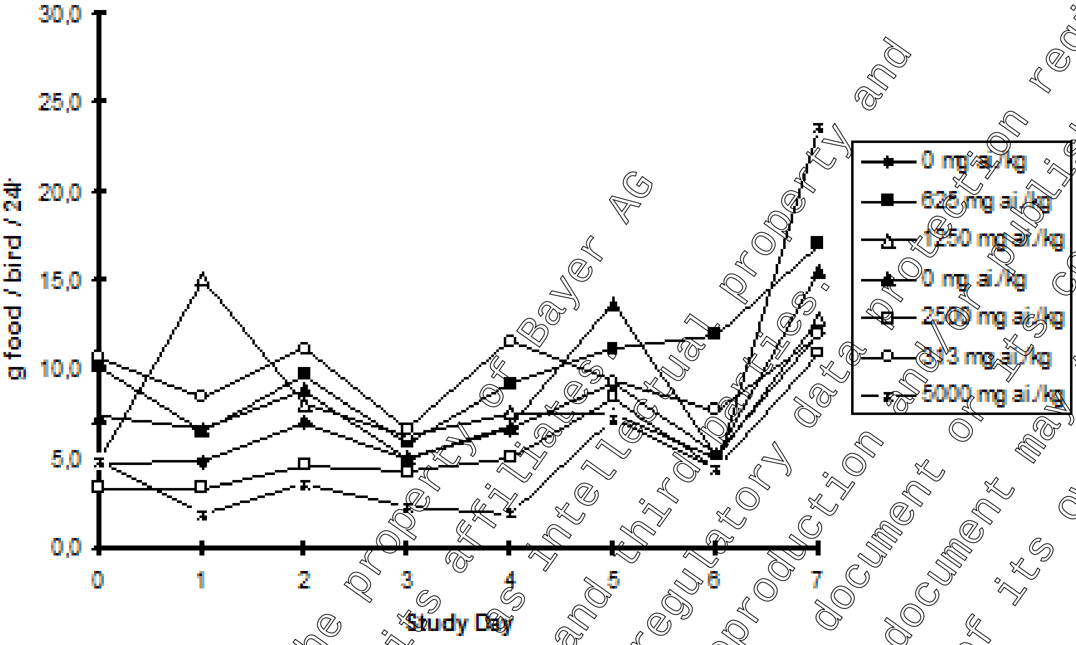

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Table CA 8.1.1.2- 2: Observed Mortalities

Nominal dietary Concentration	# Dead Birds / # Exposed Birds											Total
	d.-3	d.-2	d.-1	d.0	d.1	d.2	d.3	d.4	d.5	d.6	d.7	
0 mg ai./kg diet	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
313 mg ai./kg diet	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
625 mg ai./kg diet	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1250 mg ai./kg diet	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
2500 mg ai./kg diet	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/9	0/9	1/10
5000 mg ai./kg diet	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/9	4/8	1/4	0/3	7/10

Table CA 8.1.1.2- 3: Bodyweight at death

Concentration	Day of death	Body weight at death
2500 mg ai/kg diet	d 5	19.8g
5000 mg ai/kg diet	d 3	13.3g
5000 mg ai/kg diet	d 4	11.9g
5000 mg ai/kg diet	d 5	18.4g
5000 mg ai/kg diet	d 5	18.6g
5000 mg ai/kg diet	d 5	17.7g
5000 mg ai/kg diet	d 5	20.0g
5000 mg ai/kg diet	d 6	13.9g

Conclusion:
The LC_{50} was determined at 4090 mg/kg feed. Based on the measured concentrations the 5-d lethal dietary dose (5-d LDD50) of 603 mg/kg bw/day was calculated by [redacted]S; 2006; M-268832-02-1, KCA 8.1.1.2/04.
Effect profile and time course suggest that mortality occurred only after multiple dosing over several days, and is associated with increasing weight loss and starvation over the treatment duration.
Therefore the results of this study are not meaningful in the acute risk assessment which is intended to address a single day oral exposure event.

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 substanceprothioconazole are reported in the toxicology section MCA 5.

Table CA 8.1.2- 1: Endpoints used in risk assessment for prothioconazole and its metabolites

Test substance	Test species	Ecotoxicological endpoint	Reference
Prothioconazole	Acute, oral Rat	**LD_{50} > 6200 mg a.s./kg bw**	[redacted] (1998) M-012312-01-1 KCA 5.2.1/01
	Long-term (2-gen.-repro study) Rat	**NO(A)EL 95.6 mg a.s./kg bw/d**	[redacted] (2001) M-036206-01-1 KCA 5.6.1/02
JAU 6476-desthio	Acute, oral Mouse	**LD_{50} (male) 2225 mg p.m./kg bw** LD_{50} (female) 1459 mg p.m./kg bw	[redacted] (1991) M-008521-01-1 KCA 5.8.1/34
	Long-term (2-gen.-repro study) Rat	**NO(A)EL 10 mg p.m./kg bw/d**	[redacted] & [redacted] (2001) M-036130-01-1 KCA 5.8.1/23

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

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 8.1.3 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 prothioconazole is below the trigger, the potential for bioaccumulation is low and an evaluation of secondary poisoning is not required.
Since prothioconazole metabolites JAU 6476-desthio and JAU 6476-S-methly are above the trigget, the potential risk for bioaccumulation due to feeding on contaminated prey like fish or earthwroms are evaluated in the ecotoxicological section MCP section 10 CP 10.1.1 & CP 10.1.2. The log P_{OW} value for the metabolites triazolylketone, thiazocine and 1,2,4-triazole are below the trigger value, indicating a very low risk of secondary poisoning.

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

Information on effects of prothioconazole on reptiles is not available and not expected of additional value for the re-evaluating the active substance. Data on amphibians is given under CA 8.2.8.

CA 8.1.5 Endocrine disrupting properties

Birds
The population relevant effects of prothioconazole on birds were studied in reproductive toxicity studies on Bobwhite quail and Mallard duck. Mallard ducks proved to be more sensitive than Bobwhite quails. No statistically significant effects on adult birds, offspring or reproductive parameters were found at 700 mg prothioconazole/kg diet in Mallard ducks and 1000 mg prothioconazole/kg diet in Bobwhite quails. The effect determining the NOEC was "% 14-day survivors of normal hatchlings". This effect is likely caused by general toxicity rather than by an endocrine mediated mechanism. Based on the absence of any indication of relevant effects it can be concluded that prothioconazole is not a (potential) endocrine disrupter in birds.

Wild Mammals
A detailed analysis of all the apical toxicological studies (subchronic, chronic / onco-genicity, reproduction and developmental toxicity) on prothioconazole revealed no endocrine disrupting effect. Slight effects at high dose levels on thyroid related hormone levels were without any histopathological correlate. They were considered as a compensated thyroid status, secondary to increased thyroid hormone excretion due to increased liver enzyme induction. Effects on some reproductive and progeny parameters in the rat reproductive toxicity study were seen only at dose levels that caused severe maternal toxicity and retarded development of the offspring due to that maternal toxicity. Therefore, based on a complete toxicological data set, there is no evidence for endocrine disrupting properties of prothioconazole in mammals.

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

As a conclusion, 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. According to the current data requirements from the EU Regulation 283/2013, no studies on marine organisms are necessary to assess edge of field risk of plant protection products to the aquatic organisms. However, data for marine species have been generated for registration in the USA, which were not evaluated during the first EU Review of this compound. Additional studies with marine species will be summarized, and as a conservative approach, marine studies resulting in lower endpoints will be considered for risk assessment.
For studies already evaluated during the first EU Review of prothioconazole, please refer to corresponding section in the Monograph, amendments to the Monograph and to the studies in the baseline dossier provided by Bayer CropScience.
Two new major metabolites were identified: JAU 6476-thiazocine (M12) and JAU 6476-triazolylketone (M42). JAU 6476-thiazocine (M12) can be formed by in the aquatic environment by photolytic degradation of the parent compound. JAU 6476-triazolylketone (M42) 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”. Several new studies were conducted with JAU 6476-triazolylketone (M42). Summaries of these new aquatic studies are provided below. No new studies were conducted for the photolytic metabolite JAU 6476-thiazocine (M12). Indeed, information is available on the structural properties of this metabolite and on its residual pesticidal activity, as detailed in [redacted] K., 2015 (M-536612-01-1, KCA 8.2/01). This information clearly shows that the toxophore is lost and that M12 has no residual fungicidal, herbicidal or insecticidal properties. Consequently, no JAU 6476-thiazocine-specific endpoints are available which could be used in the risk assessment. For metabolites with such properties, the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013; cited in the following paragraphs as “EFSA AGD (2013)”, prescribes to assume that the acute and chronic toxicity of the metabolite is equal to the toxicity of the a.s. (parent compound) for all first tier taxonomic groups. Therefore, the endpoints of the parent compound prothioconazole were used in the risk assessment of M12.

Endpoint used in risk assessment

The relevant endpoint from each aquatic study was defined according to the current data requirements from the EU Regulation 283/2013 and the EFSA AGD (2013) and based on recommendations from the relevant standard test guideline e.g. Growth rate (r) is the most suitable endpoint from algae inhibition tests for use in risk assessment, as stated by OECD Guideline 201 and the EFSA AGD (2013). TER and RAC calculations presented in this dossier are thus based on the E_rC_{50} values. Indeed, processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) appears more suitable to measure effects in algae. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for biomass. Moreover, the current test guidelines OECD TG 201, the EU-Method C3, the EC regulation for Classification and Labelling (EC regulation 1272/2008) and the PPR Opinion (EFSA Journal 461, 1-44; 2007) list growth rate as the most suitable endpoint of the algae inhibition test.

In accordance with Regulation (EC) No 1107/2009 and with the EFSA AGD (2013), studies resulting in lower endpoints were used for the risk assessment. Although Regulation (EC) No 1107/2009 place no data requirement on marine species, marine studies resulting in lower endpoints compared to freshwater studies were considered for risk assessment as a conservative approach.

Table CA 8.2- 1: Aquatic toxicity data for prothioconazole and its metabolites and additional laboratory studies conducted to enhance the prothioconazole database

Test substance	Test species	Endpoint		Reference
Prothio-conazole	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	**LC_{50}**	**1.83 mg a.s./L**	[redacted] (1999) M-015215-01-1 KCA 8.2.1/01
	Fish, acute *Lepomis macrochirus* (Bluegill sunfish)	LC_{50}	4.59 mg a.s./L	[redacted] (1999) M-020269-01-1 KCA 8.2.1/02
	Fish, acute *Cyprinus carpio* (Common carp)	LC_{50}	6.91 mg a.s./L	[redacted] (2000) M-037387-01-1 KCA 8.2.1/03
	Fish, acute *Cyprinodon variegatus* (Sheepshead minnow)	LC_{50}	> 10.3 mg a.s./L	[redacted] & [redacted] (2004) M-107721-01-1 KCA 8.2.1/09
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	NOEC	1.308 mg a.s./L [1)]	[redacted] & [redacted] (2001) M-088492-01-1 KCA 8.2.2.1/01
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	**NOEC**	**0.49 mg a.s./L** [1)]	[redacted] & [redacted] (2007) M-291414-01-1 KCA 8.2.2.1/03
	Fish, bioconcentration, *Lepomis macrochirus* (Bluegill sunfish)	BCF whole fish	19[redacted]	[redacted] & [redacted] (2001) M-087902-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna* (Cladoceran)	**EC_{50}**	**[redacted] mg a.s./L**	[redacted] (1999) M-013690-01-1 KCA 8.2.4.1/01
	Invertebrate, acute *Crassostrea virginica* (Eastern oyster)	EC_{50}	2[redacted] mg a.s./L	[redacted] et al. (2001) M-055051-01-1 KCA 8.2.4.2/03
	Invertebrate, acute *Americamysis bahia* (Mysid shrimp)	**LC_{50}**	**2.4 mg a.s./L**	[redacted] et al. (2002) M-083057-01-1 KCA 8.2.4.2/02
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	**NOEC**	**0.56 mg a.s./L**	[redacted] & [redacted] (2001) M-055997-01-1 KCA 8.2.5.1/01
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	**NOEC**	**[redacted].14 mg a.s./L**	[redacted] (2000) M-047356-01-1 KCA 8.2.5.4/01
	Pseudokirchneriella subcapitata (green alga)	E_rC_{50}	2.18 mg a.s./L	[redacted] (2000) M-027625-01-1 KCA 8.2.6.1/01
	Skeletonema costatum (marine diatom)	**E_rC_{50}**	**0.046 mg a.s./L**	[redacted] & [redacted] (2004) M-000954-01-1 KCA 8.2.6.2/01
	Navicula pelliculosa (freshwater diatom)	E_rC_{50}	0.355 mg a.s./L	[redacted] & [redacted] (2004) M-001064-01-1 KCA 8.2.6.2/02
	Anabaena flos-aquae (blue-green alga)	E_rC_{50}	>9.12 mg a.s./L	[redacted] et al. (2004) M-000348-01-1 KCA 8.2.6.2/03
	Lemna gibba (Duckweed)	**E_rC_{50}**	**>0.404 mg a.s./L**	[redacted] et al. (2004) M-000532-01-1 KCA 8.2.7/01

Test substance	Test species	Endpoint		Reference
JAU 6476-desthio	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	**LC_{50}**	**6.63 mg p.m./L**	[redacted] (1990) M-013303-01-1 KCA 8.2.1/04
	Fish, acute *Pimephales promelas* (Fathead minnow)	LC_{50}	11.4 mg p.m./L	[redacted] & [redacted] (2003) M-104769-01-1 KCA 8.2.1/10
	Fish, acute *Leuciscus idus melanotus* (Golden orfe)	LC_{50}	13.2 mg p.m./L	[redacted] (1991) M-013301-01-1 KCA 8.2.1/03
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	**NOEC**	**0.00334 mg p.m./L**	[redacted] (2002) M-038386-01-1 KCA 8.2.2.1/01
	Fish, chronic Pimephales promelas (Fathead minnow)	NOEC	0.074 [2]	[redacted] et al. (2004) [2] M-001562-01-1 KCA 8.2.2.2/01 and [redacted] (2006), M-279573-01-1 KCA 8.2.2.2/02
	Fish, bioconcentration, *Lepomis macrochirus* (Bluegill sunfish)	BCF whole fish	66	[redacted] (2001) M-136749-01-1 KCA 8.2.2.3/02
	Invertebrate, acute *Daphnia magna* (Cladoceran)	**EC_{50}**	**>1.0 mg p.m./L**	[redacted] (1990) M-013308-01-1 KCA 8.2.4.1/02
	Invertebrate, acute *Americamysis bahia* (Mysid shrimp)	**LC_{50}**	**>1.009 mg p.m./L**	[redacted] et al. (2003) M-104620-01-1 KCA 8.2.5.2/02
	Invertebrate, acute *Americamysis bahia* (Mysid shrimp)	LC_{50}	0.060 mg p.m./L [3]	[redacted] et al. (2002) M-083055-01-1 KCA 8.2.5.2/01
	Invertebrate, acute *Procambarus clarkii* (Crayfish)	LC_{50}	> 26 mg p.m./L	[redacted] (2004) M-001051-01-1 KCA 8.2.4.2/01
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	**NOEC**	**0.10 mg p.m./L**	[redacted] & [redacted] (2001) M-073861-01-1 KCA 8.2.5.1/02
	Invertebrate, chronic *Americamysis bahia* (Mysid shrimp)	**NOEC**	**0.064 mg p.m./L**	[redacted] et al. (2003) M-104620-01-1 KCA 8.2.5.2/02
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	**NOEC**	**2.0 mg p.m./L [4]**	[redacted] (2000) M-023234-01-1 KCA 8.2.5.4/02
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	NOEC	50 mg p.m./kg dw (development rate)	[redacted] (2008) M-312780-01-1 KCA 8.2.5.4/03
	Scenedesmus subspicatus (green alga)	**E_rC_{50}**	**0.55 mg p.m./L**	[redacted] (1990) M-013305-01-1 KCA 8.2.6.1/02
	Lemna gibba (Duckweed)	**E_rC_{50}**	**0.0809 mg p.m./L**	[redacted] et al. (2003) M-104599-01-1 KCA 8.2.7/02

Test substance	Test species	Endpoint	Reference
JAU 6476-S-methyl	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50} **1.79 mg p.m./L**	[redacted] & [redacted] (2001) M-074388-01-1 KCA 8.2.1/05
	Fish, bioconcentration (estimated value)	BCF whole fish 319.3 [8)]	[redacted] (2013) M-459145-01-1 KCA 8.2.2.3/04
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50} **2.8 mg p.m./L**	[redacted] (2001) M-071853-01-1 KCA 8.2.4.1/03
	Pseudokirchneriella subcapitata (Green alga)	E_rC_{50} **47.4 mg p.m./L**	[redacted] & [redacted] (2001) M-061047-01-1 KCA 8.2.6.1/03
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	**NOEC 0.1 mg p.m./L**	[redacted] (2006) M-266605-01-1 KCA 8.2.5.4/04
1,2,4-Triazole	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50} **498 mg p.m./L**	[redacted] (1983) M-046022-01-1 KCA 8.2.1/06
	Fish, juvenile growth test *Oncorhynchus mykiss* (Rainbow trout)	**NOEC 3.2 mg p.m./L**	[redacted] & [redacted] (2002) M-030491-01-1 KCA 8.2.2/01
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50} **> 100 mg p.m./L** [5)]	[redacted] (1995) M-088901-01-1 KCA 8.2.4.1/06
	Pseudokirchneriella subcapitata (green alga)	E_rC_{50} **>31 mg p.m./L** [6)]	[redacted] et al. (2001) M-077067-01-1 KCA 8.2.6.1/04
JAU 6476-triazolyl-ketone	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	**LC_{50} >100 mg p.m./L**	[redacted] (2006) M-266572-01-1 KCA 8.2.1/11
	Invertebrate, acute *Daphnia magna* (Cladoceran)	**EC_{50} >100 mg p.m./L**	[redacted] (2006) M-266597-01-1 KCA 8.2.4.1/07
	Pseudokirchneriella subcapitata (green alga)	**E_rC_{50} >100 mg p.m./L**	[redacted] (2006) M-266567-01-1 KCA 8.2.6.1/05
JAU 6476-thiazocine	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50} **1.83 mg a.s./L** [7)]	[redacted] (1999) M-015215-01-1 KCA 8.2.1/01
	Fish, early life stage *Oncorhynchus mykiss* (Rainbow trout)	**NOEC 0.49 mg a.s./L** [7)]	[redacted] & [redacted] (2007) M-291414-01-1 KCA 8.2.2.1/03
	Invertebrate, acute *Daphnia magna* (Cladoceran)	EC_{50} **1.3 mg a.s./L** [7)]	[redacted] (1999) M-013690-01-1 KCA 8.2.4.1/01

Test substance	Test species	Endpoint	Reference
	Invertebrate, chronic *Daphnia magna* (Cladoceran)	NOEC **0.56 mg a.s./L** [7]	[redacted] & [redacted] (2001) M-055997-01-1 KCA 8.2.5.1/01
	Pseudokirchneriella subcapitata (green alga)	E_rC_{50} **2.18 mg a.s./L** [7]	[redacted] (2000) M-027625-01-1 KCA 8.2.6.1/01

a.s.: active substance; p.m.: pure metabolite

Bold values: Endpoints considered relevant for risk assessment.

[1] The fish early life stage study submitted in the Baseline Dossier under the number M-088492-01-1 (KCA 8.2.2.1/01) has been qualified as invalid by some authorities since the egg hatching rate in the control was judged too low. A new study has been requested by these authorities as confirmatory data. Therefore, a new valid study has been conducted in 2007 ([redacted], D.; [redacted], C. V.; 2007; M-291414-01-1, KCA 8.2.2.1/03), which is used in the risk assessment.

[2] Revised NOEC, based on a delayed time to first spawning at 0.148 mg/L

[3] The LC_{50} of 0.06 mg/L from this acute study was inconsistent with results from the full life-cycle toxicity test conducted later on in the same laboratory ([redacted], A. S.; [redacted], T. Z.; [redacted], H. O.; 2003; M-104620-01-1, KCA 8.2.5.2/02). Reasons for this inconstancy were investigated in [redacted] *et al.* 2003 (M-104620-01-1, KCA 8.2.5.2/02): two additional acute studies were conducted, that yielded LC_{50} values > 2 mg/L (nominal) and > 1.009 mg /L (mean measured). These data suggest that the results from the first acute test by [redacted] *et al.* (2002) are not repeatable. Therefore, the LC_{50} of 0.06 mg/L is deemed unreliable. The LC_{50} of > 1.009 mg /L from the [redacted] *et al.* study (2003) is thus used for the risk assessment.

[4] NOEC according to the list of endpoints given in the EFSA conclusion on prothioconazole (2007), the original study endpoint is the EC_{15} = 4.4 mg/L; the cited NOEC was not statistically derived as was explained in the DAR by the RMS but proposed as a conservative endpoint.

[5] EU agreed endpoint for 1,2,4-triazole derived from the PRAPeR expert meeting on triazole metabolites (PRAPeR 13, 2007)

[6] EU agreed endpoint is derived from the EFSA Scientific Report (2014) 12(1):3485, Conclusion on the peer review of tebuconazole.

[7] JAU 6476-thiazocine has lost the toxophore and shows no pesticidal activity, as explained in detail in a statement by [redacted] 2015 (M-536612-01-1, KCA 8.2/01). For metabolites with such properties, the 'EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge of field surface waters (2013)' prescribes to assume "that the acute and chronic toxicity of the metabolite is equal to the toxicity of the a.s. (parent compound) for all first tier taxonomic groups". Therefore, the endpoints of the parent compound prothioconazole from studies on first tier species were used for the acute and chronic risk assessment of JAU 6476-thiazocine.

[8] This BCF value has been estimated based on the current EU agreed value of log P_{ow} for prothioconazole (i.e. 3.82, as estimated using the method OECD 107 by [redacted]s; 2001; M-067502-01-1). [redacted] and [redacted] (2014; M-492539-01-1) re-estimated the log Pow of prothioconazole according to OECD 117 and found a value of 2.0. Therefore, the proposed BCF value for JAU 6476-S-methyl is worst-case and should be re-estimated based on the new value of log Pow for prothioconazole.

Statement on the structural properties of JAU 6476-thiazocine

Report:	KCA 8.2/01 [redacted]; 2015; M-536612-01-1
Title:	Report on predictive molecular modelling results and on biological test results of the prothioconazole derivative JAU 6476-thiazocine (M12).
Report No.:	M-536612-01-1
Document No.:	M-536612-01-1
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

Potential properties and biological activities of the prothioconazole photolytic degradation product JAU 6476-thiazocine were calculated by diverse computational methods. The structural conformation of JAU 6476-thiazocine is different from prothioconazole and other azole fungicides. Docking of JAU 6476-thiazocine into a protein model of cyp51, the target protein of azole fungicides, demonstrates that a binding like other azoles is impossible. Forced binding of JAU 6476-thiazocine into cyp51 requires a totally different docking pose from other azoles and therefore is unlikely.

Actual pesticidal biological activities of JAU 6476-thiazocine were retrieved from Bayer CropScience's internal biological database 'Bioquick'. Pesticidal activity of JAU 6476-thiazocine was tested against eight diverse fungi and ten other types of organisms. With the exception of two likely technical failures, JAU 6476-thiazocine was proved inactive in all biological tests.

In conclusion, inhibition of sterol biosynthesis by JAU 6476-thiazocine, like by prothioconazole, is highly unlikely based on structural considerations. This theoretical finding is strongly corroborated by experimental results. JAU 6476-thiazocine is pesticidally inactive against fungi and other organisms (oomycetes, insects, acari, nematodes, plants) under sensitive screening conditions.

CA 8.2.1 Acute toxicity to fish

Three additional studies have been performed. The corresponding study summaries are presented below. Existing studies have been evaluated during the Annex I inclusion. They have been summarized in the Monograph and are included in the baseline dossier.

Report:	KCA 8.2.1/09 [redacted] L; [redacted]; 2004; M-107721-01-1
Title:	Acute toxicity of JAU 6476 technical to the Sheepshead minnow (Cyprinodon variegatus) under static-renewal conditions
Report No.:	200615
Document No.:	M-107721-01-1
Guideline(s):	FIFRA 72-3 (a)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
A 96-hour static-renewal test was conducted to determine the acute toxicity of prothioconazole technical to the sheepshead minnow (*Cyprinodon variegatus*). The primary endpoint for acute toxicity was mortality. Sublethal and behavioural effects were also assessed during the course of the study. Results of the test were expressed as a 96-hour median lethal concentration (LC_{50}).

Materials and methods:
Test item: JAU 6476 technical (prothioconazole), CAS number #178928-70-6; Purity 97.8%, Batch No. Fl.6233/0031.

The test was conducting according to the FIFRA Guideline 72-3(a). Sheepshead minnow (*Cyprinodon variegatus;* approximately 68 days old at test start) were exposed under static-renewal conditions (renewal after 2 days) for 96 hours to the following nominal concentrations of the test item: control , solvent control, 0.75, 1.5 , 3.0 , 6.0 , and 12.0 mg a.s./L. Water samples were collected from all test vessels on Day 0, Day 2 and Day 4 and were analysed to measure actual exposure concentrations. One replicate of 20 fish was used in the control, solvent control (acetone), and the five tested concentrations.

The light cycle was programmed to produce an overall photoperiod of 16-hours light and 8-hours dark. Fish were not fed during the test and test solutions were not aerated. Salinity and pH were measured in all test vessels on Day 0, Day 2 and Day 4, whereas temperature and dissolved oxygen were measured daily. Water quality parameters were adequate and remained within expected ranges during the whole experiment. The test temperature ranged from 21.6 to 21.9°C (mean = 21.7°C) as measured hourly by the datalogger. Dissolved oxygen concentrations ranged from 6.0 to 7.6 mg/L, corresponding to 75 to 95% saturation, respectively. The pH values ranged from 7.4 to 7.9 and the salinity ranged from 16 to 17‰ throughout the test.
Daily observations were made for mortality and sublethal effects.

Findings:

Validity criteria:

The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:

The mean measured concentrations of JAU 6476 during the test period were 0.69, 1.34, 2.51, 5.42 and 10.3 mg a.s./L, which represented 84 to 92 percent of the nominal concentrations. All reported results refer to mean measured concentrations of the test solutions.

Biological results:

No compound related mortalities or sublethal effects were noted at any test level during the 96-hour exposure to JAU 6476. Since no differences were observed between either of the control groups and any JAU 6476 test level, no statistical comparisons could be made. The LC_{50}, NOEC and LOEC values were thus determined empirically.
Based on the mortality data collected and the mean measured JAU 6476 concentrations the 96-hour LC_{50} was >10.3 mg a.s./L, the 96-hour lowest-observed effect-concentration (LOEC) was >10.3 mg a.s./L and the 96-hour no-observed effect-concentration (NOEC) was 10.3 mg a.s./L.

Table CA 8.2.1- 1: Acute toxicity of JAU 6476 (technical) to Sheepshead minnow

Test substance	JAU 6476
Test object	Sheepshead Minnow
Exposure	96 hour, Static-Renewal
LC_{50}	>10.3 mg a.s./L
Lowest Observed Effect Concentration (LOEC)	>10.3 mg a.s./L
Highest Test Concentration without Toxic Effect (NOEC)	10.3 mg a.s./L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	>10.3 mg a.s./L

Conclusion:

Sheepshead minnow were exposed for 96 hours in a static renewal system to prothioconazole. prothioconazole caused no adverse effects to sheepshead minnow near the practical limit of solubility in the test system (measured concentration = 10.3 mg a.s./L). The LC_{50} was >10.3 mg a.s./L and the NOEC was 10.3 mg a.s./L.

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Report:	KCA 8.2.1/10 [redacted],; [redacted]; 2003; M-104709-01-1
Title:	Acute toxicity of JAU 6476-Desthio to the fathead minnow (Pimephales promelas) under static-renewal conditions
Report No.:	200151
Document No.:	M-104709-01-1
Guideline(s):	FIFRA Guideline 72-1 Acute Toxicity Test for Freshwater Fish
Guideline deviation(s):	no significant deviations
GLP/GEP:	**yes**

Objective:

A 96-hour static-renewal test was conducted to determine the acute toxicity of JAU 6476-desthio to the fathead minnow (*Pimephales promelas*). The primary endpoint for acute toxicity was mortality. Sublethal and behavioural effects were also assessed during the course of the study. Results of the test were expressed as a 96-hour median lethal concentration (LC_{50}). If possible, estimates of the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) were made.

Materials and methods:

Test item: JAU 6476-desthio (metabolite of prothioconazole); CAS # 120983-64-4, Purity 96.5%, Batch No. RUX76-105/8a.

The test was conducted according to the FIFRA Guideline 72-1. Fathead minnows *(Pimephales promelas*; approximately 78 days old at test start) were exposed under static-renewal conditions for 96 hours to nominal concentrations of 0.94, 1.88, 3.75, 7.50 and 15.0 mg metabolite/L, and to a water and solvent control (acetone). Test solutions were renewed at approximately 48 hours. Water samples were collected from all test vessels on Day 0, Day 2 and Day 4 and were analysed to measure actual exposure concentrations.
One replicate of 20 fish was used in the control, solvent control, and in the five toxicant levels. Fish were not fed during the test and test solutions were not aerated. The light cycle was programmed to produce an overall photoperiod of 16-hours light and 8-hours dark. Hardness, pH, conductivity, and alkalinity were measured in all test vessels every other day. Dissolved oxygen was measured daily. The test temperature during the 96-hour exposure ranged from 21.3 to 23.2°C (mean = 21.7°C) as measured hourly by the datalogger. Dissolved oxygen concentrations ranged from 5.6 to 8.6 mg/L corresponding to 64 to 98% saturation at 22°C, respectively. The pH values ranged from 7.4 to 7.7.
Daily observations were made for mortality and sublethal effects (loss of equilibrium, quiescent, on bottom of test vessel, at surface of test vessel, darkened coloration and erratic swimming behavior).

Findings:

Validity Criteria:

The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:

The mean measured concentrations of JAU 6476-desthio during the test period were 0.96, 2.06, 3.85, 7.99, and 16.3 mg metabolite/L, which represented 102 to 110 percent of the nominal concentrations. All reported results refer to mean measured concentrations of the test solutions.

Biological results:

Behavioral/sublethal effects were noted in the 7.99 mg metabolite/L test level. No behavioral/sublethal effects were observed in control, solvent control and lower test levels. Behavioral/sublethal effects could not be observed in the 16.3 mg metabolite/L test level since all fish died at Day 0.
No dose related mortalities occurred at the control, solvent control, 0.96, 2.06, 3.85, or 7.99 mg metabolite/L test levels during the exposure period. One dead fish was found at the 3.85 mg metabolite/L test concentration on Day 1. Since no sublethal effects were observed at this concentration and no mortalities occurred at the next higher concentration, this mortality was not considered to be dose related. All fish at the 16.3 mg metabolite/L test level died on Day 0 resulting in a cumulative mortality of 100%.

Table CA 8.2.1- 2: Cumulative mortality and behavioral observations in the fathead minnow exposed to JAU 6476-desthio

Mean measured concentration (mg metab./L)	4 hours		24 hours		48 hours		72 hours		96 hours	
	D	Obs.	D	Obs.	D	Obs.	D	Obs.	D	Obs.
Control	0	20N	0	20N	0	20N	0	20N	0	20N
Solvent control	0	20N	0	20N	0	20N	0	20N	0	20N
0.96	0	20N	0	20N	0	20N	0	20N	0	20N
2.06	0	20N	0	20N	0	20N	0	20N	0	20N
3.85	0	20N	1	19N	1	19N	1	19N	1	19N
7.99	0	8 LE,Q; 12 Q	0	5 OB, LE, Q; 2 AS, LE, Q; 1 LE; 3 Q; 9 N	0	3 AS, DC, Q, LE; 2 OB, DC, Q, LE; 4 Q; 11 N	0	1 AS, Q, LE; 1 OB, Q, LE; 1 E, LE; 1 Q; 1 DC; 15 N	0	1 OB, LE, Q; 1 AS, LE, Q; 1 E, LE; 17 N
16.3	20	na	20	na	20	na	20	na	20	na

D = Dead = Cumulative number of dead, Obs. = Observations (number of individuals observed plus observation)
N = Normal, LE = Loss of equilibrium, Q = Quiescent, OB = On bottom of tank, AS = At surface,
DC = Darkened coloration, E= Erratic behavior
metab. = metabolite

Since mortality only occurred at the 16.3 mg metabolite/L test level, neither the moving average nor the probit method can be used for the statistical analysis of mortality data. Therefore, a binomial probability analysis was performed. Based on the mortality data, the estimated 96-hour LC_{50} was 11.4 mg metabolite/L.
The NOEC and LOEC were determined by visual examination of mortality and clinical observations. The no-observed-effect concentration (NOEC), based upon sublethal effects, was 3.85 mg metabolite/L and the lowest-observed-effect-concentration (LOEC) was 7.99 mg metabolite/L. The threshold effect concentration, TEC (geometric mean of LOEC and NOEC), calculated based on sublethal effects was 5.5 mg metabolite/L.

Table CA 8.2.1- 3: Acute toxicity of JAU 6476-desthio to fathead minnow

Test substance	JAU 6476-desthio
Test object	Fathead Minnow
Exposure	96 hour, Static-Renewal
LC_{50}	11.4 mg metabolite/L
Lowest Observed Effect Concentration (LOEC)	7.99 mg metabolite/L
Highest Test Concentration without Toxic Effect (NOEC)	3.85 mg metabolite/L
Threshold Effect Concentration, TEC (geometric mean of LOEC and NOEC)	5.5 mg metabolite/L

Conclusion:
Based on the mortality data collected and the mean measured JAU 6476-desthio concentrations, the 96-hour LC_{50} was 11.4 mg metabolite/L. Based on sublethal effects, the 96- hour NOEC and LOEC were 3.85 and 7.99 mg metabolite/L, respectively.

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Report:	KCA 8.2.1/11 [redacted]; 2006; M-266572-01-1
Title:	Acute toxicity of JAU 6476-triazolylketone (tech.) to fish (Oncorhynchus mykiss) under static conditions
Report No.:	EBJAX306
Document No.:	M-266572-01-1
Guideline(s):	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev.1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
A limit test at 100 mg metabolite/L was performed in order to show that rainbow trouts (*Oncorhynchus mykiss*) were not affected at this test level by the metabolite JAU 6476-triazolylketone, so the 96h-LC_{50} is above this limit concentration. Mortality was the core test endpoint. Behavioural effects were also monitored during the whole exposure period.

Materials and methods:
Test item: JAU 6476-triazolylketone (tech.) (metabolite of prothioconazole), Purity: 99.5%, Batch No. HSRM 595.

The test was conducting according to the FIFRA Guideline 72-1, OPPTS 850.1075 (Draft) and OECD No. 203 (rev.1992). Test organisms (*Oncorhynchus mykiss*) had a mean body length 4.1 cm and a mean body weight 0.6 g at test initiation. Thirty fish were exposed (as a single replicate) in a limit test for 96 hours under static test conditions to a nominal concentration of 100 mg pure metabolite/L and a water control (with also 30 fish). Recoveries of JAU 6476-triazolylketone were measured in all test levels at the beginning of the test (day 0), after 48h (day 2) and at test termination (day 4) to confirm nominal concentrations.
Dissolved oxygen, water temperature and pH values were determined daily in each aquarium. Water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. Dissolved oxygen concentrations ranged from 91 to 100 % oxygen saturation, the pH values ranged

from 7.0 to 7.4 and the water temperature ranged from 11.7°C to 12.3°C in all aquaria over the whole testing period.

Findings:

Validity criteria:

All validity criteria for this study were met as given by the mentioned guidelines.

Analytical results:

Based on analytical determination of JAU 6476-triazolylketone, 95 to 99% (mean 98%) of the nominal concentration (100 mg metabolite/L) were found over the testing period of 96 hours. All reported results are based on nominal concentrations of the pure metabolite.

Biological results:

There were neither any sub-lethal effects nor any mortality in the control group. No sub-lethal or lethal effects occurred in treated fish after 96h of exposure.

Conclusion:

In a limit test, the metabolite JAU 6476-triazolylketone (tech.) did not cause any mortality to the rainbow trout (*Oncorhynchus mykiss*) at 100 mg metabolite/L. Therefore, the 96h-LC_{50} was clearly above 100 mg pure metabolite/L. There were no behavioral effects observed during the whole exposure period. Therefore, the NOEC after 96 hours is considered to be ≥ 100 mg metabolite/L.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The study submitted in the Baseline Dossier under the number M-088492-01-1 (KCA 8.2.2.1/01) has been qualified as invalid by some authorities since the egg hatching rate in the control was judged too low. A new study has been requested by these authorities as confirmatory data. Therefore, a new valid study is available under the number M-291414-01-1 (KCA 8.2.2.1/03), which should be used in the risk assessment. A summary of this new study is provided below.

Report:	KCA 8.2.2.1/03 [redacted]; [redacted]; 2007; M-291414-01-1
Title:	Early life stage toxicity of prothioconazole technical to the rainbow trout (Oncorhynchus mykiss) under flow through conditions
Report No.:	EBJAX313
Document No.:	M-291414-01-1
Guideline(s):	FIFRA Guideline 72-4 OPPTS Guideline 850.1400 (draft) OECD Guideline 210
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

A flow-through early life stage test was conducted to determine effects of prothioconazole technical on the rainbow trout (*Oncorhynchus mykiss*) over a 91 days period. This study was designed to establish a no-observed-effect-concentration (NOEC), a lowest-effect-observed-concentration (LOEC) and a Maximum Acceptable Toxicant Concentration (MATC), which equals the geometric mean of the NOEC and LOEC.

Materials and methods:
Test item: JAU 6476 technical (prothioconazole), CAS number #178928-70-6, Batch No. PFV0672333, Purity: 98.3%.

The test was conducting according to the FIFRA Guideline 72-4, the OPPTS Guideline 850.1400 (draft) and the OECD Guideline 210. Freshly fertilized rainbow trout (*Oncorhynchus mykiss*) eggs (age <24 hours) were observed for time to hatch and hatchability, [redacted] fish were assessed for abnormal behaviour, physical changes, swim-up behaviour, mortality and growth (standard length, dry weight). Study duration was 91 days under flow through conditions.
Nominal concentrations were: control, solvent control, 0.0625, 0.125, 0.25, 0.50 and 1.00 mg a.s./L. During the exposure period, test solution samples were collected weekly from two alternating test vessels to confirm exposure concentrations of prothioconazole. Each treatment was replicated four times with 30 eggs at initiation (thinned to 15 alevin after hatching phase). Photoperiod was set to 16 hours light and 8 hours dark (577-849 lux). Developing embryos/larvae were shielded from light exposure until one week post hatch.
Water quality parameters including pH, hardness, alkalinity, conductivity and dissolved oxygen levels were measured weekly. They were adequate and remained within expected ranges during the whole experiment. The test temperature ranged from 10.5 to 12.0°C (mean = 11.1°C). Dissolved oxygen concentrations ranged from 7.4 to 11.4 mg/L corresponding to 69 and 100% saturation, respectively. The pH values ranged from 7.7 to 8.2 throughout the test.

Findings:
Validity criteria:
All validity criteria for this study were met as given by the mentioned Guidelines. The oxygen level dropped for a short time < 60% saturation in two replicates of the 0.5 ppm test level. This occurred very late in the study (one day before study termination) and did not influence the outcome of the study. Therefore the study is considered to be valid.

Analytical results:
Recovery ranged from 84% to 98% and mean measured test concentration were as follow: control (<0.005), solvent control (<0.005), 0.052, 0.107, 0.22, 0.49 and 0.94 mg a.s./L All reported results were based on mean measured test concentrations.

Biological results:
With the exception of one fish in the control (exophthalmic) all other symptoms only occurred in the highest test level and were considered to be dose related. The symptoms were either transient in nature (study days 33-45; light-colored) or being associated with fish prior to death. At study termination all surviving fish showed normal behaviour and were without malformations.

Table CA 8.2.2.1- 1: Results from the Fish early life stage toxicity test exposing rainbow trout (*Oncorhynchus mykiss*) to prothioconazole technical

Test substance	Prothioconazole Technical			
Test object	Rainbow trout (*Oncorhynchus mykiss*)			
Exposure	91 Day, flow-through ELS			
Fry survival (Study Day 91):	NOEC	0.49 mg a.s./L	LOEC	0.94 mg a.s./L
Percent Hatch:	NOEC	0.94 mg a.s./L	LOEC	> 0.94 mg a.s./L
Time to Hatch:	NOEC	0.94 mg a.s./L	LOEC	> 0.94 mg a.s./L
Time to Swim-up (Study Days 46-48):	NOEC	0.49 mg a.s./L	LOEC	0.94 mg a.s./L
Growth (Standard Length):	NOEC	0.94 mg a.s./L	LOEC	> 0.94 mg a.s./L
Growth (Dry Weight):	NOEC	0.94 mg a.s./L	LOEC	> 0.94 mg a.s./L
Morphological & Behavioural Effects:	NOEC	0.49 mg a.s./L	LOEC	0.94 mg a.s./L
Maximum Acceptable Toxicant Concentration (MATC)	0.68 mg a.s./L (based on fry survival, swim-up and morphological/behavioural effects)			

Conclusion:
The 91-day exposure of rainbow trout (*Oncorhynchus mykiss*) to prothioconazole technical resulted in an overall NOEC of 0.49 mg a.s./L and a LOEC of 0.94 mg a.s./L based on fry survival, swim-up and morphological / behavioural effects. The maximum acceptable toxicant concentration (MATC) was 0.68 mg a.s./L.

CA 8.2.2.2 Fish full life cycle test

No additional studies have been performed, existing studies have been evaluated during the Annex I inclusion. They have been summarized in the Monograph and are included in the Baseline Dossier.

CA 8.2.2.3 Bioconcentration in fish

Report:	KCA 8.2.2.3/04 [redacted].; 2013; M-459145-01-1
Title:	Statement regarding the derivation of a bioconcentration factor (BCF) in fish for the metabolite prothioconazole-S-methyl
Report No.:	M-459145-01-1
Document No.:	M-459145-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Objective:
Exposure concentrations of prothioconazole-S-methyl in surface water bodies leading to critical bioconcentration in fish are not likely to occur. However, prothioconazole-S-methyl has a log Pow >3 which triggers the need to address its bioaccumulation potential. In order to reduce vertebrate testing, this was done using a non-testing approach. First, information from existing bioaccumulation studies with the parent compound (Prothioconazole) and another metabolite which has a very similar structure (Prothioconazole-desthio) was reviewed in order to evaluate the a priori bioaccumulation potential of prothioconazole-S-methyl. Second, the bioconcentration factor (BCF) value for prothioconazole-S-methyl was modelled using Quantitative Structure Activity Relationships (QSAR).

Information from existing BCF studies:
A bioconcentration and biotransformation study with the parent compound prothioconazole ([redacted].;[redacted]; 2001; M-087902-01-1, KCA 8.2.2.3/01) detected only low levels of prothioconazole-S-methyl in edibles and viscera of the bluegill sunfish. No concentration increase was

observed during the exposure phase between day 7 and day 14. This clearly indicated that formation and degradation of the metabolites were in balance after 7 days already. The BCF for the parent compound prothioconazole for the whole fish was determined to be 18.8 (normalized to 6% lipid content). Based on the total radioactive residues (TRR), a BCF of 57.8 was determined, indicating low accumulation of labeled metabolites.

Another bioconcentration and biotransformation study was conducted with the metabolite prothioconazole-desthio ([redacted].,; [redacted]; [redacted]; 2001; M-136749-01-1, KCA 8.2.2.3/02). This study resulted in a BCF of 45 for the whole fish (normalized to 6% lipid content). It was concluded that the accumulation potential in fish is low for both the parent compound as well as the metabolite prothioconazole-desthio. Since the structure of prothioconazole-S-methyl is very similar to that of prothioconazole-desthio, a comparable bioconcentration in fish can be expected for this metabolite.

QSAR modelling of the BCF using the US EPA- EPISUITE toolbox:

QSAR calculations of the bioconcentration factor were conducted with EPISUITE. Estimation Program Interface (EPI)Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation program developed by the US EPA.

Calculation of the bioconcentration factor and its logarithm was done with the program BCFBAF™ of the EPISUITE toolbox using two different methods. The first is the traditional regression based on log Pow (and any applicable correction factors), and is analogous to the WSKOWWIN™ method. The second is the Arnot-Gobas method, which calculates BCF values from mechanistic first principles. BCFBAF also incorporates prediction of apparent metabolism half-life in fish, and estimates BCF and BAF (bioaccumulation factor) for three trophic levels.

Based on the chemical structure and taking into account the experimentally determined log Pow values for the different substances, BCF values for fish were estimated with BCFBAF (v.3.01) for the parent compound prothioconazole, as well as for the metabolites prothioconazole-desthio and prothioconazole-S-methyl.

Regression-based BCF values for prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl were 154, 47.08 and 319.3, respectively. The BCF values predicted by the regression-based method matched the values which were actually measured in fish. The regression-based approach thus gives a realistic, yet conservative estimation of the bioconcentration factor in fish.

Conclusion:

Based on information from Efate studies and predicted environmental concentrations in surface waters, exposure levels of prothioconazole-S-methyl leading to critical bioconcentration in fish are not likely to occur in the environment. Bioconcentration and biotransformation studies with the parent prothioconazole and the metabolite prothioconazole-desthio (which have a very similar structure as prothioconazole-S-methyl) indicated a low bioaccumulation potential of prothioconazole-S-methyl in fish. To reduce vertebrate testing, the BCF value was estimated based using QSAR models. The regression-based models predicted a BCF value of 319.3 for prothioconazole-S-methyl.

CA 8.2.3 Endocrine disrupting properties

Population relevant effects of prothioconazole on fish were studied in two early life-stage tests (ELS) with rainbow trout (*O. mykiss,* M-088492-01-1, M-291414-01-1). The studies show NOEC values of 0.308 µg/L (endpoint: time to reach the swim up stage) and 0.49 µg/L (endpoints: time to reach the swim-up stage, survival, swimming ability, discoloration). Both studies indicate that sublethal symptoms occur at or close to lethal concentrations.

No specific studies on endocrine effects of prothioconazole in fish are available. However, as there is no indication of an endocrine disrupter potential in mammals and birds no further testing is indicated to evaluate the endocrine disrupter potential of prothioconazole to fish.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Report:	KCA 8.2.4.1/07 [redacted] Z; 2006; M-266597-01-1
Title:	Acute toxicity of JAU 6476-triazolylketone (tech.) to the waterflea Daphnia magna in a static laboratory test system
Report No.:	EBJAX305
Document No.:	M-266597-01-1
Guideline(s):	OECD - 202 (1984) and corresponding revised draft proposal, dated February 01, 2004 U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72.2 (1982) EEC Directive 92/69/EEC, part C.2 (1992) OPPTS Guideline 850.1010 Draft 1996 (modified) JMAFF 12 Nousan No. 8147 (2000)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine possible acute effects of JAU 6476-triazolylketone on mobility of *Daphnia magna* after 48 hours of exposure in a static laboratory test system, expressed as EC_{50} for immobilisation. Mortality was the primary test endpoint. Sublethal effects were also monitored along the test.

Materials and methods:
Test item: JAU 6476-triazolylketone (tech.) (metabolite of prothioconazole); Purity 99.5%, Batch No. HSRM 595.

The test was conducting according to the FIFRA Guideline 72-2, the OPPTS Guideline 850.1010, the JMAFF 12 Nousan Guideline No. 8147 and the OECD Guideline 202. *Daphnia magna* (1st instars < 24 h old) were exposed in a static test system for 48 hours to nominal concentrations of 0 (control), 0.399, 0.878, 1.93, 4.25, 9.34, 20.6, 45.2 and 100 mg pure metabolite/L, respectively. In addition, a solvent control (test medium, containing 100 µL of the solvent dimethylformamide/L) was tested. Recoveries of JAU 6476-triazolylketone were measured at start and end of the 48 hours exposure period.
The test vessels consisted of chemically clean 100 mL glass beakers, individually labelled and filled with 50 mL of the test solution, corresponding to a fluid level of approximately 3 cm height. Four vessels (replicates), each containing five daphnids were utilised per treatment group and control (= 20 animals per study group). The water fleas were not fed and the test solutions were not artificially aerated during exposure.
After 24 and 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements (slight movements of antennae were not interpreted as swimming movement) within approximately 15 seconds after gentle agitation of the test vessel. Additionally, all possible signs of sublethal effects were recorded.

Water quality parameters were monitored along the test duration (i.e. temperature, pH, O_2 concentration, conductivity, hardness and alkalinity). The measured values for the physical/chemical parameters met the required range and yielded no deviation from guideline recommendations.

Findings:

Validity criteria:

All validity criteria were met, as given by the mentioned guidelines.

Analytical results:

The measured concentrations of JAU 6476-triazolylketone in the freshly prepared test solutions at test initiation revealed an average recovery of 99% of the targetted nominal concentrations. At the end of the 48 hours exposure period, the average recovery reached 100% of the initial measured concentrations, demonstrating stability of the test item in the test system. No residues of JAU 6476-triazolylketone were detected in samples from untreated water control. All reported results were based on nominal concentrations of the pure metabolite.

Biological results:

During 48 hours of static exposure, no immobilities occurred at or below the highest tested concentration of 100 mg pure metabolite/L. Minor sublethal effects, such as being predominantly situated on the bottom of the beaker (due to distinctly decreased frequency of antennae movements) were observed after 24h and 48h for four and two exposed daphnids, respectively.

Conclusion:

Acute (48 hours) static exposure of juvenile *Daphnia magna* to JAU 6476-triazolylketone (tech.) in aqueous solution revealed no immobilisation at or below the highest tested concentration of 100 mg pure metabolite/L. Based on nominal exposure concentrations, the EC_{50} and the NOEC for immobilisation after 48 hours of static exposure were above 100 mg pure metabolite/L.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Report:	KCA 8.2.4.2/01 [redacted]., 2004; M-001051-01-1
Title:	JAU 6476-Desthio - Acute toxicity to crayfish (Procambarus clarkii) under static-renewal conditions
Report No.:	200985
Document No.:	M-001051-01-1
Guideline(s):	OPPTS 850.1075; Crayfish Acute Toxicity Test, Freshwater and Marine; "Public Draft" EPA 712-C-96-118; April 1996
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to estimate the acute toxicity, as expressed by a LC_{50}, of JAU 6476-desthio to crayfish (*Procambarus clarkii*) under static-renewal conditions. Mortality was the primary test endpoint.

Material and methods:

Test item: Triazole (JAU 6476-desthio), Purity:98.5%, Batch No. RUX76-105-1G.

Procedures used in this toxicity test followed those described in Springborn Smithers Protocol No.: 031003/EPA/STR-Crayfish/Bayer (Appendix I). The methods described in this protocol meet the testing requirements of the U.S. EPA's Pesticide Assessment Guidelines for other freshwater species.
Procambarus clarkii (mean wet weight: 0.97-6.3g, mean total length: 31-65mm) were exposed in a static test system for 96 h to nominal concentrations of 1.6, 3.1 6.3, 13 and 25 mg a.s./L, respectively. After 48 h, crayfish were transferred to a new set of test solutions. Water samples were regularly removed from the replicate solutions of each treatment level and the controls in order to confirm exposure to the targeted nominal concentration.
Ten animals were used per treatment level and controls (two replicates of five animals). All aquaria were examined at 0, 24, 48, 72 and 96 h of exposure for mortalities, sublethal effects and physical characteristics of the test solutions. Dead crayfish were removed and the findings recorded.
The pH, dissolved oxygen concentration and temperature were measured daily in each test vessel. Temperature ranged between 23°C and 25°C. The photoperiod was set to 16 h light and 8 h darkness (320 to 430 lux). All water quality parameters remained within acceptable levels for the survival of crayfish over the test duration.

Findings:

Validity criteria:

The protocol states that mortality in the control at test termination should not exceed 10%. During this test, mortality in the control and solvent control was 20% at test termination. Mortality was directly related to molting and subsequent cannibalization, and is thus not considered to be related to the health of the organisms or the suitability of the exposure system. Therefore, the test was considered acceptable.

Analytical results:

Test item recovery ranged between 100 and 120%. Mean measured concentrations were 1.9, 3.3, 6.8, 13 and 26 mg a.s./L, respectively. All results were thus based on mean measured concentrations.

Biological results:

At test termination (96 hours), 20% mortality was observed in the control and solvent control. Mortality of 40, 10, 10, 10 and 20% was observed among crayfish exposed to the 1.9, 3.3, 6.8, 13 and 26 mg a.s./L treatment levels, respectively. All of the observed mortality (controls and treatment levels) was directly related to molting and subsequent cannibalization. None of the observed mortality is considered related to exposure to JAU 6476-desthio.

Table CA 8.2.4.2- 1: Mean measured concentrations tested, corresponding cumulative mortalities, and observations made during the 96-hour static renewal toxicity test exposing crayfish (*Procambarus clarkii*) to JAU 6476-Desthio.

Mean measured Concentration mg a.s./L	Cumulative Morality											
	24 h			48 h			72 h			96 h		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0 (0)	0 (0)	0[b]	0 (0)	20 (1)	10[ce]	20 (1)	20 (1)	20[c]	20 (1)	20 (1)	20
Solvent control	0 (0)	20 (1)	10[cd]	0 (0)	20 (1)	10	0 (0)	20 (1)	10	20 (1)	20 (1)	20[c]
1.9	20 (1)	20 (1)	20[cd]	20 (1)	20 (1)	20	40 (2)	20 (1)	30[c]	60 (3)	20 (1)	40[c]
3.3	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	20 (1)	10[c]
6.8	0 (0)	20 (1)	10[cd]	0 (0)	20 (1)	10	0 (0)	20 (1)	10	0 (0)	20 (1)	10
13	20 (1)	0 (0)	10[c]	20 (1)	0 (0)	10	20 (1)	0 (0)	10	20 (1)	0 (0)	10
26	20 (1)	20 (1)	20[c]	20 (1)	20 (1)	20	20 (1)	20 (1)	20	20 (1)	20 (1)	20

[a] The actual number of mortalities is presented in parentheses.
[b] One live crayfish observed being cannibalized, appeared to be molting.
[c] Molts observed in tank. The crayfish that had molted were cannibalized.
[d] One live crayfish in each tank observed being cannibalized approximately two hours after test initiation. Crayfish that were being cannibalized appeared to be molting.
[e] One live crayfish observed being cannibalized.

Based on the results of this study, the 96-hour LC_{50} value was empirically estimated to be >26 mg a.s./L, the highest mean measured concentration tested. The No-Observed-Effect Concentration (NOEC) for this study was 26 mg a.s./L.

Conclusion:
The test item JAU 6476-desthio showed no adverse effects on adult mortality of the crayfish (*Procambarus clarkii*) after 96 h exposure. The NOEC was 26 mg a.s./L and the LC_{50} was >26 mg a.s./L.

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Report:	KCA 8.2.4.2/02 [redacted] か;[redacted];[redacted];[redacted]; 2002; M-083057-01-1
Title:	JAU 6476: A 96 hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia)
Report No.:	110983
Document No.:	M-083057-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the acute effects, as expressed by the LC_{50} values, of JAU 6476 (prothioconazole) on the saltwater mysid (*Mysidopsis bahia*) during a 96-hour exposure period

under flow-through test conditions. Mortality was the primary test endpoint. Sublethal effects were also monitored along the test.

Materials and methods:

Test item: JAU 6476 (technical), Batch No. 6233/0031, (Original) Purity: 98.4%, expiration August 22, 2001. (Subsequent recertification resulted in a purity of 97.8% with an expiration date of February 21, 2002. The original purity of 98.4% was used in all calculations for this study.)

The test was conducting according to the OPPTS Guideline 850.1035, US EPA guideline EPA-540/9-85-010 and ASTM guideline E729-88a. Juvenile saltwater mysids (*Mysidopsis bahia*, <24 hours old) were exposed for 96 hours to a geometric series of five test item concentrations under flow through conditions. The nominal test concentrations were: 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L, respectively. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at test initiation, at approximately 48 hours and at test termination. A negative control (filtered saltwater) as well as a solvent control (0.1 mL dimethylformamide/L) were tested.

Two replicate test chambers (9-L glass aquaria, filled with approx. 5-L of test solution) were maintained in each treatment and control group, with 10 saltwater mysids in each test chamber for a total of 20 saltwater mysids per test concentration. Observations of mortality and other clinical signs were made approximately 5, 24, 48, 72 and 96 hours after test initiation. The juvenile mysids were fed live brine shrimp (*Artemia* sp.) nauplii daily during the test to prevent cannibalism.

Water quality parameters were monitored daily. A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. Water temperatures were within the range of 25 ± 2°C. Dissolved oxygen concentrations remained ≥5.6 mg/L (76% saturation) throughout the test. Measurements of pH ranged from 8.1 to 8.3. Salinity of the dilution water at test initiation was 21‰.

Cumulative percent mortality observed in the treatment group was used to calculate LC_{50} values at 24, 48, 72 and 96 hours. The no-observed-effect-concentration (NOEC) was determined by visual examination of the mortality and clinical observation data.

Findings:

Validity Criteria:

The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:

The measured concentrations of JAU 6476 at test initiation ranged from 101 to 105% of nominal. In samples collected at 48 and 96 hours, measured concentrations ranged from 96 to 102 and 98 to 101% of nominal, respectively. Mean measured test concentrations were 0.25, 0.51, 0.99, 2.0 and 4.1 mg a.s./L which represented 100, 102, 99, 100 and 103% of the nominal concentrations, respectively. All results are presented based on mean measured concentrations.

Biological results:

Daily observations of mortality and other signs of toxicity observed during the test are shown in the table below.

Table CA 8.2.4.2- 2: Cumulative percent mortality and treatment-related effects in saltwater mysids exposed to JAU 6476

Mean measured concen-tration (mg a.s./L)	Rep*	5 hours		24 hour		48 hour		72 hour		96 hour		Cum. % Mor-tality
		#D	Observ.	#D	Observ.	#D	Observ.	#D	Observ.	#D	Observ.	
Negative control	A	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	0
	B	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	
Solvent control	A	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	0
	B	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	
0.25	A	0	10AN	0	10AN	0	10AN	0	10AN	1	9AN	5
	B	0	10AN	0	10AN	0	10AN	0	10AN	0	9AN,1E	
0.51	A	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	0
	B	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	
0.99	A	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	0
	B	0	10AN	0	10AN	0	10AN	0	10AN	0	10AN	
2.0	A	0	10AN	0	10AN	0	9AN,1C	1	7AN,2E	2	6AN,2E,1M	25
	B	0	10AN	0	10AN	0	8C,2E	1	8AN,1E	3	5AN,2E,1M	
4.1	A	0	10AN	1	8AN,1E	6	3C,1E	10	3M	10	--	100
	B	0	10AN	0	10AN	5	5C	10	2M	10	--	

#D = Cumulative number of dead mysids;
Observ. = Observed effects: AN = Appear Normal, C = Lethargic, E= Erratic swimming, M = Missing & assumed dead
* Rep = Replicate (10 mysids per replicate)

Saltwater mysids in the negative and solvent control appeared healthy and normal throughout the test. After 96 hours of exposure, mortality in the 0.25, 0.51, 0.99, 2.0 and 4.0 mg a.s./L treatment groups was 5, 0, 0, 25 and 100%, respectively. Based on the guideline (US-EPA, OPPTS 850.1035), up to 10% mortality is allowed for normal control performance. Consequently, the mortality in the 0.25 mg a.s./L treatment was not considered to be treatment related. LC_{50} values and 95% confidence limits were calculated from the mortality data and are as follows:

Table CA 8.2.4.2- 3: LC_{50} values for saltwater mysids exposed to JAU 6476

	LC_{50}	Lower 95% confidence limits	Upper 95% confidence limits	Statistical method
	mg a.s./L	mg a.s./L	mg a.s./L	
24	> 4.1	---	---[1]	Visual interpretation
48	3.9	> 2.0	---[1]	Binomial probability
72	2.6	2.0	4.1	Binomial probability
96	2.4	2.0	4.1	Binomial probability

[1] 95% confidence intervals could not be calculated from the data

Conclusion:

The 72-hour and 96-hour LC_{50} values for saltwater mysids (*Mysidopsis bahia*) exposed to JAU 6476 were 2.6 and 2.4 mg a.s./L, respectively. The lower and upper 95% confidence limits were 2.0 and 4.1 mg a.s./L, respectively for both the 72h and 96h-endpoint. The NOEC was 0.99 mg a.s./L.

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Report:	KCA 8.2.4.2/03 [redacted],; [redacted]; [redacted]; 2001; M-055051-01-1
Title:	JAU 6476: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
Report No.:	110956
Document No.:	M-055051-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the acute effects, as expressed by an EC_{50} , of JAU 6476 on shell deposition of the eastern oyster (*Crassostrea virginica*) during a 96-hour exposure period, under flow-through test conditions.

Materials and methods:

Test item: JAU 6476 (technical); Batch No. 6233/0031, (Original) Purity: 98.4%, expiration August 22, 2001. (Subsequent recertification resulted in a purity of 97.8% with an expiration date of February 21, 2002. The original purity of 98.4% was used for all study calculations.)

The test was conducting according to the OPPTS Guideline 850.1025, US EPA guideline EPA-540/9-85-011 and ASTM guideline E729-88a. Eastern oysters (*Crassostrea virginica*) were exposed to a geometric series of five test concentrations, a negative (unfiltered seawater) control and a solvent (0.10 mL dimethylformamide/L) control. One test chamber was maintained for each treatment and control group, with 20 oysters in each test chamber.

Nominal test concentrations were 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Mean measured test concentrations were determined from samples of test water collected from the treatment and control groups at the beginning and end of the test.

A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. Water quality parameters were monitored daily. Water temperatures were within the limits of the 22 ± 1°C range established for the test. Dissolved oxygen concentrations remained ≥ 6.8 mg/L (88% of saturation) throughout the test. Measurements of pH ranged from 8.1 to 8.3. The salinity of the dilution water measured at test initiation and termination ranged from 21 to 22‰.

Measurements of shell deposition (i.e. growth) for each oyster were made at 96 hours and were used to estimate the EC_{50} value and the no-observed-effect-concentration (NOEC).

Results:

Validity Criteria:

The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:

Initially measured concentrations ranged from 97 to 115% of nominal. Samples collected at 48 and 96 hours had measured concentrations that ranged from 111 to 133% and 102 to 118% of nominal, respectively. Mean measured test concentrations were 0.37, 0.76, 1.4, 2.8 and 5.4 mg a.s./L. All reported results were based on the mean measured concentrations.

Biological results:

Oysters in the negative control and solvent control were normal and healthy throughout the test. When the shell deposition data for the negative control was compared with that in the solvent control, no statistically significant differences were found at the 95% level of confidence. Therefore, the control groups were pooled and percent inhibition was calculated relative to the pooled control data.

Oysters in the JAU 6476 treatment groups also appeared normal and healthy with no mortalities or sublethal effects observed. Shell growth inhibition for the 0.37, 0.76, 1.4, 2.8 and 5.4 mg a.s./L treatment groups was calculated to be 10, 22, 29, 47 and 98%, respectively. The 96-hour EC_{50} value was estimated to be 2.9 mg a.s./L with 95% confidence limits of 1.9 and 3.6 mg a.s./L. Wilcoxon's rank sum test showed that shell growth was significantly reduced in the 1.4, 2.8 and 5.4 mg a.s./L treatment groups in comparison to the pooled controls ($p \leq 0.05$).

Table CA 8.2.4.2- 4: Shell deposition and shell growth inhibition during a 96-hour test with JAU 6476

Mean measured test concentration	Shell deposition (mm)	Shell growth inhibition
(mg a.s./L)	Mean ± SD [1]	(%) [2]
Negative control	2.31 ± 1.35	--
Solvent control	2.16 ± 0.805	--
Pooled controls	2.23 ± 1.10	--
0.37	2.00 ± 1.16	10
0.76	1.74 ± 0.621	22
1.4	1.58* ± 0.756	29
2.8	1.18* ± 1.14	47
5.4	0.0450* ± 0.201	98

[1] Mean and standard deviation for 20 oysters
[2] Percent inhibition relative to the pooled controls
* Indicates a significant difference from the pooled controls using Wilcoxon's rank sum test ($p \leq 0.05$).

Conclusion:

The 96-hour EC_{50} value for eastern oysters (*Crassostrea virginica*) exposed to JAU 6476 was 2.9 mg a.s./L. The 95% confidence limits were 1.9 and 3.6 mg a.s./L, respectively. Based on a statistically significant reduction in shell growth of the 1.4, 2.8 and 5.4 mg a.s./L treatment groups, the NOEC was 0.76 mg a.s./L.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

No additional studies have been performed; existing studies have been evaluated during the Annex I inclusion. They have been summarised in the Monograph and are included in the Baseline Dossier.

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

Report:	KCA 8.2.5.2/01 [redacted],; [redacted]; [redacted]; [redacted]; 2002; M-083055-01-1
Title:	Desthio JAU 6476: A 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia)
Report No.:	110979
Document No.:	M-083055-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the acute effects of JAU 6476-desthio on the saltwater mysid (*Mysidopsis bahia*) during a 96-hour exposure period under flow-through test conditions, as expressed by a LC_{50}. Mortality was the core test endpoint.

Materials and methods:
Test item: JAU 6476-Desthio, Batch No. RUX76-105-8a, Purity of 96.5%.

The test was conducting according to the OPPTS Guideline 850.1035, US EPA guideline EPA-540/9-85-010 and ASTM guideline E729-88a. Saltwater mysids were exposed to nominal concentrations of 0.013, 0.025, 0.050, 0.10 and 0.20 mg metabolite / L, a negative (filtered saltwater) control and a solvent (0.1 mL dimethylformamide/L) control. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at test initiation, after 48 hours, and at test termination.
Two replicate test chambers were maintained in each treatment and control group, with 10 saltwater mysids in each test chamber for a total of 20 saltwater mysids per test concentration. Observations of mortality and other clinical signs were made 4, 24, 48, 72, and 96 hours after test initiation.
A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. Water quality parameters were regularly measured along the test. Water temperatures were within the 25 ± 2°C range established for the test. Dissolved oxygen concentrations remained ≥ 6.0 mg/L (82% of saturation) throughout the test. Measurements of pH ranged from 8.2 to 8.3. Salinity of the dilution water at test initiation was 21‰.

Findings:
Validity criteria:
The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:
The mean measured test concentrations were 0.013, 0.026, 0.050, 0.099 and 0.20 mg a.s./L which represented 100, 104, 100, 99 and 100% of the nominal concentrations, respectively. All results were reported based on mean measured concentrations.

Biological results:
Saltwater mysids in the negative control appeared healthy and normal throughout the test. One mysid in the solvent control appeared lethargic at 72 hours; however, at 96 hours, all mysids in the solvent control appeared normal.
After 96-hours of exposure, mortality in the 0.013, 0.026, 0.050, 0.099 and 0.20 mg a.s./L treatment groups was 10, 5, 15, 95 and 100%, respectively. The 96-hour LC_{50} value for saltwater mysids exposed

to JAU 6476-desthio was 0.060 mg metabolite / L. The 95% confidence limits were 0.046 and 0.079 mg a.s./L.

Table CA 8.2.5.2- 1: Mean measured concentrations tested, corresponding cumulative mortalities during the 96-hour flow-through toxicity test exposing saltwater mysids (*Mysidopsis bahia*) to JAU 6476-desthio

Mean measured Concentration mg metabolite/L	Cumulative number of dead mysids										Cumulative percent mortality
	4 h		24 h		48 h		72 h		96 h		
	A	B	A	B	A	B	A	B	A	B	
Control	0	0	0	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0	0	0	0
0.013	0	0	0	0	1	0	1	0	1	1	10
0.026	0	0	0	0	1	0	1	0	1	0	5
0.050	0	0	0	0	0	0	1	0	1	2	15
0.099	0	0	10	5	10	9	10	9	10	9	95
0.20	0	0	9	10	10	10	10	10	10	10	100

Conclusion:

The 96-hour LC_{50} value for saltwater mysids (*Mysidopsis bahia*) exposed to JAU 6476-desthio was 0.060 mg metabolite / L. The 95% confidence limits were 0.046 and 0.079 mg metabolite / L.

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Report:	KCA 8.2.5.2/02 [redacted]; 2003; M-104620-01-1
Title:	Desthio JAU 6476: A flow-through life-cycle toxicity test with the saltwater mysid (Mysidopsis bahia)
Report No.:	200485
Document No.:	M-104620-01-1
Guideline(s):	USEPA OPPTS 850.1350
Guideline deviation(s):	--
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the effects of JAU 6476-desthio on the survival, growth and reproduction of the saltwater mysid (*Mysidopsis bahia*) in a life-cycle toxicity test under flow-through test conditions. NOEC, LOEC and MATC (geometric mean of the NOEC and LOEC) values were determined for each of these test endpoints.

Materials and methods:

Test item: JAU 6476-desthio (metabolite of prothioconazole), Batch No. RUX76-105/8a, (Original) Purity of 96.4%, expiration date: December 21, 2000. (Subsequent recertification resulted in a purity of 97.0% with an expiration date of January 29, 2003. The most recent purity of 97.0% was used in all calculations for this study).

The test was conducting according to the OPPTS Guideline 850.1350 and ASTM Standard E1191-90. *Mysidopsis bahia* neonates (age <24 h) were exposed to a geometric series of five test concentrations, a negative (saltwater) control and a solvent (dimethylformamide) control for 29 days. Nominal test concentrations were based upon the results of an exploratory range-finding toxicity test and an acute

definitive study. Nominal test concentrations were 16, 32, 63, 125 and 250 µg metabolite/L. Mean measured test concentrations were determined from samples of test water collected from the treatment groups and control groups at the beginning of the test, at weekly intervals during the test and at test termination.
Four replicate test chambers, each containing one compartment with 15 mysids, were maintained for each treatment and control group. A total of 60 mysids were exposed in each treatment and control group. On day 14 of the test, female and male adults were paired, and the reproduction of the paired mysids was monitored until Day 29. Observations of mortality, clinical signs of toxicity, and reproduction were made daily. At test termination, the total body lengths and dry weights of all surviving first-generation mysids were measured.
A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. Light intensity was 40 lux over the surface of one representative test chamber at test initiation. Water quality parameters were regularly monitored along the test. Measurements of salinity in the negative control were 20‰ throughout the test. Measurements of pH ranged from 8.0 to 8.3 and temperature was maintained within the 25 ± 2°C range established for the test. Dissolved oxygen concentrations remained >5.8 mg/L (79% of saturation) throughout the test.

Findings:
Validity Criteria:
The Guidelines used as reference do not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:
Mean measured concentrations for the entire study ranged from 88 to 105% of nominal concentrations and were as follows: 16, 32, 64, 128, and 252 µg metabolite/L, which represented 100, 100, 102, 102, and 101% of nominal concentrations, respectively. The results of the study are based on the mean measured test concentrations.

Biological results:
Survival to Pairing (Days 0-14):
There were no statistically significant differences ($p > 0.05$) in survival from test initiation until pairing between the negative and solvent control groups. Therefore, the control data were pooled for comparisons among the JAU 6476-desthio treatment groups.
Fischer's exact test was used to evaluate the survival data. After 14 days of exposure, survival in both the negative control and solvent control group was 95 and 98%, respectively. Survival in the 16, 32, 64, 128, and 252 µg metabolite/L treatment groups was 95, 92, 90, 92, and 97%, respectively, and was not statistically different from the pooled controls ($p > 0.05$). Consequently, the NOEC for survival from Days 0-14 was 252 µg metabolite/L, the highest concentration tested.

Survival After Pairing (Days 15-29):
All surviving mysids during that time appeared normal. There were no statistically significant differences ($p > 0.05$) in survival to pairing between the negative and solvent control groups. Therefore, the control data were pooled for comparisons among the JAU 6476-desthio treatment groups.
Fischer's exact test was used to evaluate the survival data. After 29 days of exposure, survival in both the negative control and solvent control group was 94%. Survival in the 16, 32, 64, 128, and 252 µg metabolite/L treatment groups was 91, 95, 88, 95, and 90%, respectively, and was not statistically different from the pooled controls ($p > 0.05$). Consequently, the NOEC for survival from Days 15-29 also was 252 µg metabolite / L.

Reproduction:
For each female, the number of reproductive days was defined as the number of days that the female was alive from the day of first brood release of any female in the test to the end of the test. The day of first brood release in this study was Day 17. The mean number of young produced per reproductive day in the negative control and solvent control groups was 0.592 and 0.573, respectively.
Reproduction rates in the 16, 32, 64, 128, and 252 µg metabolite/L treatment groups were 0.527, 0.610, 0.615, 0.398, and 0.407 young per reproductive day, respectively. There were no statistically significant differences ($p > 0.05$) in survival to pairing between the negative and solvent control groups. Therefore, the control data were pooled for comparisons among the JAU 6476-desthio treatment groups.
Bonferroni's test showed that reproduction in the JAU 6476-desthio treatment groups at concentrations < 252 µg metabolite/L was not significantly different in comparison to the pooled controls ($p > 0.05$). However, there was a 32 and 31% reduction in reproduction in the 128 and 252 µg a.s./L treatment groups, respectively, compared to the pooled controls (see table below). Although this reduction in reproduction was not statistically significant, it was concentration-dose dependant and is thus believed to be treatment related. Consequently, the NOEC for reproduction was 64 µg a.s./L and the LOEC was 128 µg a.s./L.

Table CA 8.2.5.2- 2: Mean Number of Young Produced by *Mysidopsis bahia* Per Reproductive Day

Mean measured test concentration [µg a.s./L]	Total number of young produced	Total number of reproductive days	Mean number of young/reproductive day [1, 2] ± SD
Negative control	127	221	0.592 ±0.139
Solvent control	149	260	0.573 ±0.168
16	137	260	0.527 ±0.034
32	144	234	0.610 ±0.177
64	160	260	0.615 ±0.173
128	91	221	0.398 ±0.117
252	105	258	0.407 ±0.088

[1] There were no statistically significant ($p > 0.05$) differences from the pooled controls (Bonferroni's t-test)
[2] Results were generated using Excel 2000. Manual calculations may differ slightly

Growth:
The mean total length and mean dry weight in the negative control and solvent control groups were 7.70 mm and 0.817 mg, and 7.79 mm and 0.861 mg, respectively. Mysids in the 16, 32, 64, 128, and 252 µg metabolite/L had mean total lengths of 7.68, 7.78, 7.74, 7.75, and 7.76 mm, respectively, and mean dry weights of 0.838, 0.852, 0.863, 0.853, and 0.815 mg, respectively. There were no statistically significant differences ($p > 0.05$) in survival to pairing between the negative and solvent control groups. Therefore, the control data were pooled for comparisons among the JAU 6476-desthio treatment groups.
Bonferroni's test showed that reproduction in the JAU 6476-desthio treatment groups at concentrations < 252 µg metabolite/L was not significantly different in comparison to the pooled controls ($p > 0.05$). Consequently, the NOEC for growth was 252 µg metabolite / L.

Additional confirmation acute toxicity testing:
Based on the results of the present study, additional toxicity testing was conducted to address the difference in toxicity shown in the 96-hour acute mysid study from [redacted]C; [redacted]; [redacted]; [redacted]; 2002; M-083055-01-1, see KCA 8.2.5.2/01. It run in November of year 2001, whereas the present life-cycle toxicity study run in the fall of year 2002.

While the 96-hour acute study had an LC_{50} value of 60 µg metabolite/L, there were no treatment related effects on survival in the 16, 32, 64, 128, and 252 µg metabolite/L treatment groups in the life cycle test. Two additional acute tests, an exploratory non-GLP study and a GLP study were run late in the fall of 2001 to confirm the results of the earlier acute test. A summary of the GLP acute study and non-GLP acute biological data are presented in Appendix 9 of the present study report. The LC_{50} of the non-GLP acute study was > 2000 µg a.s./L (nominal), and the LC_{50} of the GLP acute study was > 1009 µg a.s./L (mean measured).

These data suggest that the results from the first acute test were not repeatable. The more recent acute tests are consistent with the toxicity observed in the life cycle test. The reason for the differences observed between the earlier and later acute tests could not be determined. However, these tests were run with different batches of saltwater, food, and organisms.

Conclusion:

Saltwater mysids (*Mysidopsis bahia*) were exposed to mean measured concentrations of 16, 32, 64, 128, and 252 µg metabolite/L of JAU6476-desthio for 29 days. There were no treatment related effects on survival or growth of the mysid shrimp exposed to concentrations ≤ 252 µg metabolite/L, the highest concentration tested. Although not statistically significant, there was a reduction in reproduction of the mysid shrimp exposed to concentrations > 128 µg metabolite /L. Consequently, the NOEC was 64 µg a.s./L. The LOEC was 128 µg a.s./L and the MATC was 91 µg a.s./L.

CA 8.2.5.3 Development and emergence in Chironomus species

No additional studies have been performed, existing studies have been evaluated during the Annex I inclusion: They have been summarised in the Monograph and are included in the Baseline Dossier.

CA 8.2.5.4 Sediment dwelling organisms

Report:	KCA 8.2.5.4/03 [redacted].; 2008; M-312780-01-1
Title:	JAU 6476-desthio - Full life-cycle toxicity test with sediment-dwelling midges (Chironomus riparius) under static conditions, following OECD guideline 218
Report No.:	EBJAY008
Document No.:	M-312780-01-1
Guideline(s):	OECD Guideline 218
Guideline deviation(s):	Routine food and water screening analyses were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Since the analyses were conducted following standard validated methods, this exeption has no impact on the study result.
GLP/GEP:	**yes**

Objective:

The objective of this study was to determine the effects of JAU 6476-desthio on the survival, growth and maturation of larvae to adult midge (i.e., percent emergence and development rate) for a period of 28 days in a static water-sediment system (spiked sediment exposure), expressed as NOEC, LOEC and ECx for emergence ratio and development rate.

Materials and methods:

Test item: JAU 6476-desthio-triazole-3,5-[^{14}C], Lot No. 2000BRP213-183, radiochemical purity: 97.4% and JAU 6476-Desthio, Batch No. RUX76-105-1E, purity: 98.8%.

The test was conducting according to the OECD Guideline 218 for spiked-sediment tests. Eight replicates of 20 *Chironomus riparius* larvae (three days old) were exposed to spiked sediment in a static water-sediment test system for 28 days. Artificial sediment was prepared according to OECD Guideline 218 (organic carbon: 2.4%, particle size distribution: 80% sand, 2% silt, and 18% clay, pH: 7, solids: 69.24%). Nominal concentrations in the artificial sediment were 6.3, 13, 25, 50 and 100 mg/kg dry weight. Pore water samples of selected test vessels were collected on day 0, 10 and 28 and were analyzed by liquid scintillation counting (LSC) for total [^{14}C]residues. Sediment samples of selected test vessels were also collected on day 0, 10 and 28 and were analyzed by combustion followed by LSC for total [^{14}C]residues and high performance liquid chromatography with radiochemical detection (HPLC/RAM). Photoperiod was 16 h light and 8 h darkness (590-630 lux). Temperature and dissolved oxygen concentration were measured continuously. Total hardness, alkalinity, specific conductivity, and total ammonia of the test solutions were determined at test initiation and at test termination in a composite sample from the highest treatment level and the control solution.
Midge larval survival was determined in four randomly selected replicates on day 10. The growth of surviving midges was recorded and dry weight was estimated in each of the four replicates. For the remaining vessels, general behavior and emergence was recorded on daily basis until day 28.

Findings:

Validity criteria:

Water quality did not meet the validity criteria in some instances. Minor deviations to the guideline validity criteria occurred, which were temporary and remained within the tolerance of *C. riparius*. These deviations did not have a negative impact on the results or interpretation of the study, as shown by the fact that biological validity criteria (survival, emergence) were met. Therefore, the study is deemed valid.

Analytical results:

Based on the analytical results of sediment, pore water and overlying water, JAU 6476-desthio that was applied to sediment remained bound to the sediment and 100% of the measured radioactivity in the sediment was associated with JAU 6476-desthio. Therefore, all reported results refer to nominal concentrations.

Biological results:

On test day 10, there was a statistically significant difference in survival among midges exposed to the 6.3 mg/kg treatment level compared to the survival of the pooled control (99%). Although statistically significant, this effect was not believed to be biologically relevant due to the lack of a dose-response at higher concentrations.
No statistically significant difference in growth was observed in any of the treatment levels compared to the solvent control.
At test termination, no statistically significant difference was determined for mean percentage emergence. However there was a significant difference in the mean development rate of male and female midge in the 100 mg/kg treatment level compared to the solvent control.

Table CA 8.2.5.4- 1: Influence on emergence and development rate of *Chironomus riparius* after 28 days of exposure JAU 6476-desthio

Nominal concentration (sediment) mg metabolite/kg	Mean percent emerged	Mean Development rate (male/female midge)
Control	88	0.0541
Solvent control	71	0.0630
Pooled Control	79	NA[a]
6.3	81	0.0694
13	78	0.0610
25	66	0.0614
50	71	0.0589
100	78	0.0466[b]

[a] NA = Not Applicable. Treatment data was compared to solvent control data for this endpoint.
[b] Significantly reduced compared to the solvent control based on Dunnett's Test.

Conclusion:

Based on the nominal concentrations of applied test substance and midge development rate (male/female combined), the 28d-NOEC was established to be 50 mg/kg dry weight and the 28d-LOEC was established to be 100 mg/kg dry weight.
Since no concentration tested resulted in ≥ 50% inhibition of midge emergence or development rate, the 28-day EC_{50} values were empirically estimated to be > 100 mg/kg dry weight, the highest concentration tested.

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Report:	KCA 8.2.5.4/04 [redacted]; 2006; M-266605-01-1
Title:	Chironomus riparius 28-day chronic toxicity test with JAU 6476-S-methyl in a water-sediment system using spiked water
Report No.:	EBJAX303
Document No.:	M-266605-01-1
Guideline(s):	OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (adopted 13 April 2004)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the influence of JAU 6476-S-methyl on the development and emergence of *Chironomus riparius* for 28 days in a static water-sediment-system (spiked water exposure), expressed as NOEC, LOEC and ECx for emergence ratio and development rate.

Materials and methods:

Test item: JAU 6476-S-methyl (metabolite of JAU 6476); Batch No. HUPP0658-MP, Purity of 98.9%.

The test was conducting according to the OECD Guideline 219 for spiked-water tests. First instar of *Chironomus riparius* larvae were installed in 4 beakers per test concentration and control with 20 animals each. Larvae were exposed in a static test system for 28 days to nominal concentrations in the overlying medium (spiked water application) of 0.001, 0.01, 0.10, 1.00 and 10.0 mg metabolite/L. In addition, an untreated control and a solvent control (dimethylformamide; DMF) were tested. Recoveries of JAU 6476-S-methyl were measured three times during the study: 1 hour, 7 days and 28 days after application, in one additional test container of each nominal initial test concentrations of

0.001, 0.10 and 10.0 mg metabolite/L and control (only on day 0) of the overlying water and the pore water of the sediment.
Water quality parameters were measured in several beakers of each test concentration over the whole period of testing. Dissolved oxygen concentrations ranged in the water phase from 8.0 to 9.3 mg O$_2$/L, the water pH values ranged from 8.4 to 8.7 and the water temperature ranged from 20.0°C to 20.3°C.

Findings:
Validity criteria:
Test conditions met all validity criteria, given by the mentioned guideline OECD 219. Biological validity criteria were also met.

Analytical results:
Chemical analysis of overlying water and pore water over time reflected expected aquatic fate data with high recoveries of 92% to 100% for test concentration of 0.001 and 0.10 mg metabolite/L at the beginning of the exposure period in the overlying water. For the highest test concentration of 10.0 mg metabolite/L, only 68% of nominal was found on Day 0. The relatively low recovery for the highest concentration is related to the water solubility under exposure conditions. The mean recovery on Day 0 was 86.6% of nominal, which is within the acceptable range of ± 20%. Therefore, all reported results were based on nominal concentrations.

Biological results:
One dead pupae and one not fully emerged midge out of 80 inserted larvae were observed in the test concentration of 0.1 mg metabolite/L.
Emergence started on Days 14 - 15 for the control and test concentrations from 0.001 to 1.00 mg metabolite/L. The start of emergence was delayed for four days at the highest test concentration of 10.0 mg metabolite/L. 89.4% of the inserted (n= 160) larvae maturated to adults in the controls (control and solvent control pooled) after 28 days, fulfilling the guideline requirements.

Table CA 8.2.5.4- 2: Emergence and the development rate of *Chironomus riparius* after 28 days of exposure to JAU 6476-S-methyl

Nominal concentration (overlying water) mg p.m./L	Number of emerged midges	Emergence of inserted larvae			Development Rate (pooled sex)(1 / d)
		total (%)	male (%)	female (%)	
Control (pooled)	143	89.4	58.8	30.6	0.060
0.001	65	81.3	53.8	27.5	0.058
0.01	55	68.8	46.3	22.5	0.059
0.10	67	83.7	46.2	37.5	0.060
1.00	54	67.5	41.3	26.2	0.056
10.0	5	6.3	5.0	1.3	0.050

p.m. = pure metabolite

For further statistical analyses of emergence male and female results were pooled to increase the statistical power. Statistically significant effects ($p < 0.05$) were observed on the emergence ratio and on the development rate of males (and of pooled population: male and females) exposed to 1.00 mg metabolite/L (= LOEC), resulting in a NOEC of 0.10 mg metabolite/L. For the development rate of females, the LOEC was 10.0 mg metabolite/L, resulting in an NOEC of 1.00 mg metabolite/L.

ECx values were determined based on nominal concentrations of JAU 6476-S-methyl/L in the overlying water and are as follows:

Table CA 8.2.5.4- 3: EC_{15} and EC_{50} values for emergence ratio and development rate

Endpoints	EC_{15}	EC_{50}
emergence ratio (pooled sex)	**0.126**	**1.41**
development rate (pooled sex)	**8.66**	**> 10.0**

Further ECx values (x = 1-99) are listed in Appendix A of the study report.

Conclusion:
Based on the nominal concentrations of JAU 6476-S-methyl in overlying water and midge development rate (male/female combined), the 28d-NOEC was established to be 0.1 mg metabolite/L weight and the 28d-LOEC was established to be 1 mg metabolite/L.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Report:	KCA 8.2.6.1/05 [redacted]; 2006; M-266567-01-1
Title:	Pseudokirchneriella subcapitata: growth inhibition test with prothioconazole-triazolylketone
Report No.:	EBJAX304
Document No.:	M-266567-01-1
Guideline(s):	Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (October 22, 2004)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine the influence of prothioconazole–triazolylketone on exponentially growing *Peudokirchneriella subcapitata*, expressed as NOEC, LOEC and ECx for growth rate of algal biomass.

Materials and methods:
Test item: Prothioconazole–triazolylketone (JAU 6476-triazolylketone), Purity of 99.5%, Batch No. HSRM 595.

The test was conducting according to the OECD Guideline 201. *Peudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*) was exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 0.954, 3.05, 9.77, 31.3 and 100 mg pure metabolite/L, in comparison to a negative control. Concentrations of prothioconazole-triazolylketone were measured in all treatment groups and in the control on Day 0 and Day 3 of the exposure period.
Water quality parameters were regularly monitored during the test. The pH values ranged from 7.9 to 8.4 in the controls and the incubation temperature ranged from 22.2°C to 23.5°C (measured in an

additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7,929 lux.

Findings:
Validity criteria:
All validity criteria given by the mentioned guideline were met.

Analytical results:
Concentrations of prothioconazole-triazolylketone in the treatment levels at Day 0 were 96 to 109% of targeted nominal values (average 101%). On Day 3, concentrations of 97 to 109% of nominal (average 102%) were found. Therefore, all reported results are based on nominal concentrations of the pure metabolite.

Biological results:
Biomass increased in the control by a factor of 23.6 within 3 days. The growth rate in controls was homogenous along the study duration and among replicates. Similar results were observed for every tested concentration. Algae growth pattern and biomass after 72 hour of exposure were as follows:

Table CA 8.2.6.1- 1: Effects of prothioconazole–triazolylketone on *Pseudokirchneriella subcapitata* after 72 h

Nominal Concentration [mg metabolite/L]	Cell Number after 72 h (mean) per mL	(0-72h)-Average Specific Growth Rates [$days^{-1}$]	Inhibition of Average Specific Growth Rate [%]	Doubling Time of Algae Cells [days]
control	236260	1.054	-	0.658
0.954	252980	1.077	-2.2	0.644
3.05	266170	1.090	-3.4	0.636
9.77	245920	1.067	-1.3	0.650
31.3	233780	1.049	0.4	0.661
100	223400	1.033	2.0	0.671

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

Based on these results, the $NOEr_rC$ for the Average Growth Rate (0 - 72 h) was empirically estimated to be ≥ 100 mg metabolite/L. The $LOEr_rC$ and the ErC_{50} were empirically estimated to be > 100 mg metabolite/L.

Conclusion:
Prothioconazole–triazolylketone has no significant toxic effects on the green alga *Peudokirchneriella subcapitata* at concentrations up to 100 mg metabolite/L. The 72-hour E_rC_{50} was > 100 mg metabolite/L.

CA 8.2.6.2 Effects on growth of an additional algal species

Report:	KCA 8.2.6.2/01 [redacted]; [redacted]; 2004; M-000954-01-1
Title:	Toxicity of JAU 6476 technical to the saltwater diatom Skeletonema costatum
Report No.:	200434
Document No.:	M-000954-01-1
Guideline(s):	USEPA Guideline l23-2, Growth and Reproduction of Aquatic Plants (Tier 2)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
A static 96-hour algal growth test was conducted to determine the effects of prothioconazole (JAU 6476 technical) to the saltwater diatom *Skeletonema costatum*. The primary objective of this growth study was to estimate the fifty percent effective concentration (ErC_{50}), which represents the concentration that produces a fifty percent reduction in growth. A secondary objective was to determine the no-observed-effect-concentration (NOEC), which equals the lowest concentration without a statistically significant ($p > 0.05$) reduction from the control for the measured parameters. The response parameters used in this study were cell density (standing crop), cumulative biomass, and growth rate. The variable used to calculate the response parameters was cell density based on daily cell counts.

Materials and methods:
Test item: JAU 6476 technical, Batch No. 6233/0031, Purity of 98.2%.

The test was conducting according to the FIFRA Guideline 123-2. *Skeletonema costatum* was exposed under static conditions (shaken cultures) for 96 hours to the following nominal concentrations: 3.1, 7.7, 19.2, 48.0 and 120 µg a.s./L. A water control and a solvent (acetone) control were also implemented. Samples of test solutions, control and solvent control, were taken on Day 0 and Day 4 to measure actual exposure concentrations.
Each replicate was inoculated with *Skeletonema costatum* cells at a nominal density of 10,000 cells/mL. Three replicate vessels were prepared for each concentration and used to determine daily cell density. An array of cool white fluorescent lights produced a 16 hours of illumination and a light intensity of approximately 392 foot-candles (4.2 klux). Water quality parameters were monitored regularly along the test. The test temperature ranged from 19.3 to 20.3 °C (mean = 19.8 °C), as recorded hourly by the datalogger. The pH measurements ranged from 7.9 to 8.8 for all test levels during the exposure period. The salinity was maintained at 26 ppt.

Findings:
Validity Criteria:
The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:
The measured concentrations of JAU 6476 on Day 0 were 3.00, 7.30, 17.5, 46.8, and 117 µg a.s./L, which represented 91 to 98% of the nominal test concentrations. The test compound dissipated rapidly from the test. Indeed, measured concentrations of JAU 6476 on Day 4 were 0.7, 1.66, 7.85, 22.1 and 98.8 µg a.s./L, which represented 22 to 82% of nominal concentrations. No undissolved test substance was visually observed in the test vessels throughout the test period. All results refer to the initially measured concentrations.

Biological results:
No physical abnormalities were observed in the controls or treatment groups during the study. Biomass increased exponentially in the control by a factor of 118 within 3 days. Algae growth pattern and biomass after 72 and 96 hours of exposure were as follows:

Table CA 8.2.6.2- 1: 72-hour and 96-hour cell density, cumulative biomass and growth rate during the exposure of *Skeletonema costatum* to JAU 6476 (technical)

Initial measured concentration [µg a.s./L]	Mean density [cells/mL x 10^4]	Percent (%) inhibition [a)]	Mean calculated cumulative biomass [b)]	Percent (%) inhibition [a)]	Mean Growth rate [c)]	Percent (%) inhibition [a)]
72-hour						
Control [d)]	118.1	-	-	-	-	-
Solvent control	141.2	-	-	-	-	-
Pooled control	132.0	-	2667.6	-	0.06761	-
3.0	128.2	3	2496.4	6	0.06740	0
7.3	139.5	-6	2579.2	3	0.06848	-1
17.5	67.8*	49	1270.4*	52	0.05848*	14
46.8	8.6*	93	190.4*	93	0.02950*	56
117.0	0.9*	99	-3.2*	100	-0.00443*	100
96-hour						
Control [d)]	195.4					
Solvent control	219.3					
Pooled control	209.7	-	6744.0	-	0.05549	-
3.0	207.8	1	6504.0	4	0.05536	0
7.3	203.5	3	6672.0	1	0.05532	1
17.5	164.4	22	4033.2*	40	0.05310	5
46.8	23.9*	89	556.8*	92	0.03232*	42
117.0	1.1*	99	-2.8*	100	0.00068*	99

* Statistically significant from control (Dunnett's one-tailed test; $p \leq 0.05$)

[a] % Inhibition=100-((Treatment group mean density/pooled control mean density)*100)

[b] Cumulative biomass is equal to the area under the growth curve

[c] Growth rate is calculated from the cell density data

[d] Replicate A was excluded from calculations due to reduced growth

EC_{50}, NOEC, $LOEC_{\mu}$ and EC_{μ} values were determined for each endpoint based on these results, and were as follows:

Table CA 8.2.6.2- 2: Toxicity of JAU 6476 (technical) to *Skeletonema costatum*

Test substance	JAU 6476
Test object	*Skeletonema costatum*
Exposure	96 hour, static

Endpoint description:	Endpoint:	95% Confidence Interval:
96-h EC_{50} - cell density	25.6 µg a.s./L	23.6 - 27.6 µg a.s./L
96-h EC_{50} - cumulative biomass	20.1 µg a.s./L	19.0 - 21.2 µg a.s./L
96-h EC_{50} - growth rate	49.9 µg a.s./L	45.5 - 54.2 µg a.s./L
72-h EC_{50} - cell density	18.0 µg a.s./L	16.7 - 19.4 µg a.s./L
72-h EC_{50} - cumulative biomass	17.1 µg a.s./L	15.7 - 18.4 µg a.s./L
72-h EC_{50} - growth rate	45.6 µg a.s./L	43.6 - 47.6 µg a.s./L
96-h Lowest Concentration with an effect (LOE_rC)	46.8 µg a.s./L (growth rate)	
96-h Highest Concentration without toxic effect (NOE_rC)	17.5 µg a.s./L (growth rate)	
72-h Lowest Concentration with an effect (LOE_rC)	17.5 µg a.s./L (growth rate)	
72-h Highest Concentration without toxic effect (NOE_rC)	7.3 µg a.s./L (growth rate)	

Conclusion:

Skeletonema costatum were exposed under static conditions for 96 hours to JAU 6476 (technical). The 72-hour E_rC_{50} value was 45.6 µg a.s./L (95% confidence interval: 43.6 – 47.6 µg a.s./L) and the 96-hour E_rC_{50} value was 49.9 µg a.s./L (95% confidence interval: 45.5 – 54.2 µg a.s./L) based on initially measured test concentrations.

The 72-hour NOE_rC and LOE_rC were 17.5 and 46.8 µg a.s./L, respectively.
The 96-hour NOE_rC and LOE_rC were 7.3 and 17.5 µg a.s./L, respectively.

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Report: KCA 8.2.6.2/02 [redacted]; [redacted]; 2004; M-001064-01-1
Title: Toxicity of JAU 6476 technical to the freshwater diatom Navicula pelliculosa
Report No.: 200252
Document No.: M-001064-01-1
Guideline(s): - ASTM; Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae; E1218; 1997.
- US EPA; Pesticide Assessment Guidelines, Subdivision J - Hazard Evaluation: Nontarget Plants; EPA-540/9-82-020; 1982.
- US EPA; Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; EPA 600/4-89/001; 1985.
-US EPA; Standard Evaluation Procedure, Non-Target Plants: Growth and Reproduction of Aquatic Plants - Tiers 1 and 2; EPA-540/9-86-134; 1986.
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

A static 96-hour growth test was conducted to determine the effects of prothioconazole (JAU 6476 technical) to the freshwater diatom *Navicula pelliculosa*. The objective of this growth study was to estimate the fifty percent effective concentration (EC_{50}), which represents the concentration that produces a fifty percent reduction in growth. The response parameters used in this study were standing

crop (cell density), cumulative biomass, and growth rate. The variable used to calculate the response parameters was cell density based on daily cell counts.

Materials and methods:
Test item: JAU 6476 technical (prothioconazole), Batch No. 6233/0031, Purity of 97.5%.

The test was conducting according to the Guidelines EPA-540/9-82-020, EPA 600/4-89/001, EPA-540/9-86-134 and ASTM Standard E1218. *Navicula pelliculosa* was exposed under static conditions (shaken cultures) for 96 hours to the following nominal concentrations: 26, 64, 160, 400 and 1000 mg a.s./L. A water control and a solvent (acetone) control were also implemented. Samples of test solutions, control and solvent control were taken on Day 0 and Day 4 to measure actual exposure concentrations. Each replicate was inoculated with *Navicula pelliculosa* cells at a nominal density of 10,000 cells/mL. Four replicate vessels were prepared for each concentration and used to determine daily cell density. An array of cool white fluorescent lights produced 24-hour illumination and a light intensity of approximately 412 foot-candles (4.4 klux).
The quality of water parameters was regularly monitored along the study. The test temperature exposure ranged from 24.4 to 25.3 °C (mean = 24.9 °C), as recorded hourly by the datalogger. The pH measurements ranged from 6.5 to 7.5 for all test levels during the exposure period. The conductivity ranged from 127 to 139 µmhos/cm.

Results:
Validity Criteria:
The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:
Measured concentrations of JAU 6476 on Day 0 were: Control (<2.6), Solvent Control (<2.6), 23.5, 56.6, 146.3, 356.4 and 889.5 mg a.s./L, which represented 89 to 91% of the nominal test concentrations. The test compound rapidly dissipated from the test water. Indeed, measured concentrations of JAU 6476 on Day 4 were <LOQ, 8.3, 34.8, and 206.2, and 720.7 µg a.s./L ,which represented 0 to 72% of the nominal test concentrations. No undissolved test substance was visually observed in the test vessels throughout the test period. All results refer to the initially measured concentrations.

Biological results:
No physical abnormalities were observed in the controls or treatment groups during the study. Biomass increased exponentially in the control by a factor of 109 within 3 days. Algae growth pattern and biomass after 72 and 96 hour of exposure were as follows:

Table CA 8.2.6.2- 3: 72-hour and 96-hour cell density, cumulative biomass and growth rate during the exposure of *Navicula pelliculosa* to JAU 6476 (technical)

Initial measured concentration [µg a.s./L]	Mean density [cells/mL x 10^4]	Percent (%) inhibition [a)]	Mean calculated cumulative biomass [b)]	Percent (%) inhibition [a)]	Mean Growth rate [c)]	Percent (%) inhibition [a)]
72-hour						
Pooled control [d)]	108.7	-	1584.6	-	0.06507	-
23.5	94.9(*)	13	1347.6(*)	15	0.06319(*)	3
56.6	88.2*	19	1220.4*	23	0.06220(*)	4
146.3	51.4*	53	718.2*	55	0.05450*	16
356.4	9.9*	91	151.2*	90	0.03176*	51
889.5	0.38*	100	-24.9*	102	-0.01472*	123
96-hour						
Pooled control [d)]	206.4	-	5341.8	-	0.5547	-
3.0	170.2*	18	4503.6*	16	0.05349 (*)	4
7.3	182.8*	11	4448.7*	17	0.05424 (*)	2
17.5	142.4*	31	3019.2*	43	0.05160*	7
46.8	53.2*	74	884.1*	83	0.04107*	26
117.0	0.13*	100	-42.9*	101	-0.02218*	140

* Statistically significant from controls (Dunnett's one-tailed test; p # 0.05)
(*) Statistically significant from controls (Dunnett's one-tailed test; p # 0.05), but determined not to be biologically significantly different
[a] % Inhibition=100-((Treatment group mean parameter/pooled control mean parameter)*100).
[b] Cumulative biomass is equal to the area under the growth curve.
[c] Growth rate is calculated from the cell density data.
[d] Two-tailed planned comparison t-test indicated that the control and solvent control groups could be pooled. Comparisons were made to the pooled controls.

EC_{50} values were determined for each endpoint based on these results, and were as follows:

Table CA 8.2.6.2- 4: Toxicity of JAU 6476 (technical) to *Navicula pelliculosa*

Test substance	JAU 6476	
Test object	*Navicula pelliculosa*	
Exposure	96 hours, static	
Endpoint description:	**Endpoint (µg a.s./L):**	**95% Confidence Interval (µg a.s./L):**
96-h EC_{50} - cell density	215.0	193.1 - 237.0
96-h EC_{50} - cumulative biomass	163.8	152.6 - 175.1
96-h EC_{50} - growth rate	395.3	317.4 - 473.2
72-h EC_{50} - cell density	136.3	124.7 - 147.8
72-h EC_{50} - cumulative biomass	128.6	115.9 - 141.4
72-h EC_{50} - growth rate	354.7	331.5 - 377.9

Conclusion:
Navicula pelliculosa were exposed to JAU 6476 technical for 96 hours under static conditions.
The 72-hour E_rC_{50} value was 354.7 µg a.s./L (95% confidence interval: 331.5 - 377.9 µg a.s./L) and the 96-hour E_rC_{50} value was 395.3 µg a.s./L (95% confidence interval: 317.4 – 473.2 µg a.s./L).

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Report:	KCA 8.2.6.2/03 [redacted] T; [redacted]; [redacted]; 2004; M-000348-01-1
Title:	Toxicity of JAU 6476 technical to the blue-green alga Anabaena flos-aquae
Report No.:	200497
Document No.:	M-000348-01-1
Guideline(s):	USEPA Guideline l23-2, Growth and Reproduction of Aquatic Plants (Tier 2)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

A static 4-day algal growth test was conducted to determine the effects of prothioconazole (JAU 6476 technical) to the blue-green alga *Anabaena flos-aquae*. The primary objective of this growth study was to estimate the fifty percent effective concentration (EC_{50}), which represents the concentration that produces a fifty percent reduction in growth. A secondary objective was to determine the no-observed-effect-concentration (NOEC). The response parameters used in this study were cell density (standing crop), cumulative biomass, and growth rate. The variable used to calculate the response parameters was cell density based on daily cell counts.

Materials and methods:

Test item: JAU 6476 technical (prothioconazole), Batch No. 6233/0031, Purity of 98.2%.

The test was conducting according to the FIFRA Guideline 123-2. *Anabaena flos-aquae* were exposed under static conditions (shaken cultures) for 96 hours to the following nominal concentrations: 0.02, 0.08, 0.27, 0.90, 3.00 and 10.00 mg a.s./L. A water control and a solvent (acetone) control were also implemented. Samples of test solutions, control and solvent control, were taken on Day 0 and Day 4 to measure actual exposure concentrations.
Three replicate vessels were prepared for each concentration and used to determine daily cell density. Each replicate was inoculated with *Anabaena flos-aquae* cells at a nominal density of 10,000 cells/mL. Testing was conducted in an environmental chamber which was programmed to maintain a test temperature of 24 ± 2.0°C and a 24 hour light photoperiod. A light intensity of approximately 200 foot-candles (2.2 klux) was maintained. Water quality parameters were regularly monitored along the test. The actual test temperature during the 4-day exposure ranged from 24.1 to 25.2°C (mean = 24.6°C), as recorded hourly by the datalogger. The pH measurements ranged from 7.3 to 8.7 for all test levels during the exposure period. The conductivity measurements ranged from 86.9 to 99.5 µmhos/cm.
Each day, cell density was determined in the three replicates at each test concentration using a light microscope and an improved Neubauer haemocytometer. The growth rate was analysed by comparing the change in cell density from Day 0 to Day 3 or 4. The cumulative biomass, or area under the growth curve, was determined by plotting the daily cell density from Day 0 to Day 3 or 4.

Findings:

Validity Criteria:

The Guideline used as reference does not state validity criteria. No significant deviations to the Guideline recommendations occurred, so that the study is deemed valid.

Analytical results:

The measured concentrations of JAU 6476 on Day 0 were 0.02, 0.08, 0.22, 0.82, 2.97, and 9.12 mg a.s./L, which represented 81 to 103% of the nominal test concentrations. The test compound was stable in the test system for all but the 0.02, 0.08 and 0.27 mg a.s./L test levels. Therefore, all results refer to these initially measured concentrations. The measured concentrations of JAU 6476 on Day 4

were 0.01, 0.05, 0.19, 0.86, 2.81, and 9.32 mg a.s./L, which represented 58 to 96% of the nominal test concentrations. No undissolved test substance was visually observed in the test vessels throughout the test period.

Biological results:

No physical abnormalities were observed in the controls or treatment groups during the study. Biomass increased exponentially in the control by a factor of 48 within 3 days. Algae growth pattern and biomass after 72 and 96 hour of exposure were as follows:

Table CA 8.2.6.2- 5: 72-hour and 96-hour cell density, cumulative biomass and growth rate during the exposure of *Anabaena flos-aquae* to JAU 6476 (technical)

Initial measured concentration [mg a.s./L]	Mean density [cells/mL x 10^4]	Percent (%) inhibition [1]	Mean calculated cumulative biomass [2]	Percent (%) inhibition [1]	Mean Growth rate [3]	Percent (%) inhibition [1]
72-hour						
Control	48.1	-	-	-	-	-
Solvent control	14.9	-	352.8	-	0.03227	-
0.02	23.3	-56.4	468.0	-32.7	0.04008	-24.2
0.08	18.6	-24.8	389.2	-10.3	0.03079	4.6
0.22	42.1	-183	717.2	-103.3	0.05119	-58.6
0.82	22.7	-52.3	430.0	-21.9	0.04295	-33.1
2.97	14.0**	6.0	360.4**	-2.2	0.03642	-12.9
9.12	6.3**	57.7	186.0**	47.3	0.02470**	23.5
96-hour						
Control	74.1	-	-	-	-	-
Solvent control	83.4	-	-	-	-	-
Pooled control	78.8	-	1901.0	-	0.04602	-
0.02	83.7	-6.2	1728.0	9.1	0.04601	-1.6
0.08	93.4	-18.5	1708.4	10.1	0.04708	-3.9
0.22	92.4	-17.3	2306.8	-21.3	0.04693	-3.6
0.82	74.2	5.8	1568.4	17.5	0.04480	1.1
2.97	50.6*	35.8	1111.6	41.5	0.04074*	10.0
9.12	6.8*	91.4	318.8*	83.2	0.01803*	60.2

* Statistically significant from control (Dunnett's one-tailed test; $p \leq 0.05$)

** Values considered biologically significant from the control

[1] % Inhibition=100-((Treatment group mean cell density/pooled control mean cell density or solvent control mean cell density)*100).

[2] Cumulative biomass is equal to the area under the growth curve.

[3] Growth rate is calculated from the cell density data.

EC_{50}, NOEC, LOEC, and TEC (threshold effect concentration, relevant for the US risk assessment) values were determined for each endpoint based on these results, and were as follows:

Table CA 8.2.6.2- 6: Toxicity of JAU 6476 (technical) to *Anabaena flos-aquae*

Test substance	JAU 6476	
Test object	*Anabaena flos-aquae*	
Exposure	96 hour, static	
Endpoint description:	**Endpoint (mg a.s./L):**	**95% Confidence Interval (mg a.s./L):**
96-h EC_{50} - cell density	3.71	3.35 – 4.08
96-h EC_{50} - cumulative biomass	3.55	3.00 – 4.10
96-h EC_{50} - growth rate	9.12	7.82 – 10.42
72-h EC_{50} - cell density	>9.12	NC
72-h EC_{50} - cumulative biomass	>9.12	NC
72-h EC_{50} - growth rate	>9.12	NC
96-h Lowest Concentration with an effect (LOEC)	2.97 mg a.s./L (growth rate)	
96-h Highest Concentration without toxic effect (NOEC)	0.82 mg a.s./L (growth rate)	
72-h Lowest Concentration with an effect (LOEC)	9.12 mg a.s./L (growth rate)	
72-h Highest Concentration without toxic effect (NOEC)	2.97 mg a.s./L (growth rate)	

NC = Not calculable

Conclusion:
Anabaena flos-aquae were exposed to JAU 6476 (technical) for 96 hours under static conditions. The 72-hour E_rC_{50} value was >9.12 mg a.s./L (95% confidence interval: not calculable) and the 96-hour E_rC_{50} value was 9.12 mg a.s./L (95% confidence interval: 7.82 – 10.42 mg a.s./L).
The 72-hour NOE_rC and LOE_rC were 2.97 and 9.12 mg a.s./L, respectively.
The 96-hour NOE_rC and LOE_rC were 0.82 and 2.97 mg a.s./L, respectively.

CA 8.2.7 Effects on aquatic macrophytes

Report:	KCA 8.2.7/01 [redacted]; 2004; M-000532-01-1
Title:	Toxicity of JAU 6476 technical to duckweed (Lemna gibba G3) under static-renewal conditions
Report No.:	200488
Document No.:	M-000532-01-1
Guideline(s):	U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4400
Guideline deviation(s):	no major deviations
GLP/GEP:	**yes**

Objective:
A 7-day static-renewal duckweed growth test was conducted to determine the effects of prothioconazole (JAU 6476) technical on *Lemna gibba* G3. The primary objective of this study was to estimate the fifty percent effective concentration (E_rC_{50}) which represents.the concentration that produces a fifty percent reduction in growth when compared to controls. A secondary objective was to determine the no-observed-effect-concentration (NOEC), which equals the lowest concentration without a statistically significant ($p > 0.05$) reduction from the control for the measured parameters. For the parameter frond number, standing crop, growth rate and cumulative biomass (as area under the growth curve) were calculated. The endpoint calculation for the second parameter frond dry weight was confined to measurements at termination of the test.

Materials and methods:
Test item: JAU 6476 technical (prothioconazole), Batch No. 6233/0031; Purity of 98.2%.

The test was conducted according to OPPTS 850.4400 guideline.
The duckweed *Lemna gibba* G3 was exposed for 7 days under static-renewal conditions (renewals on day 3 and day 5). Nominal concentrations were control, solvent control, 0.97, 3.24, 10.8, 36.0, 120 and 400 ug a.s./L. Growth was determined by frond counts on study days 0, 3, 5, and 7. In addition, frond dry weight was determined after 7 days of exposure. The used solvent was acetone.

The test temperature ranged from 24.4 to 25.6°C with a mean of 24.6°C, as recorded hourly by the datalogger. The pH measurements ranged from 7.5 to 9.0. Conductivity ranged from 1428 to 1533 µmhos/cm with a mean of 1467 µmhos/cm.

Findings:
Validity Criteria:
The Guideline used as reference does not state validity criteria. However, both in the water and in the solvent control a 17.5fold increase in frond number was observed over the 7 days study period which indicates good growing conditions.

Analytical results:
Mean measured calculations were based on the recoveries of the newly prepared test solutions on days 0 and 5 since reduced recoveries were observed on day 3 and 7, indicating degradation in the test system. The mean measured concentrations of JAU 6476 technical were 1.01, 3.34, 10.4, 35.1, 106.4 and 404.0 µg a.s./L which represents 89 to 104% of the nominal test concentrations. Recoveries for the control and solvent control test solutions were below the limit of quantitation (0.5 µg/L). No undissolved test substance was visually observed in the test vessels throughout the exposure period. All results refer to the mean measured concentrations.

Biological results:
Observations made on Day 5 and Day 7 showed treatment related effects with regards to frond size at the 35.1, 106.4 and 404.0 µg a.s./L treatment levels. Observations on Day 7 showed treatment related effects with regards to frond color at the 35.1, 106.4 and 404.0 µg a.s./L treatment levels.

The effects of prothioconazole (JAU 6476 technical) on frond number, cumulative biomass and growth rate are summarized in the following table:

Table CA 8.2.7- 1: Day 7 frond count, cumulative biomass and growth rate during the exposure of *Lemna gibba* G3 to JAU 6476 technical

Mean measured concentration [µg a.s./L]	Mean frond counts	Percent (%) inhibition[a)]	Mean calculated cumulative biomass [b)]	Percent (%) inhibition[a)]	Mean Growth rate [c)]	Percent (%) inhibition[a)]
Control	279	-	12360	-	0.01701	-
Solvent control	281	-	12520	-	0.01705	-
Pooled controls	280	-	12440	-	0.01703	-
1.01	280	0	12172	2	0.01702	0
3.34	280	0	12872	-3	0.01703	0
10.4	253*	10	11388*	8	0.01644	3
35.1	172*	39	8656*	30	0.01413*	17
106.4	101*	64	6044*	51	0.01095*	36
404.0	80*	71	5032*	60	0.00958*	44

* Statistically significant from control (Dunnett's one-tailed test; $p \leq 0.05$)

[a)] Percent inhibition = 100 - ((mean parameter per test level/mean parameter of pooled controls) x 100).

[b)] Cumulative biomass is equal to the area under the growth curve.

[c)] Growth rate is calculated from the frond count data.

Calculated EC_{50}, LOEC and NOEC values were as follows:

Table CA 8.2.7- 2: Effects of JAU 6476 technical on *Lemna gibba* G3

Test substance	JAU 6476
Test object	*Lemna gibba* G3
Exposure	7 days, static renewal (day 3 & day 5)
Endpoint description:	**Endpoint (µg a.s./L):**
7-day EC_{50} – standing crop	74.0
7-day EC_{50} - growth rate	> 404
7-day EC_{50} - cumulative biomass	404
7-day EC_{50} – frond dry weight	404
Lowest Concentration with an effect (LOEC)	10.4 (standing crop and cumulative biomass)
Highest Concentration without toxic effect (NOEC)	3.34 (standing crop and cumulative biomass)

Conclusion:

Lemna gibba G3 was exposed to prothioconazole (JAU 6476 technical) for a 7-day period under static-renewal conditions. The most sensitive measurement variable proved to be frond number. The 7d-E_rC_{50} value for frond number was determined as >404 µg a.s./L.

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Report:	KCA 8.2.7/02 [redacted],; [redacted]; [redacted]; 2003; M-104599-01-1
Title:	Toxicity of JAU 6476-Desthio to duckweed (Lemna gibba G3) under static-renewal conditions
Report No.:	200469
Document No.:	M-104599-01-1
Guideline(s):	USEPA OPPTS 850.4400
Guideline deviation(s):	--
GLP/GEP:	**yes**

Objective:
A 7-day static-renewal duckweed growth test was conducted to determine the effects of JAU 6476-desthio on *Lemna gibba* G3. The primary objective of this growth study was to estimate the fifty percent effective concentration (EC_{50}) for JAU 6476-desthio which representes the concentration that produces a fifty percent reduction in growth when compared to controls. A secondary objective was to determine the no-observed-effect-concentration (NOEC), which equals the lowest concentration without a statistically significant ($p > 0.05$) reduction from the control for the measured parameters. For the parameter frond number, standing crop, growth rate and cumulative biomass (as area under the growth curve) were calculated. The endpoint calculation for the second parameter frond dry weight was confined to measurements at termination of the test.

Materials and methods:
Test item: JAU 6476-desthio (metabolite of prothioconazole), Purity of 97.0% metabolite, Batch No. RUX76-105/8a; CAS # 120983-64-4.

The test was conducted according to OPPTS 850.4400 guideline.
The duckweed *Lemna gibba* G3 was exposed for 7 days under static-renewal conditions (renewal on day 3). Nominal concentrations were control, solvent control, 2.56, 6.40, 16.0, 40.0 and 100 µg metabolite/L. The used solvent was acetone.
Each replicate was impartially inoculated with three *Lemna* plants for a total of 16 fronds at study initiation. The study was conducted under axenic conditions. Three replicate vessels were prepared for each treatment group.
Growth was determined by frond counts on study days 0, 3, 5, and 7. At the same time, phytotoxicity observations were performed to determine the health of the plants. Frond dry weight was determined after 7 days of exposure.
The test temperature during the 7-day exposure ranged from 23.6 to 25.6°C with a mean of 24.0°C, as recorded hourly by the datalogger. The pH measurements ranged from 7.7 to 8.9. Conductivity measurements ranged from 1437 to 1470 µmhos/cm with a mean conductivity of 1455 µmhos/cm.

Findings:
Validity Criteria:
The Guideline used as reference does not state validity criteria. However, in the water and in the solvent control, 13.5 and 12fold increases in frond number, respectively, were observed over the 7 days study period which indicates good growing conditions.

Analytical results:
Mean measured concentrations (mean of new solutions from Day 0 and Day 3, as well as composite old solutions from Day 7) of JAU 6476-Desthio were 2.42, 5.78, 14.30, 35.60, 89.77 µg metabolite/Lwhich

represents 89 to 94% of the nominal test concentrations. Thus, the test material was stable in the test system throughout the exposure period. Recoveries for the control and solvent control test solutions were below the limit of quantitation (0.5 µg/L). No undissolved test substance was visually observed in the test vessels. All results refer to the mean measured concentrations.

Biological results:
Observations made on Day 5 showed a reduction in frond size in the 35.60 and 89.77 µg metabolite/L treatment levels and observations on Day 7 showed a reduction in frond size at the 14.30, 35.60 and 89.77 µg metabolite/L treatment levels.

The effects of JAU 6476-desthio on frond number, cumulative biomass and growth rate are summarized in the following table:

Table CA 8.2.7- 3: Day 7 frond count, cumulative biomass and growth rate during the exposure of *Lemna gibba* G3 to JAU 6476-desthio

Mean measured concentration [µg metab./L]	Frond counts	Percent (%) inhibition [1]	Mean calculated cumulative biomass [2]	Percent (%) inhibition [1]	Mean Growth rate [3]	Percent (%) inhibition [1]
Control	216	-	10104	-	0.01550	-
Solvent control [4]	187	-	8460	-	0.01462	-
2.4	195	-4	9192	-9	0.01487	-2
5.8	187	0	8696	-3	0.01463	0
14.3	160*	14	7740*	9	0.01369*	6
35.6	90*	52	5020*	41	0.01030*	30
89.8	51*	73	3192*	62	0.00693*	53

* Statistically significant difference from solvent control (Dunnett's one-tailed test; $p \leq 0.05$)
[1] % Inhibition = 100-((Treatment group endpoint mean/solvent control mean)* 100).
[2] Cumulative biomass is equal to the area under the growth curve.
[3] Growth rate is calculated from the frond count data.
[4] Two-tailed planned comparison t-test indicated that the control and solvent control groups should not be pooled. Comparisons for each endpoint were made to the solvent control group only.
metab.= metabolite

Calculated EC_{50}, LOEC and NOEC values were as follows:

Table CA 8.2.7- 4: Effects of JAU 6476-desthio on *Lemna gibba* G3

Test substance	JAU 6476-desthio
Test object	*Lemna gibba* G3
Exposure	7 days, static renewal (day 3)
Endpoint description:	**Endpoint (µg metabolite/L):**
7-day EC_{50} – standing crop	39.4
7-day EC_{50} - growth rate	80.9
7-day EC_{50} - cumulative biomass	56.8
7-day EC_{50} – frond dry weight	41.1
Lowest Concentration with an effect (LOEC)	14.3 (all endpoints)
Highest Concentration without toxic effect (NOEC)	5.8 (all endpoints)

Conclusion:
Lemna gibba G3 was exposed to JAU 6476-desthio for a 7-day period under static-renewal conditions. The most sensitive measurement variable proved to be frond number. The 7d-E_rC_{50} value for frond number was determined as 80.9 µg metabolite/L.

CA 8.2.8 Further testing on aquatic organisms

No additional studies have been performed, existing studies have been evaluated during the Annex I inclusion and have been summarised in the Monograph and are included in the Baseline Dossier.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

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

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

- Acute oral and contact toxicity of prothioconazole
- Acute oral and contact toxicity of JAU 6476-desthio (metabolite of prothioconazole),
- Acute contact toxicity of prothioconazole to adult bumble bees under laboratory conditions,
- Chronic 10 day toxicity test with of Prothioconazole SC 480 on adult bees under laboratory conditions,
- Colony feeding study with Prothioconazole SC 480 according to [redacted]. 1992 (using a realistic worse case spray solution concentration and covering exposure for effects on brood (eggs, young and old larvae) and their development, nurse bee on-going behaviour in brood care and colony strength),
- Semi-field brood feeding study with Prothioconazole EC 250 following OECD guidance document 75 (using a more realistic spray scenario onto flowering *Phacelia tanacetifolia* at the maximum application rate for the approval/renewal of prothioconazole and covering exposure for effects on brood (eggs) and their development and colony parameters).

These studies were not submitted during the first Annex I inclusion process and are submitted within this Supplementary Dossier for the prothioconazole Annex I Renewal. The studies are summarized below and a full list of the relevant ecotoxicological endpoints for prothioconazole and its metabolite JAU 6476-desthio and bees are presented in the following table.

Table CA 8.3.1- 1: EU evaluated and additional studies on bee toxicity of prothioconazole, JAU 6476-desthio and prothioconazole formulations

Test substance	Test species	Test method	Ecotoxicological endpoint	Reference
Prothioconazole	Honey bee (*Apis mellifera*)	Laboratory, acute, 48 h oral acute, 48 h contact	LD_{50} >71 µg a.s./bee **LD_{50} >200 µg a.s./bee**	[redacted] (1999) M-023105-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Honey bee (*Apis mellifera*)	Laboratory, acute, 48 h oral acute, 48 h contact	**LD_{50} >105.1 µg a.s./bee** LD_{50} >100.0 µg a.s./bee	[redacted] (2014) M-505379-01-1 KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02
	Bumble bee (*Bombus terrestris*)	Laboratory, acute, 48 h contact	LD_{50} > 100 µg a.s./bumble bee	[redacted] (2015) M-521802-01-1 KCA 8.3.1.1.2/04
JAU 6476-desthio	Honey bee (*Apis mellifera*)	Laboratory, acute, 48 h oral acute, 48 h contact	**LD_{50} >106.5 µg p.m./bee** **LD_{50} >100 µg p.m./bee**	[redacted] (2015) M-528139-01-1 KCA 8.3.1.1.1/03 KCA 8.3.1.1.2/03
Prothioconazole SC 480	Honey bee (*Apis mellifera*)	Laboratory, chronic, 10-day feeding (*ad libitum*)	LC_{50} > 100 mg a.s./kg LDD_{50} > 3.8 µg a.s./bee/day NOEC 100 mg a.s./kg NOEDD 3.8 µg a.s./bee/day	[redacted] (2015) M-528888-01-1 KCA 8.3.1.2/01
	Honey bee (*Apis mellifera*)	Bee brood feeding test (Oomen *et al.*)	No adverse effects on brood development, mortality and behaviour after feeding honey bee colonies sugar syrup at 0.47 g a.s./L.	[redacted] & [redacted] (2014) M-478670-01-1 KCA 8.3.1.3/01
Prothioconazole EC 250	Honey bee (*Apis mellifera*)	Semi-field brood study (OECD 75)	No adverse effects on brood development, mortality, foraging activity, behaviour, colony condition and strength after application of 187.5 g a.s./ha onto flowering *Phacelia tanacetifolia*	[redacted] (2015) M-532419-01-1 KCA 8.3.1.3/02

a.s.: active substance; p.m. : pure metabolite

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	KCA 8.3.1.1.1/02 [redacted] ; 2014; M-505379-01-1
Title:	Effects of prothioconazole tech. (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89491035
Document No.:	M-505379-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine the acute contact and oral toxicity of prothioconazole tech. to the honey bee *Apis mellifera* L. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and methods:
Test item: Prothioconazole tech., purity: 96.7% w/w (analytical), Batch No.: HEC 21597-1-1; TOX10687-00; Specification No. 102000014040.
Reference item: dimethoate

Under laboratory conditions *Apis mellifera* 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test). The test item was applied as one 5 nL droplet of prothioconazole tech. dissolved in acetone, placed on the dorsal bee thorax.

For the oral limit test, 50 worker bees were fed with a single dose of treated 50% sucrose solution for a maximum of 6 hours. Test dose was 105.1 µg a.s. per bee (value based on the actual intake of the test item). Bees were observed up to 48 h after end of exposure.

Findings:
Validity criteria:
The contact and oral tests are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item dimethoate (0.16 and 0.12 µg a.s./bee for the contact and oral 48h-LD_{50}, respectively), were within the required ranges.

Biological results:
At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg a.s./bee.

In the oral toxicity test, the maximal nominal test level of prothioconazole tech. (i.e. 100 µg a.s./bee (corresponded to an actual intake of 105.1 µg a.s./bee) led to no mortality after 48 h.

No test item induced behavioural effects were observed at any time in the contact and oral toxicity tests.

Table CA 8.3.1.1.1- 1: Toxicity of prothioconazole technical to honey bees; laboratory test

Test Item	Prothioconazole tech.	
Test Species	*Apis mellifera*	
Exposure	**contact (solution in acetone)**	**oral (50 % w/v sucrose solution containing 1 % Tween 80 + 4 % acetone)**
Application dose [µg a.s./bee]	100.0	105.1
LD_{50} [µg a.s./bee]	> 100.0	> 105.1

Conclusion:
The toxicity of prothioconazole tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees.
The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 105.1 µg a.s./bee.

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Report:	KCA 8.3.1.1.1/03[redacted],; 2015; M-528139-01-1
Title:	Prothioconazole-desthio (BCS-AA53879): Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	100071035
Document No.:	M-528139-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine the acute contact and oral toxicity of prothioconazole-desthio (BCS-AA53879) to the honey bee (*A. mellifera* L.). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and methods:
Test item: Prothioconazole-desthio (BCS-AA53879), purity: AE 1194888, 99.5% w/w (analytical), Batch No. KTS9616-4-2;

Under laboratory conditions *Apis mellifera* 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg p.m. per bee by topical application (contact limit test). The test item was applied as one 5 µL droplet of prothioconazole-desthio dissolved in acetone, placed on the dorsal bee thorax using a calibrated pipette.

For the oral limit test, 50 worker bees were fed with a single dose of treated 50% sucrose sultion for a maximum of 3 hours and 25 minutes. Test dose was 106.5 µg p.m. per bee by feeding value based on the actual intake of the test item.

Findings:
Validity criteria:
The contact and oral tests are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item dimethoate (0.19 and 0.16 µg a.s./bee for the contact and oral 48h-LD_{50}, respectively), were within the required ranges.

Biological results:
At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg p.m./bee.

In the oral toxicity test, the maximum nominal test level of prothioconazole-desthio (BCS-AA53879) (i.e. 100 µg p.m./bee) corresponded to an actual intake of 106.5 µg p.m./bee. This dose level led to 8.0% mortality after 48 hours.

No test item induced behavioural effects were observed at any time in the both toxicity tests.

Table CA 8.3.1.1.1- 2: Toxicity of prothioconazole-desthio to honey bees; laboratory tests

Test Item	Prothioconazole-desthio	
Test Species	*Apis mellifera* L.	
Exposure	**contact (solution in acetone)**	**oral (sugar/acetone/water solution)**
Test dose [µg p.m./bee]	100.0	106.5
LD_{50} [µg p.m./bee]	> 100.0	> 106.5

Conclusion:
The toxicity of prothioconazole-desthio (BCS-AA53879) was tested in both, an acute contact and an acute oral toxicity test on honey bees.
The contact LD_{50} (48 h) was > 100.0 µg p.m./bee.
The oral LD_{50} (48 h) was > 106.5 µg p.m./bee.

CA 8.3.1.1.2 Acute contact toxicity

Report: KCA 8.3.1.1.2/02 [redacted], 2014; M-505379-01-1
Title: Effects of prothioconazole tech. (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.: 89491035
Document No.: M-505379-01-1
Guideline(s): OECD 213 and 214 (1998)
Guideline deviation(s): not specified
GLP/GEP: **yes**

Please refer to CA 8.3.1.1.1.

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Report: KCA 8.3.1.1.2/03 [redacted]L; 2015; M-528139-01-1
Title: Prothioconazole-desthio (BCS-AA53879): Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.: 100071035
Document No.: M-528139-01-1
Guideline(s): OECD 213 and 214 (1998)
Guideline deviation(s): not specified
GLP/GEP: **yes**

Please refer to CA 8.3.1.1.1.

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Report: KCA 8.3.1.1.2/04 [redacted]; 2015; M-521802-01-1
Title: Prothioconazole technical: Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.: S14-00616
Document No.: M-521802-01-1
Guideline(s): no specific guidelines available, based on OEPP/EPPO 170 (4) (2010), OECD Guideline No. 214 (1998) and on the review article of VAN DER STEEN (2001)
Guideline deviation(s): not applicable
GLP/GEP: **yes**

Objective:
The objectives of this study were to determine possible effects of prothioconazole technical on the bumble bee, *Bombus terrestris* L., from contact exposure and to determine whether the LD_{50} value was greater or lower than the tested dose.

Material and methods:
Test item: Prothioconazole technical (Short code: HEC 21597-1-1), purity: 96.7% prothioconazole (analysed), Batch No. HEC 21597-1-1, TOX10687-00.

The contact toxicity of prothioconazole technical to the bumble bee (*Bombus terrestris* L.) was determined in a limit test based on OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of VAN DER STEEN (2001).
In the laboratory, the bumble bees were exposed to 100 µg prothioconazole/bumble bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after application. One control group was exposed for the same period of time under identical exposure conditions to acetone, the other control group was exposed to tap water.
The test item treatment group contained 50 test organisms, divided in 5 parallel replicates, each containing 10 test organisms. The control groups, contained 30 test organisms, divided in 3 parallel replicates, each containing 10 organisms.
During the experimental phase, the test organisms were kept in constant darkness except during the application and the assessments which were conducted under day light. The temperature during the test period was between 23.8 and 26.2°C, the relative humidity was between 47.0 and 61.8%, recorded with a calibrated data logger.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CA 8.3.1.1.2- 1: Validity criteria

Validity criteria	Recommended	Obtained
Mean mortality in the (solvent control) control group	≤ 10%	(3.33%) 0.0%
Mean mortality in the reference item treatment	≥ 50%	86.67%

Biological results:
In the solvent control group, treated with acetone a mortality of 3.33% could be observed. In the control group treated with tap water, no mortality was observed during the 48 h test period.
The bumble bees of the reference item group were treated with 13 µg dimethoate/bumblebee in the contact test. The reference item mortality of 86.67% at the end of the test (48 hours after application) was within the required range. The validity criteria were met, thus the test is considered to be valid.
In the test item treatment group, no mortality was observed at the dose level corresponding to 100 µg prothioconazole-technical/bumble bee at the final assessment after 48 hours.

Table CA 8.3.1.1.2- 2: Effect of prothioconazole tech. on the bumble bee (*Bombus terrestris*) – contact test

Test item	Prothioconazole technical			
Test species	Bumble bee (*Bombus terrestris*)			
Exposure	Topical application			
	Mortality [%]		**Corrected Mortality [%]**	
Treatment	**24 h**	**48 h**	**24 h**	**48 h**
Control (acetone)	3.33	3.33	-	-
Control (tap water)	0.0	0.0	-	-
Prothioconazole technical: 100 µg a.s./bumble bee	0.0	0.0	-3.4	-3.4
Reference item: Perfekthion	80.0	86.67	-	-
LD_{50} (24 h)	>100 µg a.s./bumble bee			
LD_{50} (48 h)	>100 µg a.s./bumble bee			
NOED (48 h)	100 µg a.s./bumble bee			

In the test item treatment group no sublethal effects were observed during the entire observation period. The NOED (No Observed Effect Dose) was determined to be 100 µg prothioconazole technical/bumble bee.

Conclusion:
The 48 hour contact LD_{50} value for prothioconazole technical was determined to be > 100 µg prothioconazole/bumble bee.
The contact NOED (48 h) was determined as 100 µg prothioconazole technical/bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

Report:	KCA 8.3.1.2/01 [redacted]; 2015; M-528888-01-1
Title:	Prothioconazole SC 480 - Assessment of effects on the adult honey bee, Apis mellifera L., in a 10 days chronic feeding test under laboratory conditions
Report No.:	S14-00176
Document No.:	M-528888-01-1
Guideline(s):	(GLP compliant study based on OECD 213 (1998) and CEB No. 230 with modifications and current recommendations of the ring test group (2014))
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The objective of this study was to determine the effects of the test item Prothioconazole SC 480 G on the adult honey bee, *Apis mellifera* L., in a 10-day chronic feeding test in the laboratory.

Material and methods:
Test item: Prothioconazole SC 480 G, Analyzed a.s. content: 471.8 g/L (39.6% w/w), Batch No. EM4L0J3333, TOX10578-00, Specification No. 10200007878.

The chronic effects of the test item Prothioconazole SC 480 G on the honey bee, *Apis mellifera* L., were assessed in a 10-days chronic feeding test under laboratory conditions.
Over a period of 10 consecutive days, honey bees were exposed to 50% (w/v) aqueous sucrose feeding solution, with a nominal concentration of 100 mg prothioconazole/kg feeding solution by continuous

and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution.
Mortality and sub-lethal effects were assessed every day throughout the 10-day exposure period. Furthermore, the daily consumption of feeding solution, the mean uptake of test item and the accumulated mean uptake of test item were determined.
Samples of the feeding solutions prepared freshly every day throughout the 10-day exposure period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item.

Findings:

Validity criteria:
All validity criteria were met as presented below:

Table CA 8.3.1.2- 1: Validity criteria

Validity criteria	Recommended	Obtained
Mean mortality in the control group	≤ 15%	2.5%
Mean mortality in the reference item treatment	≥ 50%	100%

Analytical results:
The actual concentration of prothioconazole in the application (feeding) solutions, determined for each preparation day, was in the range from 75 to 92% of the nominal concentration. No residues of prothioconazole above the LOQ (10 µg/kg) were found in any of the control samples. The average actual concentration of prothioconazole over a period of 10 consecutive days accounted to 84% of nominal.

Biological results:
The cumulative mortality at the concentration level of 100 mg prothioconazole/kg feeding solution was 5.0% (corrected: 2.6%), at the final assessment.
In the control group, no sub-lethal effects were observed. In the test item treatment group at the concentration level of 100 mg prothioconazole/kg feeding solution one single affected bee was observed at assessment E6.
The overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) in the test item treatment group was not statistically significantly different (lower) when compared to the untreated control group (38.0 mg/bee/day at 100 mg prothioconazole/kg feeding solution, compared to 41.2 mg/bee/day in the control group). In the toxic reference item group, the overall mean daily consumption of feeding solution was 35.2 mg/bee/day.
At the end of the 10-day exposure period, the mean accumulated uptake of the test item at the concentration level 100 mg prothioconazole/kg feeding solution was 38.0 µg a.s./bee (based on the actual consumption of feeding solution by the honey bees). The corresponding daily mean uptake was therefore 3.80 µg a.s./bee/day.

Table CA 8.3.1.2- 2: Effects of Prothioconazole SC 480 G on adult honey bee (*Apis mellifera* L.) in a 10-day chronic feeding test in the laboratory (*ad libitum*)

Test item		Prothioconazole SC 480 G		
Test species		Honey bees (*Apis mellifera* L.)		
Exposure		*via* treated sugar solution (10 days)		
Treatment [mg a.s./kg feeding solution]	**10-days cumulative mortality (M_{corr}[4]) [%]**	**Overall mean consumption of feeding solution [mg/bee/day]**	**Dietary dose (DD)[5] [µg a.s./bee]**	**Accumulated mean uptake of test item [µg a.s./bee]**
C[1] (0.0)	2.5	41.2	-	-
R[2] (0.9)	100 (100)	35.2	0.03	0.41
Prothioconazole SC 480 G[3]				
100	5.0 (2.6)	38.0	3.80	38.0
LC_{50}		> 100 mg a.s./kg		
LDD_{50}		> 3.8 µg a.s./bee/day		
NOEC		100 mg a.s./kg		
NOEDD		3.8 µg a.s./bee/day		

[1] Feeding solution: 50 % w/v aqueous sucrose solution
[2] Feeding solution: 50 % w/v aqueous sucrose solution containing Perfekthion (a.s. dimethoate)
[3] Feeding solution: 50 % w/v aqueous sucrose solution containing Prothioconazole SC 480 G
[4] Corrected morality according to SCHNEIDER-ORELLI (1947)
[5] Dietary Dose (DD): mean uptake of test item (calculation based on the replicate values)
LC: Lethal Concentration
LDD: Lethal Dietary Dose
NOEC: No Observed Effect Concentration based on mortality (not significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $\alpha = 0.05$)
NOEDD: No Observed Effect Dietary Dose based on mortality (not significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $\alpha = 0.05$)

Conculsion:

It can be concluded that the continuous *ad libitum* feeding of adult honey bees in the laboratory over a period of 10 consecutive days with the test item Prothioconazole SC 480 G at the treatment level of 100 mg prothioconazole/kg feeding solution caused no adverse effect regarding mortality and sub-lethal effects.

The NOEC for mortality was determined to be 100 mg prothioconazole/kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 3.8 µg a.s./bee/day.

The LC_{50} after 10 days was determined to be > 100 mg prothioconazole/kg feeding solution. The corresponding LDD_{50} (Lethal Dietary Dose), based on the actual consumption of the respective feeding solutions, was determined to be > 3.8 µg a.s./bee/day.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	KCA 8.3.1.3/01 [redacted].,; [redacted]; 2014; M-478670-01-1
Title:	Prothioconazole SC 480 G (480 g/L): Effects on honey bee brood (Apis mellifera L.) - Brood feeding test
Report No.:	79051031
Document No.:	M-478670-01-1
Guideline(s):	GLP compliant study based on the method according to [redacted]. (1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

This study provides information on the brood development under the influence of food contaminated with Prothioconazole SC 480 G, comparable to standard rates for normal field use and information on the potential effect of Prothioconazole SC 480 G to honey bee brood. The employed method of investigating the development of the honey bee brood is based on the method of [redacted]. (1992). Ontogenesis of eggs, young and old larvae of honey bees were observed. Mortality of the honey bees and sublethal effects, such as changes in behaviour, were also monitored.

Material and methods:

Test item: Prothioconazole SC 480 G, analyzed a.s. content: 40.9 % w/w, 487.8 g/L; Batch No. EM4L011663; TOX10161-00; Specification No. 102000007878 – 04; Density: 1.193 g/mL (20 °C).

Honey bee colonies (*Apis mellifera* L.) were maintained according to normal beekeeping practice, containing two magazines with 11 combs, each. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply of nectar and pollen. The mean strength of the colonies per treatment group, two days before application, ranged between 10035 and 15030 adult bees. Colonies were free flying, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops or flowering weeds in the surrounding area.
An untreated control and a toxic reference (3.0 g Insegar; 25% fenoxycarbin) were included in the study. Three bee colonies were used per treatment group. The test item and reference item solutions were mixed with ready-to-use sugar syrup (Apiinvert; 30% sucrose, 31% glucose, 39% fructose) and applied to the bee colonies via a feeding trough, which was put directly into the colony on top of the second magazine. Test concentration was 0.47 g prothioconazole/L. Pure sugar syrup (Apiinvert) was used for the controls. Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a period of 21 days following the application for each treatment group and colony. This was assessed one day before the application, by selecting one (or several) brood comb(s) of each colony and by taking a digital photo of this (these) brood comb(s). After saving the photo-file on a computer, eggs, young- and old larvae were marked at this first Brood area Fixing Day (BFD0). For each subsequent brood assessment (BFDn), again, the same comb(s) was (were) selected from the respective colony and another digital photo was taken, in order to investigate the progress of brood development. Ontogenesis of the bee brood was observed for a period of 21 days after application (i.e. 22 days following BFD 0). Mortality of adult bees and pupae was also assessed.

Findings:

Validity criteria:

The reference item treatment (Insegar, a.s. = fenoxycarb) resulted in an egg termination rate of 100% and a statistically significantly increase of unsuccessful young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Climatic conditions:

The experimental phase of this study took place at a settled, constant weather period with sunny and warm days. Mean temperatures over the course of the study ranged from 19.7 °C to 28.6 °C. Rain occurred only on a few occasions.

Biological results:

The mean termination rate of eggs was slightly lower in the test item treatment group (16.0%) when compared to the values of the control group (17.8%). There was no statistically significant difference when compared to the control. Thus, there was no effect on the development of eggs following the consumption of the test item.

There was also no effect on the development of young larvae after consumption of the test item via treated sugar solution. The development success of the young larvae in the test item treatment group was slightly higher and resulted in a mean termination rate of 12.4% compared to 10.2% in the control group. This difference was not statistically significant compared to the control group.

No effect on the development of old larvae was observed after consumption of the test item treated sugar solution. The mean termination rate of old larvae in the test item treatment group was lower with a mean of 3.6% compared to 6.4% in the control group. Accordingly, this was not statistically significant compared to the control group.

Adult bee mortality in the test item treatment group was lower (mean of 8.8 dead bees per day) when compared to the control group (21.2 dead bees per day) and not statistically significantly different.

Nearly no dead larvae and pupae were found in the dead bee traps after treatment with Prothioconazole SC 480 G. Thus, there was no effect of the test item on honey bee pupae and larvae.

Table CA 8.3.1.3- 1: Effects of Prothioconazole SC 480 G on honey bee brood

Test item	Prothioconazole SC 480 G		
Test species	Honey bees (*Apis mellifera* L.) (complete colonies)		
Exposure	*via* treated sugar solution		
Treatment	**Untreated control**	**Prothioconazole SC 480 G**	**Reference Item (Insegar, a.s. = fenoxycarb)**
Rate per L sugar solution [product][1)]	-	1.15 g/L	3.0 g/L
Rate per L sugar solution [a.s.][1)]	-	0.47 g/L	0.75 g a.s./L
Termination rate of the eggs [%] [2)]	17.8 %	16.0 % (n.s.)	100.0 % (n.d.)
Termination rate of the young larvae [%] [2)]	10.2 %	12.4 % (n.s.)	99.6 % (*)
Termination rate of the old larvae [%] [2)]	6.4 %	3.6 % (n.s.)	43.8 % (*)
Mean brood termination rate over all stages	11.5 %	10.7 % (n.s.)	81.1% (*)
Mean mortality of worker bees/colony/day during pre-application phase [3)] during the entire post-application phase [3)]	20.3 21.2	4.4 (n.s.) 8.8 (n.s.)	5.8 (n.s.) 25.4 (n.s.)
Mean mortality of pupae + larvae/colony/day during pre-application phase [4)] during the entire post-application phase [4)]	0.9 0.9	0.1 (n.s.) 0.0 (n.s.)	2.0 (n.s.) 1.3 (n.s.)
Mean Number of Bees before Application[5)]	15030	12630	10035

[1)] test and reference item were mixed with sugar solution
[2)] mean termination rate of 3 colonies per treatment group
[3)] mean number of dead honeybees per day and colony found in dead bee traps
[4)] mean number of dead pupae/larvae per day and colony found in dead bee traps
[5)] mean number of bees per colony

Statistics: n.s. = not statistically significant compared to the control;* = statistically significant compared to the control; n.d. = not determined; Student t-test, $\alpha = 0.05$, pairwise comparison, two-sided (before application), one-sided greater (after application)

Conclusion:

Overall, it can be concluded according to the results of this study that the administration of Prothioconazole SC 480 G fortified sugar syrup (470 ppm prothioconazole) to honey bee colonies does neither adversely affect honey bee colonies nor bee brood development.

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Report:	KCA 8.3.1.3/02 [redacted]; 2015; M-532419-01-1
Title:	Assessment of side effects of prothioconazole EC 250 G on the honeybee (Apis mellifera L.) in the semi-field after one application on Phacelia tanacetifolia in Germany 2015
Report No.:	S15-02997
Document No.:	M-532419-01-1
Guideline(s):	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (PISTORIUS et al., 2012); OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s):	no major deviations
GLP/GEP:	**yes**

Objective:

The aim of the study was to evaluate potential side effects of a spray application of Prothioconazole EC 250 G on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the OECD guidance document No. 75 (2007), with methodological improvements by the AG Bienenschutz ([redacted]., 2012).

Material and methods:
Test item: Prothioconazole EC 250 G; analyzed a.s. content: 246.9 g/L; Batch No. ECE2101729; Specification No.: 102000008022, Density: 1.005 g/cm³ (at 20°C).

The crop used was full-flowering *Phacelia tanacetifolia*, the study was conducted in [redacted] in [redacted], Germany.
The study included three treatment groups with four replicates (tunnels) each: one tap-water treated control group (C), one test-item group (T) and one reference item group (R).
Applications were made at full-flowering (BBCH 64 – 65) with honeybees actively foraging on the crop. The target application rate of the test item Prothioconazole EC 250 G was 187.5 g a.s./ha (actual mean rate applied 199.2 g a.s./ha). Tap water was applied in the control group and Insegar was applied at a target rate of 1200 g product/ha in the reference item group (corresponding to 300 g fenoxycarb/ha). The spray volume was 400 L/ha in all treatment groups. The initial mean colony sizes per treatment group were in the range of 8109 to 8759 bees. The honeybees remained in the tunnels for 12 days and colonies were assessed twice during the confined phase and four times afterwards.

The following endpoints were assessed:
- Total and mean number of dead bees on the linen sheets in tunnels, in the dead bee traps and in the dead bee bottoms before as well as after the start of exposure in T and the application in C and R, respectively.
- Flight intensity (mean number of forager bees/m² *Phacelia tanacetifolia*) before as well as after the start of exposure in T and the application in C and R, respectively.
- Behaviour of the bees in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony and assessment date).
- Development of the bee brood assessed in individual brood cells. For this particular assessment, between 206 and 385 individually marked cells per colony were selected.

Findings:
Biological results:
Mortality:
Throughout the study (before and following exposure) worker bee mortality was similar across all treatments, indicating no effect of the test item. Statistically significant higher values in T were found on 7DAA and 23DAA but these were only minor in nature and not related to the treatment.
The pupal mortality in T and C was on a very low level throughout the study. There were no statistically significant differences between C and T on any individual day of the pre- or post-application period.
The mean value for the entire confinement period (0DAA to 7DAA) was on a very low level in T (0.5 dead pupae/day). Although this value was statistically significantly different from the control (0.2 dead pupae/day), it was within the range of natural variability, only slightly higher than the pre-application mortality in T (0.2 dead pupae/day) and even lower than the pre-application pupal mortality in the untreated R (0.9 dead pupae/day recorded from 4DBA to 0DBA) and it is therefore not considered as biologically relevant and treatment related.
For the whole post-application period (0DAA to 26DAA), no statistically significant difference between C (0.4 dead pupae/day) and T (0.3 dead pupae/day) was observed.
In contrast, a clear and statistically significant effect of the reference item treatment R on pupal mortality was observed for the periods from 0DAA to 7DAA (0.8 dead pupae/day) and from 0DAA to 26DAA (35.3 dead pupae/day). Moreover, dead malformed pupae with white eyes or sickle shaped (rimmed)

eyes were observed in R on most days from 9DAA to 26DAA. Effects on pupae of the reference substance are a well-known effect.

Table CA 8.3.1.3- 2: Mortality

Assessment timing	Control (C)	Test Item (T)	Reference Item (R)
Daily mean mortality (dead worker bees/colony) ± STD			
4DBA – 0DBA)	49.0 ± 19.6	56.4 ± 20.9	55.5 ± 18.8
0DAA	30.0 ± 6.6	38.0 ± 13.0	16.5 ± 4.4
0DAA – 7DAA	40.9 ± 5.2	52.0 ± 13.8	24.7 ± 5.5
08DBA2 – 26DAA	23.8 ± 8.2	24.6 ± 6.4	17.8 ± 3.3
Daily mean mortality (dead larvae+pupae/ colony) ± STD			
4DBA – 0DBA)	0.3 ± 0.4	0.2 ± 0.2	0.9 ± 1.0
0DAA	0.0 ± 0.0	1.3 ± 1.5	0.0 ± 0.0
0DAA – 7DAA	0.2 ± 0.2	0.5 ± 0.2*	0.8 ± 0.4*
08DBA2 – 26DAA	0.4 ± 0.3	0.3 ± 0.3	35.3 ± 20.9*

DAA: days after application; DBA: days before application; STD: standard deviation
*: statistically significantly higher than control group

Flight intensity:

During the pre-application period (4DBA to 0DBA), flight activity in T was slightly though statistically significantly lower than the control (Tukey's test, two-sided, α = 0.05). Since T was still untreated at this time, this difference is not related to the test item. Pre-application flight activity in T was on the same level as R which may be used for comparison since it was also still untreated at this time (not significantly different; Tukey's test, two-sided, α = 0.05). Therefore, pre-application flight activity in T was on a normal level for this kind of crop and within the natural range of variability.
After the application until the end of the confinement period (0DAA to 7DAA), foraging rates in the test item treatment were slightly lower but not statistically significantly different from the control. Actually, flight activity in T was higher after the application than before, and no repellence effect could be discerned.
On the day of the application (0DAA), the mean daily flight intensity, assessed over a period of 6 hours, accounted to 26.2, 21.3 and 25.2 forager bees/m², for C, T, and R, respectively (no statistically significant differences; Student's t-test, method pooled, one-sided, α = 0.05). The slight difference of flight activity in T compared to the control has no biological relevance and was on a normal level (higher than before application at 0DBA) in T throughout this day.
Overall, none of these slight differences between C and T is considered as biologically relevant or treatment-related.

Table CA 8.3.1.3- 3: Flight intensity

Assessment timing	Control (C)	Test Item (T)	Reference Item (R)
Daily mean flight intensity (bees/m²) ± STD			
4DBA – 0DBA)	13.3 ± 3.3	8.2 ± 1.6*	9.4 ± 2.0
0DAA	26.2 ± 4.4	21.3 ± 3.0	25.2 ± 4.6
0DAA – 7DAA[1)]	19.0 ± 3.1	15.6 ± 2.0	19.8 ± 1.0

DAA: days after application; DBA: days before application; STD: standard deviation
*: statistically significantly lower than control group
[1)] Data on 4DAA and 6DAA were excluded from the calculation of mean values and STD because there was hardly any flight activity in any treatment due to bad weather

Behaviour of the Bees:
Small numbers of bees displaying unusual behaviour were observed in T on several days after the application (0DAA, 1DAA, 3DAA, 7DAA, 11DAA, 16DAA, 18DAA, 21DAA, 26DAA), but similar observations were also made in the control during this period, and in few cases also in the untreated C and T before application (4DBA to 0DBA). Therefore, no test item related adverse effect on honeybee behaviour was discerned.

Development of Honeybee Brood in Individual Cells:
In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+23) was acceptable at 30.57%.
In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were clearly lower than those observed in the control, indicating a strong adverse effect.
The mean brood and compensation indices as well as the mean termination rates in R were statistically significantly different from the respective values in the control for all post treatment assessments (Student's t-Test, method pooled, one-sided, $\alpha = 0.05$). The mean termination rate at the end of the observation period (BFD+23) was 97.54 %, indicating that the majority of the initially marked eggs had not completed its development.
In the test item treatment group T the brood and compensation indices were slightly lower and mean termination rates were slightly higher than in the control on all assessment dates after BFD 0. The mean termination rate at the end of the observation period (BFD+23) was at 46.63%. No statistically significant differences between control and test item were found.

Table CA 8.3.1.3- 4: Brood and compensation indices and termination rates

Replicate	Brood index / Compensation index at x days after brood area fixing day (BFD)					Termination rate (BFD +23)
	0	+6	+11	+16	+23	
Control	1.00/1.00	2.48/2.51	2.97/3.01	2.93/3.03	3.47/3.98	30.57
STD	0.00/0.00	0.65/0.63	0.71/0.68	0.72/0.64	0.98/0.73	19.61
Test item T	1.00/1.00	1.88/1.96	2.32/2.38	2.30/2.61	2.67/3.79	46.63
STD	0.00/0.00	0.14/0.14	0.17/0.15	0.20/0.27	0.33/0.34	6.47
Reference item R	1.00/1.00	0.12*/0.18*	0.12*/0.23*	0.10*/1.09*	0.12*/2.57*	97.54*
STD	0.00/0.00	0.11/0.15	0.11/0.17	0.12/0.47	0.14/0.70	2.75

BFD: Brood area fixing day; STD: Standard deviation
*: Statistically significantly lower (brood and compensation indices) or higher (termination rate) compared to the control

Strength of the Colonies:
The overall development of colony strength of all treatment groups showed fluctuations in a typical and normal range. The colony strength values of the test item group were on approximately the same level during the entire study than the corresponding values of the control group, except colony Td where a slight decrease of the colony size from 1DBA to 5DAA was observed while all other colonies were growing. No similar observation was made in the other colonies of treatment T and colony Td developed normal on all following assessments. Therefore, no test-item related adverse effects on colony strength were observed.

Development of the Brood Area:

The mean amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) was assessed.
Overall, on the level of whole colonies, honeybee brood development in the test item treatment group T was not affected when compared to the control.

Development of the Food Storage Area:
The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed. All colonies were well provided during the course of the study and there was no lack of pollen or nectar in any colony at any assessment date. No test-item related adverse effects on the development of the food storage area were observed.

Conclusion:
Prothioconazole EC 250 G was applied at a target rate corresponding to 187.5 g a.s./ha at full-flowering *Phacelia tanacetifolia* during honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated.
No test-item related adverse effects on mortality of adult worker bees, flight intensity and behaviour were observed. No biologically relevant effect in pupae mortality was observed over the entire test period.
The quantitative assessments of brood development in individually marked cells containing eggs did not result in statistically significant differences on honeybee brood development.
No test-item related adverse effects on colony strength (mean number of bees per colony), amount of brood (mean number of cells covered with the different types of brood) or on the development of the food storage area were observed.

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

A number of studies on non-target arthropods were evaluated in the monograph, many of these studies used the previous representative formulation, for summaries of the studies please refer to the Monograph.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Studies on *Aphidius rhopalosiphi* have been conducted with the representative formulations of prothioconazole and are presented in MCP documents, Annex point 10.6.2.

CA 8.3.2.2 Effects on Typhlodromus pyri

Studies on *Typhlodromus pyri* have been conducted with the representative formulations of prothioconazole and are presented in MCP documents, Annex point 10.6.2.

CA 8.4 Effects on non-target soil mesoand macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

For information on studies already evaluated during the first EU review of prothioconazole, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. Additional studies on earthworms were performed with the representative formulations and soil metabolites of prothioconazole and are submitted within this Supplementary Dossier:

Table CA 8.4.1- 1: Endpoints used in risk assessment for earthworms for prothioconazole and its metabolites

Test substance	Test species	Ecotoxicological endpoint	Reference
Prothioconazole	*Eisenia fetida* acute 14 days, mixed	LC_{50} >1000 mg a.s./kg dws	[redacted] (2000) M-031137-02-1 KCA 8.4.1/01
JAU 6476-desthio	*Eisenia fetida* acute 14 days, mixed	LC_{50} >1000 mg a.s./kg dws	[redacted] (2000) M-038880-01-1 KCA 8.4.1/02
JAU 6476-methyl	*Eisenia fetida* acute 14 days, mixed	LC_{50} >1000 mg a.s./kg dws	[redacted] (2000) M-020680-01-1 KCA 8.4.1/03
Prothioconazole EC 250	*Eisenia fetida* reproduction 56 d, sprayed	NOER ≥ 4.0 kg prod./ha NOER ≥ 1.0 kg a.s./ha NOEC ≥ 3.96 mg a.s./kg dws	[redacted] (2002) M-033501-02-1 KCA 8.4.1/04
JAU 6476-desthio	*Eisenia fetida* reproduction 56 d, mixed	NOEC **0.5 mg p.m./kg dws***	[redacted] (2000) M-026193-01-2 KCA 8.4.1/05
JAU 6476-S-methyl	*Eisenia fetida* reproduction 56 d, mixed	NOEC **250 mg p.m./kg dws***	[redacted] (2000) M-021370-01-1 KCA 8.4.1/06
Prothioconazole FS 100	*Eisenia fetida* reproduction 56 d, wheat seedlings	NOER ≥ 1150 kg seed/ha ≥ 122 g a.s./ha	[redacted] & [redacted] (2001) M-088126-01-1 KCA 8.4.1/07
Prothioconazole FS 300 G	*Eisenia fetida* reproduction 56 d, mixed	NOEC ≥1000 mg prod./kg dws **≥ 257 mg a.s./kg dws**	[redacted] (2007) M-287144-01-1 KCA 8.4.1/09
Prothioconazole EC 250	Natural earthworm populations, Field study up to 11 months, spraying	NOEAER **≥ 3 × 200 g a.s./ha**	[redacted] - [redacted] (2005) M-040814-03-1 KCA 8.4.1/08

[1)] Study endpoint refined with the actual test conditions: area 198 cm^2 and 500 g dry weight soil
* Adjusted by a factor of 2 to address the log Pow and the high organic matter content of 10% in the study
Bold values: Endpoints considered relevant for risk assessment

The EU-agreed endpoint for prothioconazole was derived from a study where PTZ EC 250 was sprayed onto the soil surface and the NOEC represents the highest application rate tested. This endpoint does not reflect the intrinsic toxicity of prothioconazole active substance to *E. fetida*. An earthworm reproduction study with Prothioconazole FS 100 (application of treated seeds) was evaluated during the EU review (2007) with a NOER of ≥122 g a.s./ha, however, a study where the test item was mixed homogeneously into the soil is not available with Prothioconazole FS 100. An earthworm reproduction study where the test item was mixed into soil is available with Prothioconazole FS 300 which is a slightly

different formulation compared to Prothioconazole FS 100. This study is considered to better describe the low intrinsic toxicity of prothioconazole to *E. fetida*. A summary is presented below.

Report:	KCA 8.4.1/09 [redacted],; 2007; M-287144-01-1
Title:	Prothioconazole FS 300 G: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5 % peat
Report No.:	LRT-RG-R-30/07
Document No.:	M-287144-01-1
Guideline(s):	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to assess the effect of Prothioconazole FS 300 G on survival, growth, and reproduction on the earthworm *Eisenia fetida* during an exposure into an artificial soil with five different test concentrations. The method of application and the test species are recommended by the international test guidelines (ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004).

Material and methods:

Test item: Prothioconazole FS 300 G, analyzed a.s. content: 296.8 g/L (25.7% w/w), Batch No. 2006-006218, TOX07688-00, Specification No. 102000014331.

Adult *Eisenia fetida* (approx. 7 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 test concentrations of 100 – 178 – 316 – 562 – 1000 mg test item/kg dry weight artificial soil. Non-re-usable plastic boxes (length x width x height ca. 16.5 cm x 12 cm x 6 cm, area approximately 200 cm2) were used as test vessels.

Each test vessel contained an amount of approximately 500 g artificial soil (dry weight) to obtain a depth of approximately 5 cm soil in the test vessels. The test item was mixed into the soil.

The vessels were kept in a temperature-controlled room at 20 ± 2°C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 - 800 Lux.

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:

Validity criteria:

All validity criteria were met as presented below:

Table CA 8.4.1- 2: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10%	0%
Mean change in growth of the adult earthworms in the control during the exposure period of four weeks	>-20%	+56.8%
Mean rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	175.0
Coefficient of variance of reproduction in the control	≤ 30%	17.5%

Biological results:
No mortality of adult earthworms was observed after 28 days of exposure at the control group and the test concentrations of 100, 178, 316 and 1000 mg prod./kg dry weight artificial soil. Mortality of 2.5% was determined at the test concentration of 562 mg prod./kg dry weight artificial soil. This mortality is not considered as treatment related, but rather a sporadic event.

No statistically significant different values for the growth relative to the control were observed at any test concentration including the highest concentration of 1000 mg prod./kg dry weight artificial soil.

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at any test concentration including the highest concentration of 1000 mg prod./kg dry weight artificial soil.

Table CA 8.4.1- 3: Effects on mortality and change in body weight of adult earthworms (*Eisenia fetida*) after an exposure of 28 days to Prothioconazole FS 300 G and the number of offspring per test vessel after 56 days.

Test item		Prothioconazole FS 300 G					
Test species		Earthworm (*Eisenia fetida*)					
Exposure		Mixed soil					
Treatment **[mg prod./kg dry weight soil]**	**Mortality of adult earthworms [%]**	**Mean change of body weight of the adults from day 0 to day 28 [%] (± Standard Deviation)**			**Mean number of offspring per test vessel after 56 days (±Standard Deviation)**		
Control	0	+56.8	± 7.9	---	175.0	± 30.7	---
100	0	+50.3	± 8.7	n.s. A	165.0	± 33.3	n.s. B
178	0	+51.6	± 3.3	n.s. A	165.0	± 12.9	n.s. B
316	0	+56.5	± 11.5	n.s. A	153.5	± 35.5	n.s. B
562	2.5	+54.9	± 14.4	n.s. A	166.5	± 10.1	n.s. B
1000	0	+54.2	± 6.9	n.s. A	154.3	± 16.4	n.s. B
NOEC related to growth:		≥ 1000 mg test item/kg dry weight artificial soil					
LOEC related to growth:		> 1000 mg test item/kg dry weight artificial soil					
NOEC related to reproduction:		≥ 1000 mg test item/kg dry weight artificial soil					
LOEC related to reproduction:		> 1000 mg test item/kg dry weight artificial soil					

A Result of a Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$
B Result of a Wiliams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$
n.s.: mean value not statistically significant different compared to the control ($p \geq 0.05$)

Conclusion:
The overall NOEC is determined to be ≥ 1000 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be > 1000 mg test item/kg dry weight artificial soil.

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

For information on studies already evaluated during the first EU review of prothioconazole, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. Additional studies on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) were performed with the representative formulations and soil metabolites of prothioconazole and are submitted within this Supplemental Dossier:

Table CA 8.4.2- 1: Endpoints used in risk assessment for Collembola and soil mites and additional studies for prothioconazole and its metabolites

Test substance	Test species	Ecotoxicological endpoint	Reference
Prothioconazole	*Folsomia candida* Reproduction 28 d, mixed	NOEC **≥1000mg a.s./kg dws[1)]**	[redacted] (2011) M-405273-01-1 KCA 8.4.2.1/06
	Folsomia candida Reproduction 28 d, mixed	NOEC ≥ 64 mg a.s./kg dws[1)]*	[redacted] (2002) M-034235-01-1 KCA 8.4.2.1/01
	Hypoaspis aculeifer Reproduction 34 d, mixed Lufa 2.1	NOEC ≥ 100 mg a.s./kg dws	[redacted] (2000) M-037786-02-1 KCA 8.4.2.1/02
JAU 6476-desthio	*Folsomia candida* Reproduction 28 d, mixed	NOEC 31.3 mg p.m./kg dws*	[redacted] & [redacted] (2002) M-035070-03-1 KCA 8.4.2.1/03
	Hypoaspis aculeifer Reproduction 14 d, mixed	NOEC **≥ 100 mg p.m./kg dws**	[redacted] (2014) M-491764-01-1 KCA 8.4.2.1/07
JAU 6476-S-methyl	*Folsomia candida,* Reproduction 28 d, mixed	NOEC ≥ 15.8 mg p.m./kg dws*	[redacted] & [redacted] (2001) M-087207-01-1 KCA 8.4.2.1/04
	Hypoaspis aculeifer Reproduction 14 d, mixed	NOEC **≥ 100 mg p.m./kg dws**	[redacted] (2014) M-491804-01-1 KCA 8.4.2.1/08

* Adjusted by a factor of 2 to address the log Pow and the high organic matter content of 10% in the study

1) The corrected NOEC of ≥ 32 mg/kg dws in the old *Folsomia candida* reproduction study with prothioconazole active substance (M-034235-01-1, KCA 8.4.2.1/01) was set above the highest concentration tested (64 mg/kg dws (no effects seen), which had to becorrected due to the peat content (10%)). The new study was conducted with test concentrations up to 1000 mg a.s./kg dws where as well no effects were observed up to the highest concentration tested. This endpoint better reflects the overall low toxicity of Prothioconazole to *Folsomia candida*

dw s = dry weight soil

Bold values: Endpoints considered relevant for risk assessment

CA 8.4.2.1 Species level testing

Report:	KCA 8.4.2.1/06 [redacted],; 2011; M-405273-01-1
Title:	Prothioconazole a.s.: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.:	FRM-COLL-118/11
Document No.:	M-405273-01-1
Guideline(s):	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
Guideline deviation(s):	minor deviations
GLP/GEP:	**yes**

Objective:

The purpose of this study was to assess the effect of prothioconazole a.s. on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Material and methods:
Test item: Prothioconazole a.s., prutiy: 97.1 % w/w (analytical), Batch No. EDFL004807, TOX09215-00, Specification No. 102000014040.
Toxic standard (Boric acid): 44, 67, 100, 150 and 225 mg Boric acid/kg artificial soil dry weight; control: untreated, solvent control: none.

10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 62.5, 125, 250, 500 and 1000 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CA 8.4.2.1- 1: Validity criteria

Validity criteria	Recommended	Obtained
Mean adult mortality	≤20 %	5 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1570
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	12 %

The most recent non-GLP-test (FRM-Coll-Ref-15/11, U. [redacted], March 08, 2011) with the reference item Boric showed an EC_{50} of 91 mg test item/kg artificial soil dry weight (95 % confidence limits from 80 mg to 104 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

Biological results:
A LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg artificial soil dry weight.

Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, $\alpha = 0.05$) revealed a statistically significant difference between control and the lowest treatment group with 62.5 mg test item/kg artificial soil dry weight. Because the other test concentrations up to 1000 mg test item/kg artificial soil dry weight revealed no significant difference to the control the NOEC is determined to be ≥ 1000 mg test item/kg artificial soil dry weight.

Table CA 8.4.2.1- 2: Effects of prothioconazole technical on *Folsomia candida* after 28 days exposure (nominal concentration)

Test item **Test object** **Exposure**	Prothioconazole a.s. *Folsomia candida* Artificial soil		
Nominal concentration (mg test item/kg soil dry weight)	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	5	1570 ± 188	-
62.5	5	1389 ± 176	89*
125	0	1510 ± 183	96 n.s.
250	5	1593 ± 111	102 n.s.
500	5	1658 ± 123	106 n.s.
1000	5	1608 ± 131	102 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)		≥1000	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)		>1000	

The calculations were performed with un-rounded 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)

Conclusions:

The test item prothioconazole technical showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at all test concentration.

Therefore, the $NOEC_{reproduction}$ was determined to be ≥ 1000 mg a.s./kg soil dry weight, and the overall $LOEC_{reproduction}$ was determined to be > 1000 mg a.s./kg soil dry weight.

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Report:	KCA 8.4.2.1/07 [redacted] E; 2014; M-491764-01-1
Title:	Prothioconazole-desthio (BCS-AA53879): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.:	14 10 48 102 S
Document No.:	M-491764-01-1
Guideline(s):	OECD 226 (2008)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine potential effects of prothioconazole-desthio 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 according to the OECD guideline 226 (2008).

Material and methods:

Test item: Prothioconazole-desthio (BCS-AA53879), purity: 99.5% w/w (analytical), Batch No. KTS9616-4-2.

Toxic standard (Dimethoate EC 400): 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg soil d.w.; control: deionised water, solvent control: none.

10 adult soil mites (females) were exposed to 10, 18, 32, 56 and 100 mg pure metabolite/kg dry weight (d.w.) of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.2% $CaCO_3$, at

19.7 - 21.1 °C and a photoperiod: light : dark = 16 h : 8 h (510 lx) and were fed every 2 - 3 days with *Tyrophagus putrescentiae* (SCHRANK).
Mortality and reproduction were determined after 14 days of exposure. Eigth replicates were performed in the control group and four replicates were done in the treated groups.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CA 8.4.2.1- 3: Validity criteria

Validity criteria	Recommended	Obtained
Mean mortality of adult females	≤ 20%	0.0%
Mean number of juvenile per replicate	≥ 50	201.8
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	13.6%

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

Biological results:
In the control group a parental mortality of 0.0% could be observed. The mortality in the test item treatment groups ranged between 0.0 and 2.5%.
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 201.8 in the control and 183.5, 215.3, 191.8, 191.8, 239.0 and 181.8 at concentrations of 10, 18, 32, 56 and 100 mg pure metabolite/kg soil d.w., respectively.

Table CA 8.4.2.1- 4: Effects of prothioconazole-desthio on *Hypoaspis aculeifer* after 14 days exposure (nominal concentration)

Test item **Test object** **Exposure**	Prothioconazole-desthio (BCS-AA53879) *Hypoasis aculeifer* Artificial soil					
Endpoint	**Treatment group (mg pure metabolite/kg soil d.w.)**					
	Control	**10**	**18**	**32**	**56**	**100**
Mortality of soil mites after 14 days (%)	0.0	2.5	0.0	2.5	2.5	0.0
Mean number of juveniles after 14 days	201.8	183.5	215.3	191.8	239.0	181.8
CV (%)	13.6	27.1	20.7	13.4	7.6	9.3
Reproduction (% of control)	100	91	107	95	118	90
	Reproduction			**Adult mortality**		
	(mg pure metabolite/kg soil d.w.)					
NOEC	≥ 100			≥ 100		
LOEC	> 100			> 100		
EC_{10}	> 100			> 100		
EC_{20}	> 100			> 100		

No statistically significant differences compared to the control (Fisher's Exact Binomial with Bonferroni Correction for mortality, $\alpha = 0.05$, one-sided greater and Dunnett-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)
Calculations were done using unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
Rt = mean number of juvenile mites in the treated group(s)
Rc = mean number of juvenile mites in the control group
CV (%) = Coefficient of variation

Conclusion:

The test item Prothioconazole-desthio (BCS-AA53879) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at all tested concentrations.
Therefore, the NOEC and LOEC for mortality and reproduction were determined to be ≥ 100 mg and > 100 mg pure metabolite/kg soil d.w., respectively.

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Report:	KCA 8.4.2.1/08 [redacted],; 2014; M-491804-01-1
Title:	Prothioconazole-S-methyl (BCS-AB94480): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.:	14 10 48 103 S
Document No.:	M-491804-01-1
Guideline(s):	OECD 226 (2008)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine potential effects of prothioconazole-S-methyl 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: Prothioconazole-S-methyl (BCS-AB94480), purity: 99.7 % w/w (analytical), Batch No. SES 12549-3-1, TOX10378-00.
Toxic standard (Dimethoate EC 400): 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg soil d.w.; control: untreated, solvent control: none.

10 adult soil mites (females) were exposed to 100 mg pure metabolite/kg dry weight (d.w.) of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.2 % $CaCO_3$, at 19.7 - 21.1°C and a photoperiod: light : dark = 16 h : 8 h (510 lx) and were fed every 2 - 3 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure. Eight repliactes were performed for each treatment.

Findings:
Validity criteria:
All validity criteria were met as presented below:

Table CA 8.4.2.1- 5: Validity criteria

Validity criteria	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	3.8 %
Mean number of juvenile per replicate	≥ 50	244.1
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	11.0 %

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

Biological results:
The test item caused no statistically significantly adverse effects on adult mortality (Fisher´s Exact Binomial test, $\alpha = 0.05$, one-sided greater) and reproduction (Student t-test, $\alpha = 0.05$, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg pure metabolite/kg soil dry weight.

Table CA 8.4.2.1- 6: Effects of prothioconazole-S-methyl on *Hypoaspis aculeifer* after 14 days exposure (nominal concentration)

Test item **Test object** **Exposure**	Prothioconazole-S-methyl (BCS-AB94480) *Hypoasis aculeifer* Artificial soil	
	Treatment group (mg pure metabolite/kg soil d.w.)	
	Control	**100**
Mortality of soil mites after 14 days (%)	3.8	2.5
Mean number of juveniles after 14 days	244.1	247.5
CV (%)	11.0	15.0
Reproduction (% of control)	100	101
	Reproduction	**Adult mortality**
	(mg pure metabolite/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC_{10}	> 100	> 100
EC_{20}	> 100	> 100

No statistically significant differences compared to the control (Fisher's Exact Binomial with Bonferroni Correction for mortality, α = 0.05, one-sided greater and Dunnett-t-test for reproduction, α = 0.05, one-sided smaller)
Calculations were done using unrounded values
Percent reproduction: (Rt / Rc) * 100 %
Rt = mean number of juvenile mites in the treated group(s)
Rc = mean number of juvenile mites in the control group
CV (%) = Coefficient of variation

Conclusion:

The test item prothioconazole-S-methyl (BCS-AB94480) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg pure metabolite/kg soil dry weight.
Therefore, the overall NOEC was determined to be ≥ 100 mg pure metabolite/kg soil dry weight, and the overall LOEC was determined to be > 100 mg pure metabolite/kg soil dry weight.

CA 8.5 Effects on soil nitrogen transformation

For information on studies already evaluated during the first EU review of prothioconazole, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. No additional studies were conducted as N-cycle studies for the active substance and the metabolites were already evaluated in the first EU Dossier (Baseline Dossier), which resulted in the Annex I inclusion under Directive 91/414/EEC in 2007, and found suitable for being used in the risk assessment. The endpoints are provided in the table below.

Table CA 8.5- 1: Studies on nitrogen transformation for prothioconazole and its metabolites

Test species	Test item	Test design	Ecotoxicological endpoint	Reference
N-cycle	Prothioconazole	28 d	no influence **≥ 2.71 mg a.s./kg dws**	[redacted] (1999) M-024673-01-1 KCA 8.5/01
C-cycle		28 d	No influence ≥ 2.71 mg a.s./kg dws	[redacted] (1999) M-024679-01-1 KCA 8.5/02
N-cycle	JAU 6476-S-methyl	28 d	no influence **≥ 2.69 mg a.s./kg dws**	[redacted] (1999) M-024931-01-1 KCA 8.5/03
C-cycle		28 d	no influence ≥ 2.69 mg a.s./kg dws	[redacted] (1999) M-024939-01-1 KCA 8.5/04
N-cycle	JAU 6476-desthio	42 d	no influence ≥ 0.27 mg a.s./kg dws	[redacted] (2000) M-033060-01-1 KCA 8.5/05
N-cycle		28 d	no influence **≥ 1.37 mg/kg dws**	[redacted] (2001) M-057459-01-1 KCA 8.5/06

CA 8.6 Effects on terrestrial non-target higher plants

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

Studies on non-target plants (seedling emergence and vegetative vigour) have been conducted with the representative formulations of prothioconazole and are presented in MCP documents, Annex point 10.6.2.

CA 8.6.1 Summary of screening data

Please refer to CA 8.6.

CA 8.6.2 Testing on non-target plants

Please refer to CA 8.6.

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

No additional studies were performed.

CA 8.8 Effects on biological methods for sewage treatment

No additional studies were performed. Please refer to the Baseline dossier (KCA 8.8 /01, M-012578-01-1) and to the original DAR for the first Annex I inclusion.

CA 8.9 Monitoring data

No monitoring data are available.