Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Isoxaflutole

Data Requirements

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

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

Date

2013-11-29

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M-472484-01-4

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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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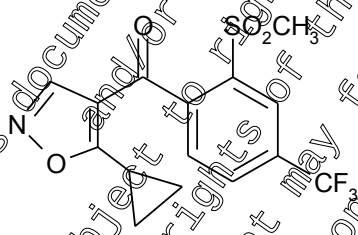
CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

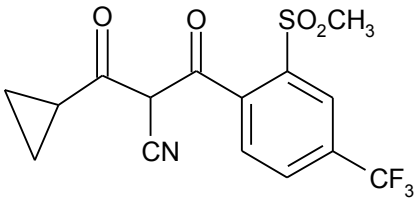
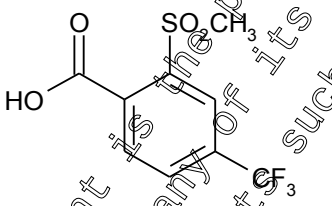
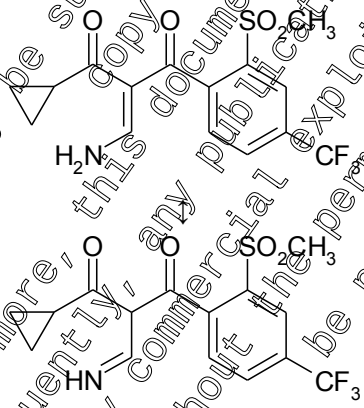
INTRODUCTION

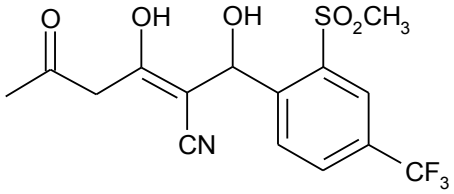
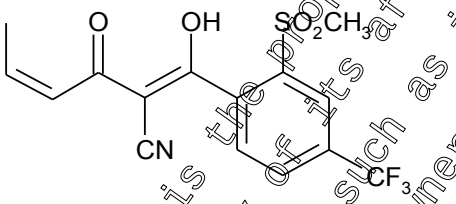
Isoxaflutole (RPA 201772) is an herbicidal active substance. In early 1996, the original Annex II dossier was submitted to the Netherlands being the designated Rapporteur Member State. The representative use supported for the peer review process was pre-emergence treatment of maize at a rate of 100 g a.s./ha in central and southern Europe.
In this renewal of approval dossier, the safe uses in maize and sweet corn will be presented under new scientific and regulatory aspects.

List of synonyms and codes

In the original study reports on metabolism of isoxaflutole the metabolites are denominated by different synonyms. In order to present a common system of nomenclature for the evaluation of this parent substance and its degradation products in the dossier a complete list of metabolites is placed in front of this Section.

Report name **Structure** **IUPAC name** **CAS name** **[CAS registry number]**	**Molecular formula** **molar mass** **Other names / codes**	**Occurrence**
Isoxaflutole (parent substance)  (5-cyclopropyl-1,2-oxazol-4-yl)[2-methylsulfonyl-4-(trifluoromethyl)phenyl]methanone (IUPAC) Methanone,(5-cyclopropyl-4-isoxazolyl)(2-(methylsulfonyl)-4-(trifluoromethyl)phenyl}- (9CI) (CAS) CAS no: 141112-29-0	$C_{15}H_{12}F_3NO_4S$ 359.32 g/mol **Isoxaflutole** (common name) RPA 201772 RPA 591428 AE B197278 BCS-AH21981	Parent substance used as test material in all reports

Report name Structure IUPAC name CAS name [CAS registry number]	Molecular formula molar mass Other names / codes	Occurrence
Diketonitrile  2-cyclopropylcarbonyl-3-(2-methylsulfonyl-4-trifluoromethylphenyl)-3-oxopropanenitrile (IUPAC) Benzenepropanenitrile, a-(cyclopropylcarbonyl)-2-(methylsulfonyl)-b-oxo-4-(trifluoromethyl)- (CAS) CAS no: 143701-75-1	$C_{15}H_{12}F_3NO_4S$ 359.32 g/mol RPA 202248 AE 0540092 BCS-AB59005 DKN	Soil, aerobic Soil, anaerobic Soil photolysis Abiotic hydrolysis
Benzoic acid  2-mesyl-4-trifluoromethylbenzoic acid (IUPAC) Benzoic acid, 2-(methylsulfonyl)-4-(trifluoromethyl)- (CAS) CAS no: 142994-06-7	$C_9H_7F_3O_4S$ 268.22 g/mol RPA 203328 AE B197555 Pyrasulfotole-benzoic acid BCS-AB49990 BA IFT acid	Soil, aerobic Soil, anaerobic Soil photolysis
RPA 205834 Enamine-amidine tautomeric forms:  2-(1-aminomethylidene)-1-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)propane-1,3-dione (IUPAC) CAS no: n.a.	$C_{15}H_{14}F_3NO_4S$ 361.34 g/mol RPA 205834 AE 0692291 BCS-BY16134	Soil, aerobic Soil, anaerobic Water/Sediment

Report name **Structure** **IUPAC name** **CAS name** **[CAS registry number]**	**Molecular formula** **molar mass** **Other names / codes**	**Occurrence**
Peak 14  (2Z)-3-hydroxy-2-{hydroxy[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methyl}-5-oxohex-2-enenitrile (IUPAC) CAS no: n.a.	$C_{15}H_{14}F_3NO_5S$ 371 g/mol Met 14 AE Code: None BCS Code: None	Photolysis, buffer.
Peak 20  (2Z,4Z)-2-{hydroxy[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methylene}-3-oxohex-4-enenitrile (IUPAC) CAS no: n.a.	$C_{15}H_{12}F_3NO_4S$ 359 g/mol Met 20 AE Code: None BCS Code: None	Photolysis, buffer

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

CA 8.1.1.1 Acute oral toxicity to birds

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

CA 8.1.1.2 Short-term dietary toxicity to birds

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

One additional study on dietary toxicity to birds was performed, which was not submitted during the first Annex I inclusion process and is submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. This study will be summarized below.

Table 8.1.1.2- 1: Additional study on avian dietary toxicity of RPA 203328

Test substance	Exposure	Test species	Endpoint	References Doc. No.
RPA 203328	5-d dietary study	Bobwhite quail	5-day LC_{50} > 5620 ppm LDD_{50} > 2619 mg p.m./kg bw/d NOEL > 5620 ppm	[redacted] et al. (1998) B004404 M-241327-01-1 KCA 8.1.1.2/04

Metabolite RPA 203328

Report:	[redacted];[redacted];[redacted];[redacted];[redacted];1998;M-241327-01
Title:	RPA 203328: A Dietary LC50 Study with the Northern Bobwhite
Report No:	B004404
Document No:	M-241327-01-1
Guidelines:	**OECD; 205; USEPA (=EPA); FIFRA 71-2; Deviation not specified**
GLP/GEP:	**yes**

Objective:

The objective of this study was to characterize dietary toxicity potential of RPA 203328 (metabolite of isoxaflutole) following feeding in the diet over 5 days to juvenile Northern Bobwhite (*Colinus virginianus*).

Materials and Methods:

Test item: RPA 203328 (metabolite of isoxaflutole) technical, Batch No: NM1874, CAS No: 142991-06-7, purity: 990 g/kg, white powder

Groups of five 10 days old Northern Bobwhite were fed in duplicate a diet containing either 562, 1000, 1780, 3160 or 5620 ppm RPA 203328 for 5 days. A control group of 30 animals was fed untreated diet. Following five-day exposure, all groups were given untreated feed for three days.

The average temperature during the test in the brooding compartment of the pens was 38 °C. Relative humidity was 63% and the photoperiod was 16 hours of light and 8 hours dark (215 lux).

Birds were observed at least twice daily for mortality and sublethal effects. Animal body weight was measured at test initiation, at the end of the exposure period on day 5 and at test termination on day 8. Feed consumption was determined by weighting the feed.

Samples of the test diets were collected at test initiation from the 562 and 5620 ppm treatment level test homogeneity of the test substance in the diet. Also samples from 1000, 1780 and 3160 ppm were taken at test initiation to verify test concentrations. At the end of the exposure period samples were taken from all treatment levels.

Dates of experimental work: August 06, 1998 to August 14, 1998

Results:

Validity criteria:

The tested parameters of the bird population used, particularly of the control pairs, were within the acceptable limits as specified in the respective testing guidelines. The definitive test criteria for control groups as set out in the respective testing guidelines and the corresponding values obtained in this study are shown in the table below.

Validity Criteria	Definitive test criteria	Present study
Mortality of control group	< 10%	0%
Stability of test item in the diet (after 5 days of test period)	> 80%	84 – 91%
Mortality at lowest treatment level	Not compound-related mortality	No mortalities

All validity criteria for the study were met.

Analytical results:

Mean values for the two test concentrations (562 and 5620 ppm) sampled at test initiation were 545 ± 9.4 ppm and 5500 ± 167 ppm. Therefore the standard deviations were 1.72 and 3.04%. Diet samples collected at test initiation for 1000, 1790 and 3110 ppm showed 99, 101 and 98% of nominal concentrations. At test termination 84, 91, 85, 88 and 90% of nominal were measured for the test levels of 562, 1000, 1780, 3160 and 5620 ppm.

Biological results:

No mortalities were observed throughout the study at any treatment level and in the control. One bird at the 562 ppm treatment level showed displayed lethargy, reduced reaction to external stimuli and a ruffled appearance on day 7. The bird had recovered by morning of the next day and was normal in appearance and behaviour. Based upon the fact that clinical signs were isolated to a single bird and were not observed at higher test concentrations, they were not considered to be treatment related.
When compared to the control group, there were no apparent treatment related effects on body weight among the birds in the treatment groups. Additionally, there were no treatment-related effects on feed consumption at any of the tested concentrations.

Conclusion:

It was concluded that the dietary 5-day 50%-lethal concentration (LC_{50}) of RPA 203328 (metabolite of isoxaflutole) in Northern Bobwhite was higher than the highest tested concentration of 5620 ppm RPA 203328. The No Observed Effect Level (NOEL) was 5620 ppm RPA 203328.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

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

One additional study on reproductive toxicity to birds with the metabolite RPA 202248 and a statement justifying the use of the derived endpoint in the long-term risk assessment were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal.

The documents will be summarized below.

Table 8.1.1.3- 1: Additional studies on reproductive toxicity of metabolites of isoxaflutole

Test substance	Time scale/Study type	Test species	Endpoint	References Doc. No.
RPA 202248	Reproductive toxicity	Bobwhite quail	NOEL ≥ 500 ppm NOEL ≥ 43.6 mg p.m./kg bw/d	[redacted] et al. (1999) B008788 M-238510-01-1 KCA 8.1.1.3/01
Isoxaflutole/ RPA 202248	Expert statement		Risk assessment is based on primary and longer available metabolite RPA 202248 instead of isoxaflutole	[redacted], A. (2005) M-254543-01-1 KCA 8.1.1.3/02

Metabolite RPA 202248

Report:	[redacted];[redacted];[redacted];[redacted];1999;M-238510-01
Title:	The Reproductive Toxicity Test of RPA-202248 with the Northern Bobwhite (Colinus virgianicus): RPA 202248
Report No:	B002788
Document No(s):	Report includes Trial Nos.: 029809 14518 M-238510-01-1
Guidelines:	**US-EPA, Subdivision E, § 71-4 (1982); Deviation not specified**
GLP/GEP:	**yes**

Objective:

The objective of this study was to characterize the reproduction toxicity potential of RPA 202248 (metabolite of isoxaflutole) following feeding in the diet over a period of 204 days to adult Bobwhite Quail (*Colinus virginianus*).

Materials and Methods:

Test item: RPA-202248 (metabolite of isoxaflutole), Batch No: DJA16-R, purity: 99.9%, CAS No: 143701-75-1

Groups of 18 pairs (1 male and 1 female) of adult Bobwhite Quail were fed diet containing 62.5, 125, 250 or 500 ppm RPA 202248 for 204 days. A control group was fed untreated diet.

Birds were examined daily for mortality and sublethal effects. Body weight was measured 4 times at regular intervals. Feed consumption was measured weekly for 23 weeks and six days. At necropsy, birds were examined externally and internally for macroscopically visible changes.

During egg laying period number of eggs was determined daily. Egg quality (number of broken or cracked eggs) was determined weekly, the shell thickness of the intact eggs from each female was measured weekly. All other intact eggs were incubated. The fertility eggs were determined after 14 days of incubation and on day 21 the number of viable embryos was recorded. Hatched chicks were counted, weighed and housed for further 14 days while they were observed daily for mortality and sublethal effects.

Throughout the test period, the mean temperature in the experimental area was 22.7 °C (range 20.0 – 27.8 °C) and the relative humidity was 49% on average (range: 21 - 76%). The lighting cycle was as follows:

First 8 weeks of test period: 7 h light : 17 h darkness
After week 8 until test termination: 17 h light : 7 h darkness

Dates of experimental work: January 27, 1999 to August 19, 1999

Results:

Biological results:

Mortality

During the test eleven birds died. These mortalities were not considered to be treatment-related. There were a number of females that died early during the egg laying period. The cause of mortality appeared to be from impacted eggs. The lot of Purina basal diet most recently mixed was analyzed and determined to have a Vitamin D deficiency. This deficiency apparently effected calcium content of the egg shells during the early days of the egg laying period. The diet mix was immediately reformulated with a different lot of feed. This corrected the problem within a day, and only one bird subsequently died from an impacted egg. There were no observations made on the post-mortem documentation regarding any toxicity-related effects.

Behaviour, feed consumption and body weight

There were no behavior abnormalities that would indicate any treatment-related effect. There were notations of cage injury and pair aggression which is typical in a bobwhite quail colony. There were no significant differences in feed consumption and body weight detected between any of the treatment groups and the corresponding controls.

Reproductive parameters

No significant differences compared to the control were detected for the number of laid eggs, impacted eggs, egg shell thickness, egg fertility, viable embryos, number of hatched chicks and surviving chicks.

A statistically significant difference was detected for the surviving chicks in the 500 ppm treatment group. The weight in this treatment group was 2.04 g less than in the control group. Based on the vigour, general health and survival of the hatchlings in the 500 ppm group, this difference was not considered to be treatment-related, nor biologically significant. This opinion was substantiated by comparing the mean 14-day survivor weights against historical control data from five previous reproduction studies. The mean body weights of the treatment groups from this study were not different from the mean body weights of historical controls.

Conclusion:

Dietary administration for 204 days of up to 500 ppm RPA 202248 (metabolite of isoxaflutole) had treatment related effect on the growth or reproductive performance of Bobwhite Quail.

The No Observed Effect Level (NOEL) was 500 ppm.

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Report:	[redacted]; [redacted];2005;M-254543-01
Title:	Long-term avian risk assessment of MERLIN - response to the Italian ministry of health
Report No:	M-254543-01-1
Document No:	M-254543-01-1
Guidelines:	**n.a., Deviation not specified**
GLP/GEP:	**n.a.**

The purpose of this statement is to provide a justification on the selected approach of Bayer CropScience why the notifier considers an avian reproduction study with parent isoxaflutole to be dispensable, also considering animal welfare aspects.

In environmental fate aspects, the normalised mean DT_{50} field of isoxaflutole in soil was determined to be about 0.6 days whereas the normalised mean $DT_{50\ field}$ of the primary metabolite RPA 202248 (which still contains the active moiety/toxophor) has been determined to be 16-times longer, i.e. 9.8 days.

In studies on plants (as potential food item) only limited uptake of isoxaflutole has been observed. Further, isoxaflutole was not detected in any plant matrix due to its rapid metabolisation. Studies on animal metabolism showed as well rapid and extensive metabolisation of isoxaflutole. No parent isoxaflutole was determined in animal excreta, tissues, eggs or milk.

Based on the rapid degradation of the active substance in the environment and in treated plants and animals, the long-term avian risk assessment for MERLIN® is based on the **NOEC of ≥ 43.6 mg/kg bw/d** derived from an avian reproduction study with the primary and longer available isoxaflutole metabolite **RPA 202248**.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

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

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

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

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

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

As the log P_{ow} of the active substance isoxaflutole and its metabolites is below the trigger (<3), no evaluation of secondary poisoning is needed. See MCP point 10.1.1.2 for more details.

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

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

CA 8.1.5 Endocrine disrupting properties

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

WHO/IPCS (2002)[1] provided the currently widely accepted definition "*An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations.*" An adverse effect has been defined also by WHO/IPCS (2009)[2]: "*Change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.*"

Wild Mammals

Based on a complete toxicological data set there is no evidence of any endocrine disrupting potential of Isoxaflutole in mammals. Furthermore isoxaflutole does not fall under the interim criteria for endocrine disruption.

Studies submitted for evaluation during the initial evaluation of Isoxaflutole demonstrated that Isoxaflutole is an inducer of hepatic phase I and phase II xenobiotic metabolizing enzymes. Secondary to this induction alterations of thyroid homeostasis through a known mechanism may be observed in some sensitive species. Isoxaflutole itself does not possess endocrine disrupting properties.

Further details of the relevant studies can be found in sections 5.3 and 5.8.2.

Birds

The population relevant effects of the primary metabolite of isoxaflutole, RPA 202248 (DKN), on birds were studied in a reproductive toxicity study on bobwhite quail. The active substance itself was not tested for reproductive effects because its environmental half-life in potential avian feed items is extremely short and exposure to the parent molecule is therefore expected very limited in time.

No effects on adult birds, offspring or reproductive parameters were seen up to and including the highest test level of 500 ppm. As reproduction was not affected in this avian species, it is concluded that there are no population relevant adverse effects of isoxaflutole to be expected. No additional studies are deemed necessary.

[1] WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2002. Global Assessment of the State-of-the-science of Endocrine Disruptors. WHO/PCS/EDC/02.2, 180 pp.

[2] WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. 689 pp.

CA 8.2 Effects on aquatic organisms

In order to complete the aquatic risk assessment and to address new data requirements according to Regulation (EC) No 1107/2009, additional studies were performed. In addition, tests on marine species and test with metabolites, which were no data requirement according to the old regulation and hence were not evaluated during the first EU review of this compound, will be summarized as well.

CA 8.2.1 Acute toxicity to fish

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

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

Table 8.2.1- 1: Additional studies for acute fish toxicity of isoxaflutole and its metabolite

Test substance	Test species/study type	Endpoint	References
Isoxaflutole	Fish, acute *Cyprinodon variegatus*	LC_{50} > 7 mg a.s./L	[redacted] (1994) R002592 M-162973-01-1 KCA 8.2.1/06
RPA 202248	Fish, acute *Cyprinodon variegatus*	LC_{50} > 78 mg p.m./L	[redacted] (2000) B002804 M-238523-01-1 KCA 8.2.1/07

Report:	[redacted];[redacted];1994;M-162973-01
Title:	RPA201772 technical - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under flow-through conditions
Report No:	R002592
Document No(s):	Report includes Trial Nos.: 10566.0194.6320.505 M-162973-01-1
Guidelines:	**USEPA (=EPA): FIFRA 72-3;Deviation not specified**
Deviations:	The guideline limit of 0.1 mL/L of solvent was exceeded in order to maximize solubility of the test substance.
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the acute toxicity of RPA 201772 (isoxaflutole) technical to sheepshead minnow (*Cyprinodon variegatus*). The study was designed as a flow-through experiment for 96 hours.

Materials and Methods:

Test item: RPA 201772 (isoxaflutole) technical, Batch code: 39 ADM 93; purity: 96.8 %; light yellow powder

Ten fish in each treatment were exposed in duplicate to nominal concentrations of 0.91, 1.5, 2.5, 4.2 and 7.0 mg a.s./L (corresponding to mean measured concentrations of 1.0, 1.6, 2.6, 3.8 and 6.4 mg a.s./L). In addition, a negative control (dilution water) and a solvent control (0.5 mL acetone/L) were tested.

The endpoints were expressed in terms of nominal concentrations.

Dilution water was natural filtered seawater with a salinity of 30 – 31 ‰ and a pH of 7.7. Water temperature was 21 - 23 °C during the test, the photoperiod was 16 hours of light and 8 hours dark (300 – 970 lux).

After 0, 24, 48, 72 and 96 hours fish were observed for mortality and sublethal effects. In the 0.91, 2.5 and 7.0 mg a.s./L test levels, the concentrations of the test substance was measured at test initiation and test termination at 96 hours.

Dates of experimental work: March 14, 1994 to March 18, 1994

Results:

Analytical results:

The results of analysis for test substance concentrations in the test solutions were 91 – 112% of nominal. Therefore, it is appropriate to use nominal test concentrations.

Biological results:

Throughout the 96 h study, no mortality or sublethal effects were observed at any treatment level.

The mortalities in the control and solvent control groups were both 0% throughout the test.

Conclusion:

It was concluded that the 96-hour 50% lethal concentration (LC_{50}) of RPA 201772 (isoxaflutole) technical in sheepheads minnow based on nominal concentration was higher than the highest tested concentration of 7 mg a.s./L.

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Metabolite RPA 202248

Report:	[redacted]; [redacted];2000;M-238523-01
Title:	RPA 202248 - Acute Toxicity to the Sheepshead Minnow (Cyprinodon variegatus) under Static Conditions
Report No:	B002804
Document No(s):	Report includes Trial Nos.: 10566.6574 GOod #18308 M-238523-01-1
Guidelines:	**USEPA (=EPA): FIFRA Guideline 72-3;Deviation not specified**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the acute toxicity of RPA 202248 (metabolite of isoxaflutole) to sheepshead minnow (*Cyprinodon variegatus*). The study was designed as a static experiment for 96 hours.

Materials and Methods:
Test item: RPA 202248 (metabolite of isoxaflutole), Batch No: DJA16-R, CAS No.: 143701-75-1, purity: 99.9 %.
Ten fish in each treatment were exposed in duplicate to nominal concentrations of 10, 17, 29, 48 and 80 mg RPA 202248/L (corresponding to mean measured concentrations of 10, 17, 29, 46 and 78 mg RPA 202248/L). In addition, a negative control (dilution water) and a solvent control (0.5 mL acetone/L) were tested. The endpoints were expressed in terms of nominal concentrations.
Dilution water was natural filtered seawater with a salinity of 33 ‰ and a pH of 7.9. Water temperature was 21 - 22 °C during the test, the photoperiod was 16 hours of light and 8 hours dark.
After 0, 24, 48, 72 and 96 hours fish were observed for mortality and sublethal effects. In all test levels, the concentrations of the test substance were measured at test initiation and after 96 hours.

Dates of experimental work: November 19, 1999 to November 23, 1999

Results:

Analytical results:
The result of analysis for test substance concentration in the test solution was 96 – 100% of nominal. Therefore, it is appropriate to use nominal test concentrations.

Biological results:
Throughout the 96 h study, no mortality or sublethal effects were observed at any treatment level.
The mortalities in the control and solvent control groups were both 0% throughout the test.

Conclusion:

It was concluded that the 96-hour 50%-lethal concentration (LC_{50}) of RPA 202248 (metabolite of isoxaflutole) in sheepheads minnow based on nominal concentration was higher than the highest tested concentration of 78 mg RPA 202248/L.

CA 8.2.2 Long-term and chronic toxicity to fish

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

An additional fish chronic study was performed, which was not submitted during the first Annex I inclusion process and is submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. This study will be summarized below.

Table 8.2.2- 1: Additional studies for chronic fish toxicity of isoxaflutole

Test substance	Test species/study type	Endpoint	References
Isoxaflutole	Fish, ELS *Pimephales promelas*	NOEC 0.102 mg a.s./L	[redacted] (2013) EBISX074 M-469327-01-1 KCA 8.2.2.1/02

CA 8.2.2.1 Fish early life stage toxicity test

Report:	[redacted]; [redacted]; 2013; M-469327-01
Title:	Early-life stage toxicity of isoxaflutole (tech.) to fish (Pimephales promelas) under flow-through conditions
Report No:	EBISX074
Document No:	M-469327-01-1
Guidelines:	**EPA-FIFRA § 72-4a/SEP-EPA-560/6-82-002 (1982)** **ASTM E 1241-92 (1992)** **OCSPP 850.1400 (1996)** **OECD No. 210 (1992);** **minor deviations, without any influence on the biological outcome of the study**
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of the test item during the early-life stages of fathead minnow (*Pimephales promelas*), expressed as NOEC, LOEC and MATC.

Materials and Methods:

Test item: isoxaflutole (tech.), purity 98.7% w/w, specified by origin batch no.: 6464/5/8/9, specification no.: 102000002961, Tox-No: 08283-02.

Test organism: Fathead minnow (*Pimephales promelas*), freshly fertilized eggs (< 24 hours old) were used at the start of exposure.

Eggs starting at <24 hours old were observed for hatch rate; young fish were assessed for abnormal behavior, physical changes, mortality and growth (length, dry weight).

Observations of fish were recorded daily throughout the study. In all test levels, the concentrations of the test substance were measured weekly (± 2 days).

Early-life stages of fathead minnow (eggs, larvae/fry) were exposed to five test concentrations, a control and a solvent control under flow-through conditions with four replicates per test level over 33 days (28 days post-hatch). The definitive study was conducted at the nominal test concentrations of 10.0, 32.0, 102.4, 327.7 and 1049 µg a.s./L.

Water temperature was 23.8 – 24.8 °C and a pH of 7.0 to 7.0 during the test. Mean dissolved oxygen (DO) concentrations ranged from 103 to 105 percent oxygen saturation. Light intensity in the room was between 404 - 791 lux with a photoperiod of 16-hours light and 8-hours dark.

Dates of experimental work: September 24 to October 10, 2013

Results:

Validity criteria:

Validity Criteria	**Recommended by guideline**	**Obtained in this study**
Mean hatching success (control)	> 66%	90.0%
Post-hatch average survival (control)	> 80%	100%
Survival per replicate (control)	> 70%	100%
Dissolved oxygen	> 60%	103-105%

The test fulfilled the validity criteria, with the exception of two minor cases. Short-term incidents (a decrease in water temperature and precipitations of the test substance) were observed during the study without resulting in any influence on the results and/or on the biological outcome of the study, as demonstrated by the overall control data.

Analytical results:

The mean measured concentrations of isoxaflutole in the test solutions during the test were 10.2, 32.7, 102.5, 302.9 and 1027 µg/L. These overall mean measured values ranged between 92 and 102 % of nominal during the test period for all test levels. Results are based on nominal concentrations.

Biological results:

Egg hatching began on study day 3 and was completed on study day 5, when 100 % of all fertilised and living embryos in the pooled control had hatched (defined as post hatch day 0). On this day mean hatching success/embryo survival (based on the number of inserted eggs) ranged overall between 89 and 93 % and showed no significant difference in any test concentration compared to the pooled control data.

Larval/fry survival was analysed between study day 6 and test termination on study day 33 (post hatch day 28). Data analysis showed a slight significant difference at the concentration of 327.7 µg a.s./L

and a clear significant difference at the concentration of 1049 µg a.s./L compared to the pooled control data. Mean larval/fry survival at test termination ranged from 83 to 100 % in all test levels including controls.

Up to the test concentration of 102.4 µg a.s./L and including the control and solvent control observations of single or few morphological and behavioural symptoms were made only in single or few fish and in single or few replicates. These observations are in line with historical control data without any test item relationship. Starting with the test concentration of 327.7 µg a.s./L and up to the highest test level (1049 µg a.s./L), it was evident, that the quantity of symptoms observed in individual fish was dose-dependently increased. Only in these two highest test level observations of spinal deformities were made, starting in most cases approximately on study day 21, which resulted in association with other symptoms in death or in an obviously reduced fitness.

Larval/fry growth, expressed as standard length and dry weight, was measured at test termination on study day 33 (post hatch day 28). Data analysis showed a statistically significant decrease in length and dry weights in comparison to the pooled control data at the two highest test concentrations of 327.7 and 1049 µg a.s./L. No significant decrease for both parameters was evident at any other test level when compared to the pooled control. Mean standard length ranged from 16.6 to 18.4 mm and mean dry weights from 14.5 to 19.1 mg over all test levels including controls.

Conclusion:

The overall chronic 33-day-NOEC observed in this study is 102 µg a.s./L and the respective overall chronic 33-day-LOEC is 328 µg a.s./L (based on survival, growth and morphological/behavioural effects).

The resulting Maximum Acceptable Toxicant Concentration (MATC) is 183.2 µg a.s./L.

CA 8.2.2.2 Fish full life cycle test

See point 8.2.2. No additional studies were performed.

CA 8.2.2.3 Bioconcentration in fish

See point 8.2.2. No additional studies were performed.

CA 8.2.3 Endocrine disrupting properties

Population relevant effects of IFT on fish were studied in a juvenile growth test with rainbow trout and in an early life-stage test (ELS) under flow-through conditions with fathead minnow *(Pimephales promelas)*. In the ELS the overall NOEC was 102 µg/L, based on the most sensitive endpoints larval/fry mortality, growth and swimming behaviour. In the juvenile growth test the NOEC is in the same range (100 µg/L (nom) or 80 µg/L (mm)) with the most sensitive endpoints being likewise mortality, growth and swimming behaviour.

Also an early life-stage test (ELS) with fathead minnow was performed for the main metabolite RPA 202248 (DKN). Up to a concentration of 10.0 (9.59 mean measured) mg/L no effects on any parameter in fish were observed.

Based on the absence of relevant effects it can be concluded that isoxaflutole is not a (potential) endocrine disrupter. No further testing is indicated to evaluate the endocrine disrupter potential of IFT to fish.

CA 8.2.4 Acute toxicity to aquatic invertebrates

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

Additional acute studies on aquatic invertebrates were performed, which were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. These studies will be summarized below.

Table 8.2.4- 1: Additional studies for acute aquatic invertebrates of isoxaflutole and its metabolites

Test substance	Test species/study type	Endpoint	References
Isoxaflutole	Invertebrate, acute *Chironomus riparius*	48 h-EC_{50} > 1.3 mg a.s./L	[redacted] (2013) EBISN014 M-468785-01-1 KCA 8.2.4.2/06
Isoxaflutole	Invertebrate, acute *Americamysis bahia*	48 h-LC_{50} 0.077 mg a.s./L	[redacted] (1994) C033878 M-227961-02-1 KCA 8.2.4.2/07
RPA 202248	Invertebrate, acute *Americamysis bahia*	48 h-LC_{50} 24 mg p.m./L	[redacted] (1995) R005386 M-170861-01-1 KCA 8.2.4.2/08
RPA 203328	Invertebrate, acute *Americamysis bahia*	48 h-LC_{50} 160 mg p.m./L	[redacted] (1998) C026471 M-211469-01-1 KCA 8.2.4.2/09

CA 8.2.4.1 Acute toxicity to Daphnia magna

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

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

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

However, studies on *Americamysis bahia* (mysid shrimp) for the parent compound and its metabolites (RPA 202248, RPA 203328) are available due to US-EPA requirements, which have not yet been submitted to the EU. In the US, the above mentioned studies on marine organisms are in support of the development of isoxaflutole for its use in field crops, particularly for those selected areas, where application is in direct vicinity of brackish or estuarine water bodies.

This situation is not relevant for the field use of a maize herbicide under European considerations. Since in the European risk assessment for plant protection products the ecosystem of concern is a freshwater body neighbouring fields of agricultural use, marine species are not considered relevant. However as *Americamysis bahia* is explicitly listed in the new data requirements (EC 283/2013), endpoints derived with this species are also taken into account for freshwater edge-of-field risk assessment.
Where the mysid endpoint is lower than the endpoint derived with *Daphnia magna* or *Chironomus riparius* the risk assessment for aquatic invertebrates exposed to isoxaflutole is based on mysid shrimp data although not required by the new data requirements (EC 283/2013) for an herbicide. This is clearly a worst case situation.

For information and to complete the data package describing the acute toxicity profile of isoxaflutole, an acute study with *Chironomus riparius* has been conducted. *Chironomus riparius* is the more relevant second invertebrate species to base a fresh water edge-of-field risk assessment on and is the preferred second species to be tested for insecticides or compounds with insecticidal activity according to the EFSA panel (EFSA Journal 461, 1-44; 2007).

The summaries of these studies are presented below.

Report:	[redacted];2013;M-468785-01
Title:	Acute toxicity of isoxaflutole (tech.) to larvae of Chironomus riparius in a 48 h static laboratory test system - LIMIT - test
Report No:	EBISN014
Document No:	M-468785-01-1
Guidelines:	**OECD Guideline No. 235 (Guideline for Testing of Chemicals, Chironomus sp., Acute Immobilisation Test, adopted July 28, 2011); EU Directive 91/414/EEC; Regulation (EC) No 1107/2009; US EPA OCSPP 850.SUPP.**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the effect of isoxaflutole on immobilisation of larvae of *Chironomus riparius*. The study was designed as a limit test under static conditions.

Materials and Methods:
Test item: isoxaflutole (tech.), purity: 98.5 % w/w was tested, specified by batch-ID.: AE B197278-01-01, TOX-no. 08283-03 and specification no.: 102000002961.
Larvae of *Chironomus riparius* (1st instars < 2-3 days old, 6 beakers for the limit test concentration and the controls, with 5 animals each) were exposed for 48 hours in a static test system (water only) to the only concentration of 1.5 mg a.s./L (practical solubility limit of isoxaflutole in the used test water). Measurements of the water temperature were done continuously in one negative control vessel and recorded hourly by a data logger (non-GLP data). Additionally water parameters (temperature, pH and oxygen) were measured in the controls and freshly prepared test solution of the limit test concentration on day 0 and on day 2 in the combined test solutions of the limit test concentration and the controls.

Quantitative amounts of isoxaflutole (tech.) and its metabolite RPA 202248 were measured in the freshly prepared test solution of the only test concentration and the controls at test start and on day 2, the end of exposure.

Dates of experimental work: September 19 to September 20, 2013

Results:

Test system:
Dissolved oxygen concentrations ranged from 8.4 to 8.7 mg O_2/L (8.7 mg O_2/L = 99 % O_2 - saturation), the water pH values ranged from 7.8 to 7.9 and the water temperature ranged from 20.3°C to 20.7°C over the whole period of testing, fulfilling the guideline requirements.

Analytical results:
The analytical findings of isoxaflutole and its metabolite RPA 202248 (DKN, AE0540092, BCS-AB59005) in the only test concentration at test start was 108 % of nominal. At test end (day 2) analytical findings of 96 % of nominal was observed. All biological results are based on nominal concentrations since the analytical measurements showed correct dosing and the test item was between 80 and 120 % over the test period.

Biological results:
Control mortality did not exceed 15 % and measured dissolved oxygen concentrations in the control and all test concentrations did not fall below 3 mg/L during exposure, fulfilling the guideline requirements.
Since no immobility occurred, no statistical evaluation of the results has to be performed. The NOEC after 24 and 48 hours was equal or higher than the limit test concentration of 1.5 mg a.s./L. Additionally, no sublethal effects were observed over the whole exposure period of 48 hours.
The EC_{50} was determined to be higher

Acute toxicity of isoxaflutole (tech.) to first instar-larvae of *Chironomus riparius* after 48 h (based on nominal concentrations)

Test concentration [mg a.s/L]	Exposed chironomids	Immobility			
		24 hours		48 hours	
		n	%	n	%
Control	30	0	0	0	0
Solvent control	30	0	0	0	0
1.5	30	0	0	0	0

Conclusion:
The EC_{50} value was determined to be greater than the limit test concentration of 1.5 mg a.s./L.

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Report:	[redacted];[redacted];1994;M-227961-02
Title:	RPA201772 technical - Acute toxicity to mysid shrimp (Mysidopsis bahia) under flow through conditions
Report No:	R002591
Document No(s):	Report includes Trial Nos.: 10566.0194.6319.515 10566.1094.6319.515 M-227961-02-1
Guidelines:	**USEPA (=EPA): FIFRA 72-3;** **Deviation not specified**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the acute toxicity of RPA 201772 (isoxaflutole) to mysid shrimp (*Americamysis bahia*). The study was designed as a flow-through experiment for 96 hours.

Materials and Methods:
Test item: RPA 201772 (isoxaflutole), Batch No. 39 ADM 93, purity: 96.8%, light yellow powder.
Ten mysid shrimps in each treatment were exposed in duplicate to nominal concentrations of 4.7, 9.3, 19, 37 and 75 µg a.s./L (corresponding to mean measured concentrations of 5.1, 9.8, 18, 36 and 77 µg a.s./L). In addition, a negative control (dilution water) and a solvent control (0.1 mL acetone/L) were tested.
The endpoints were expressed in terms of mean measured concentrations.
Dilution water was natural filtered seawater with a salinity of 30 - 31 ‰ and a pH of 7.8. Water temperature was 24 – 25 °C during the test, the photoperiod was 16 hours of light and 8 hours dark (220 - 860 lux).
At test initiation and after 24, 48, 72 and 96 h mysid shrimps were observed for mortality and sublethal effects. In the 5.1, 18 and 77 µg a.s./L test levels, the concentrations of the test substance were measured at test initiation and at test termination after 96 hours.

Dates of experimental work: April 16, 1994 to April 20, 1994

Results:

Analytical results:
The results of analysis for test substance concentration in the test solution were 93.3 - 108% of nominal. Therefore it is appropriate to use nominal concentrations. For US-EPA requirements however results were reported as mean measured.

Biological results:
At test termination (96 hours) mortality of 65, 75 and 95% was observed in the three highest test concentrations of 18, 36 and 77 µg a.s/L. At concentrations of 9.8 µg a.s./L, 25% mortality was recorded and no mortality was observed at the lowest test level of 5.1 µg a.s./L. Sublethal effects (e.g. lethargy, erratic swimming behaviour) were observed among all surviving mysid shrimps exposed to

36 and 77 µg a.s./L and among several of surviving mysid shrimps exposed to 18 µg a.s./L. In the control and solvent control no mortality was observed.

Mortality during 96-hour exposure of *Mysidopsis bahia* to RPA 201772 (isoxaflutole)

Mean measured concentration [µg a.s/L]	Replicate	Cumulative mortality after 24 h [%]	Cumulative mortality after 48 h [%]	Cumulative mortality after 72 h [%]	Cumulative mortality after 96 h [%]
Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
Solvent Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
5.1	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
9.8	A	0	0	0	30
	B	0	0	10	20
	Mean	0	0	5	25
18	A	0 [a]	0 [a]	30 [d]	80 [j]
	B	0 [b]	0	20 [f]	50 [cf]
	Mean	0	0	25	65
36	A	0 [a]	0 [ad]	40 [h]	90 [d]
	B	0 [a]	0 [d]	30 [ad]	60 [cf]
	Mean	0	0	35	75
77	A	0 [cd]	0 [af]	40 [fh]	90 [i]
	B	10 [bd]	10 [fg]	70 [i]	100
	Mean	5	5	55	95

a One of the surviving mysids was observed to be lethargic.
b One of the surviving mysids exhibited darkened pigmentation
c Two of the surviving mysids were observed to be lethargic
d One of the surviving mysids exhibited erratic swimming behaviour
e One of the surviving mysids exhibited darkened pigmentation and erratic swimming behaviour
f Two of the surviving mysids exhibited erratic swimming behaviour
g One of the surviving mysids exhibited darkened pigmentation and was observed to be lethargic
h Several of the surviving mysids exhibited erratic swimming behaviour
i All of the surviving mysids exhibited erratic swimming behaviour
j All of the surviving mysids were observed to be lethargic

The LC_{50} values (95% confidence limit) and No-Observed-Effect Concentration established during the 96-hour flow-through toxicity test exposing mysid shrimp to RPA 201772 Technical (isoxaflutole)

LC_{50} (µg a.s./L)[a]				No-Observed-Effect Concentration Through 96-Hours (µg a.s./L)
24-Hour[b]	48-Hour[b]	72-Hour[c]	96-Hour[d]	
> 77	> 77	58 (39-120)	18 (18-23)	5.1

[a] Corresponding 95% confidence limits are presented in parentheses.
[b] LC_{50} value empirically estimated as greater than the highest mean measured concentration tested.
[c] LC_{50} value and 95% confidence limit calculated by probit analysis.
[d] LC_{50} value and 95% confidence limit calculated by moving average angle analysis.

Conclusion:

It was concluded that the 96-hour 50%-lethal concentration (LC_{50}) of RPA 201772 (isoxaflutole), estimated by moving average angle analysis in mysid shrimps based on mean measured concentration was 18 µg a.s./L (95% CL: 14 – 23 µg a.s./L). The No-observed-Effect-Concentration (NOEC) after 96-hour exposure was 5.1 µg a.s./L.
According to the new data requirements for EU submissions relevant 48-hour LC_{50} value is estimated to be greater than the highest mean measured concentration tested (> 77 µg a.s./L).

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Metabolite RPA 202248

Report:	[redacted]; [redacted]; 1995; M-170861-01
Title:	RPA202248 - Acute toxicity to mysids (Mysidopsis bahia) under static renewal conditions
Report No.:	R005386
Document No(s):	Report includes Trial Nos.: 10566.0895.6369.510 M-170861-01-1
Guidelines:	**USEPA (=EPA): FIFRA, 72-3; Deviation not specified**
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the acute toxicity of RPA 202248 (metabolite of isoxaflutole) to mysid shrimp (*Americamysis bahia*). The study was designed as a static-renewal experiment for 96 hours.

Materials and Methods:

Test item: RPA 202248 (metabolite of isoxaflutole), Lot No: DJ A16-R, CAS: 143701-75-1, purity: 999g/kg, white powder

Ten mysid shrimps in each treatment were exposed in duplicate to nominal concentrations of 1.0, 5.2, 8.6, 14, 24 and 40 mg RPA 202248/L (corresponding to mean measured concentrations of 0.83, 4.5, 7.4, 11, 20 and 33 mg RPA 202248/L). In addition, a negative control (dilution water) and a solvent

control (0.5 mL acetone/L) were tested. After 24, 48 and 72 h of exposure test solutions were renewed. The endpoints were expressed in terms of mean measured concentrations.
Dilution water was natural filtered seawater with a salinity of 18 - 21 ‰ and a pH of 7.2 – 7.6. Water temperature was 24 – 26 °C during the test, the photoperiod was 16 hours of light and 8 hours dark (650 lux).
At test initiation and after 24, 48, 72 and 96 h mysid shrimps were observed for mortality and sublethal effects. In all test levels, the concentrations of the test substance were measured at test initiation and after 48 and 96 hours.

Dates of experimental work: November 27, 1995 to December 01, 1995

Results:

Analytical results:

Throughout the exposure period, a small amount of undissolved test material was observed on the bottom of the 11, 20 and 33 mg RPA 202248/L test solutions. The results of analysis for test substance concentration in the test solution were 75 - 87% of nominal. Therefore, it is appropriate to use mean measured test concentrations.

Biological results:

At test termination (96 hours) mortality of 100, 95, 95 and 100% was observed in the four highest test concentrations of 7.4, 11, 20 and 33 mg RPA 202248/L. At concentrations of 4.5 mg a.s./L, 60% mortality was recorded and no mortality was observed at the lowest test level of 0.83 mg RPA 202248/L. Among the surviving mysid shrimps in the two lowest test concentrations no sublethal effects were seen.
In the solvent control 5% mortality was observed and none in the dilution water control.

Mortality during 96-hour exposure of *Mysidopsis bahia* to RPA 202248 (metabolite of isoxaflutole)

Mean measured concentration [mg RPA 202248/L]	Replicate	Cumulative mortality after 24 h [%]	Cumulative mortality after 48 h [%]	Cumulative mortality after 72 h [%]	Cumulative mortality after 96 h [%]
Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
Solvent Control	A	0	0	0	0
	B	0	0	0	10
	Mean	0	0	0	5
0.83	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
4.5	A	0	0	0	50
	B	0	0	0	70
	Mean	0	0	0	60
7.4	A	0	10	70 [c]	100
	B	0	20	70	100
	Mean	0	15	70	100
11 [a]	A	0	30	90	90 [d]
	B	0	30	80 [c]	100
	Mean	0	30	85	95
20 [a]	A	0	40	90 [d]	100
	B	0 [b]	50	80 [c]	90
	Mean	0	45	85	95
33 [a]	A	10 [b]	50 [c]	80 [c]	100
	B	20	60	90	100
	Mean	15	55	85	100

a Small amount of undissolved test material was observed on the bottom of the test vessel
b Two of the surviving mysids were observed to be lethargic
c One of the surviving mysids was observed to be lethargic
d All of the surviving mysids was observed to be lethargic

The LC_{50} values (corresponding 95% confidence limits) and the No-Observed-Effect Concentration (NOEC) established for RPA 202248 (metabolite of isoxaflutole) and mysids (*Americamysis bahia*) during the 96-hour static renewal exposure

		95 % Confidence Limit[a]	
Observation Interval	LC 50[a] (mg a.s./L)	Lower (mg a.s./L)	Upper (mg a.s./L)
24-Hour[b]	> 33	-	-
48-Hour[c]	24	18	43
72-Hour[d]	7.7	5.5	11
96-Hour[e]	3.7	0.83	7.4
NOEC through 96 hours: 0.83 mg a.s./L			

[a] Based on mean measured concentrations of RPA 202248 (as active ingredient).
[b] LC_{50} value was estimated to be greater than the highest concentration tested, therefore, the corresponding 95% confidence limit could not be calculated.
[c] LC_{50} value and corresponding 95% confidence limit calculated by probit analysis.
[d] LC_{50} value and corresponding 95% confidence limit calculated by moving average angle analysis.
[e] LC_{50} value was estimated by nonlinear interpolation (corresponding 95% confidence limit calculated by binomial probability.

Conclusion:

It was concluded that the 96-hour LC_{50} of RPA 202248 (metabolite of isoxaflutole), estimated by nonlinear interpolation in mysid shrimps based on mean measured concentration was 3.7 mg RPA 202248/L (95% CL: 0.83 – 7.4 mg RPA 202248/L). The No-observed-Effect Concentration (NOEC) after 96 hours exposure was 0.83 mg RPA 202248/L.
According to the new data requirements for EU submissions relevant 48-hour LC_{50} value is calculated to be 24 mg RPA 202248/L.

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<u>Metabolite RPA 203328</u>

Report:	[redacted];[redacted];1998;M-211469-01
Title:	RPA 203328 - Acute toxicity to mysids (Mysidopsis bahia) under static acute conditions
Report No:	C026471
Document No(s):	Report includes Trial Nos.: 10566.0797.6436.510 M-211469-01-1
Guidelines:	**USEPA (=EPA): 72-3; Deviation not specified**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the acute toxicity of RPA 203328 (metabolite of isoxaflutole) to mysid shrimp (*Americamysis bahia*). The study was designed as a static experiment for 96 hours.

Materials and Methods:

Test item: RPA 203328 (metabolite of isoxaflutole), Log No: DA 1009, Batch No: NMI874, purity: 990 g/kg, beige powder.

Ten mysid shrimps in each treatment were exposed in duplicate to nominal concentrations of 9.3, 16, 26, 43, 72, 120 and 200 mg RPA 203328/L (corresponding to mean measured concentrations of 9.2, 15, 25, 42, 70, 120 and 200 mg RPA 203328 /L). In addition, a negative control (dilution water) and a solvent control (0.5 mL acetone/L) were tested. The endpoints were expressed in terms of nominal concentrations.

Dilution water was natural filtered seawater with a salinity of 31 - 35 ‰ and a pH of 7.0 – 8.0. Water temperature was 24 – 25 °C during the test, the photoperiod was 16 hours of light and 8 hours dark (970 lux).

At test initiation and after 24, 48, 72 and 96 h mysid shrimps were observed for mortality and sublethal effects. In all test levels, the concentrations of the test substance were measured at test initiation and at test termination after 96 hours.

Dates of experimental work: October 05, 1998 to October 09, 1998

Results:

Analytical results:

The results of analysis for test substance concentration in the test solution were 95 - 99% of nominal. Therefore, it is appropriate to use nominal test concentrations. For US-EPA requirements however results were reported as mean measured.

Biological results:

At test termination (96 hours) mortality of 15 and 95% was observed in the two highest test concentrations of 120 and 200 mg RPA 203328/L. At concentrations of 42 mg RPA 203328/L 5% mortality was recorded and no mortality was observed at the lower test level of 70, 25, 25 and 9.2 mg RPA 203328/L and the controls. Sublethal effects were observed among the surviving mysid shrimps in the 42, 70, 120 and 200 mg RPA 203328/L treatment levels.

Mortality during 96-hour exposure of *Mysidopsis bahia* to RPA 203328 (metabolite of isoxaflutole)

Mean measured concentration [mg RPA 203328 /L]	Replicate	Cumulative mortality after 24 h [%]	Cumulative mortality after 48 h [%]	Cumulative mortality after 72 h [%]	Cumulative mortality after 96 h [%]
Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
Solvent Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
9.2	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
15	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
25	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
42	A	0	0	0	0 [b]
	B	0	0	0	10 [e]
	Mean	0	0	0	5
70	A	0	0	0	0 [e]
	B	0	0	0	0 [e]
	Mean	0	0	0	0
120	A	0	0 [f]	0 [h]	10 [bjm]
	B	0 [a]	10 [ghi]	10 [bil]	20 [bin]
	Mean	0	5	5	15
200	A	10 [bc]	80 [ej]	90 [j]	90 [j]
	B	20 [bc]	90 [k]	100	100
	Mean	15	85	95	95

a One of the surviving mysids was at the surface of the test solution.
b Several of the surviving mysids were lethargic
c Two of the surviving mysids exhibited darkened pigmentation and were lethargic
d Several of the surviving mysids exhibited partial loss of equilibrium
e One of the surviving mysids was lethargic
f Two of the surviving mysids were lethargic and at the surface of the test solution
g Several of the surviving mysids were lethargic and at the surface of the test solution
h Two of the surviving mysids were lethargic
i Two of the surviving mysids exhibited complete loss of equilibrium.
j One of the surviving mysids exhibited partial loss of equilibrium
k One of the surviving mysids exhibited complete loss of equilibrium
l Two of the surviving mysids exhibited partial loss of equilibrium
m One of the surviving mysids exhibited partial loss of equilibrium and was at the surface of the test solution
n Two of the surviving mysids exhibited partial loss of equilibrium and were at the surface of the test solution

The LC_{50} values (corresponding 95% confidence limits) and the No-Observed-Effect Concentration (NOEC) established for RPA 203328 (metabolite of isoxaflutole) and mysids (*Americamysis bahia*) during the 96-hour static acute exposure.

		95 % Confidence Limit	
Observation Interval	LC_{50} (mg a.s./L)	Lower (mg a.s./L)	Upper (mg a.s./L)
24-Hour[a]	> 200	-	-
48-Hour[b]	160	120	200
72-Hour[b]	150	120	200
96-Hour[b]	150	120	200
NOEC through 96 hours: 25 mg a.s./L			

[a] LC_{50} value was estimated to be greater than the highest concentration tested, therefore, the corresponding 95% confidence limit could not be calculated.
[b] LC_{50} value was estimated by nonlinear interpolation (corresponding 95% confidence limit was calculated by binomial probability).

Conclusion:

It was concluded that the 96-hour LC_{50} of RPA 203328 (metabolite of isoxaflutole), estimated by nonlinear interpolation in mysid shrimps based on nominal concentration was 150 mg RPA 203328/L (95% CL: 120 – 200 mg RPA 203328/L). The No-observed-Effect Concentration (NOEC) after 96 hours exposure was 25 mg RPA 203328/L.
According to the new data requirements for EU submissions, the relevant endpoint is the 48-hour LC_{50} value, which is 160 mg RPA 203328/L as estimated by nonlinear interpolation.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

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

Additional chronic studies on aquatic invertebrates were performed, which were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. These studies will be summarized below.

Table 8.2.5- 1: Additional chronic studies with isoxaflutole on aquatic invertebrates

Test substance	Test species/study type	Endpoint		References
Isoxaflutole	Invertebrates, chronic *Daphnia magna*	NOEC	5.7 mg a.s./L	[redacted] (1998) 98-10-7505 M-210464-01-2 KCA 8.2.5.1/02
Isoxaflutole	Invertebrates, chronic *Americamysis bahia*	NOEC	0.001 mg a.s./L	[redacted] (1995) R004949 M-166884-01-1 KCA 8.2.5.2/01

Justification for the use of time weighted average concentrations (TWA) as refinement option in the long-term risk assessment for *Americamysis bahia* (isoxaflutole)

In chronic risk assessments a TWA may be used under certain conditions. The onset of effects plays a key role. The TWA approach cannot be followed if effects are occurring early in the test or if the acute to chronic ratio (acute EC_{50} or LC_{50} / chronic NOEC) both based on immobility or mortality is < 10. Also in case of i) indications for latency of effects; ii) co-occurrence of exposure and specific sensitive life stages the TWA approach is not appropriate. Furthermore, the chronic study needs to be performed under constant exposure and results need to be expressed as mean measured in case of > 20% loss of a.s.

The TWA approach is considered justified when assessing the risk for *Americamysis bahia* for the following reasons:

- In the case of *A. bahia* exposed to isoxaflutole the acute to chronic ratio is greater 10 (0.077 mg/L / 0.001 mg/L = 77).
- In the chronic test *A. bahia* was exposed to the a.s. in a flow through system for 28 days. The test item concentration has been verified analytically at days 0, 2, 7, 14, 21 and 28. Mean measured concentrations ranged from 66 to 117%. The results were reported as mean measured.
- No indications for latency of effects are known.
- The NOEC is based on survival of *A. bahia* in a chronic study covering all life stages.

As time window of the TWA a default value of 7 days has been proposed by ELINK. Furthermore it is stated in the aquatic guidance document (EFSA PPR Panel, Journal, 2013) that "*the PPR Panel of EFSA adopts this pragmatic approach that most likely is relatively worst case.*" Hence this approach is followed as presented in the risk assessment (MCP, point CP 10.2, Table 10.2- 10).

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

Report:	[redacted];1998;M-210464-01
Title:	IFT Technical RPA201772 - The chronic toxicity to daphnia magna under static renewal conditions
Report No:	98-10-7505
Document No(s):	Report includes Trial Nos.: 10566.0898.6516.130 M-210464-01-2
Guidelines:	**OECD: 211 (1997)**
GLP/GEP:	**yes**

Objective:

The chronic toxicity of RPA 201772 (isoxaflutole) to *Daphnia magna* was assessed in a static renewal system over 21 days.

Material and methods:

Test item: RPA 201772 (isoxaflutole), Batch No.: 05ADM95, purity: 99.43%, Ref. No.: 98048LIH.

Two daphnids (neonates; < 24 hours old) were exposed in ten replicates to nominal concentrations of 0.030, 0.095, 0.31, 0.98, 3.1 and 10 mg a.s./L (corresponding to mean measured concentrations of 0.019, 0.057, 0.18, 0.58, 2.0 and 5.7 mg a.s./L) for 21 days under static renewal conditions. Test solutions were renewed every Monday, Wednesday, and Friday of the study. Daphnids were fed once per day the freshwater green alga *Ankistrodesmus falcatus* and 50 µL yeast of cereal leaves and digested flaked fish food. In addition, a negative control (dilution water) and a solvent control (0.1 mL acetone/L) were tested.

The endpoints were expressed in terms of mean measured concentrations.

Dilution water was fortified well water with a pH of 7.9 – 8.1. Water temperature was 19 - 21 °C during the test, the photoperiod was 16 hours of light and 8 hours dark (16.5 – 18.7 $\mu E_i \times m^{-2} \times s^{-1}$). The specific conductance was 480 – 500 µS/cm and the total hardness 170 – 180 mg $CaCO_3$/L. Total alkalinity was 120 mg $CaCO_3$/L.

Survival of adult daphnids, abnormal behaviour and offspring production were recorded daily. The length of time for appearance of the first brood released was also recorded.

At test initiation and after 3, 14 and 17 days a sample of the freshly prepared test solutions was removed from every treatment level and analyzed. Samples from the aged solutions were taken on day 3, 5, 17 and 19.

Dates of experimental work: September 04, 1998 to September 25, 1998

Results:

Validity Criteria	Recommended by guideline	Obtained in this study
Control mortality of parent animals at test end	≤ 20%	max. 10%
Mean number of live offspring produced per parent animal surviving at end of test in the control	≥ 60	min. 122

All validity criteria for the study were met.

Analytical results

Undissolved test substance was observed in the highest treatment level of 10 mg a.s./L. The result of the analysis for test substance concentration in the test solutions was 56 - 63% of nominal. Therefore, it is appropriate to use mean measured test concentrations.

Biological results

Survival among daphnids exposed to concentrations all test levels ranged from 70 – 100% which is not significant compared to the controls. Number of offspring averaged between 114 and 129 offspring per female for all concentrations level. Statistics showed that this was not significantly different from the controls. No young daphnids were observed to be immobilized in any of the nominal concentrations or the controls. First brood release for all test concentration and the controls occurred on day 8.

Immobilization and reproduction of Daphnia magna during 21-day exposure to RPA 201772 (isoxaflutole)

Mean measured concentration [mg a.s./L]	Immobilization [%]	Offspring/female (mean) [-]
Control	0	134
Solvent Control	10	122
0.019	0	126
0.057	10	116
0.18	30	114
0.58	10	119
2.0	10	134
5.7	0	129

Conclusion:

It was concluded that the 21-day No Observed Effect Concentration (NOEC) of RPA 201772 (isoxaflutole) in *Daphnia magna* based on mean measured concentrations was 5.7 mg/L.

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

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

However, a study on *Americamysis bahia* (mysid shrimp) for the parent compound is available due to US-EPA requirements, which has not yet been submitted to the EU. In the US, studies on marine organisms are in support of the development of isoxaflutole for its use in field crops, particularly for those selected areas, where applications is in direct vicinity of brackish or estuarine water bodies.

This situation is not relevant for the field use of a maize herbicide under European considerations. Since in the European risk assessment for plant protection products the ecosystem of concern is a freshwater body neighbouring fields of agricultural use, marine species are not considered relevant.

However as *Americamysis bahia* is explicitly listed in the new data requirements (EC 283/2013), endpoints derived with this species are also taken into account for freshwater edge-of-field risk assessment.

Where the mysid endpoint is lower than the endpoint derived with *Daphnia magna* or *Chironomus riparius* the risk assessment for aquatic invertebrates exposed to isoxaflutole is based on mysid shrimp data although not required by the new data requirements (EC 283/2013) for an herbicide. This is clearly a worst case situation.

The summary of this study is presented below.

Report:	[redacted]; [redacted];1995;M-166884-01
Title:	Isoxaflutole - Chronic toxicity to mysids (Mysidopsis bahia) under flow-through conditions
Report No:	R004949
Document No(s):	Report includes Trial Nos.: 10566.1294.6352.530 M-166884-01-1
Guidelines:	**USEPA (=EPA): FIFRA 72-4;** **Deviation not specified**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the chronic toxicity of RPA 201772 (isoxaflutole) technical to mysid shrimp (*Mysidopsis bahia*). The study was designed as a flow-through experiment for 28 days.

Materials and Methods:
Test item: RPA 201772 (isoxaflutole) technical; Batch code: 39 ADM 93; purity: 96.8 %; light yellow powder
Thirty mysid shrimps in each treatment were exposed in duplicate to nominal concentrations of 0.31, 0.62, 1.2, 2.5 and 5.0 µg a.s./L (corresponding to mean measured concentrations of 0.30, 0.52, 1.0, 1.9 and 3.8 µg a.s./L). In addition, a negative control (dilution water) and a solvent control (0.0065 mL acetone/L) were tested. When mysids reached sexual maturity on day 15, they were redistributed within the test aquaria. Male/female pairs within each exposure aquarium were transferred to glass pairing jars. Mysid shrimps were fed twice daily with *Artemia salina*, during pairing period at least once daily additional with Selco®, a substance high in saturated fatty acids, and afterwards with *Artemia salina* and Selco® once daily.
The endpoints were expressed in terms of mean measured concentrations.
Dilution water was artificial seawater formulated by addition of a commercial salt mix to freshwater with a salinity of 25 ‰ and a pH of 8.0 – 8.3. Water temperature was 26 - 28 °C during the test, the photoperiod was 16 hours of light and 8 hours dark (220 – 750 lux).
During the first 14 days of the test, mysid shrimps were observed for mortality and sublethal effects. After the pairing on day 15 the mortality of male and female adults as well as number of offspring produced by each female and sublethal effects were recorded. At test termination individual body length and dry weight of mysid shrimps were measured. In the 0.30, 1.0 and 3.8 µg a.s./L test levels, the concentrations of the test substance was measured at test initiation and after 2, 7, 14, 21 and 28 days.

Dates of experimental work: June 20, 1995 to July 18, 1995

Results:
Analytical results:
The results of analysis for test substance concentration in the test solution was 66 – 117% of nominal. Therefore, it is appropriate to use mean measured test concentrations.

Biological results:

Mortality

At test termination after 28 days a survival of 82 and 77% was observed among organisms exposed to the control and solvent control.

At the two highest tested concentrations (1.9 and 3.8 µg a.s./L) 48% and 13% survival was observed which is statistically different from the controls. At the lower concentrations of 0.30, 0.52 and 1.0 µg/L, survival of 77%, 63% and 70%, which is not significant, was observed. Since it was determined that organism survival was adversely affected by exposure to the two highest treatment levels, reproduction and growth data for these levels were excluded from statistical analysis to determine further treatment effects. Comparison of these data established that survival was unaffected by exposure to RPA 201772 technical at concentrations ≤ 1µg a.s./L.

Reproduction

The reproductive success of females in the control and solvent control was ≥ 75% and the average number of young produced ≥ 3. No significant differences between control and solvent control were observed. Reproduction success at the two highest treatment levels of 1.9 and 3.8 µg a.s./L was 0.40 and 0.53 offspring per female per day. These values were not included in the statistical analysis for treatment effects. In the lower test concentrations of 0.30, 0.52 and 1.0 µg a.s./L, the reproductive success ranged from 0.58 to 0.73 offspring per female per days and was not significantly different from the controls. Comparison of these data established that reproduction was unaffected by exposure to RPA 201772 technical at concentrations ≤ 1µg a.s./L.

Survival and reproductive success during 28-day-life-cycle exposure of *Mysidopsis bahia* to RPA 201772 (isoxaflutole) technical

Mean measured concentration [µg a.s./L]	Survival [%] [a]	Mean survival [%] [a]	Reproductive success [offspring/female/day] [a]	Mean reproductive success [offspring/female/day] [a]
Control	77	82	0.43	0.44
	87		0.44	
Solvent Control	83	77	0.62	0.53
	70		0.43	
Pooled Controls [b]	-	79	-	0.49
0.30	77	77	0.90	0.73
	77		0.56	
0.52	70	63	0.57	0.67
	57		0.77	
1.0	67	70	0.60	0.58
	73		0.55	
1.9	40	48 [c]	0.57	0.40 [d]
	57		0.23	
3.8	67	13 [c]	0.67	0.53 [d]
	20		0.39	

a Values presented have been rounded to two significant figures
b Since control and solvent control data were not determined to be significantly different, all treatment data were compared to the pooled control data
c Significantly different ($p \leq 0.05$) from the pooled control (Williams' test)
d Since organism survival was adversely affected, this treatment level was excluded from statistical analysis to determine treatment effects on reproductive success.

Body length

Mean body length of male and female organisms after test termination in both control solutions was 7.1 and 7.0 mm. In the two highest tested concentrations of 1.9 and 3.8 µg a.s./L the mean body length was 6.9 and 7.1 mm (male) and 7.1 and 7.2 mm (female). Those values were not included in the statistical analysis for treatment effects. The mean body length of male mysids exposed to 0.30 0.52 and 1.0 µg a.s./L was 7.1, 7.3 and 7.1 mm and for females 7.1, 7.2 and 7.0 mm. This is statistically not different to the control. Comparison of these data established that body length was unaffected by exposure to RPA 201772 technical at concentrations ≤ 1 µg a.s./L.

Total body length of F_0 generation male and female mysid shrimps measured after test termination

Mean measured concentration [µg a.s./L]	Mean total body length males [mm] [a]	Mean total body length males from both replicates [mm] [a]	Mean standard deviation for both replicates [a]	Mean total body length females [mm] [a]	Mean total body length females from both replicates [mm] [a]	Mean standard deviation for both replicates [a]
Control	7.1	7.1	0.26	6.9	7.0	0.31
	7.1			7.1		
Solvent Control	7.0	7.1	0.31	7.0	7.0	0.38
	7.2			7.1		
Pooled Controls [b]	-	7.1	0.29	-	7.0	0.34
0.30	7.2	7.1	0.50	7.2	7.1	0.32
	7.0			7.1		
0.52	7.2	7.3	0.25	7.2	7.2	0.31
	7.3			7.3		
1.0	7.0	7.1	0.36	7.1	7.0	0.34
	7.2			7.0		
1.9	7.1	6.9	0.33 [c]	7.1	7.1	0.41 [c]
	6.8			7.0		
3.8	7.7	7.1	0.64 [c]	7.3	7.2	0.21 [c]
	6.8			7.2		

a Values presented have been rounded to two significant figures.
b Since control and solvent control data were not determined to be significantly different (t-Test), all treatment data were compared to the growth of the pooled control organisms.
c Since organism survival was adversely affected (Williams' test), this treatment level was excluded from statistical analysis to determine treatment effects for organisms length.

Body weight

In the control and solvent control the mean body weight of males was 0.84 and 0.85 mg which is not significantly different. Mean body weight of female organisms was 0.85 and 0.97 mg, which is significantly different. Mean body weight of male and female control and solvent control organisms, based on pooled data was 0.85 and 0.97.

For the two highest test concentrations of 1.9 and 3.8 µg a.s./L the mean body weight was 0.84 (males) and 0.99 and 0.85 mg (females), which was not included in the statistical analysis. For the lower concentrations of to 0.30 0.52 and 1.0 µg a.s./L male body weight was 0.76, 0.84, and 0.89 mg and for females 1.0, 0.96 and 1.0 mg. Those data are similar to the pooled control and solvent control data. Comparison of these data established that dry body weight was unaffected by exposure to RPA 201772 technical at concentrations ≤ 1 µg a.s./L.

Dry body weight of F_0 generation male and female mysid shrimps measured after test termination

Mean measured concentration [µg a.s./L]	Mean dry body weight males [mg] [a]	Mean dry body weight males from both replicates [mg] [a]	Mean standard deviation for both replicates [a]	Mean dry body weight females [mg] [a]	Mean dry body weight females from both replicates [mg] [a]	Mean standard deviation for both replicates [a]
Control	0.90	0.84	0.13	0.83	0.85	0.21
	0.79			0.88		
Solvent Control	0.86	0.85	0.11	0.98	0.97	0.23 [c]
	0.84			0.97		
Pooled Controls	-	0.85	0.12 [b]	-	-	-
0.30	0.76	0.76	0.17	1.1	1.0	0.19
	0.77			0.94		
0.52	0.80	0.84	0.097	0.89	0.96	0.22
	0.87			1.1		
1.0	0.93	0.89	0.13	1.1	1.0	0.21
	0.85			0.96		
1.9	0.83	0.84	0.086 [d]	1.1	0.99	0.26 [d]
	0.84			0.91		
3.8	0.91	0.84	0.17 [d]	0	0.85	0.071 [d]
	0.81			0.85		

a Values presented have been rounded to two significant figures.
b Since control and solvent control data were not determined to be significantly different (t-Test), all treatment data were compared to the growth of the pooled control organisms.
c Since control and solvent control data were determined to be significantly different (t-Test) all control data were compared to the growth of the solvent control organisms.
d Since organism survival was adversely affected (Williams' test), this treatment level was excluded from statistical analysis to determine treatment effects for body weight.

Conclusion:

The No Observed Effect Concentration (NOEC) was 1.0 µg/L and the Lowest Observed Effect Concentration (LOEC) was 1.9 µg/L based on mean measured concentrations.

CA 8.2.5.3 Development and emergence in Chironomus species

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

CA 8.2.5.4 Sediment dwelling organisms

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

CA 8.2.6 Effects on algal growth

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

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

Table 8.2.6- 1: Additional studies for algal toxicity of isoxaflutole and its metabolite

Test substance	Test species/study type	Endpoint		References
RPA 202248	Chronic/growth inhibition *Pseudokirchneriella subcapitata*	72h- E_dC_{50}	1.9 mg p.m./L	[redacted] (1997) R004952 M-166891-01-1 KCA 8.2.6.1/05
Isoxaflutole	Chronic/growth inhibition *Skeletonema costatum*	72h- E_dC_{50} 72h-E_rC_{50}	0.082 mg a.s./L 0.2079 mg a.s./L*	[redacted] (1994) R002577 M-162947-01-1 KCA 8.2.6.2/01
Isoxaflutole	Chronic/growth inhibition *Anabaena flos-aquae*	72h - E_dC_{50}	0.18 mg a.s./L	[redacted] (1994) R004947 M-166879-01-1 KCA 8.2.6.2/02
Isoxaflutole	Chronic/growth inhibition *Navicula pelliculosa*	72h- E_dC_{50}	0.20 mg a.s./L	[redacted] (1994) R004948 M-166881-01-1 KCA 8.2.6.2/03

* Re-calculation of algae endpoint based on growth rate (M-468837-01-1, KCA 8.2.6.2/04)

Selection of algae endpoint

Processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) appears most 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. After numerous discussions, the current test guidelines OECD TG 201, the EU-Method C3, the EC regulation for Classification and Labeling (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. Also in the new Aquatic Guidance Document (EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290) it is stated that growth rate is the preferred endpoint to be used.

In case of isoxaflutole the results of the newly submitted algae studies (Table 8.2.6- 1) are reported based on density only. The lowest density based endpoint amongst the newly submitted algae studies is derived from the study with *Skeletonema costatum*. Following EFSA's conclusion on the relevant algae endpoint this study has been statistically re-evaluated and the E_rC_{50} has been calculated.

As the EU agreed endpoint is lower than the E_rC_{50} of the study with *Skeletonema costatum*, the EU agreed endpoint is used for the risk assessment. The EU agreed endpoint is based on biomass (E_dC_{50}

corresponds to a biomass endpoint). As the biomass endpoint is generally lower than the growth rate this can be considered worst case.

CA 8.2.6.1 Effects on growth of green algae

Metabolite RPA 202248

Report:	[redacted]; [redacted];1997;M-166891-01
Title:	RPA202248 technical - Toxicity to the freshwater green alga, (Selenastrum capricornutum)
Report No:	R004952
Document No(s):	Report includes Trial Nos.: 10566.0797.6435.430 M-166891-01-1
Guidelines:	**USEPA (=EPA): FIFRA §122-2; FIFRA 123-2** (1982); **Deviation not specified**
GLP/GEP:	**yes**

Objectives:
The objective of this study was to determine the effect of RPA 202248 Technical, the primary degradate of the herbicide RPA 201772, on the growth of the freshwater green alga, *Pseudo-kirchneriella subcapitata* (*Selenastrum capricornutum)*.

Materials and Methods:
Test material: RPA 202248, analysed purity: 99.9 % w/w was tested, specified by origin batch no.: JYG 803A (EA5019SD1).
Pseudokirchneriella subcapitata were exposed in a chronic multi-generation test for 72 hours under static exposure conditions to the mean measured concentrations of 0.024, 0.077, 0.29, 0.86, 2.9 and 9.4 mg pure metabolite/L in comparison to a water and a solvent control [100 µL acetone (including the appropriate concentration of the test item) / 1000 mL nutrient medium was added to all concentration levels and the solvent control].
The test system consisted of three replicate vessels per test level and control. The initial cell number was 3,000 cells/mL.
Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).
The pH values in the controls ranged from 7.1 to 7.5 at test initiation and from 7.3 to 8.9 at test termination and the incubation temperature was continuously maintained at 24°C (measured in an additional incubated glass vessel) over the whole period of testing at a illumination of 3400 to 4300 lux.
Quantitative amounts of BCS - BJ39463 (CGA 357262) were measured in all treatment groups and in the control on day 0 and day 5 of the exposure period.

Dates of experimental work: August 15 1997 to August 20 1997

Results:

Analytical results:

The analytical finding of test item in the treatment level was determined on day 0 and 5. Mean measured concentrations ranged from 94 to 110% of the nominal concentrations. All results are based on mean measured test concentrations of the test item.

Biological results:

Effect of RPA 202248 on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 120 h growth inhibition test

Geom. mean measured concentration [mg p.m./L]	Day 3 (72 h)	Day 5 (120 h)	
	Mean cell number ± SD x 10^4 per mL	Mean cell number ± SD x 10^4 per mL	Reduction [%]
Control	36 ± 1.2	150 ± 5.0	n.a.
Solvent control	43 ± 7.6	154 ± 3.3	n.a.
Pooled control	40 ± 6.0	152 ± 4.4	n.a.
0.024	29 ± 2.4	154 ± 4.9	-1.2
0.077	49 ± 16	152 ± 4.8	0.58
0.29	40 ± 5.7	149 ± 3.0	2.1
0.86	34 ± 7.3	146 ± 3.2	4.0
2.9	10 ± 2.1	142 ± 3.5	6.6
9.4	3.4 ± 1.0	6.0 ± 1.1	96

test initiation with 3,000 cells/mL

At test termination, in addition to normal cells, bloated cells were observed in the 0.29, 0.86, 2.9 and 9.4 mg p.m./L test concentrations. Cell fragments were observed in the 2.9 and 9.4 mg p.m./L test concentrations. Cells exposed to the remaining treatment levels (0.024 and 0.077 mg p.m./L) and the controls were observed to be normal.

Statistical analysis (Williams' Test) of the data established a significant reduction in cell density in the 0.86, 2.9 and 9.4 mg test item/L treatment levels when compared to the performance of the pooled control.

Conclusions:

A growth inhibition test conducted with RPA 202248 on algae (*P. subcapitata)* under static exposure conditions revealed the following results:

E_dC_{50} (0 - 72h) 1.9 mg p.m./L (based on mean measured concentration) and
E_dC_{50} (0 - 120h) 5.5 mg p.m./L (based on mean measured concentration).

CA 8.2.6.2 Effects on growth of an additional algal species

Report:	[redacted];[redacted];1994;M-162947-01
Title:	RPA201772 technical - Acute toxicity to the marine diatom, Skeletonema costatum
Report No:	R002577
Document No(s):	Report includes Trial Nos.: 10566.0194.6322.450 M-162947-01-1
Guidelines:	**USEPA (=EPA): FIFRA §122-2 and §123-2 (1982); Deviation not specified**
GLP/GEP:	**yes**

Objectives:

The objective of this study was to determine the effect of RPA 201772 Technical (isoxaflutole) on the growth of the marine diatom, *Skeletonema costatum*.

Materials and Methods:

Test material: RPA 201772 (isoxaflutole), analysed purity: 96.8 % w/w was tested, specified by origin batch no.: 39 ADM 93.

Skeletonema costatum were exposed in a chronic multi-generation test for 120 hours under static exposure conditions to the mean measured concentrations of 0.0024, 0.0081, 0.027, 0.090, 0.30 and 1.0 mg test item/L (corresponding to nominal concentrations of 0.0022, 0.0074, 0.024, 0.074, 0.24 and 0.75) in comparison to a water and a solvent control [100 µL acetone (including the appropriate concentration of the test item) / 100 mL nutrient medium was added to all concentration levels and the solvent control].

The test system consisted of three replicate vessels per test level and control. The initial cell number was 1,000 cells/mL.

Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).

The pH values in the controls ranged from 8.0 to 8.1 at test initiation and from 8.6 to 8.7 at test termination and the incubation temperature ranged from 19 to 20°C (measured in an additional incubated glass vessel) over the whole period of testing at a illumination of 3200 to 4800 lux.

Quantitative amounts of RPA 201772 (isoxaflutole) were measured in all treatment groups and in the control on day 0 and day 5 of the exposure period.

Dates of experimental work: April 06 to April 11 1996

Results:

Analytical results:

The analytical finding of test item in the treatment level was determined on day 0 and 5. Mean measured concentrations ranged at test initiation from 75 to 92% and at test termination from <LOQ to 21% of the nominal concentrations.

All results are based on mean measured test concentrations of the test item.

Biological results:

Effect of RPA 201772 on marine diatom (*Skeletonema costatum*) in a 120 h growth inhibition test

Geom. mean measured concentration [mg a.s./L]	Day 3 (72 h)	Day 5 (120 h)	
	Mean cell number x 10^3 per mL	Mean cell number x 10^3 per mL	Reduction [%]
Control	21	106	n.a.
Solvent control	21	105	n.a.
Pooled control	21	106	n.a.
0.0024	22	108	-2.1
0.0081	18	94	11
0.027	17	87	17
0.090	12	64	39
0.3	5	52	51
1.0	3	4	97

test initiation with 1,000 cells/mL

Thin cell walls, cell fragments and bloated cells were observed in the 0.24 and 0.75 mg a.s./L treatment levels at test termination. Cell fragments were observed in the 0.024 and 0.074 mg a.s./L treatment levels at test termination.
Statistical analysis (Williams' Test) of the data established a significant reduction in cell density in the 0.0074, 0.024, 0.074, 0.24 and 0.75 mg a.s/L treatment levels when compared to the performance of the pooled control.

Conclusions:

A growth inhibition test conducted with RPA 201772 (isoxaflutole) on marine diatom (*Skeletonema costatum*) under static exposure conditions revealed the following results:

E_dC_{50} (0 - 72h) 0.082 mg a.s./L (based on mean measured concentration) and
E_dC_{50} (0 - 120h) 0.11 mg a.s./L (based on mean measured concentration).

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Report:	[redacted];[redacted];1994;M-166879-01
Title:	RPA201772 technical - Acute toxicity to the freshwater blue-green alga, Anabaena flos-aquae
Report No:	R004947
Document No(s):	Report includes Trial Nos.: 10566.0194.6324.420 M-166879-01-1
Guidelines:	**USEPA (=EPA): FIFRA §122-2 and §123-2 (1982); Deviation not specified**
Deviations:	At test termination, after the analytical samples were collected there was an insufficient amount of solution to measure conductivity in the 1.0 mg a.s./L test solution. This deviation had no impact on the results of this study.
GLP/GEP:	**yes**

Objectives:

The objective of this study was to determine the effect of RPA 201772 Technical (isoxaflutole) on the growth of the blue-green alga, *Anabaena flos-aquae*.

Materials and Methods:

Test material: RPA 201772 (isoxaflutole), analysed purity: 98.7% w/w was tested, specified by origin batch no.: 21 ADM 93.

Anabaena flos-aquae were exposed in a chronic multi-generation test for 120 hours under static exposure conditions to the nominal concentrations of 0.0024, 0.0081, 0.027, 0.090, 0.30 and 1.0 mg test item/L (corresponding to initial measured concentrations of 0.0020, 0.0086, 0.028, 0.087, 0.23 and 0.99) in comparison to a water and a solvent control [100 µL acetone (including the appropriate concentration of the test item) / 1000 mL nutrient medium was added to all concentration levels and the solvent control].

The test system consisted of three replicate vessels per test level and control. The initial cell number was 10,000 cells/mL.

Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).

The pH values in the controls ranged from 7.4 to 7.5 at test initiation and from 7.7 to 8.5 at test termination and the incubation temperature was 24 ± 1°C (measured in an additional incubated glass vessel) over the whole period of testing at a illumination of 1100 to 3300 lux.

Quantitative amounts of RPA 201772 (isoxaflutole) were measured in all treatment groups and in the control on day 0 and day 5 of the exposure period.

Dates of experimental work: March 16 to March 21 1994

Results:

Analytical results:

The analytical finding of test item in the treatment level was determined on day 0 and 5. Mean measured concentrations ranged at test initiation from 78 to 110% and at test termination from <LOQ to 53% of the nominal concentrations.

All results are based on mean measured test concentrations of the test item.

Biological results:

Effect of RPA 201772 on blue-green alga (*Anabaena flos-aquae*) in a 120 h growth inhibition test

Geom. mean measured concentration [mg a.s./L]	Day 3 (72 h)	Day 5 (120 h)	
	Mean cell number x 10^4 per mL	Mean cell number x 10^4 per mL	Reduction [%]
Control	16	89	n.a.
Solvent control	16	94	n.a.
Pooled control	16	92	n.a.
0.0020	16	92	-0.64
0.0086	14	82	6.8
0.028	12	73	20
0.087	12	62	32
0.23	7	49	46
0.99	3	12	87

test initiation with 10,000 cells/mL

Cell fragments were observed in the 0.23 and 0.99 mg a.s./L treatment levels throughout the exposure. Normal algal cells were observed in the remaining treatment levels (0.0020, 0.0086, 0.028 and 0.87 mg a.s./L) and the controls throughout the exposure.

Statistical analysis (Williams' Test) demonstrated a significant reduction in cell density in the 0.028, 0.087, 0.23 and 0.99 mg a.s./L treatment levels as compared to the pooled control data. No significant reduction in cell density was established at the 0.0020 and 0.0086 mg a.s./L treatment levels.

Conclusions:

A growth inhibition test conducted with RPA 201772 (isoxaflutole) on blue-green alga (*Anabaena flos-aquae)* under static exposure conditions revealed the following results:

E_dC_{50} (0 - 72h) 0.18 mg a.s./L (based on mean measured concentration) and

E_dC_{50} (0 - 120h) 0.17 mg a.s./L (based on mean measured concentration).

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Report:	[redacted];[redacted];1994;M-166881-01
Title:	RPA201772 technical - Acute toxicity to the freshwater diatom, Navicula pelliculosa
Report No:	R004948
Document No(s):	Report includes Trial Nos.: 10566.0194.6323.440 M-166881-01-1
Guidelines:	**USEPA (=EPA): FIFRA §122-2 and §123-2 (1982); Deviation not specified**
Deviations:	The Study Protocol states that when a 250-mL test flask is required, a 100 mL test solution is used. Due to an additional analytical sampling (24 hours), 10 mL of test solution was removed from each test vessel, thus reducing the solution volume to 90 mL per vessel. Analysis of the exposure solutions at 24 hours was required because two of the three QC samples associated with the day 0 analyses were outside of the acceptable range established for this laboratory. These deviation had no impact on the results of this study.
GLP/GEP:	**yes**

Objectives:
The objective of this study was to determine the effect of RPA 201772 Technical (isoxaflutole) on the growth of the diatom, *Navicula pelliculosa*.

Materials and Methods:
Test material: RPA 201772 (isoxaflutole), analysed purity: 98.7% w/w was tested, specified by origin batch no.: 21 ADM 93.
Navicula pelliculosa were exposed in a chronic multi-generation test for 120 hours under static exposure conditions to the nominal concentrations of 0.0024, 0.0081, 0.027, 0.090, 0.30 and 1.0 mg test item/L (corresponding to initial measured concentrations of 0.0031, 0.0093, 0.030, 0.096, 0.29 and 0.64) in comparison to a water and a solvent control [100 µL acetone (including the appropriate concentration of the test item) / 1000 mL nutrient medium was added to all concentration levels and the solvent control].
The test system consisted of three replicate vessels per test level and control. The initial cell number was 10,000 cells/mL.
Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).
The pH of the two highest treatment levels decreased from 7.4 at test initiation to 7.2 at test termination. This decrease in pH appears to be attributable to the degradation of RPA 201772 Technical. The pH of the remaining test solutions at test initiation ranged from 7.4 to 7.5 and increased to a range of 7.5 to 8.5 at test termination.
The incubation temperature ranged from 24 to 25°C (measured in an additional incubated glass vessel) over the whole period of testing at a illumination of 3200 to 4800 lux.
Quantitative amounts of RPA 201772 (isoxaflutole) were measured in all treatment groups and in the control on day 0 and day 5 of the exposure period.

Dates of experimental work: March 17 to March 22 1994

Results:

Analytical results:

At test initiation, measured concentrations of RPA 201772 ranged from 64 to 130% of the nominal concentrations. The decreased recovery of RPA 201772 from the 1.0 mg A.I./L test solution (64%) is believed to be due to the limited water solubility of RPA 201772 (e.g., ≤ 8.0 mg/L). At test termination, measured concentrations of RPA 201772 established for the treatment levels tested ranged from 36 to 44% of the nominal concentrations.
All results are based on mean measured test concentrations of the test item.

Biological results:

Effect of RPA 201772 on diatom (*Navicula pelliculosa*) in a 120 h growth inhibition test

Geom. mean measured concentration [mg a.s./L]	Day 3 (72 h)	Day 5 (120 h)	
	Mean cell number x 10^4 per mL	Mean cell number x 10^4 per mL	Reduction [%]
Control	25	93	n.a.
Solvent control	26	96	n.a.
Pooled control	26	95	n.a.
0.0031	26	96	-1.5
0.0093	20	89	6.3
0.030	19	83	13
0.096	15	81	15
0.29	11	66	30
0.64	10	12	87

test initiation with 10,000 cells/mL

Cell fragments and bloated cells were observed in the 0.29 and 0.64 mg a.s./L treatment levels at test termination. Normal algal cells were observed in the remaining treatment levels (0.0031, 0.0093, 0.030 and 0.096 mg a.s./L) and the controls throughout the exposure.

Statistical analysis (Williams' Test) demonstrated a significant reduction in cell density in the 0.0093, 0.030, 0.096, 0.29 and 0.64 mg a.s./L treatment levels as compared to the pooled control data. No significant reduction in cell density was established at the 0.0031 mg a.s./L treatment levels.

Conclusions:

A growth inhibition test conducted with RPA 201772 (isoxaflutole) on diatom (*Navicula pelliculosa)* under static exposure conditions revealed the following results:

E_dC_{50} (0 - 72h) 0.20 mg a.s./L (based on mean measured concentration) and
E_dC_{50} (0 - 120h) 0.38 mg a.s./L (based on mean measured concentration).

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Report:	[redacted];[redacted];2013;M-468837-01
Title:	Isoxaflutole technical: Recalculation of 72h endpoint for Skeletonema costatum (Original study report no. 94-6-5302)
Report No:	M-468837-01-1
Document No:	M-468837-01-1
Guidelines:	**not applicable**
GLP/GEP:	**n.a.**

Objective:
This statement presents the recalculated E_rC_x values based on the original study data from a 120h static marine diatom growth inhibition test by [redacted] (1994, M-162947-01-1), where results are based on cell density. According to the current guideline on algae testing (OECD, 2006) and following the new Aquatic Guidance Document the recommended response variable in the evaluation of algae study results is average specific growth rate (E_rC_x). Moreover, for EU requirements the standard test period is set to 72h.

Results & Conclusion:
Results are based on initial measured concentrations of the test item.
The 72h-EC_{50} obtained for the response variable growth rate was determined to be 0.2179 mg a.s./L.

CA 8.2.7 Effects on aquatic macrophytes
For information on studies already evaluated during the first EU review of isoxaflutole, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

Additional studies on aquatic macrophytes were performed, which were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. These studies will be summarized below.

Table 8.2.7- 1: Additional studies on aquatic macrophytes tested with isoxaflutole and its metabolites

Test substance	Test species/study type	Endpoint		References
Isoxaflutole	chronic *Lemna gibba*	9d E_rC_{50} 9d E_yC_{50}	0.01439 mg a.s./L 0.00313 mg a.s./L	[redacted] (2013) M-449195-01-1 KCA 8.2.7/04
Isoxaflutole	chronic *Myriophyllum spicatum*	14d E_rC_{50} 14d E_yC_{50}	0.429 mg a.s./L 0.238 mg a.s./L	[redacted] (2013) EBISX046 M-452561-01-1 KCA 8.2.7/05
RPA 203328	chronic *Lemna gibba*	E_bC_{50}	> 9.8 mg p.m./L	[redacted] (1997) R004951 M-166893-01-1 KCA 8.2.7/06
RPA 205834	chronic *Lemna gibba*	E_bC_{50}	1.1 mg p.m./L	[redacted], J.R. (2003) B004561 M-241470-01-1 KCA 8.2.7/07

For the risk assessment with isoxaflutole parent, new endpoints have been calculated ([redacted], 2013; M-449195-01-1). This was considered necessary because the original *Lemna* study with the compound ([redacted], 1994; M-166896-01-1) had a duration of 14 days and delivered 14-days 'biomass' endpoints, i.e. endpoints calculated from final measurements of frond number and dry weight. In contrast to this, the guideline for *Lemna* testing (OECD 221, March 2006) recommends a study duration of 7 days and the proposed response variables are growth rate and yield. A recalculation for the variable dry weight was not possible since no measurements were taken for this parameter during the course of the study. However, for the variable frond number measurements were taken on study days 3, 6, 9, 12 and 14. The endpoint recalculations were performed with the 9-days results as 9 days is close to 7 days but not shorter. Since both the guideline for *Lemna* testing (OECD 221, March 2006) and the new aquatic guidance document[3] propose growth rate as the preferred response variable, the **9d E_rC_{50} = 14.39 µg a.s./L** is considered as the relevant endpoint for the aquatic macrophyte risk assessment of isoxaflutole. This endpoint is in line with the results of the 7-days Lemna formulation study which delivered a lowest E_rC_{50} of 49.2 µg prod./L equivalent to 10.1 µg a.s./L.

It should be added that the current EU endpoint for isoxaflutole and aquatic macrophytes is a 3-days 'biomass' endpoint (3-d EC_{50} = 16 µg a.s./L) which also does not comply with recent OECD and EFSA guidance.

The new study with *Myriophyllum spicatum* ([redacted], 2013; M-452561-01-1) revealed a comparably low sensitivity of dicotyledonous macrophytes to the compound. It is therefore considered appropriate to focus on *Lemna gibba* in the risk assessment; no further testing with *M. spicatum* (metabolites, formulation) is necessary.

Comments on photo-metabolites M14 and M20

In the study on photochemical degradation in water the metabolites M14 and M20 were detected with occurrences of 9.3% and 16.8%, respectively (cf. CA 7.2.1.2). Since it is not possible to synthesize and test these metabolites, the following estimation is proposed to assess the potential risk of these degradates to aquatic macrophytes.

Due to the high similarity of chemical structures of M14 and M20 with the known metabolite RPA 202248 (cf. CA 7.2.1.2) reference is made to the ecotoxicological endpoint of this metabolite (E_bC_{50} = 55 µg/L) by following a worst-case approach for M14 and M20. The *Lemna* endpoint of RPA 202248 has been divided by a factor 10, resulting in a highly conservative E_bC_{50} of 5.5 µg/L. For the exposure side, maximum PEC_{sw} values calculated for isoxaflutole parent were multiplied with the maximum occurrences of M14 and M20 in the photolysis study.

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[3] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013: Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7): 3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

Report:	[redacted];[redacted];[redacted];2013;M-449195-01
Title:	Isoxaflutole technical: Recalculation of 9-days endpoints for Lemna gibba (Original Study Report No. 94-6-5319)
Report No:	M-449195-01-1
Document No:	M-449195-01-1
Guidelines:	**not applicable**
GLP/GEP:	**n.a.**

Objective:

This statement presents the recalculated E_rC_x values based on the original study data from a 14-days semi-static *Lemna* growth inhibition test by [redacted] (1994, M-166896-01-1), where results are derived from frond number and frond dry weight. According to the current guideline on *Lemna* testing (OECD, 2006) the recommended response variable in the evaluation of *Lemna* study results is average specific growth rate (E_rC_x). Moreover, the standard test period is set to seven days.

Results & Conclusion:

Results are based on mean measured concentrations of the test item.

The 9-day EC_{50} obtained for the response variable growth rate was determined to be 14.39 µg a.s./L.

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Report:	[redacted];[redacted];2013;M-452561-01
Title:	Toxicity of isoxaflutole technical to the aquatic macrophyte, Myriophyllum spicatum
Report No:	EBISX046
Document No:	M-452561-01-1
Guidelines:	**Higher Tier Study based on OECD 221 (2006) and OCSPP 850.4400**
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the dose-response effect of isoxaflutole tech.to the rooted aquatic macrophyte, *Myriophyllum spicatum*. The study was performed under static conditions for 14 days.

Materials and Methods:

Test item: isoxaflutole tech., Batch No.: 6464/5/8/9, purity 98.7%.

Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed for 14 days under static conditions. Five plants were exposed per replicate to nominal (mean measured) concentrations of 8.0 (7.0), 24 (20), 72 (65), 216 (167), 648 (496) and 1994 (1118) µg a.s./L. In addition a control and solvent control were tested. Mean measured recoveries measured for the combined content of isoxaflutole and the metabolite RPA 202248 (diketonitrile) ranged from 58 to 90% of nominal values. Results are based on nominal test concentrations of µg isoxaflutole/L.

Water temperature of the test medium was 19.32 to 20.26 °C during the test, with a pH of 8.1 – 9.7 and continuous illumination at 9130 to 10450 lux.

At test initiation (day 0) and test termination (day 14) plant biomass wet and dry weight were recorded. At days 0, 1, 4, 7 and 14, samples were taken from all test levels and the controls analyzed for isoxaflutole and RPA 202248 (metabolite of isoxaflutole).

Dates of experimental work: June 28 to July 19, 2012

Results:

Analytical results:
The results of analysis for test substance concentrations in the test solutions were 58 to 90% of nominal. Therefore, it is appropriate to use mean measured test concentrations.

Biological results:
Plants in the control, solvent control and four lowest treatment groups (8.0, 24, 72 and 216 µg/L) appeared normal throughout the study. Plants in the two highest treatment groups (648 and 1994 µg/L) had shoots with red tips and roots with somewhat reduced development as compared to the control and solvent control groups. Growth data for all plants was included in the data analysis.

Inhibition of *Myriophyllum spicatum* during 14-day exposure to isoxaflutole tech.

Test substance	isoxaflutole technical		
Test object	*Myriophyllum spicatum*		
Exposure	14-day – static exposure		
Endpoint units	[µg a.s./L]		
Endpoint results	Shoot length Growth rate	Wet weight Growth rate	Dry weight Growth rate
NOE_rC	8.0	72	72
E_rC_{50}	>1994	1049	429
95% C.I.	n.d.	576 to 1829	258 to 690

n.d.: not determined

Conclusion:

The lowest E_rC_{50} in the 14 day exposure of the rooted aquatic macrophyte *Myriophyllum spicatum* to isoxaflutole technical was obtained for dry weight growth rate. The statistical NOE_rC, LOE_rC and E_rC_{50} for this endpoint were 72, 216 and 429 µg a.s./L, respectively.

Metabolite RPA 203328

Report:	[redacted]; [redacted];1997;M-166893-01
Title:	RPA203328 technical - Toxicity to the duckweed, Lemna gibba
Report No:	R004953
Document No(s):	Report includes Trial Nos.: 10566.0797.6441.410 M-166893-01-1
Guidelines:	**USEPA (=EPA): FIFRA 122-2, FIFRA 123-2;** **Deviation not specified**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the toxicity of RPA 203328 (metabolite of isoxaflutole) to duckweed (*Lemna gibba*). The study was designed as a static-renewal experiment for 14 days.

Materials and Methods:
Test item: RPA 203328 (metabolite of isoxaflutole), Batch No.: NMI874, purity 99.0%
Five plants with three fronds each were exposed in three replicates to nominal concentrations of 0.10 and 10.0 mg RPA 203328/L. Dilution water was renewed after 3, 6, 9 and 12 days. In addition, a negative control (dilution water) and a solvent control (acetone) were tested. The endpoints were expressed in terms of nominal concentrations.
Dilution water was Hoagland's medium with a pH of 5.0 ± 0.1. Water temperature was 25 ± 1°C during the test, with continuous illumination at 3200 - 5400 lux.
After 3, 6, 9, 12 and 14 days reduction in frond density and biomass dry weight were recorded. At the beginning and end of one renewal period (day 0 and day 3), samples were taken from all test levels and the controls analyzed for RPA 203328.

Dates of experimental work: August 26 to September 09, 1997

Results:

Analytical results:
The results of analysis for test substance concentrations in the test solutions were 98 - 100% of nominal.

Biological results:
On day 14, fronds exposed to the 1.2, 2.4, 4.7 and 9.5 mg RPA 203328/L treatment levels were observed to be normal. Statistical analysis (Dunnett's Test) demonstrated no significant reduction in frond density in the 0.10 and 9.8 mg test item/L treatment levels as compared to the solvent control.

Inhibition of *Lemna gibba* during 14-day exposure to RPA 203328 (metabolite of isoxaflutole)

Nominal concentration [mg RPA 203328/L]	Mean frond numbers at test end	Inhibition [%]	Mean frond dry weight [mg]	Inhibition [%]
Control	448	-	60.3	-
Solvent Control	394	-	61.2	-
0.10	412	-4.6	60.1	1.8
10.0	392	0.42	60.5	6.5

Conclusion:

Therefore, the 14-day NOEC for frond density was determined to be 9.8 mg test item/L and the 14-day EC_{50} for frond density was empirically estimated to be > 9.8 mg test item/L, the highest mean measured concentration tested.

Metabolite RPA 205834

Report:	[redacted]; [redacted]; 2004; M-241470-01
Title:	RPA 205834 - Toxicity To Duckweed Lemna gibba
Report No:	B004561
Document No(s):	Report includes Trial Nos.: 13798.6107 M-241470-01-1
Guidelines:	**USEPA (=EPA): 122-2 and 123-2; Deviation not specified**
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the toxicity of RPA 205834 (metabolite of isoxaflutole) to duckweed (*Lemna gibba*). The study was designed as a static-renewal experiment for 14 days.

Materials and Methods:

Test item: RPA 205834 (metabolite of isoxaflutole), Batch No.: IBGB932, purity 99.0%

Five plants with three fronds each were exposed in three replicates to nominal concentrations of 0.63, 1.3, 2.5 and 10 mg RPA 205834/L (corresponding to mean measured concentrations of 0.59, 1.2, 2.4, 4.7 and 9.5 mg RPA 205834/L). Dilution water was renewed after 3, 6, 9 and 12 days. In addition, a negative control (dilution water) and a solvent control (0.1 mL dimethyl formamide/L) were tested. The endpoints were expressed in terms of mean measured concentrations.

Dilution water was Hoagland's medium with a pH of 4.9 – 6.2. Water temperature was 23 - 25 °C during the test, with continuous illumination at 3200 - 5400 lux.

After 3, 6, 9, 12 and 14 days reduction in frond density and biomass dry weight were recorded. At the beginning and end of one renewal period (day 0 and day 3), samples were taken from all test levels and the controls analyzed for RPA 205834.

Dates of experimental work: October 28, 2002 to November 14, 2002

Results:

Analytical results:

The results of analysis for test substance concentrations in the test solutions were 94 - 95% of nominal.

Biological results:

On day 14, fronds exposed to the 1.2, 2.4, 4.7 and 9.5 mg RPA 205834/L treatment levels were observed to be smaller, curled, chlorotic and had less root formation than the controls. Fronds exposed the 0.59 mg RPA 205834/L treatment level were observed to be smaller and slightly chlorotic as compared to the controls. No significant differences in frond density compared to the controls were observed in any test concentration.

The frond production and frond biomass (dry weight) were significantly reduced in the 1.2, 2.4, 4.7 and 9.5 mg RPA 205834/L treatment levels.

Inhibition of *Lemna gibba* during 14-days exposure to RPA 205834 (metabolite of isoxaflutole)

Mean measured concentration [mg RPA 205834/L]	Mean frond numbers at test end	Inhibition [a] [%]	Mean frond dry weight [mg]	Inhibition [b] [%]
Control	823	-	167	-
Solvent Control	815	-	172	-
Pooled control	819	-	169	-
0.59	792	3.4	155	8.6
1.2	712	13 [c]	76	55 [c]
2.4	575	30 [c]	55	68 [c]
4.7	547	33 [c]	36	79 [c]
9.5	134	84 [c]	17	90 [c]

a Percent inhibition relative to the pooled control
b Percent inhibition relative to the control
c Significantly reduced compared to pooled control, based on Bonferroni's Test

Conclusion:

The 14-days NOEC for both measurement variables (frond number, dry weight) was determined to be 0.59 mg test item/L. The lowest 14-days EC_{50} value was obtained for the measurement variable dry weight and was calculated as 1.1 mg test item/L.

CA 8.2.8 Further testing on aquatic organisms

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

Additional studies on other aquatic species were performed, which were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. These studies will be summarized below.

Table 8.2.8- 1: Additional studies on other aquatic organisms tested with isoxaflutole

Test substance	Test species/study type	Endpoint	References
Isoxaflutole	Amphibian, chronic *Xenopus laevis*	EC_{50} > 3.7 mg a.s./L	[redacted] et al. (2011) M-410610-01-1 EBISY004 KCA 8.2.8/01

Report:	[redacted];[redacted];[redacted];[redacted];2011;M-410610-01
Title:	Acute toxicity of isoxaflutole to Xenopus laevis under flow-through conditions
Report No:	EBISY004
Document No:	M-410610-01-1
Guidelines:	**FIFRA Guideline 72-1 (1982), OECD 203 (1992)**
GLP/GEP:	**yes**

Objective:
The objective of this study was to evaluate the toxicity of isoxaflutole to *Xenopus laevis*. The study was designed as a flow-through experiment for 48 hours.

Materials and Methods:
Test item: isoxaflutole, Batch No.: 6464/5/8/9, purity 98.7%.
Xenopus laevis tadpoles were exposed under flow-through conditions to determine the 48-hour LC_{50}. The following nominal (mean measured) concentrations were included in the study: control (<LOQ), solvent control (<LOQ), 0.31 (0.26), 0.63 (0.50), 1.25 (0.87), 2.5 (1.7) and 5.0 (3.7) mg a.s./L. Since no toxicity was expected, the high test levels were set up to the practical limit of solubility of 5.0 mg a.s./L in dilution water. There were three replicates of 10 tadpoles in the control and each toxicant level.
Water temperature was 21.5 – 21.9 °C and a pH of 8.1 to 8.4 during the test, the photoperiod was 16 hours of light and 8 hours dark.
Survival and sublethal behavioral effects of tadpoles were recorded after 6, 24 and 48 hours. The concentrations of the test substance was measured at test initiation (day 0) and at test termination (day 2).

Dates of experimental work: June 22 to June 24, 2010

Results:

Validity criteria

Validity Criteria	Recommended by guideline	Obtained in this study
Mortality during domestication period	< 5%	< 5%
Mortality of control group	< 10%	0%
Dissolved oxygen	> 5.8 mg/L	8.1 to 9.0 mg/L
pH value during the test	constant	8.1 – 8.4

Analytical results:

The mean measured recoveries ranged from 68 to 84% of the nominal test concentrations. The results of the study are based on the mean measured test concentrations.

Biological results:

There we no mortalities or sublethal effects at any test concentration and the organisms in all test levels appeared normal throughout the exposure period.

Acute toxicity to *Xenopus laevis* exposed to isoxaflutole (48 h)

Test substance	isoxaflutole
Test object	*Xenopus laevis*
Exposure	48-hour, flow-through
LC_{50} 48 hours (95% CL)	> 3.7 mg a.s./L (practical limit of solubility)
LOEC	> 3.7 mg a.s./L
NOEC	> 3.7 mg a.s./L

Conclusion:

Based on the results presented above, the 48h-LC_{50} is determined to be > 3.7 mg a.s./L.

CA.3 Effect on arthropods

CA 8.3.1 Effects on bees

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

An additional laboratory study on acute oral and contact toxicity to honey bees has been performed with technical isoxaflutole according to current guidelines and requirements.
Further, a chronic 10-day adult feeding limit test was conducted with Isoxaflutole WG 75. Moreover, in order to investigate the intrinsic properties of isoxaflutole on immature honey bee live stages, a honey bee brood feeding study has been performed with Isoxaflutole WG 75 (mixed together with the herbicide safener cyprosulfamide).
These additional studies were not submitted during the first Annex I inclusion process and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. The studies will be summarized below.

Table 8.3.1- 1: Additional studies on bee toxicity of isoxaflutole

Test substance	Test species/study type	Endpoint	References
Isoxaflutole	Honey bee, acute *Apis mellifera*	Oral. LC_{50} > 108.9 µg a.s./bee Contact: LC_{50} > 100 µg a.s./bee	[redacted] (2012) 72931035 M-441348-01-1 KCA 8.3.1.1.1/02
Isoxaflutole WG 75	Honey bee, 10 d chronic adult feeding study	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted] (2013) S13-00146 M-470650-01-1 KCA 8.3.1.2/01
Isoxaflutole WG 75	Honey bee, Bee brood /development *Apis mellifera*	No adverse effects on bee colonies and bee brood	[redacted] (2013) 71401031 M-454689-01-1 KCA 8.3.1.3/01

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	[redacted];[redacted];2012;M-441348-01
Title:	Effects of isoxaflutole tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	72931035
Document No:	M-441348-01-1
Guidelines:	**OECD 213 and 214 (1998);none**
Deviations:	Yes: For the contact toxicity test a 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.
GLP/GEP:	**yes**

Objective:

The purpose of this study was to investigate the acute contact and oral toxicity of isoxaflutole tech. to the honey bee (*Apis mellifera* L.).

Materials and Methods:

Isoxaflutole tech. (Batch No: 6464/5/8/9, Batch code: AE B197278-01-01, Customer Order No.: TOX 08283-02, Material: isoxaflutole technical substance, CAS 141112-29-0, LIMS No 1210230); Purity: 98.7% w/w analytical).

Under laboratory conditions, *Apis mellifera* (50 worker bees per dose; 10 individuals in 5 replicates per test item dose level, controls and reference item doses) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 108.9 µg a.s./bee by feeding (oral limit test; value based on the actual intake of the test item). In addition a water control group (water + 0.5% Adhäsit), a solvent control group (acetone) and a reference item (Perfekthion EC (= 400 g/L dimethoate) was tested. The test was conducted in the dark, temperature during the test was 25 °C and relative humidity 50 – 75%. Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing.

Oral toxicity study

Appropriate amounts of isoxaflutole tech. dilutions in acetone were mixed with syrup (ready-to-use syrup, sugar component: 30% sucrose, 31% glucose, 39% fructose) in order to achieve the required test concentrations in a final dilution of 50% syrup solution (45% water, 50% syrup and 5% acetone (w/w)). For the solvent control the same proportion of syrup water and acetone was used. The water control consisted of 50% water and 50% syrup.

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 2 hours 55 minutes for the test item treatments). After a maximum of 2 hours 55 minutes, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food.

The target dose levels (e.g. 100.0 µg a.s./bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested. In practice, higher (or lower) dose levels were obtained as the bees had a higher or lower uptake of the test solutions than the nominal 20 mg/bee. Therefore, results are based on measured concentrations of the a.s./ bee.

Contact toxicity study

A single 5 µL droplet of isoxaflutole tech. in an appropriate carrier (acetone) was placed on the dorsal bee thorax.

For the water control one 5 µL droplet of tap water containing 0.5% Adhäsit[4] and for the solvent control pure acetone was used. The reference item was also applied in 5 µL tap water (dimethoate made up in acetone). Results are based on nominal concentrations of the product per bee.

Dates of experimental work: May 14, 2012 to May 17, 2012

[4] Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees.

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality in water control	≤ 10%	0%
Mortality in solvent control	≤ 10%	0%
Contact test LD_{50} (24 h) of reference item	0.10 – 0.30 µg a.s./bee	0.24 µg a.s./bee
Oral test LD_{50} (24 h) of reference item	0.10 – 0.35 µg a.s./bee	0.10 µg a.s./bee

All validity criteria for the study were met.

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

dosage [µg a.s./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %
test item 100.0	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
solvent	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.30	2.0	2.0	80.0	14.0	90.0	8.0
0.20	0.0	0.0	30.0	16.0	50.0	2.0
0.15	0.0	0.0	12.0	16.0	26.0	8.0
0.10	0.0	2.0	2.0	2.0	4.0	0.0

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

dosage [µg a.i./bee]	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %
test item 108.9	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
solvent	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.27	34.0	54.0	100.0	0.0	100.0	0.0
0.16	6.0	28.0	96.0	2.0	98.0	0.0
0.08	0.0	0.0	30.0	2.0	38.0	0.0
0.05	0.0	0.0	0.0	0.0	0.0	0.0

Toxicity to Honey Bees; laboratory tests

Test Item	Isoxaflutole tech.	
Test object	*Apis mellifera*	
Application rate [µg a.s./bee]	108.9	100.0
Exposure	oral (sugar/acetone solution)	contact (solution in acetone)
LD_{50} [µg a.s./bee]	> 108.9	> 100.0
LD_{20} [µg a.s./bee]	> 108.9	> 100.0
LD_{10} [µg a.s./bee]	> 108.9	> 100.0
NOEC [µg a.s./bee]*	108.9	100.0

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

Contact toxicity test:

At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg a.i./bee. Also no mortality occurred in the water control group (water + 0.5% Adhäsit) and in the solvent control (acetone) group.

Oral toxicity test:

In the oral toxicity test, the maximum nominal test level of isoxaflutole, (i.e. 100 µg a.s./bee) corresponded to an actual intake of 108.9µg a.i./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the solvent control group and in the water control group (50% sugar syrup-solution), respectively.

Conclusion:

The toxicity of isoxaflutole 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 and the oral LD_{50} (48 h) was > 108.9 µg a.s./bee.

CA 8.3.1.1.2 Acute contact toxicity

See point 8.3.1.1.1 above.

CA 8.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Isoxaflutole WG 75 as technical isoxaflutole is only slightly soluble in water.

Report:	[redacted];[redacted];2013;M-470650-01
Title:	Isoxaflutole WG 75 W - Assessment of chronic effects to the honeybee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test
Report No:	S13-00146
Document No:	M-470650-01-1
Guidelines:	**not applicable** (No agreed and ring tested guideline available)
GLP/GEP:	**yes**

Objective:

To investigate the potential chronic effects of isoxaflutole on the honeybee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory and to investigate whether the LC50-/NOEC- value is greater than the tested concentration.

Materials and methods:

Over a period of 10 days, honey bees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing nominally 120 mg a.s./kg of the test item Isoxaflutole WG 75 W by continuous and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50 % (w/v) aqueous sucrose application (feeding) solution. Mortality, sub-lethal effects and behavioural observations were assessed every day throughout the 10 days continuous exposure period. Furthermore, the daily food uptake was determined.

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

Results:

After 10 days of continuous exposure, mortality at the test item treatment level of 120 mg a.s./kg of Isoxaflutole WG 75 W was not statistically significantly different when compared to the control group. The cumulative control mortality was 0.0 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg Isoxaflutole WG 75 W was 0.0 % at the final assessement. At 120 mg a.s./kg Isoxaflutole WG 75 W, no remarkable sub-lethal effects or behavioural abnormalities were observed throughout the entire observation period of 10 days.

After 10 days of continuous exposure, by considering the actual food consumption of the honey bees, the accumulated nominal intake of the test item Isoxaflutole WG 75 W at the treatment level of 120 mg a.s./kg was 49.08 µg a.s./bee, the corresponding average daily dose was therefore 4.9 µg a.s./bee.

The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different (lower) when compared to the untreated control group (40.9 mg/bee at 120 mg a.s./kg, compared to 42.9 mg/bee in the control group). The mean daily consumption of the aqueous sucrose application (feeding) solution was not statistically significantly different (lower) between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison) except for the 9th day of exposure.

Conclusions:

It can be concluded that the continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item Isoxaflutole WG 75 W at the treatment level of 120 mg a.s./kg caused no adverse effect regarding mortality, sub-lethal effects and behaviour.
The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different when compared to the untreated control group. Further, on every single day during the 10-day continuous exposure period the mean food consumption per bee was not statistically significantly different (lower) in the test item treatment group compared to the control group except for the 9th day of exposure.
As the overall mean daily food uptake in the test item treatment group was not significantly lower compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 120 mg a.s./kg.
The NOEC for mortality was determined at the end of the test period to be 120 mg a.s./kg (nominal).
The LC_{50} was determined to be > 120 mg a.s./kg (nominal).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	[redacted];2013;M-454689-01
Title:	Study on the effects of a test item mix of isoxaflutole WG 75 W + cyprosulfamide SC 500 G on honey bee brood (Apis mellifera L.) - Brood feeding test -
Report No:	71401031
Document No:	M-454689-01-1
Guidelines:	**GLP compliant study based on the method according to Oomen et al. (1992)** **Deviation: not specified**
GLP/GEP:	**yes**

Objective:
The purpose of this study was to investigate the effect of the test item mix Isoxaflutole WG 75 W + Cyprosulfamide SC 500 G to honey bee brood when exposed by oral ingestion.

Materials and Methods:
Isoxaflutole WG 75 W (Batch ID: ESID202014, sample description: TOX09745-00, purity: 74.9% w/w isoxaflutole (AE B197278), material no: 05923271, specification no: 102000001698-03)
Cyprosulfamide SC 500 G (Batch ID: 2012-002411, sample description: TOX09783-00, purity: 42.6 % w/w cyprosulfamide (AE 001789), specification no: 102000014017-01, density: 1.158 g/mL (20 °C))
Isoxaflutole WG 75 W + Cyprosulfamide SC 500 G mixed with 1 L ready-to-use sugar syrup was fed to bee colonies and mortality of adult bees, pupae and larvae observed at test end (21 days after test initiation). The mixing ratio was 0.334 g Isoxaflutole WG 75 W (= 0.25 g isoxaflutole) + 0.587 g Cyprosulfamide SC 500 G (= 0.25 g cyprosulfamide). Also bee brood development (eggs, young and old larvae) were recorded at test initiation and after 4, 8, 15 and 21 days. As control pure sugar syrup

(30% sucrose, 31% glucose, 39% fructose) was used. 3.0 g/L syrup Insegar (25% fenoxycarb, 0.75 g fenoxycarb/L) was used as reference substance.
Bee colonies were free flying in natural field conditions, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops of flowering weeds in the surrounding area.

Dates of experimental work: July 02, 2012 to July 26, 2012

Results:

Effect of Isoxaflutole WG 75 W + Cyprosulfamide SC 500 G on honey bees (*Apis mellifera*) in a bee brood study

Test item Test object Exposure		Isoxaflutole WG 75 W + Cyprosulfamide SC 500 G *Honey bees (Apis mellifera L.)*, complete colonies Natural conditions		
		Control	**Test item**	**Reference item**
Termination rate [%]	eggs	18.9	18.4 n.s.	100*
	young larvae	14.7	16.0 n.s.	97.8**
	old larvae	3.3	1.8 n.s.	54.3**
Mean brood termination rate over all stages [%] 1)		12.3	12.1	12.1 n.s.
Mean mortality of worker bees/colony/day during 2)	pre-application phase	23.1	20.0 n.s.	24.3 n.s.
	during entire post application phase	54.0	45.7 n.s.	33.9 n.s.
Mean mortality of worker pupae/colony/day during 3)	pre-application phase	0.6	0.2 n.s.	0.1 n.s.
	during entire post application phase	3.6	3.2 n.s.	4.0 n.s.
Mean number of bees before application		15300	19590	13665

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

There was no statistically significant difference in the termination rate of eggs, young larvae and old larvae in the test item treatment group when compared to the values of the control group. Adult bee mortality in the test item treatment group was not statistically significantly different when compared to the control group. No statistically significant effects of the test item on honey bee pupae were observed.

Conclusion:

Overall, it can be concluded according to the results of this study that a test item mix of isoxaflutole and cyprosulfamide does neither adversely affect honey bee colonies nor bee brood development.

CA 8.3.1.4 Sub-lethal effects

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

CA 8.3.2 Effects on non-target arthropods other than bees

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

Studies on non-target arthropods have been performed with the representative formulation IFT + CSA SC 480 and are presented in MCP, Dossier number D-009257-01-1, Annex point 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

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

CA 8.3.2.2 Effects on Typhlodromus pyri

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

CA 8.4 Effects on non-target soil mesoand macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

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

In order to address new data requirements according to Regulation (EC) No 1107/2009, several additional studies on chronic exposure to earthworm have been performed with isoxaflutole and two major soil metabolites and are submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. These studies will be summarized below.

RPA 205834 is a major soil metabolite only in anaerobic soil. Isoxaflutole is only applied in the spring/summer months when anaerobic conditions would not occur. Since RPA 205834 is only formed directly from isoxaflutole and isoxaflutole is rapidly degraded in soil no formation of RPA 205834 would be likely in the winter period when anaerobic conditions could occur. Therefore no studies with RPA 205834 on soil organisms are considered necessary.

Table 8.4.1- 1: Additional chronic earthworm studies of isoxaflutole and its metabolites

Test substance	Test species/study type	Endpoint		References Doc. No.
Isoxaflutole	*Eisenia fetida* reproduction 56 d	NOEC	17.8 mg a.s./kg dws	[redacted] (2013) kra-Rg-R-129/12 M-450435-01-1 KCA 8.4.1/01
RPA 202248	*Eisenia fetida* reproduction 56 d	NOEC	16 mg p.m./kg dws	[redacted] (2012) kra-Rg-R-132/12 M-442776-01-1 KCA 8.4.1/02
RPA 203328	*Eisenia fetida* reproduction 56 d	NOEC	≥ 1000 mg p.m./kg dws	[redacted] (2004) C041342 M-230530-01-2 KCA 8.4.1/03

Report:	[redacted];[redacted];2013;M-450435-01
Title:	Isoxaflutole (AE B197278) technical: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No:	kra-Rg-R-129/12
Document No:	M-450435-01-1
Guidelines:	**ISO 11268-2: 1998 (E); OECD 222: April 13, 2004; Deviation: not specified**
GLP/GEP:	**yes**

Objective:

The purpose of this study was to assess the effects of isoxaflutole technical on mortality, reproduction and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake during an exposure of 56 days in an artificial soil.

Materials and Methods:

Test item: isoxaflutole technical; (Batch code: AE B197278-01-01, Origin Batch No.: 6464/5/8/9; specification no: 102000029614, CAS 141112-29-0, Article No. 06180779, purity: 98.7% w/w).

Adult earthworms (*Eisenia fetida*, approx. 3 months old in the 1st test run and 5 months old in the 2nd test run, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed to 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight (d.w.) in a first test run and to 5.6, 10.0, 17.8, 31.7 and 56.0 mg test item/kg soil d.w. containing 73.8% industrial quartz sand, 20% kaolin clay, 5% sphagnum peat, 0.2 – 0.22% $CaCO_3$ and 1% dried ground cow manure as food. The temperature was 20 ± 2 °C and a light/dark cycle = 16 h : 8 h and a light intensity of 400 – 800 lux. As toxic reference Carbendazim EC 360 G (CAS 10605-21-7) was tested. Mortality and biomass change were determined after 28 days and reproduction was determined after 56 days.

Dates of experimental work: First run: October 17, 2011 to March 09, 2012
Second run: December 20, 2011 to May 14, 2012

Results:

Validity Criteria	**Recommended**	**Obtained**	
		1st test run	**2nd test run**
Adult mortality in control	≤ 10%	0%	0%
Number of juveniles per replicate	≥ 30	233.4	279.1
Coefficient of variation of reproduction	≤ 30%	9.0%	18.2%

All validity criteria for the study were met.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	Isoxaflutole technical *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change [mg test item /kg soil d.w.]	Reproduction
LOEC	> 1000	56.0	31.7
NOEC	≥ 1000	31.7	17.8

Isoxaflutole technical, first run [mg test item /kg soil d.w.]						
	Control	100	178	316	562	1000
	Mortality of adult worms after 28 days					
Mortality [%]	0	0	2.5	0	0	0
	Biomass change (Mean change of body weight from day 0 to day 28)					
Mean [%]	47.8	19.06 [a]	27.42 [a]	22.49 [a]	7.34 [a]	14.55 [a]
Standard deviation	4.37	9.96	7.48	3.25	12.02	5.75
	Number of juveniles per test vessel after 56 days					
Mean	233.4	147.8 [b]	126.0 [b]	144.5 [b]	106.0 [b]	111.5 [b]
Standard deviation	20.9	25.7	19.5	26.1	19.3	24.9
Coefficient of variance [%]	9.0	21.8	15.5	18.1	18.2	22.3
	Reproduction compared to control [%]					
% to control	-	50.5	54.0	61.9	45.4	47.8

[a] Williams multiple sequential t-test, two-sided, $\alpha = 0.05$)
[b] Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$)

Isoxaflutole technical, second run [mg test item /kg soil d.w.]						
	Control	**5.6**	**10.0**	**17.8**	**31.7**	**56.0**
	Mortality of adult worms after 28 days					
Mortality [%]	0	0	0	0	0	0
	Biomass change (Mean change of body weight from day 0 to day 28)					
Mean [%]	51.06	60.72	60.84	61.31	51.55	37.72[a]
Standard deviation	5.81	3.33	5.95	8.31	13.05	2.21
	Number of juveniles per test vessel after 56 days					
Mean	279.1	322.5	259.3	260.5	206.8[b]	184.3 [b]
Standard deviation	50.9	11.7	28.5	17.9	46.1	7.3
Coefficient of variance [%]	18.2	3.6	11.0	6.9	22.3	3.9
Reproduction compared to control [%]						
% to control	-	115.5	92.9	93.3	74.1	66.0

[a] Statistical significant compared to control (Welch t-test for inhomogenous variances with Bonferroni-Holm-Adjustment)
[b] Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$)

Mortality:
No mortality occurred in any tested concentration except for one dead earthworm at 178 mg test item/kg soil d.w. In the control groups 0 % mortality, which is below the allowed maximum of ≤ 10%, was recorded. The LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg soil d.w.

Biomass:
Body weight change was significantly different to the control at and above concentrations of 56.0 mg test item/kg soil d.w.

Reproduction:
Reproduction was significantly different to the control at and above concentrations of 31.7 mg test item/kg soil d.w.

Conclusion:

For isoxaflutole technical, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 17.8 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 31.7 mg test item/kg soil d.w.

Reference test:
The most recent non-GLP-test (Study No: Rg 07/12, Report No. kra-Rg-R-Ref-16/12, February 24, 2012 – May 02, 2012) with the reference item carbendazim was performed at test concentrations 1.25, 2.5 and 5.0 mg a.s./kg soil d.w.

No mortality was observed during the study at any concentration tested. Carbendazim showed an EC_{50} (reproduction) of 1.66 mg a.s./kg soil d.w. based on Williams multiple sequential t-test, $\alpha = 0.05$. This is in the recommended range of the guideline of 1 – 5 mg a.s./kg soil d.w.

Metabolite RPA 202248

Report:	[redacted]; [redacted];2012;M-442776-01
Title:	Isoxaflutole-RPA202248: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No:	KRA-RG-R-132/12
Document No:	M-442776-01-1
Guidelines:	**ISO 11268-2: 1998(E) and OECD 222: April 13, 2004; Deviation: not specified**
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effects of RPA 202248 (metabolite of isoxaflutole) on mortality, reproduction and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake during an exposure of 56 days in an artificial soil.

Materials and Methods:
Test item: RPA 202248 (AE 0540092); (Batch code: AE 0540092; Origin Batch No.: BCOO5951-1-1; Material: AE 0540092, pure substance; purity: 99.9 %w/w).

Adult earthworms (*Eisenia fetida andrei*) were exposed to 100 mg test item/kg soil dry weight (d.w.) in a first test run and to 9.0, 16.0, 28.4, 50.6 and 90.0 mg test item/kg soil d.w. containing 73.77-73.82% industrial quartz sand, 20% kaolin clay, 5% sphagnum peat, 0.18 - 0.22% $CaCO_3$ and 1% dried ground cow manure as food. The earthworms in the first run were 6 month old and in the second run 5 month old. The temperature was 20 ± 2 °C and a light/dark cycle = 16 h : 8 h and a light intensity of 400 – 800 lux. For each group (treatment and control), 8 replicates with 10 worms each were used in the first run and in the second run 4 replicates with 10 animals were used for the test groups and 8 replicates with 10 animals for the control. As toxic reference carbendazim (CAS 10605-21-7) was tested.
Mortality and biomass change were determined after 28 days and reproduction was determined after 56 days.

Dates of experimental work: First run: September 01, 2011 to November 03, 2011
Second run: February 15, 2012 to April 23, 2012

Results:

Validity Criteria	Recommended	Obtained	
		1st test run	2nd test run
Adult mortality in control	≤ 10%	1.25%	0%
Number of juveniles per replicate	≥ 30	370.4 (300 – 431)	298.4 (221 – 396)
Coefficient of variation of reproduction	≤ 30%	13.3%	20.6%

All validity criteria for the study were met.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	RPA 202248 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change [mg test item /kg soil d.w.]	Reproduction
LOEC	100	28.4	28.4
NOEC	≥ 100	16.0	16.0

Isoxaflutole-RPA 202248 [mg test item /kg soil d.w.]								
	Control	9.0	16.0	28.4	50.6	90.0	Control	100
	Mortality of adult worms after 28 days							
Mortality [%]	0	0	0	0	0	0	1.25	1.25
	Biomass change (Mean change of body weight from day 0 to day 28)							
Mean [%]	91.61	96.53	86.19	75.52[a]	72.43[a]	86.51[a]	91.43	54.08[b]
Standard deviation	6.81	9.18	8.48	5.57	9.83	18.49	10.57	14.92
	Number of juveniles per test vessel after 56 days							
Mean	298.4	282.0	258.3	195.8[c]	196.0[c]	148.5[c]	370.1	188.0[d]
Standard deviation	61.5	31.9	45.0	22.5	38.2	27.9	49.3	21.8
Coefficient of variance [%]	20.6	11.3	17.6	11.5	19.5	18.8	13.3	11.6
	Reproduction compared to control [%]							
% to control	-	94.5	85.5	65.6	65.7	49.8	-	50.8

[a] Williams multiple sequential t-test, two-sided, α = 0.05)
[b] Statistical significant compared to control (Student-t test, two-sided, α = 0.05)
[c] Williams multiple sequential t-test, one-sided smaller, α = 0.05)
[d] Statistical significant compared to control (Student-t test, one-sided smaller, α = 0.05)

Mortality:
No mortality occurred at all concentrations except 100 mg test item/kg soil d.w. At this concentration 1.25% mortality was observed at the tested concentration and in the control. Therefore the corrected mortality is also 0%.
In the control groups 0 - 1.25% mortality, which is below the allowed maximum of ≤ 10%, was recorded. The LC_{50} could not be calculated and is considered to be > 100 mg test item/kg dry weight artificial soil.

Biomass:
Body weight change was significantly different to the control at and above concentrations of 28.4 mg test item/kg soil d.w.

Reproduction:
Reproduction was significantly different to the control at and above concentrations of 28.4 mg test item/kg soil d.w.

Conclusion:

For RPA 202248 (metabolite of isoxaflutole) the overall No-Observed-Effect-Concentration (NOEC) was determined to be 16.0 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 28.4 mg test item/kg soil d.w.

Reference test:
The most recent non-GLP-test (Document Number: Rg 18/101, Report No. LRT-Rg-R-Ref-15/11, January 31, 2001 – April 05, 2011) with the reference item carbendazim was performed at test concentrations 1.25, 2.5 and 5.0 mg a.s./kg soil d.w.
No mortality was observed during the study at any concentration tested. Carbendazim showed an EC_{50} (reproduction) of 1.66 mg a.s./kg soil d.w. (95% confidence limits from 1.62 to 1.69 mg a.s./kg soil d.w.) based on Williams multiple sequential t-test, $\alpha = 0.05$.
This is in the recommended range of the guideline of 1 - 5 mg a.s./kg soil d.w.

Metabolite RPA 203328

Report:	KCA 8.4.1 /03; M[redacted], A.;2004;M-230530-01
Title:	Isoxaflutole-RPA203328 (AE B197555): Reproduction toxicity to earthworm Eisenia fetida in artificial soil
Report No:	C041342
Document No.	M-230530-01-2
Guidelines:	**ISO: 11268-2;** **Deviation: not specified**
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effects of RPA 203328 (metabolite of isoxaflutole) on mortality, reproduction and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake during an exposure of 56 days in an artificial soil.

Materials and Methods:
Test item: RPA 203328; Batch No: IGB947, CAS No.: 142994-06-7, purity: 99.6%, white powder

Adult earthworms (*Eisenia fetida*) were exposed to nominal concentrations of 10.0, 31.6, 100.0, 316.0 and 1000.0 mg RPA 203328/kg soil dry weight (d.w.) containing 69.6% industrial quartz sand, 20% kaolin clay, 10% sphagnum peat, 0.4% $CaCO_3$. Worms were fed with 5 g finely ground cow manure per test vessel weekly. The temperature was 18 - 22°C and a light:dark cycle = 16 h : 8 h and a light intensity of 494 - 574 lux. Four replicates with 10 animals were used for the test groups and the control. As toxic reference Carbendazim SC (CAS 10605-21-7) was tested.
Mortality, biomass change and morphological or behavioural changes were determined after 28 days and the number of juveniles was determined after 56 days.

Dates of experimental work: February 12, 2004 to April 08, 2004

Results:

Validity Criteria	Recommended	Obtained
Adult mortality in control	≤ 10%	0%
Number of juveniles per replicate	≥ 30	285.8 ± 9.5
Coefficient of variation of reproduction	≤ 30%	3.3%

All validity criteria for the study were met.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	RPA 203328 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change [mg test item /kg soil d.w.]	Reproduction
LOEC	> 1000	> 1000	> 1000
NOEC	≥ 1000	≥ 1000	≥ 1000

RPA 203328 [mg test item /kg soil d.w.]						
	Control	**10**	**31.6**	**100**	**316**	**100**
	Mortality of adult worms after 28 days					
Mortality [%]	0	0	0	0	0	0
	Biomass change (Mean change of body weight from day 0 to day 28)					
Mean [%]	132.1	132.7	133.2	132.4	135.3	143.0
	Number of juveniles per test vessel after 56 days					
Mean	285.8	317.3	270.0	320.8	251.3	289.0

Mortality:
No mortality occurred in any tested concentration and the control. The LC_{50} could not be calculated and is considered to be > 1000 mg RPA 203328/kg soil d.w.

Biomass:
Body weight change was not significantly different to the control at any concentration tested.

Reproduction:
Reproduction was not significantly different to the control at any tested concentration.

Conclusion:

For RPA 203328 (metabolite of isoxaflutole), the No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil d.w.

Reference test:
The most recent test (December, 2003, ECT Study No. RR1003) with the reference item carbendazim was performed at test concentrations 1, 3 and 5 mg a.s./kg soil d.w.
A reduction in reproduction of 100% compared to the control was found at treatment levels of 3 and 5 mg a.s./kg soil d.w. The NOEC for reproduction was 1.0 mg a.s./kg soil d.w.

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

Testing on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) was performed with the parent compound and two soil metabolites of isoxaflutole. The corresponding summaries are provided below under point 8.4.2.1.

RPA 205834 is a major soil metabolite only in anaerobic soil. Isoxaflutole is only applied in the spring/summer months when anaerobic conditions would not occur. Since RPA 205834 is only formed direct from isoxaflutole and isoxaflutole is rapidly degraded in soil no formation of RPA 205834 would be likely in the winter period when anaerobic conditions could occur. Therefore no studies with soil organisms are considered necessary.

Table 8.4.2- 1 Additional studies on soil organisms with isoxaflutole and its metabolites

Test substance	Test species/study type	Endpoint		References
Collembola, reproduction				
Isoxaflutole	*Folsomia candida* reproduction, 28 d	NOEC	≥ 1000 mg a.s./kg dws	[redacted] (2011) FRM-COLL-124/11 M-416012-01-1 KCA 8.4.2/02
RPA 202248	*Folsomia candida* reproduction, 28 d	NOEC	≥ 100 mg p.m./kg dws	[redacted] (2011) FRM-Coll-134/11 M-420112-01-1 KCA 8.4.2/04
RPA 203328	*Folsomia candida* reproduction, 28 d	NOEC	≥ 100 mg p.m./kg dws	[redacted] (2011) FRM-COLL-135/11 M-420062-01-1 KCA 8.4.2/06
Soil mites, reproduction				
Isoxaflutole	*Hypoaspis aculeifer* reproduction, 14 d	NOEC	562 mg a.s./kg dws	[redacted] (2011) kra-HR-46/11 M-416751-01-1 KCA 8.4.2/03
RPA 202248	*Hypoaspis aculeifer* reproduction, 14 d	NOEC	≥ 100 mg p.m./kg dws	[redacted] (2011) kra-HR-63/11 M-417912-01-1 KCA 8.4.2/05
RPA 203328	*Hypoaspis aculeifer* reproduction, 14 d	NOEC	≥ 100 mg p.m./kg dws	[redacted] (2011) kra-HR-64/11 M-419849-01-1 KCA 8.4.2/07

CA 8.4.2.1 Species level testing

Report:	[redacted];[redacted];2011;M-416012-01
Title:	Isoxaflutole a.s.: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-124/11
Document No:	M-416012-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil; Deviation: none**
GLP/GEP:	**yes**

Objective:

The purpose of this study was to assess the effect of isoxaflutole 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.

Materials and Methods:

Isoxaflutole a.s. (analytical findings: 98.7 % w/w isoxaflutole, origin batch no.: 6464/5/8/9, customer order no.: TOX 08283-01, specification no.: 102000002961, LIMS no.: 1013108.

Ten collembolans (11-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 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.

Dates of experimental work: August 05, 2011 to September 07, 2011

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20 %	8.8 %
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 100	1476.4
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	6.1 %

All validity criteria for the study were met.

Effect of isoxaflutole on Collembola (*Folsomia candida*) in a 28-day reproduction study

Test item **Test object** **Exposure**	**Isoxaflutole a.s.** ***Folsomia candida*** **Artificial soil**		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles ± SD	Reproduction (% of control)
Control	8.8	1476.4 ± 90.7	-
100	2.5	1581.0 ± 115.3	107.1 n.s.
178	2.5	1572.0 ± 132.9	106.5 n.s.
316	7.5	1641.5 ± 68.0	111.2 n.s.
562	0.0	1568.5 ± 129.7	106.2 n.s.
1000	2.5	1524.8 ± 195.7	103.3 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 unrounded values
n.s. = statistically not significant (William's-t test one-sided-smaller, α = 0.05)

Mortality:

In the control group 8.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg artificial soil dry weight.

Reproduction:

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

Conclusion:

The $NOEC_{reproduction}$ for reproduction is ≥ 1000 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ is > 1000 mg test item/kg soil d.w. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 1000 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 1000 mg test item/kg artificial soil dry weight.

Reference test:

The most recent non-GLP-test (FRM-Coll-Ref-15/1, U. [redacted], March 08, 2011) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg boric acid/kg artificial soil dry weight.

Boric acid showed an EC_{50} of 91 mg test item/kg artificial soil dry weight (95% confidence limits from 80 mg to 104 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.

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

The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg artificial soil dry weight according to Williams-Test multiple t-test procedure, α = 0.05, one-sided smaller.

This shows that the test organisms are sufficiently sensitive.

Report:	[redacted];[redacted];2011;M-416751-01
Title:	Isoxaflutole a. s.: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-46/11
Document No:	M-416751-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; Minor deviations**
Deviations:	Minor deviations
GLP/GEP:	**yes**

Objective:

The purpose of the study was to assess the effects of isoxaflutole a.s. on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:

Test item: isoxaflutole a.s.; (Batch code: AE B197278-01-01; Origin Batch No.: 6464/5/8/9; Customer Order No.: TOX08283-01; purity 98.7 %w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for test item concentration) were exposed to control and one treatment. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (29 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$).

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

Dates of experimental work: August 05, 2011 to August 26, 2011

Results:

Validity Criteria	**Recommended**	**Obtained**
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	274.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	18.4 %

All validity criteria for the study were met.

Effect of isoxaflutole a.s. on *Hypoaspis aculeifer* in a 14-day reproduction study

Test item **Test object** **Exposure**	**Isoxaflutole a.s.** ***Hypoaspis aculeifer*** **Artificial soil**				
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles ± SD**			**Reproduction (% of control)**
Control	3.8	274.5	±	50.6	-
100	5.0	285.0	±	48.3	103.8
178	7.5	265.0	±	45.5	96.5
316	10.0	253.3	±	34.4	92.3
562	7.5	236.0	±	14.3	86.0
1000	12.5	195.0	±	10.7	71.0
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)					562
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)					1000

Statistical significance (William-t-test, one-sided smaller, α = 0.05 was found.

Mortality:

In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg dry weight artificial soil.

Reproduction:

Concerning the number of juveniles statistical analysis (William-t-test, one-sided smaller, α = 0.05) revealed significant differences between the control and the highest concentration of the test item. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg test item/kg dry weight artificial soil. EC_{50} could not be calculated.

Conclusion:

The $NOEC_{reproduction}$ is 562 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ 1000 mg test item/kg soil d.w.

Reference test:

The most recent non-GLP test ([redacted], kra/HR-O-10/11, March 21, 2011) with the reference item dimethoate was performed at test concentrations 0.990, 1.780, 3.156, 5.517 and 9.853 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.051 mg a.s./kg (95 % confidence limits from 3.222 to 5.313 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The $NOEC_{reproduction}$ was calculated to be 3.156 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.517 mg a.s./kg dry weight artificial soil according Williams-Test multiple t-test procedure, α = 0.05, one-sided. Dimethoate showed a EC_{50} of 6.445 mg a.s./kg dry weight artificial soil (95 % confidence limits from 6.022 to 8.022 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.

Metabolite RPA 202248

Report:	[redacted];[redacted];2011;M-420112-01
Title:	Isoxaflutole-RPA202248 (AE 0540092): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-134/11
Document No:	M-420112-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil; Minor deviations**
Deviations:	Minor deviations (feeding and re-moistening took place after 23 days instead of 14 days, no randomizing after 14 days)
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effect of RPA 202248 (metabolite of isoxaflutole) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test item: RPA 202248 (analytical findings: 99.9 % w/w AE 0540092, origin batch no.: BCOO 5951-1-1, certificate no.: AZ 16522, LIMS no.: 1009623, batch code: AE 0540092-PU-01.
10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 lux, 16h light: 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Dates of experimental work: October 07, 2011 to November 08, 2011

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20 %	11.3 %
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 100	1388
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	14.0 %

All validity criteria for the study were met.

Effect of RPA 202248 on Collembola (*Folsomia candida*) in a 28-day reproduction study

Test item **Test object** **Exposure**	**RPA 202248** ***Folsomia candida*** **Artificial soil**		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	11.3	1388.3 ± 194.2	-
100	12.5	1484.0 ± 143.5	106.9 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			> 100

The calculations were performed with unrounded values
n.s. = statistically not significant (Student t-test one-sided-smaller, $\alpha = 0.05$)

Mortality:

In the control group 11.3 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:

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

Conclusion:

The $NOEC_{reproduction}$ is ≥ 100 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ > 100 mg test item/kg soil d.w. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight.

Reference test:

The most recent non-GLP-test (FRM-Coll-Ref-15/11, U. [redacted], March 08, 2011) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.

Boric acid showed an EC_{50} of 91 mg test item/kg artificial soil dry weight (95 % confidence limits from 80 mg to 104 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.

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

The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.

This shows that the test organisms are sufficiently sensitive.

Report:	[redacted];[redacted];2011;M-417912-01
Title:	Isoxaflutole-RPA202248 (AE 0540092): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-63/11
Document No:	M-417912-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; Deviation: none**
GLP/GEP:	**yes**

Objective:

The purpose of the study was to assess the effects of RPA 202248 (metabolite of isoxaflutole) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:

Test item: RPA 202248; (Batch code: AE 0540092-PU-01; Origin Batch No.: BCOO.5951-1-1; Material: AE 0540092; Certificate No.: AZ 16522; purity: 99.9 %w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 100 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$).
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Dates of experimental work: October 07, 2011 to October 26, 2011

Results:

Validity Criteria	**Recommended**	**Obtained**
Mean adult mortality	≤ 20 %	3.8 %
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	314.3
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	13.9 %

All validity criteria for the study were met.

Effect of RPA 202248 on *Hypoaspis aculeifer* in a 14-days reproduction study

Test item **Test object** **Exposure**	**RPA 202248** ***Hypoaspis aculeifer*** **Artificial soil**		
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	3.8	314.3 ± 43.6	-
100	6.3	326.3 ± 37.9	103.8
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

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

Mortality:

In the control group 3.8 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality. The LC_{50} could not be calculated and is considered to be > 100 mg test item/kg dry weight artificial soil.

Reproduction:

Concerning the number of juveniles statistical analysis (Student-t-test, one-sided smaller, α = 0.05) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil.

Conclusion:

The $NOEC_{reproduction}$ is ≥ 100 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ > 100 mg test item/kg soil d.w. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_x-values could not be calculated.

Reference test:

The most recent non-GLP-test ([redacted], kra/HR-O-10/11, March 21, 2011) with the reference item dimethoate was performed at test concentrations 0.990, 1.780, 3.156, 5.517 and 9.853 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.051 mg a.s./kg (95 % confidence limits from 3.222 to 5.313 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The $NOEC_{reproduction}$ was calculated to be 3.156 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.517 mg a.s./kg dry weight artificial soil according Williams-Test multiple t-test procedure, α = 0.05, one-sided. Dimethoate showed a EC_{50} of 6.445 mg a.s./kg dry weight artificial soil (95 % confidence limits from 6.022 to 8.022 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.

Metabolite RPA 203328

Report:	[redacted];[redacted];2011;M-420062-01
Title:	Isoxaflutole-RPA203328 (AE B197555): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-135/11
Document No:	M-420062-01-1
Guidelines:	**OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil; ISO 11267 (1999)**
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effect of RPA 203328 (metabolite of isoxaflutole) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test item: RPA 203328, analytical findings: 99.6 % w/w, origin batch no.: IGB947, certificate no.: AZ 16135, LIMS no.: 0926554, batch code: AE B197555 00 1B99 0001.
10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control (water treated) and 100 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 - 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

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

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	8.8%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 100	1338
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	10%

All validity criteria for the study were met.

Effect of RPA 203328 on Collembola (*Folsomia candida*) in a 28-day reproduction study

Test item **Test object** **Exposure**	**RPA 203328** ***Folsomia candida*** **Artificial soil**		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles±SD**	**Reproduction (% of control)**
Control	8.8	1338.0 ± 134.3	-
100	12.5	1267.8 ± 95.3	94.7 n.s.
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥100
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			>100

The calculations were performed with un-rounded values
n.s. = statistically not significant (Student-t test one-sided-smaller, $\alpha = 0.05$)

Mortality:

In the control group 8.8 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality. A LC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Reproduction:

Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the treatment group.

Conclusion:

The $NOEC_{reproduction}$ is ≥100 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ >100 mg test item/kg soil d.w. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Reference test:

The most recent non-GLP-test (FRM-Coll-Ref-15/11, U. [redacted], March 08, 2011) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.

Boric acid showed an EC_{50} of 91 mg test item/kg artificial soil dry weight (95 % confidence limits from 80 mg to 104 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression.

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

The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure $\alpha = 0.05$, one-sided smaller.

This shows that the test organisms are sufficiently sensitive.

Report:	[redacted];[redacted];2011;M-419849-01
Title:	Isoxaflutole-RPA203328 (AE B197555): Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-64/11
Document No:	M-419849-01-1
Guidelines:	**OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil**
Deviations:	Yes (15 days of exposure instead of 14 days)
GLP/GEP:	**yes**

Objective:
The purpose of the study was to assess the effects of RPA 203328 (metabolite of isoxaflutole) on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 15 days in artificial soil comparing control and treatment.

Materials and Methods:
Test item: RPA 203328, (Batch code: AE B197555 00 1B99 0001; Origin Batch No.: IGB947; Material: AE B197555, pure substance; Certificate No.: AZ 16135; purity: 99.6 %w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control and one treatment. The concentration of 100 mg test item/kg dry weight artificial soil was tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (29 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8 % fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20 % Kaolin clay and approximately 0.2 % Calcium carbonate ($CaCO_3$).
After a period of 15 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Dates of experimental work: October 14, 2011 to November 04, 2011

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20 %	1.3 %
Mean number of juveniles per replicate (with 10 adult females introduced)	≥ 50	392
Coefficient of variation calculated for the number of juvenile mites per replicate	≤ 30 %	8.0%

All validity criteria for the study were met.

Effects of RPA 203328 on *Hypoaspis aculeifer* in a 14-days reproduction study

Test item **Test object** **Exposure**	**RPA 203328** ***Hypoaspis aculeifer*** **Artificial soil**		
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	1.3	392.0 ± 31.2	
100	6.3	380.9 ± 42.0	97.2
NOEC (mg test item/kg dry weight artificial soil)			≥ 100
LOEC (mg test item/kg dry weight artificial soil)			> 100

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

Mortality:

In the control group 1.3% of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20% mortality. The LC_{50} could not be calculated and is considered to be > 100 mg test item/kg dry weight artificial soil.

Reproduction:

Concerning the number of juveniles statistical analysis (Student-t-test, one-sided smaller, α = 0.05) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil.

Conclusion:

The $NOEC_{reproduction}$ is ≥ 100 mg test item/kg soil d.w. and the $LOEC_{reproduction}$ > 100 mg test item/kg soil d.w. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_x-values could not be calculated.

Reference test:

The most recent non-GLP-test ([redacted], kra/HR-O-10/11, March 21, 2011) with the reference item dimethoate was performed at test concentrations 0.990, 1.780, 3.156, 5.517 and 9.853 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.051 mg a.s./kg (95% confidence limits from 3.222 to 5.313 mg a.s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The $NOEC_{reproduction}$ was calculated to be 3.156 mg a.s./kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 5.517 mg a.s./kg dry weight artificial soil according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided. Dimethoate showed a EC_{50} of 6.445 mg a.s./kg dry weight artificial soil (95% confidence limits from 6.022 to 8.022 mg a.s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil.

CA 8.5 Effects on soil nitrogen transformation

For information on studies already evaluated during the first EU review of isoxaflutole, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.
An N-transformation study with the metabolite RPA 202248 was performed, which was not submitted during the first Annex I inclusion process and is submitted within this Supplemental Dossier for the isoxaflutole Annex I Renewal. This study will be summarized below.

Table 8.8- 1: Additional studies on nitrogen transformation with isoxaflutole metabolites

Test substance	Test species/study type	Endpoint		References
RPA 202248	Nitrogen transformation, 84 d	No influence	0.13 mg p.m./kg soil 0.67 mg p.m./kg soil	[redacted] (2013) M-469915-01-1 KCA 8.5/04

Report:	[redacted]; [redacted]; 2013; M-469915-01
Title:	Isoxaflutole-RPA202248 (BCS-AB59005): Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	13 10 48 084 N
Document No:	M-469915-01-1
Guidelines:	**OECD 216 (2000)**
GLP/GEP:	**yes**

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

Materials and Methods:
Test item: RPA202248 (metabolite of isoxaflutole), BCS-code: BCS-AB59005, Batch code: AE 0540092-01-01, Origin Batch No.: GSE 61005-35-1, Customer order No: TOX 09986-01, CAS No.: 143701-75-1, LIMS No.: 1328350, Certificate No.: MZ 00730, analysed purity: 99.5 % w/w
A loamy sand soil (DIN 4220) was exposed for 84 days to 0.13 and 0.67 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration

in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14, 28, 42, 56, 70 and 84 days after treatment).

Dates of experimental work: August 16 to November 08, 2013

Results:

Validity criteria:

Validity Criteria	Recommended by the guideline	Obtained in this study
coefficient of variation (CV) between nitrate-N concentration in replicate control samples	15%	6.5%

All validity criteria for the study were met.

Effects on nitrogen transformation in soil after treatment with RPA 202248 (BCS-AB59005)

Time Interval	Control			0.13 mg test item/kg soil dry weight equivalent to 0.1 kg test item/ha				0.67 mg test item/kg soil dry weight equivalent to 0.5 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.58	±	0.06	3.17	±	0.07	**-11.3** *s.	3.24	±	0.46	**-9.3** n.w.
7-14	1.51	±	0.19	1.05	±	0.19	**-30.5** *s.	1.65	±	0.12	**+8.8** n.s.
14-28	1.00	±	0.10	1.38	±	0.07	**+38.5** *s.	1.00	±	0.01	**+0.2** n.w.
28-42	0.70	±	0.20	1.19	±	0.37	**+69.5** n.s.	0.84	±	0.14	**+19.3** n.s.
42-56	0.42	±	0.21	0.20	±	0.13	**-52.8** n.s.	0.52	±	0.22	**+23.3** n.s.
56-70	0.48	±	0.20	0.65	±	0.13	**+37.5** n.s.	0.69	±	0.10	**+44.0** n.s.
70-84	0.86	±	0.08	0.65	±	0.28	**-24.1** n.s.	0.78	±	0.13	**-9.1** n.s.

The calculations were performed with unrounded values

1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

n.w. = No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

*s. = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application.

Observations:

The test item RPA 202248 (BCS-AB59005) caused temporary stimulations and inhibitions of the daily nitrate rate at the tested concentration of 0.13 mg/kg soil dry weight up to time interval 56-70 days after application.

However, no adverse effects of RPA202248 (BCS-AB59005) on nitrogen transformation in soil could be observed at a test concentration of 0.13 mg/kg dry soil at the end of the test, 84 days after application (time interval 70-84 days after application).
A difference from control of -24.1 % (test concentration 0.13 mg/kg dry soil) was measured 84 days after application (time interval 70-84 days after application).

The test item RPA 202248 (BCS-AB59005) caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.67 mg/kg soil dry weight at time interval 56-70 days after application. However, no adverse effects of RPA202248 (BCS-AB59005) on nitrogen transformation in soil could be observed at the tested concentration of 0.67 mg/kg dry at the end of the test, 84 days after application (time interval 70-84 days).
A difference from the control of -9.1 % (test concentration 0.67 mg/kg dry soil) was measured at the end of the 84-day incubation period (time interval 70-84).

Conclusion:

RPA 202248 (BCS-AB59005) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 84-day incubation period. The study was performed in a field soil at concentrations up to 0.67 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.5 kg test item/ha.

CA 8.6 Effects on terrestrial non-target higher plants

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

CA 8.6.1 Summary of screening data

According to the data requirements for plant protection products (Commission Regulation No 284/2013) screening data shall only be required for plant protection products other than those exhibiting herbicidal or plant growth regulator activity. Since isoxaflutole is an herbicide and a complete set of Tier 2 terrestrial non-target plant studies is available (see MCP, Dossier number D-009257-01-2), no further data is considered necessary.

CA 8.6.2 Testing on non-target plants

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

Studies on non-target plants (seedling emergence and vegetative vigour) have been conducted with the representative formulation Isoxaflutole + Cyprosulfamide SC 480 and are presented in MCP, Dossier number D-009257-01-2, Annex point 10.6.2.

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

No studies on other terrestrial organisms were necessary.

CA 8.8 Effects on biological methods for sewage treatment

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

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

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

Test substance	Test species/ study type	Endpoint		References
Isoxaflutole, tech.	Activated sludge, 3 h	EC_{50}	> 1000 mg a.s./L	[redacted] (2001) M-240627-01-1 KCA 8.8/01

Report:	[redacted]; [redacted]; 2001; M-240627-01
Title:	Toxicity of Isoxaflutole, substance technical; Code AE B197278 00 1D99 0001 to Activated Sludge in a Respiration Test
Report No:	B003605
Document No:	M-240627-01-1
Guidelines:	**OECD: 209 (1984)** **Deviations: not specified**
GLP/GEP:	**yes**

Objective:

The influence of the test item Isoxaflutole; substance technical; Code: AE B1972 78 00 1D99 0001 on the activity of activated sludge was evaluated by measuring the respiration rate under defined conditions.

Materials and Methods:

Test item: Isoxaflutole tech., Code: AE B197278 00 1D99 0001, Batch No.: 279, analysed purity: 98.7 % w/w.

The respiration rate (oxygen consumption) of an aerobic activated sludge fed with a standard amount of synthetic sewage was measured in the presence of various concentrations of the test item after an incubation period of 3 hours. Test concentrations were 10, 32, 100, 320 and 1000 mg Isoxaflutole; substance technical; /L; 3.2, 10 and 32 mg 3,5-Dichlorophenol/L as reference substance and two inoculum controls.

Dates of experimental work: July 12 to July 12, 2001

Results:

Influence of Isoxaflutole technical on oxygen consumption of activated sludge

Treatment [mg test item/L]	Oxygen consumption [mg O_2/L min]	Inhibition [%]	Oxygen concentration (mg O_2/L)	
			Start*	End*
Control	0.457	-	6.9	7.1
Control	0.533		6.0	6.7
Pooled control	0.495	-	-	-
1000	0.520	-5.1	6.5	6.7
320	0.492	0.6	6.5	6.9
100	0.513	-3.6	6.3	6.8
32	0.482	2.6	6.2	6.9
10	0.520	-5.1	6.2	6.6

*: start and end of 3-hour aeration

Observations:

In comparison to the inoculum controls the respiration rate of the activated sludge was not inhibited (- 5.1 % to 2.6 %) up to the highest test concentration of 1000 mg test item/L. Concentrations exceeding 1000 mg/L nominal were not tested.

Based on measured inhibition rates, the 3-hour EC_{20} and EC_{50} could not be quantified because up to the highest nominal test concentration of 1000 mg/L less than 20 % inhibition was noted after three hours incubation.

The 3-hour EC_{50} for the positive control 3,5-Dichlorophenol, which was tested in the same way as the test item, was found to be 5.2 mg/L and is within the range of 5 - 30 mg/L recommended by the test guidelines; thus, confirming suitability of the activated sludge.

Conclusion:

The 3-hour EC_{20} and EC_{50} for isoxaflutole are clearly higher than 1000 mg/L under the present test conditions.

CA 8.9 Monitoring data

No monitoring data are available.