Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Trifloxystrobin

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

2013-12-04

Author(s)

[redacted]

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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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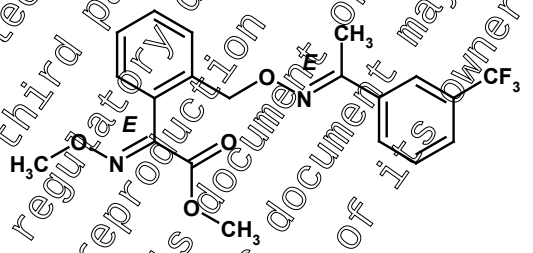
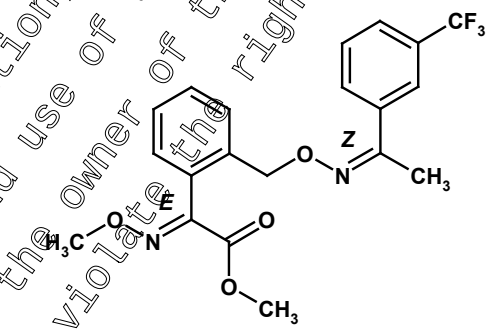
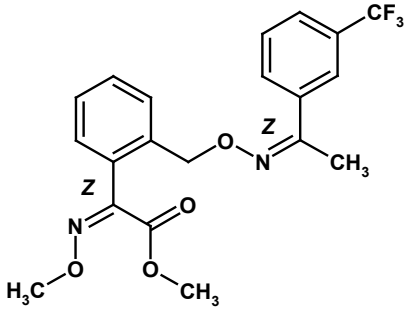
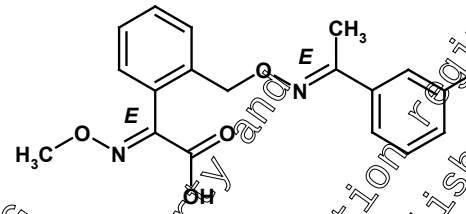
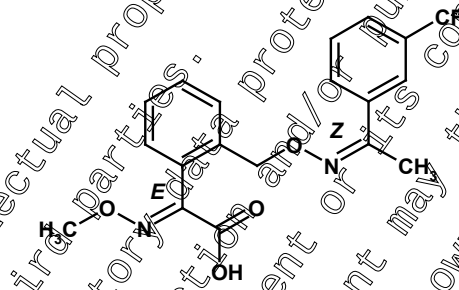
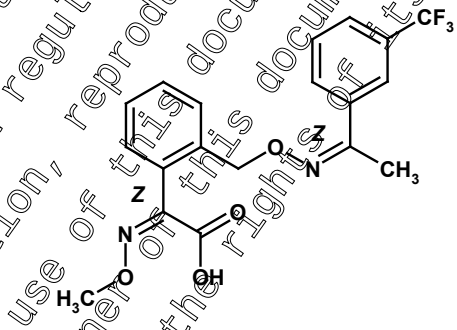
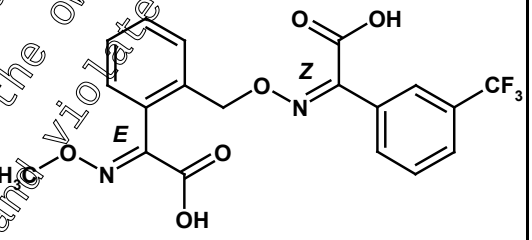
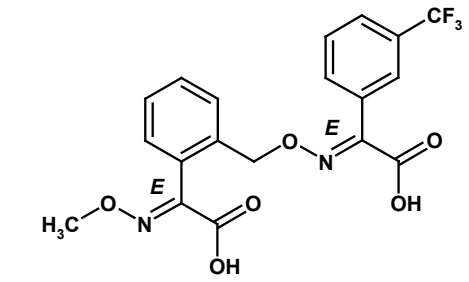
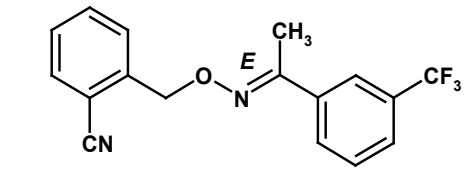
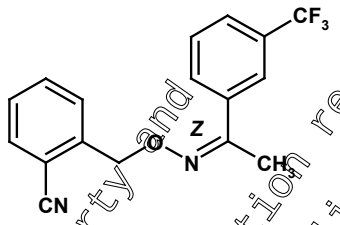
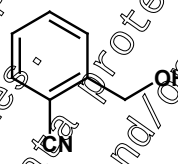
CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Data on the ecotoxicological data of trifloxystrobin and its major metabolites had been submitted within the EU Dossier (Baseline Dossier), which resulted in the Annex inclusion under Directive 91/414/EEC in 2003. In the Supplemental Dossier for renewal of approval of trifloxystrobin presented here only those ecotoxicological studies are described, which had not been submitted within the Baseline Dossier. The codes and structures of trifloxystrobin and its metabolites are presented in Table 8 – 1.

Table 8 - 1: List of codes and structures

Structural formula of trifloxystrobin (CGA 279202, *EE*-isomer):	
Structural formula of CGA 357261 (*ZE*-isomer):	
Structural formula of CGA 357262 (*ZZ*-isomer)	

Structural formula of CGA 321113 (*EE*-isomer):	
Structural formula of CGA 373466 (*ZE*-isomer):	
Structural formula of CGA 381318 (*ZZ*-isomer):	
Structural formula of NOA 413161 (*ZE*-isomer):	
Structural formula of NOA 413163 (*EE*-isomer):	
Structural formula of CGA 357276 (*E*-isomer):	

Structural formula of NOA 409480 (*Z*-isomer):	
Structural formula of 2-hydroxymethylbenzonitrile Note: 2-Hydroxymethylbenzonitrile is present in a tautomeric equilibrium with 2-Benzofuran-1(3H)-imine, for further details please refer to the study report M-442300-01-1	

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

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.1.1.1- 1: Avian acute oral toxicity data of trifloxystrobin

Test substance	Exposure	Species	Endpoint		Reference
trifloxystrobin	Acute risk assessment	Bobwhite quail	LD_{50}	>2000 mg a.s./kg bw	[redacted], 1995 M-032008-01-1 KCA 8.1.1.1/01

CA 8.1.1.2 Short-term dietary toxicity to birds

For information on studies already evaluated during the first EU review of trifloxystrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. No new studies have been performed but the calculation of the achieved daily dietary dose in these studies has been performed in M-469005-01-1 (KCA 8.1.1.2/03)

Table 8.1.1.2- 1: Daily dietary dose in avian short term dietary toxicity studies with trifloxystrobin

Test substance	Exposure	Species	Endpoint		Reference
trifloxystrobin	Short-term dietary	Bobwhite quail	LDD_{50}	>1568 (nom.) > 1396 (meas.) mg a.s./kg bw/d	[redacted], 1995 M-032010-01-1 KCA 8.1.1.2/01
		Mallard duck	LDD_{50}	>1486 (nom.) > 1443 (meas.) mg a.s./kg bw/d	[redacted], 1995 M-032012-01-1 KCA 8.1.1.2/02

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

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

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.1.1.3- 1: Avian long-term toxicity of trifloxystrobin

Test substance	Exposure	Species	Endpoint		Reference
trifloxystrobin	Reproductive risk assessment	Bobwhite quail	NOEL	≥ 32 [a] mg a.s./kg bw/d	[redacted], 1996 M-032013-01-2 KCA 8.1.1.3/01

[a] Dose calculation from 320 ppm with conversion factor 0.1 according to EFSA GD (2009) section 2.3.1.1

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

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 10.1.2.1- 1: Acute oral toxicity data for mammals exposed to trifloxystrobin

Test substance	Exposure	Species/Origin	Endpoint		Reference
Trifloxystrobin	Acute risk assessment	Rat	LD_{50}	>5000 mg a.s./kg bw	[redacted], 1994 M-039034-01-1 KCA 5.2.1/01

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

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

An additional statement is submitted within this Supplemental Dossier for renewal of approval of trifloxystrobin. A summary of the statement discussing the long-term endpoint for mammals in the risk assessment is given below.

Table 8.1.2.2- 1: Mammals long-term toxicity of trifloxystrobin

Test substance	Exposure	species/ origin	Endpoint [mg a.s./kg bw/d]		Reference
Trifloxystrobin	Long-term risk assessment	EFSA GD Screening level	ADI NOAEL	9.8	List of endpoints EU-review report (2003)
		FSA GD Tier 1 level	NOAEL 2-gen repro	2.3	List of endpoints EU-review report (2003)
		EFSA GD Tier 2 level	BMD_5 pup weight	38.3	KCA 8.1.2.2 / 01

Report:	**KCA 8.1.2.2/01; [redacted] K, [redacted] M & [redacted] L, 2013**
Title:	Trifloxystrobin: Toxicity endpoint for the Wild Mammal Chronic/Reproductive Risk Assessment
Report No.:	EnSa-13-0869
Document No:	M-468788-01-1
Guidelines:	EFSA GD 2009
Deviations:	None
GLP:	no

The toxicity endpoint that has been adopted by the EU for the wild mammal risk assessment for trifloxystrobin is not unequivocally reported in the official List of Endpoints (SANCO/4339/2000-Final, 2003) where both the NOAEL of a 90-day subchronic feeding study in rats is included as a 'short-term' endpoint (100 ppm, equivalent to 6.4 mg/kg bw/day), and the no-observed effect concentration (NOEC) for reproductive toxicity in the 2-generation reproduction study is given as >1500 ppm in this endpoint list. Additionally some authorities have also considered the overall NOAEL established in this reproduction toxicity as being directly relevant for the wild mammal risk assessment (50 ppm, equivalent to 2.3 mg/kg bw/day).

According to the EFSA GD (2009) the focus of the longterm risk assessment for wild mammals is on a reproductive endpoint.

Therefore a comprehensive evaluation has been conducted to analyze the toxicological studies available for trifloxystrobin with regard to the relevance of findings for the wild mammal reproductive risk assessment. Especially information from the rat reproduction study and the rat and rabbit developmental toxicity studies are to be considered. Additionally effects on adult body weight have been evaluated in studies that include exposure and assessment approaches similar to the conditions in the 90-d rat study.

Retardation of body weight development was the primary toxic effect of trifloxystrobin in adult rats. A similar finding was obtained in mice and rabbits but at higher dose levels. Most obviously, lower body weights were causally related to lower food intake rates but not to direct systemic toxicity of trifloxystrobin. There was no evidence of a teratogenic effect in developmental toxicity studies in rats or rabbits. In the rat 2-generation reproduction study reproductive performance of parent animals and viability of pups were not adversely affected by trifloxystrobin up to highest dose tested (1500 ppm). Only lower pup weights seen at feed concentrations >750 ppm during and at the end of the lactation phase (21-d) were considered to be of possible ecotoxicological relevance, although at later life stages no correlations were evident to lower survival rates or impaired reproductive performance.

The benchmark dose for lower 21-d pup weights was calculated to be 38.3 mg/kg bw/day at an effect level of 5% (BMDs). This value is considered to be protective also for potential effects on parental bodyweight, and is proposed as an appropriately conservative endpoint for trifloxystrobin wild mammal chronic and reproductive risk assessment.

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

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

As the log P_{ow} of the active substance trifloxystrobin (but not for its metabolites) is above the trigger (>3), 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 trifloxystrobin is of low toxicity in birds and laboratory rodents, no risk for reptiles and amphibians is to be expected.

Results from literature review

Report:	**KCA 8.1.4/01; [redacted], [redacted], [redacted], and [redacted]. (2010)**
Title:	Acute toxicity of fungicide formulations to amphibians at environmentally relevant concentrations
Source:	Environmental Toxicology and Chemistry, Vol. 29, No. 11, pp. 2477-2480, 2010.
DOI No:	10.1002/etc.297
Document No:	M-400506-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The present study provides toxicity rates to amphibians at short-term (72 h). Mortality was the selected endpoint. Stratego, a product containing the active substances trifloxystrobin and propiconazole, killed 40% of exposed tadpoles on average at the corn label rate, but only 7% of the juveniles. In the maximum label rate for field corn (880 ml/ha) and the 0.10x label rate (Low), toxicity was significantly lower than at the highest concentration level (10x field rate) tested.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Stratego
Active substance(s):	Trifloxystrobin (+ propiconazole)
Chemical state and description:	Not reported
Source of test item:	U.S. EPA Reg. 264-779, Bayer CropScience
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Vehicle/solvent:	Formulations were mixed in reagent [redacted] water similar to labeling instructions

3. Test organism(s)

Species:	Juvenile *Bufo cognatus*
Common name:	Great Plains toads
Source of test species:	wild caught near wetlands in western Texas in July and held until September when experiments began. To obtain tadpoles, five unrelated wild-caught *B. cognatus* males and five females were paired and induced to breed by injecting 20jxg LHRHa/10g body

weight into the dorsal lymph sacs

4. Culture conditions of test organism(s)

Culture medium:	Aquaria (~400 cm^2 floor surface area) with 1 cm of sterilized soil
Temperature:	26 ± 1°C
Photoperiod:	13:11 h light:dark
Light intensity:	Not reported
pH:	Between 7.0 and 7.2
Oxygen saturation:	above 5.5 mg/L
Food and feeding regime:	Juvenile toads were fed 0.625 cm (0.25 inch) crickets dusted with Miner-All (Sticky Tongue Farms) ad libitum.
Acclimatisation prior to testing:	2 month between caught of species and test start
Observations during acclimatisation:	natural mortality (<20%) was observed in the holding facility

B. Study design and methods

1. Test procedure

Test system:	Laboratory: Tadpoles were 6 d old at the start of the toxicity test
Test concentration(s):	maximum label rate for field corn (880 ml/ha), 0.10x label rate (Low), and 10x label rate (High). (0.11, 1.1 and 11µg trifloxystrobin/cm^2)
Control(s):	exposed to the chemical vehicle only (water).
Number of replicates:	n=3 (see header of figure 1 in the paper)
Test conditions:	Laboratory-reared tadpoles were placed in the same type of aquaria as above but with 16 cm of carbonfiltered water immediately after thoroughly mixing in 1 ml of spray mix (20 tadpoles in 6 L).
Feeding:	Tadpoles were fed a mix of commercial rabbit chow and TetraMin® (Tetra)
Medium renewal:	None
Frequency of test item application:	once at the beginning of test application
Test duration:	72 h
Endpoints:	mortality
Statistics:	ANOVA and Tukey´s multiple range test

2. Measurements during the test

Water/medium parameters:	Water quality was tested within tadpole toxicity tests at the beginning and end of experiments. Dissolved oxygen was always above 5 mg/L, and pH was maintained between 7.0 and 7.2. Concentration of each fungicide active ingredient was measured within the spray mix used for juveniles and within the water used for tadpole testing at the beginning and end of the test.

3. Sampling

Sampling frequency:	checked every 12 h
Transport/storage of samples:	Not reported

4. Chemical analysis

Guideline/protocol:	None
Method:	gas chromatography (GC) / mass spectrometry (Agilent 5975c). Concentrations were determined using liquid-liquid solvent extraction.
Pre-treatment of samples:	For juvenile studies, 1 ml of spray mix was extracted with 10 ml pesticide [redacted] hexane (Burdick and Jackson). For the tadpole studies, 50 ml of water from the tadpole tanks was extracted with

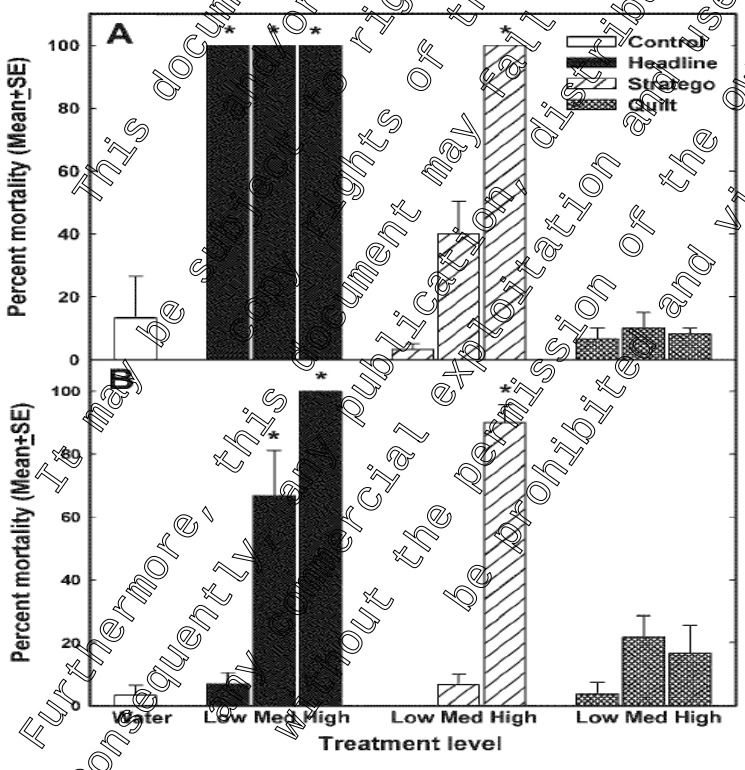
	10 ml of hexane. Extraction efficiencies for the tadpole technique were greater than 85% efficient (n = 4 in reagent [redacted] water).
Conduction:	Quantitation ion for Trifloxystrobin 116
Reference item:	Not reported
Recovery:	63% (after 3h) and 41% (after 72h) of nominal concentrations
Limit of detection:	Not reported
Limit of quantification:	Not reported

RESULTS

1. Biological findings:

Stratego was toxic to tadpoles ($F_{3,8} = 26.1$; $p < 0.001$) and juveniles ($F_{3,8} = 135.7$; $p < 0.001$). Stratego killed 100 and 90% of the tadpoles and juveniles, respectively, exposed to the highest concentration evaluated (10 x the application rate). In maximum label rate for field corn (880 ml/ha) and the 0.10 x label rate (Low), toxicity was lower (Figure 1).

Figure 1: Mean (± standard error) percent mortality of *Bufo cognatus* tadpoles (A) and juveniles (B) 72 h after a single exposure to either Headline, Stratego, or Quilt fungicide at one of three concentrations. Fungicide concentrations were maximum label rate for corn (Med), 0.10x label rate (Low), and 10x label rate (High). Control animals were exposed to an equivalent volume of chemical vehicle (water). Each treatment consisted of three replicates (n = 20 tadpoles and n = 9-10 juveniles per replicate). Asterisk (*) indicates significantly ($p < 0.05$) different from control and among concentrations within a specified fungicide.



RESULTS SUMMARY

Stratego killed 100% and 90% of the tadpoles and juveniles, respectively, exposed to the highest concentration evaluated (10 x the application rate) and 40% of exposed tadpoles on average at the corn label rate, but only 7% of the juveniles. Toxicity increased with the applied concentration rate.

Effects on terrestrial life-stages of amphibians
Comment by the Notifier

[redacted] et al. (2010), see KCA 8.1.4/01, published experiments where wild caught Great Plains toad (*Bufo cognatus*) have been exposed to direct overspray of fungicide formulations, one of which was Stratego® containing trifloxystrobin and propiconazole. The test rates were 0.11 plus 0.11, 1.1 plus 1.1 and 11 plus 11 µg trifloxystrobin plus propiconazole/cm². The medium test rate refers to the rate recommended according to good agricultural practice. The mortalities at the low and the medium rate did not differ significantly from the control. The high rate (corresponding to 10-times field rate) led to 90% mortality.

The exposure scenario used in these experiments can be regarded as unrealistic worst case for the following reasons:

1. The experimental set-up (glass tanks with a 1cm layer of soil) did not provide any shelter that would have mitigated the exposure
2. The test organisms were juvenile toads. The surface:volume ratio is larger in small specimens compared to adult individuals from the same species. Thus, small organisms receive more test substance per bodyweight than large ones.
3. Activity patterns of amphibians in on-crop areas only partly overlap with the time of the application of pesticides, because:
    a. Agricultural fields are not suitable habitats for amphibians (soil moisture, availability of food, opportunities for shelter)
    b. Migrating amphibians, that are passing open areas, are mostly active at night or during rainfall. Applications of plant protection products mostly take place during day-time under dry weather conditions

Therefore these effect data on terrestrial life stages of amphibians published by [redacted] et al. (2010), see KCA 8.1.4/01, are presented here as supplemental data and are not used for a risk assessment. Moreover, the results indicate that the field rate of Stratego® has no significant effects on terrestrial life stages of amphibians.

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

CA 8.1.5 Endocrine disrupting properties

Wild Mammals

Effects of trifloxystrobin on mammals were studied in 90-d, chronic, and reproductive studies in rats, 90-d and chronic studies in mice, 90-d and 1-year studies in dogs, and in prenatal development studies in rats and rabbits.

In these apical toxicological studies no evidence of any endocrine effect was seen. Therefore, based on a complete toxicological data set, there is no evidence of an endocrine potential.

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

No further testing for endocrine disrupting properties is warranted.

Birds

The population relevant effects of trifloxystrobin on birds were studied in reproductive toxicity studies with bobwhite quail and mallard ducks. There were no effects on reproductive parameters up to and including the respective highest tested dietary concentration of 320 mg/kg feed in quail and 500 mg/kg feed in mallard.

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

No further testing for endocrine disrupting properties is warranted.

CA 8.2 Effects on aquatic organisms

In order to complete the aquatic risk assessment and to address new data requirements according to Regulation (EC) No 1107/2009, additional studies were performed. In addition, tests on marine species 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.

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

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

Metabolite CGA 381318 (*ZZ*) is found as a metabolite in soil solely under photolytic conditions (max. 6.2% AR, see MCA Section 7.1.1). The compound was synthesized by photoisomerization and ester cleavage of the active substance, however the compound is not stable. All attempts to isolate the free acid failed, only the sodium salt could be isolated. Isomerization to the more stable *ZE*-Isomer takes place very rapidly, not only under influence of light, but also under already mild acidic conditions. This isomerization to the more stable isomers will also take place under the conditions of the aquatic studies, where mild acidic conditions and light are present. Thus the aquatic toxicity of CGA 381318 (*ZZ*) is covered by the corresponding *EE* (CGA 321113) and *ZE* (CGA 373466) isomers, which are all non-toxic to aquatic organisms.

As can be seen in the following tables, all metabolites are clearly less toxic compared to the parent, with the lowest ratio being 32-fold less toxic than the parent (for *Daphnia magna* and CGA 357276), and the highest ratio > 5000 for all metabolites with endpoints ≥ 100 mg/L.

Figure 8.2-1: Proposed degradation pathway of trifloxystrobin in soil (major degradation products)

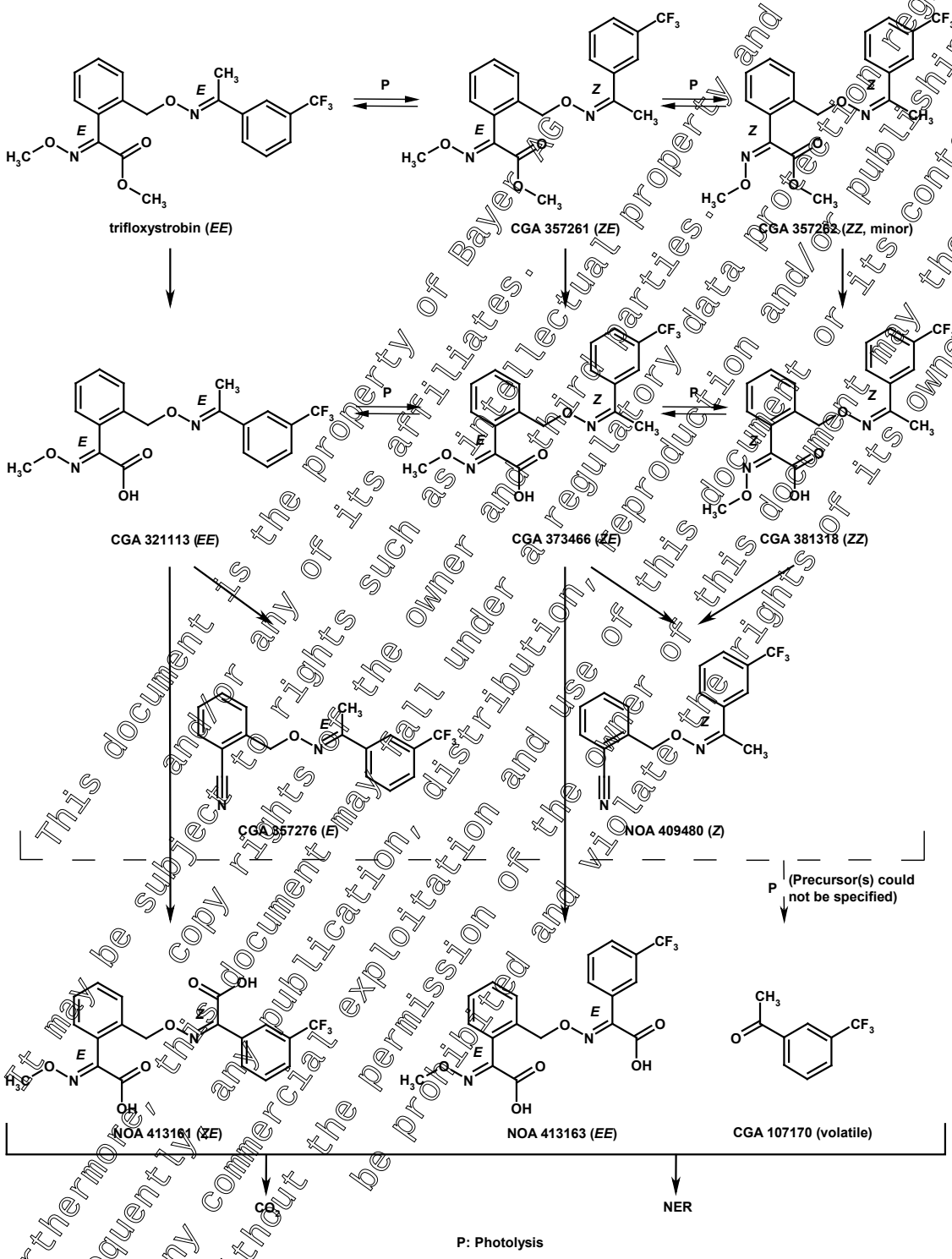
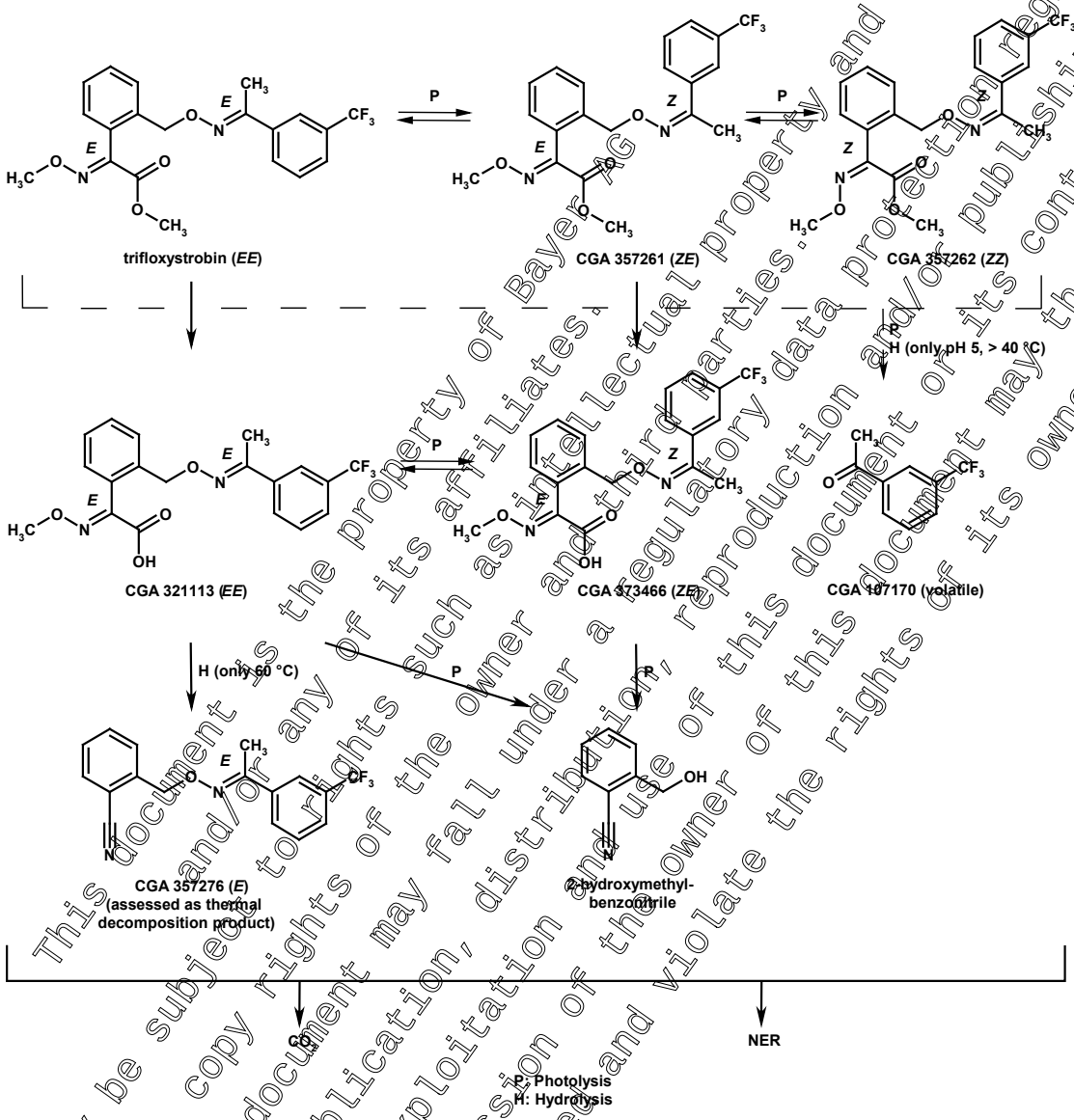


Figure 8.2-2: Proposed degradation pathway of trifloxystrobin in water and sediment (major degradation products)



CA 8.2.1 Acute toxicity to fish

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

The endpoints from the following table have been evaluated during the first EU review (SANCO/4339/2000-Final) and are used in the risk assessment. An additional repeated peak exposure study with the formulation and the most sensitive fish species (rainbow trout) and life stage (alevin stage larvae) under lab conditions covers both, acute and chronic effects of Trifloxystrobin, and thus is

also used to derive the acute endpoint ([redacted] & Sommer, 2002, M-056670-01-1, KCP 10.2.3/02).

Table 8.2.1- 1: Acute toxicity to fish exposed to trifloxystrobin and its metabolites

Test substance	Test species	Endpoint		Reference
Trifloxystrobin	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**0.015 mg a.s./L**	[redacted] (1997) M-032048-01-1 KCA 8.2.1/01
CGA 357261	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**0.9 mg p.m./L**	[redacted] (1997) M-032074-01-1 KCA 8.2.1/12
CGA 321113	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**> 100 mg p.m./L**	[redacted] (1996) M-032076-01-1 KCA 8.2.1/14
CGA 373466	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**> 200 mg p.m./L**	[redacted] (1997). M-032078-01-1 KCA 8.2.1/15
NOA 413161	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**> 100 mg p.m./L**	[redacted] (1998) M-033964-01-1 KCA 8.2.1/17
NOA 413163	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**> 100 (nom)**	[redacted] (1998) M-033967-01-1 KCA 8.2.1/18
CGA 107170	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**13.6 mg p.m./L**	[redacted] (1997) M-032079-01-1 KCA 8.2.1/16

An additional acute fish study has been performed for the metabolite CGA 357262 and is submitted within this Supplemental Dossier for renewal of approval of trifloxystrobin. A study summary is given below.

Table 8.2.1- 2: Additional acute fish study of trifloxystrobin metabolites according to new data requirements

Test substance	Test species	Endpoint		Reference
CGA 357262	Fish, acute *Oncorhynchus mykiss*	**LC_{50}**	**> 10.1 mg a.s./L**	[redacted] (2012) EBTFL017 M-430569-01-1 KCA 8.2.1/22

Metabolite CGA 357262

Report:	**KCA 8.2.1/22; [redacted], 2012**
Title:	Acute toxicity of BCSBJ39463 (tech.) to fish (*Oncorhynchus mykiss*) under static conditions (limit test)
Report No.:	EBTFL017
Document No:	M-430569-01-1
Guidelines:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Council Regulation (EC) No 440/2008, C.1 (2008) OECD Guideline No. 203 (1992) 12 Nousan Notification No. 8147 (2000)
Deviations:	None
GLP:	Yes (certified laboratory)

Objective:

A limit test at 10.1 (10.0) mg test item / L was performed in order to demonstrate that the concentration which kills 50 percent of the fish (96h-LC_{50}) exceeds the limit test concentration of 10.1 (10.0) mg (a.s.). The limit test concentration was chosen based on a non-GLP range-finder test (10.0 mg / L).

Materials and Methods:

Test item: BCSBJ39463 (CGA 357262, metabolite of trifloxystrobin), analyzed content of active substance: 99.4% w/w; specified by batch code: AE 1344146-01-01; Origin batch number: SES 10487-2-1; tox no.: 09326-00 (AZ 17373).

Test organism: Rainbow trout (*Oncorhynchus mykiss*), mean body length 4.4 cm, mean body weight 0.9 g. The biomass loading for this test was 0.68 g fish / L test medium.

Thirty fish were exposed in a limit test for 96 h under static test conditions to a nominal concentration of 10.1 (10.0) mg test item (a.s.) / L against a control (dilution water) and a solvent control (DMF) with further 30 fish.

During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning. Within the study the pH-value, the oxygen saturation level and the temperature were measured with commercial measurement devices, daily. Dissolved oxygen concentrations ranged from 78 to 88% oxygen saturation, the pH values ranged from 6.7 to 7.1 and the water temperature ranged from 12.0°C to 12.5°C in all aquaria over the whole testing period. The photoperiod was 16 hours of light and 8 hours dark.

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

The endpoints were expressed in terms of nominal and mean measured concentrations.

Dates of experimental work: October 24 to March 12, 2012

Results:

Validity criteria:

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

All validity criteria for the study were met.

Analytical results:

The analytical determination of BCSBJ39463 (CGA 357262) (in water by HPLC - UV) revealed recoveries of 526% on day 0, 57.3% on day 2 and 52.8% on day 4 of nominal test concentration of 10.0 mg a.s. / L. The results of day 2 and day 4 represent the solubility of approximately 5 to 6 mg / L under exposure conditions. The arithmetic mean was 5.51 mg / L. The recoveries observed on day 0 exceed by far the nominal concentration. The reason for these values which are far above the expectations is that the nominal concentration exceeds the water solubility limit. This might have led to inhomogeneities within the water samples.
Therefore, in the present study, the measured concentrations were used to evaluate the test results.

Biological results:

All surviving fish showed at this test level the following symptoms after 96 hours:

- fish were dead
- fish remained for unusually long periods on the bottom of the aquarium
- laid on their sides or backs
- turned dark in coloration
- open mouth
- showed labored respiration

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

LC_{50} values for rainbow trout exposed to BCSBJ39463 (CGA 357262) technical based on nominal and mean measured concentrations

Test substance:	BCSBJ39463 (CGA 357262)
Test object:	Rainbow trout (*Oncorhynchus mykiss*)
Exposure:	96 hours, static test design (limit)
LC_{50} 96 h (95% C.I.):	**> 10.1 (5.51) mg (mean measured) test item / L**

Conclusions:

The LC_{50} (96h) of BCSBJ39463 (CGA 357262) to Rainbow trout (*Oncorhynchus mykiss*) in a static 96-hour-test was determined to be > 10.1 (5.51) mg nominal (mean measured) / L.

CA 8.2.2 Long-term and chronic toxicity to fish

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

The endpoints from the following table have been evaluated during the first EU review (SANCO/4339/2000-Final) and are used in the risk assessment. An additional repeated peak exposure study with the formulation and the most sensitive fish species (rainbow trout) and life stage (alevin stage larvae) under lab conditions is used to derive the chronic endpoint ([redacted] [redacted] 2002, M-056670-01-1, KCP 10.2.3/02).

Table 8.2.2- 1: Chronic fish toxicity of trifloxystrobin and its metabolite CGA 321113

Test substance	Test species	Endpoint	Reference
Trifloxystrobin	Fish, chronic *Oncorhynchus mykiss*	**NOEC 0.0077 mg a.s./L**	[redacted] (1997) M-022080-02-1 KCA 8.2.2.2/01
Trifloxystrobin	Fish, chronic *Oncorhynchus mykiss*	**NOEC 3 x 0.0253 mg a.s./L**	[redacted] & [redacted] (2002) M-056670-01-1, KCP 10.2.3/02
CGA 321113	Fish, chronic *Oncorhynchus mykiss*	**NOEC ≥ 100 mg p.m./L**	[redacted] (1999) M-070819-01-1 KCA 8.2.2.1/01

CA 8.2.2.1 Fish early life stage toxicity test

See point 8.2.2. No additional studies were performed.

CA 8.2.2.2 Fish full life cycle test

See point 8.2.2. No additional studies were performed.

CA 8.2.2.3 Bioconcentration in fish

See point 8.2.2. No additional studies were performed.

CA 8.2.3 Endocrine disrupting properties

The aquatic profile of Trifloxystrobin is characterized by fast degradation in natural water and thus lack of chronic exposure.

Population relevant effects of Trifloxystrobin on fish were studied in a flow-through early life-stage test (ELS) with rainbow trout. The lowest NOEC of 7.7 µg/L was found for the parameters survival

and hatching success, with no effects on weight or length. This chronic NOEC is very close to the acute LC50 of 15 µg/L for rainbow trout, also determined under flow-through conditions.

An outdoor repeated peak exposure pond study with fish (bluegill sunfish) showed no effect on survival, fish length and fish weight up to the highest tested concentration of 12.3 µg/L and a repeated peak exposure laboratory ELS with rainbow trout showed no effects up to 23.5 µg/L and lethal as well as sublethal effects at the highest concentration of 38 µg/L.

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

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

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

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

The following endpoints from studies evaluated during the first EU review (SANCO/4339/2000-Final) are used in the risk assessment:

Table 8.2.4.1- 1: Acute toxicity to *Daphnia magna* exposed to trifloxystrobin and its metabolites

Test substance	Test species	Endpoint		Reference
Trifloxystrobin	Invertebrate, acute *Daphnia magna*	EC_{50}	0.016 mg a.s./L	[redacted] (1997) 963542 M-032085-01-1 KCA 8.2.4.1/02
CGA 357261	Invertebrate, acute *Daphnia magna*	EC_{50}	1.4 mg p.m./L	[redacted] (1997) 649293 M-032090-01-1 KCA 8.2.4.1/04
CGA 321113	Invertebrate, acute *Daphnia magna*	EC_{50}	> 100 mg p.m./L	[redacted] (1996) 953569 M-032091-01-1 KCA 8.2.4.1/05
CGA 373466	Invertebrate, acute *Daphnia magna*	EC_{50}	>100 mg p.m./L	[redacted] (1997) 649350 M-032092-01-1 KCA 8.2.4.1/06
NOA 413161	Invertebrate, acute *Daphnia magna*	EC_{50}	> 100 mg p.m./L	[redacted] (1998) G 528 14 M-033972-01-1 KCA 8.2.4.1/08
NOA 413163	Invertebrate, acute *Daphnia magna*	EC_{50}	> 100 (nom)	[redacted] (1998) G 529 14 M-033975-01-1 KCA 8.2.4.1/09
CGA 107170	Invertebrate, acute *Daphnia magna*	EC_{50}	22.7 mg p.m./L	[redacted] (1997) 649236 M-032096-01-1 KCA 8.2.4.1/07

Additional acute studies on *Daphnia magna* have been performed for several metabolites of trifloxystrobin and are submitted within this Supplemental Dossier for renewal of approval of trifloxystrobin. Study summaries are given below.

Table 8.2.4.1- 2: Additional studies for acute toxicity to *Daphnia magna*

Test substance	Test species	Endpoint	Reference
CGA 357262	Invertebrate, acute *Daphnia magna*	EC_{50} 3.6 mg p.m./L	[redacted] (2012) EBTFL019 M-431690-01-1 KCA 8.2.4.1/16
CGA 357276	Invertebrate, acute *Daphnia magna*	EC_{50} 0.51 mg p.m./L	[redacted] (2012) EBTFX195 M-433856-01-1 KCA 8.2.4.1/17
NOA 409480	Invertebrate, acute *Daphnia magna*	EC_{50} 2.25 mg p.m./L	[redacted] (2012) EBTFX201 M-432300-01-1 KCA 8.2.4.1/18
2-Hydroxymethyl-benzonitrile [a]	Invertebrate, acute *Daphnia magna*	EC_{50} 9.9 mg p.m./L	[redacted] (2012) EBTFX197 M-432300-01-1 KCA 8.2.4.1/19

[a] 2-Hydroxymethylbenzonitrile is present in a tautomeric equilibrium with 2-Benzofuran-1(3H)-imine, for further details please refer to the study report (M-442300-01-1).

Metabolite CGA 357262

Report: **KCA 8.2.4.1/16; [redacted] (2012)**
Title: Acute toxicity of BCS-BJ39463 (tech.) to the waterflea *Daphnia magna* in a static laboratory test system
Report No: EBTFL019
Document No: M-431690-01-1
Guidelines: OECD Guideline 202 (2004)
U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982)
EEC Regulation No 440/2008, Method C.2 (2008)
OPPTS Guideline 850.1010, modified (1996)
JMAFF 12 Nousan No. 8147 (2000)
Deviations: None
GLP: Yes (certified laboratory)

Objective:

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

Materials and methods:

Test item: BCS-BJ39463 (CGA 357262), batch SES 10487-2-1, (BCS-batch code: AE 1344146-01-01), purity: 99.4% w/w (TOX 09326-00).

Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0, 0.625, 1.25, 2.50, 5.00 and 10.00 mg pure

metabolite/L (corresponding to mean-measured concentrations of 0.577, 1.37, 2.24, 3.99 and 6.27 mg pure metabolite / L) and a solvent control without feeding.
The content of BCS-BJ39463 in exposure media was measured for verification of the test item concentrations at start and end of the exposure period.
After 24 and 48 hours, the behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements within approx. 15 seconds after gentle agitation of the test vessel. Additionally, all visible features of the test item in water as well as possible signs on sublethal affected daphnids had to be recorded.
Adequate sensitivity of the used test-organisms was verified by simultaneous testing of appropriate aqueous solutions of the reference-substance potassium dichromate.

Dates of experimental work: October 17, 2011 to February 09, 2012

Results:

Analytical findings:
The actually dissolved and analytically determined amounts of BCS-BJ39463 in the freshly prepared test solutions at test initiation ranged between 85.6% and 126% (mean: 108%) of the aspired nominal test concentrations.
The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 39.7% and 92.0% (mean: 65.2%) of nominal.
No contaminations of BCS-BJ39463 were detected in samples from untreated water control.
Due to the limited solubility of BCS-BJ39463 (tech.) under test conditions, the measured test concentrations at the end of the 48 hours exposure interval partially fell below 80% of nominal. Therefore all reported results were based on mean measured concentrations

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

Toxicity of BCS-BJ39463 to *Daphnia magna*:

Nominal test concentration (mg p.m./L)	Mean measured test concentration (mg p.m./L)	Exposed daphnids (=100%)	Immobilised daphnids			
			24 h.		48 h.	
			n	%	n	%
Control	Control	30	0	0.0	**0**	**0.0**
Solvent control *)	Solvent control	30	0	0.0	**0**	**0.0**
0.625	0.577	30	0	0.0	**0**	**0.0**
1.25	1.37	30	1	3.3	**0**	**0.0**
2.50	2.24	30	2	6.7	**12**	**40.0**
5.00	3.99	30	2	6.7	**20**	**66.7**
10.00	6.27	30	5	16.7	**17**	**56.7**

p.m. = pure metabolite
*) DMF (0.1 mL/L dimethylformamide)

Conclusions:

Based on mean-measured concentrations of BCS-BJ39463 (tech.), the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed:

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

Probit analysis for data obtained after	EC_{50} mg pure metabolite / L (mean measured)	lower 95% cl mg pure metabolite / L (mean measured)	upper 95% cl mg pure metabolite / L (mean measured)
24 hours	**13.1**	n.d.	n.d.
48 hours	**3.6**	2.9	4.7

n.d.: not determined due to mathematical reasons

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Metabolite CGA 357276

Report: **KCA 8.2.4.1/17; [redacted] (2012)**
Title: Acute toxicity of BCS-AB39835 (tech.) to the waterflea *Daphnia magna* in a static laboratory test system
Report No: EBTFX195
Document No: M-433856-01-1
Guidelines: OECD Guideline 202 (2004)
U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982)
EEC Regulation No 440/2008, Method C.2 (2008)
OPPTS Guideline 850.1010, modified (1996)
JMAFF 12 Nousan No. 8147 (2000)
Deviations: None
GLP: Yes (certified laboratory)

Objective:

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

Materials and methods:

Test item: BCS-AB39835 (CGA 357276, metabolite of trifloxystrobin), origin batch no.: BCOO 6204-3-3 (BCS-batch code: BCS-AB39835-PU-01), purity: 97.8% w/w (AZ 16891).

Daphnia magna (1^{st} instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0, 0.10, 0.17, 0.31, 0.56 and 1.00 mg pure metabolite/L (corresponding to mean-measured concentrations of 0, 99.8, 172, 304, 520 and 954 µg pure metabolite / L) without feeding.

The content of BCS-AB39835 in exposure media was measured for verification of the test item concentrations at start and end of the exposure period.

After 24 and 48 hours, the behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements within approx. 15 seconds after gentle

agitation of the test vessel. Additionally, all visible features of the test item in water as well as possible signs on sublethal affected daphnids had to be recorded.
Adequate sensitivity of the used test-organisms was verified by simultaneous testing of appropriate aqueous solutions of the reference-substance potassium dichromate.

Dates of experimental work: October 03, 2011 to February 15, 2012

Results:

Analytical findings:

The actually dissolved and analytically determined amounts of BCS-AB39835 (CGA 357276) in the freshly prepared test solutions at test initiation ranged between 104% and 110% (mean: 107%) of the aspired nominal test concentrations.
The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 78% and 96% (mean: 88%) of nominal.
No contaminations of BCS-AB39835 (CGA 357276) were detected in samples from untreated water control.
Since the measured content of BCS-AB39835 (CGA 357276) in one test solution fell below 80% of nominal at the end of the 48 hours exposure interval, all reported results were based on mean measured concentrations.

Biological findings:

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

Toxicity of BCS-AB39835 (CGA 357276) to *Daphnia magna*:

Nominal test concentration (mg p.m./L)	Mean measured test concentration (ug p.m./L)	Exposed daphnids (=100%)	Immobilised daphnids			
			24 h.		48 h.	
			n	%	n	%
Control	Control	30	0	0.0	0	0.0
Solvent control *)	Solvent control	30	0	0.0	0	0.0
0.10	99.8	30	0	0.0	0	0.0
0.18	172	30	0	0.0	0	0.0
0.31	304	30	0	0.0	5	16.7
0.56	520	30	3	10.0	15	50.0
1.00	954	30	26	86.7	30	100

p.m. = pure metabolite
*) DMF (0.1 mL/L dimethylformamide)

Conclusions:

Based on mean-measured concentrations of BCS-AB39835 (CGA 357276), the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed:

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

Probit analysis for data obtained after	EC_{50} µg pure metabolite / L (mean measured)	lower 95% cl µg pure metabolite / L (mean measured)	upper 95% cl µg pure metabolite / L (mean measured)
24 hours	**718**	642	802
48 hours	**514**	409	647

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Metabolite NOA 409480

Report:	**KCA 8.2.4.1/18; [redacted] (2012)**
Title:	Acute toxicity of BCS-CR74871 (tech.) to the waterflea *Daphnia magna* in a static laboratory test system
Report No:	EBTFX201
Document No:	M-432300-01-1
Guidelines:	OECD Guideline 202 (2004) U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982) EEC Regulation No 440/2008, Method C.2 (2008) OPPTS Guideline 850.1010, modified (1996) JMAFF 12 Nousan No. 8147 (2000)
Deviations:	None
GLP:	Yes (certified laboratory)

Objective:

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

Materials and methods:

Test item: BCS-CR74871 (NOA409480, metabolite of trifloxystrobin), origin batch no.: BCOO 6263-3-4, (BCS-batch code: BCS-CR74871-01-01), purity: 98.7% w/w (AZ 17177 / TOX09206-00).

Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 0, 0.625, 1.25, 2.50, 5.00 and 10.00 mg pure metabolite/L (corresponding to mean-measured concentrations of 0.660, 1.33, 2.41, 3.90, 6.83 mg pure metabolite / L) without feeding.

The content of BCS-CR74871 in exposure media was measured for verification of the test item concentrations at start and end of the exposure period.

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

Adequate sensitivity of the used test-organisms was verified by simultaneous testing of appropriate aqueous solutions of the reference-substance potassium dichromate.

Dates of experimental work: October 10, 2011 to March 05, 2012

Results:

Analytical findings:

The accompanying chemical analysis of BCS-CR74871 (NOA409480) in freshly prepared test solutions revealed measured concentrations between 108% and 99% (mean: 103%) of nominal.

The corresponding concentrations in the aged test solutions at the end of the 48 hours exposure period ranged between 106% and 37% (mean: 91%) of nominal.

No contaminations of BCS-CR74871 (NOA409480) were detected in samples from untreated water control.

Due to the limited solubility of BCS-CR74871 (NOA409480) under test conditions, the measured test concentrations at the end of the 48 hours exposure interval partially fell below 80% of nominal. Therefore all reported results were based on mean measured concentrations.

Biological findings:

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

Toxicity of BCS-CR74871 (NOA409480) to *Daphnia magna*:

Nominal test concentration (mg p.m./L)	Mean measured test concentration (mg p.m./L)	Exposed daphnids (=100%)	Immobilised daphnids			
			24 h.		48 h.	
			n	%	n	%
Control	Control	30	0	0.0	**0**	**0.0**
Solvent control *)	Solvent control	30	0	0.0	**0**	**0.0**
0.625	0.660	30	0	0.0	**0**	**0.0**
1.25	1.33	30	4	13.3	**4**	**13.3**
2.50	2.41	30	6	20.0	**16**	**53.3**
5.00	3.90	30	19	63.3	**27**	**90.0**
10.00	6.83	30	28	93.3	**30**	**100**

p.m. = pure metabolite
*) DMF (0.1 mL/L dimethylformamide)

Conclusions:

Based on mean-measured concentrations of BCS-CR74871 (NOA409480), the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed:

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

Probit analysis for data obtained after	**EC_{50}** mg pure metabolite / L (mean measured)	lower 95% cl mg pure metabolite / L (mean measured)	upper 95% cl mg pure metabolite / L (mean measured)
24 hours	**3.20**	2.74	3.74
48 hours	**2.25**	1.97	2.56

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Metabolites 2-Hydroxymethylbenzonitrile + 2-Benzofuran-1(3H)-imine (tautomeric mixture)

Report:	**KCA 8.2.4.1/19; [redacted] (2012)**
Title:	Acute toxicity of BCS-AR14212 + BCS-CR34532 (tech.) to the waterflea *Daphnia magna* in a static laboratory test system
Report No:	EBTFX197
Document No:	M-442300-01-1
Guidelines:	OECD Guideline 202 (2004) U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982) EEC Regulation No 440/2008, Method C.2 (2008) OPPTS Guideline 850.1010, modified (1996) JMAFF 12 Nousan No. 8147 (2000)
Deviations:	None
GLP:	Yes (certified laboratory)

Objective:

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

Materials and methods:

Test item: BCS-AR14212 + BCS-CR34532 (2-Hydroxymethylbenzonitrile + 2-Benzofuran-1(3H)-imine, metabolites of trifloxystrobin), origin batch no.: BCOO 6206-4-2 (BCS-Batch code: BCS-AR14212-01-01), content: 31.8% w/w BCS-AR14212, 63.8% w/w BCS-CR34532 (AZ16949).

Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static test system for 48 hours to nominal concentrations of 2.50, 5.00, 10.00, 20.00 and 40.00 mg pure metabolites/L (corresponding to mean-measured concentrations of 0.832, 1.66, 3.33, 6.65 and 13.3 mg BCS-AR14212 / L) without feeding. A water and solvent control group were run in parallel.

The test item is a tautomeric mixture of BCS-AR14212 (2-(hydroxymethyl)benzonitrile) and BCS-CR34532 (2-benzofuran-1(3H)-imine), and each tautomer can be formed from the other in aqueous solution with an equilibrium ratio of ca. 1:2. Therefore analysis was performed for 2-(hydroxymethyl)benzonitrile and results are expressed as initial measured concentrations of the whole

mixture (assuming a ratio of 1:2).
The content of BCS-AR14212 + BCS-CR34532 in exposure media was measured for verification of the test item concentrations at start and end of the exposure period.
After 24 and 48 hours, the behaviour of the water fleas was visually evaluated by counting mobile daphnids, defined as animals with swimming movements within approx. 15 seconds after gentle agitation of the test vessel. Additionally, all visible features of the test item in water as well as possible signs on sublethal affected daphnids had to be recorded.
Adequate sensitivity of the used test-organisms was verified by simultaneous testing of appropriate aqueous solutions of the reference-substance potassium dichromate.

Dates of experimental work: October 04, 2011 to April 18, 2012

Results:

Analytical findings:
The accompanying chemical analysis of BCS-AR14212 in freshly prepared test solutions revealed measured concentrations between 93% and 97% (mean: 95%) of nominal.
The corresponding concentrations in the aged test solutions at the end of the 48-hour exposure period, based on analytics for 2-(hydroxymethyl)benzonitrile and expressed as the mixture (assuming a ratio of 1:2, as above), ranged between 8% and 21% (mean: 15%) of nominal. Because of the continuous equilibration between the two tautomeric compounds in the test and also during analysis, the endpoints are based on initial measured values, and reflect the toxicity of the tautomeric mixture including any further degradation products that may have formed during the test.
No contaminations of BCS-AR14212 were detected in samples from the untreated water and solvent control.

Biological findings:
No immobilities or other effects on behaviour occurred in the untreated untreated water and solvent control within 48 hours of exposure.

Toxicity of BCS-AR14212 to *Daphnia magna*:

Nominal test concentration (mg p.m./L)	initial measured test concentration (mg p.m./L)	Exposed daphnids (=100%)	Immobilised daphnids			
			24 h.		48 h.	
			n	%	n	%
Control	Control	30	0	0.0	**0**	**0.0**
Solvent control *)	Solvent control	30	0	0.0	**0**	**0.0**
2.5	2.33	30	0	0.0	**0**	**0.0**
5.0	4.66	30	0	0.0	**2**	**6.67**
10.0	9.69	30	5	16.5	12	**40.0**
20.0	19.0	30	27	90.0	29	**96.7**
40.0	37.6	30	30	100	30	**100**

p.m. = pure metabolite
*) DMF (0.1 mL/L dimethylformamide)

Conclusions:

Based on initial measured test concentrations, the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were assessed:

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

Probit analysis for data obtained after	**EC_{50}** mg pure metabolite / L (mean measured)	lower 95% cl mg pure metabolite / L (mean measured)	upper 95% cl mg pure metabolite / L (mean measured)
24 hours	12.95	11.40	14.71
48 hours	**9.90**	8.48	11.51

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Results from literature review

Report: **KIIA 8.2.4.1/20; [redacted], S.; [redacted], S.; [redacted], L.; [redacted], J. (2013)**
Title: Acute Toxicity of pyraclostrobin and trifloxystrobin to *Hyalella azteca*
Source: Environmental Toxicology and Chemistry, Volume 32, Issue 7, pp. 1516–1525
DOI No: 10.1002/etc.2228
Document No: M-462365-01-1
Guidelines: None
GLP: No
Classification: b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

To investigate fungicide toxicity, *Hyalella azteca* amphipods was exposed to the fungicide formulation, Stratego, and its active ingredient trifloxystrobin. Water-only exposures resulted in a lethal concentration 20/4 µg/L values for the formulation. These values were below concentrations that could occur following spray drift over embedded cropland wetlands. When fungicides were added to overlying water of sediment-water microcosms, toxicity was reduced by 160% for Stratego, compared with water-only exposures, based on the total amount of fungicide added to the systems. In addition, when fungicide was added to sediment prior to the addition of water, the reduction in toxicity was even greater, with no toxicity occurring at environmentally relevant levels. Differences in toxicity among exposure groups were explained by dissipation from water as toxicity values based on measured water concentrations were within 20% between all systems. The present study reinforces previous studies that Stratego is toxic to nontarget aquatic organisms. However, the presence of sediment is likely to ameliorate some toxicity of fungicide formulations, especially if spraying occurs prior to wetland inundation.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Stratego
Active substance(s): trifloxystrobin

Chemical state and description:	n/a
Source of test item:	Sigma-Aldrich
Batch number:	n/a
Purity:	99.5%

2. Test solutions

Vehicle/solvent:	acetone

3. Test organism

Species:	*Hyalella azteca*
Common name:	waterflea
Source of test species:	Oklahoma State University

4. Culture conditions of test organism(s)

Culture medium:	Cultures were kept in a flow-through system with dechlorinated water and coarse sand substrate
Food and feeding regime:	Organisms were fed a combination of Hikari tropical algae wafers (Kyorin USA) and Tetramin (Tetra) fish food every other day.
Acclimatisation prior to testing:	n/a
Observations during acclimatisation:	n/a
Other specifications of test organism:	Juvenile amphipods were haphazardly collected for testing by selecting individuals collected between 250- and 500-mm stainless-steel sieves

B. Study design and methods

A 1. Test procedure — *Water-only toxicity tests*

Test system:	n/a
Test concentration(s):	150 µL, 74 µL, 37 µL, 18 µL and 9 µL
Control(s):	yes
Number of replicates:	Four replicates
Test conditions:	Light cycle was kept at 16:8 light:dark, and water-quality measurements were taken every 24 h for the duration of the experiments.
Feeding:	Organisms were fed daily by adding 1.0 mL of 1800 mg/L stock solution of ground Tetramin fish food into each experimental unit for all experiments.
Medium renewal:	n/a
Frequency of test item application:	n/a
Test duration:	10 d
Endpoints:	LC_{50}; LC_{10}
Statistics:	IBM SPSS Statistics Data Editor

A 2. Measurements during the test

Water/medium parameters:	Dissolved oxygen concentrations ranged from 3.2 mg/L to 9.0 mg/L, temperature averaged 23°C (+/- 1°C), and pH ranged from 7.2 to 7.5.

A 3. Sampling

Sampling frequency:	n/a
Transport/storage of samples:	n/a

B 1. Test procedure — *Microcosm toxicity tests*

Test system:	Microcosm exposures were conducted in glass jars containing 800 mL dechlorinated water and 100 g of sediment.
Test concentration(s):	293 µg/L, 103 µg/L, 39 µg/L, 14 µg/Land 6 µg/L
Control(s):	yes
Number of replicates:	Six replicates
Test conditions:	Light cycle was kept at 16:8 light:dark, and water-quality measurements were taken every 24 h for the duration of the experiments.
Feeding:	Organisms were fed daily by adding 1.0 mL of 1800 mg/L stock solution of ground Tetramin fish food into each experimental unit for all experiments.
Medium renewal:	n/a
Frequency of test item application:	n/a
Test duration:	7 d
Endpoints:	LC_{50}; LC_{10}
Statistics:	IBM SPSS Statistics Data Editor

B 2. Measurements during the test

Water/medium parameters:	Dissolved oxygen concentrations ranged from 3.2 mg/L to 9.0 mg/L, temperature averaged 23°C (+/- 1°C), and pH ranged from 7.2 to 7.5.

B 3. Sampling

Sampling frequency:	n/a
Transport/storage of samples:	n/a

C 1. Test procedure *Microcosm fungicide fate tests*

Test system:	
Test concentration(s):	water concentration of 300 µg/L (assuming full water incorporation) or a sediment concentration of 2300 µg/kg (assuming complete adsorption to the sediment)
Control(s):	yes
Number of replicates:	three replicates
Test duration:	7 d
Endpoints:	n/a
Statistics:	IBM SPSS Statistics Data Editor

4. Chemical analysis

Guideline/protocol:	n/a
Method:	GC-MS
Pre-treatment of samples:	n/a
Conduction:	n/a
Reference item:	n/a
Recovery:	n/a
Limit of detection:	n/a
Limit of quantification:	n/a

RESULTS

1. Validity criteria:

No validity criteria defined.

2. Analytical findings:

Measured water concentrations for trifloxystrobin were 122% (+/- 28%), 78% (+/- 32%), and 136% (+/- 38%), of targeted concentrations over the entire test.

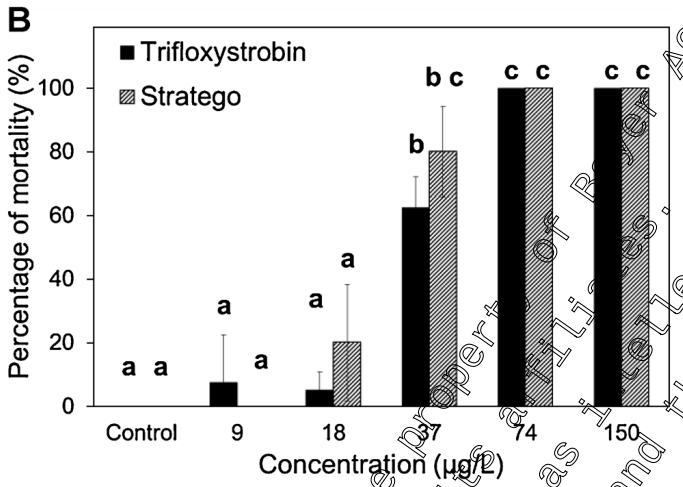

Figure 1. Mean (+/- standard deviation) percentage of mortality of *Hyalella azteca* for water-only exposures for Stratego or trifloxystrobin for 5 concentrations and a control. Provided mortalities correspond to the 96-h assessment, which represents the timepoint when the majority of toxicity had taken place. Each treatment consisted of 4 replicates (n = 4). Categorical letters represent statistical differences between concentrations and treatments ($p < 0.05$).

3. Biological findings:

Exposure to Stratego formulation and trifloxystrobin resulted in LOECs of 37 µg/L ($p < 0.001$); however, there was no significant difference between the 2 treatments ($p = 0.271$; Figure 1). This is supported by overlapping LC_{50}s and LC_{10} 95% CIs for Stratego and trifloxystrobin (Table 1).

Table 1. The median lethal concentration (LC_{50}) and 10% lethal concentration (LC_{10}) values for *Hyalella azteca* comparing toxicity of Trifloxystrobin and formulation for water-only exposures with 95% confidence intervals shown[a]

Fungicide treatment	Concentrations based on total added to the system (nominal)		96-h average water concentrations (measured)	
	LC_{50} (µg/L)	LC_{10} (µg/L)	LC_{50} (µg/L)	LC_{10} (µg/L)
Trifloxystrobin	29.9 (21.0-43.8)	15.0 (6.7-21.34)	24.7 (17.9-37.9)	12.9 (5.5-17.8)
Stratego	25.8 (22.7-29.3)	15.6 (12.2-18.2)	20.4 (18.6-22.5)	14.2 (11.3-16.0)

[a]Because most of the toxicity occurred within 96 h, measured concentrations are based on the first 96 h, while final mortality assessment was at 168 h.

Table 2. The median lethal concentration (LC_{50}) and 10% lethal concentration (LC_{10}) values for *Hyalella azteca* in sediment-water microcosms comparing fungicide formulation application to water versus sediment with 95% confidence intervals shown[a]

Fungicide treatment	Concentrations based on total added to the system (nominal)		96-h average water concentrations (measured)	
	LC_{50} (µg/L)	LC_{10} (µg/L)	LC_{50} (µg/L)	LC_{10} (µg/L)
Stratego water overspray	43.1 (36.0-51.8)	13.8 (10.1-17.4)	16.8 (10.2-25.7)	4.0 (1.4-7.0)
Stratego sediment overspray	284 (166-756)	32.4 (15.1-56.4)	17.8 (11.7-32.7)	2.6 (1.5-3.8)

[a]Mortality was assessed after 168 h, Headline sediment-treated microcosms did not have significant enough mortality to determine lethal concentration values.

Trifloxystrobin rapidly dissipated from the water following application of Stratego to the overlying water within the microcosms. Trifloxystrobin concentrations across all treatment concentrations were 73% (+/- 11%) of that expected based on full water incorporation at the initial time point (4 h) during microcosm toxicity tests (Figure 2). Water concentrations continued to decline throughout the test with 25% (+/- 2%) remaining after 96 h and 13% (+/- 3%) after 168 h (Figure 2). Only the highest application of Stratego to the sediment was significantly more toxic as compared to controls. No statistical differences in mortality were observed between controls and microcosms receiving sediment treated with Stratego (p = 1.00) with the exception of the highest treatment concentration ($p < 0.001$; Figure 3).

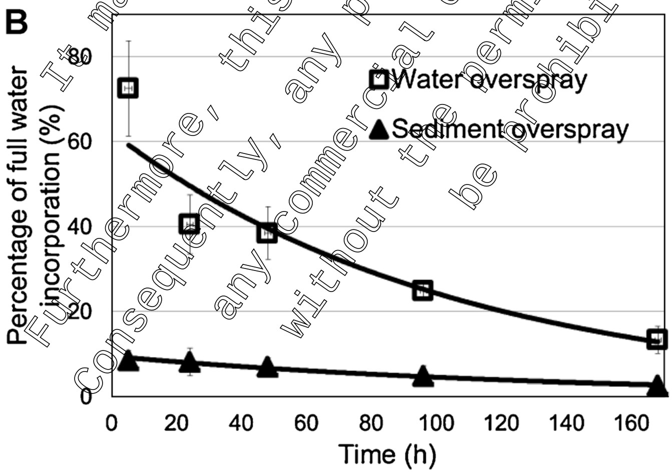

Figure 2. Mean (+/- standard deviation) shown for water concentrations of trifloxystrobin across all 5 treatment concentrations in sediment and water microcosm toxicity tests following the application of Stratego, to either overlying water or sediment. Water concentrations are expressed as percentage of full water incorporation, assuming complete water partitioning of the total amount of fungicide applied to the system. First-order exponential decay curves are fitted through the data to provide a visualization of how water concentrations varied during microcosm toxicity tests.

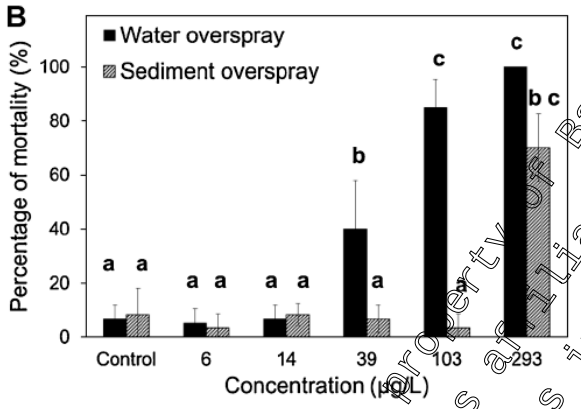

Figure 3. Mean (+/- standard deviation) percentage of mortality of *Hyalella azteca* for sediment-water microcosm exposures comparing toxicity between sediment and water applications of Stratego at 5 concentrations and a control. Formulations were applied to sediment-treated microcosms 24 h prior to water addition. Formulations were applied to water treated microcosms following the addition of *H. azteca*. Each treatment consisted of 6 replicates (n = 6). Categorical letters represent statistical differences between concentrations and/or treatments ($p < 0.05$).

RESULTS SUMMARY

Trifloxystrobin has an LC_{50} of 24.7 µg/L and Stratego has an LC_{50} of 44.2 µg/L based on 96-h average water concentrations. The LC_{50} of Stratego based on 96-h average water concentrations, water overspray was 16.3 µg/L and based on sediment overspray 17.8 µg/L.

Comment by the Notifier

The publication is well documented, however the study is not performed according to a Guideline and is not used for risk assessment. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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Report: **KCA 8.2.4.1/21 [redacted], H.; [redacted], W.; [redacted], G., L.(2009)**
Title: Toxicity of soybean rust fungicides to freshwater algae and Daphnia magna.
Source: Ecotoxicology, Volume 18, Issue 4, p. 440-446
DOI No: DOI 10.1007/s10646-009-0298-1
Document No: M-459634-01-1
Guidelines: none
GLP: No
Classification: b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Soybeans are intensively grown over large swaths of land in the Midwestern US. Introduction of the pathogenic fungus responsible for Soybean Rust (*Phakopsora pachyrhizi*) will likely result in a significant increase in the environmental load of strobilurin and conazole fungicides. The toxicity of trifloxystrobin was determined to the unicellular algae *Pseudokirchneriella subcapitata* and the aquatic invertebrate, *Daphnia magna*. For Algae the endpoints were determined as IC_{50} (72 h) = 120 µg/L and IC_{10} (72 h) = 5.7 µ/L. For Daphnia the endpoint were determined as: LC_{50} (48 h) = 740 µg/L; LC_{10} (48 h) = 380 µg/L; LC_{50} (96 h) = 530 µg/L and LC_{10} (96 h) = 290 µg/L.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	trifloxystrobin
Active substance(s):	n/a
Chemical state and description:	n/a
Source of test item:	AccuStandard, Inc. (New Haven, CT, USA)
Batch number:	n/a
Purity:	n/a
Storage conditions:	n/a
Water solubility:	n/a

2. Test solutions

Vehicle/solvent:	No solvent used
Source of vehicle/solvent:	n/a
Concentration of vehicle/solvent:	n/a
Method of preparation:	n/a
Evidence of unsolved material:	n/a

3. Test organism(s)

Species:	*Pseudokirchneriella subcapitata*
Common name:	Green algae
Source of test species:	Carolina Biological Supplies (Burlington, NC)
Species:	*Daphnia magna*
Common name:	Water flea
Source of test species:	Aquatic Biosystems Inc. (Fort Collins, CO, USA)

4. Culture conditions of test organism(s)

	Pseudokirchneriella subcapitata
Culture medium:	standard medium, as proposed by the United States Environmental Protection Agency (USEPA) (2002)
Temperature:	25 +/- 1°C
Photoperiod:	n/a
Light intensity:	4000 lux
pH:	n/a
Oxygen saturation:	n/a
Food and feeding regime:	n/a
Acclimatisation prior to testing:	n/a
Observations during acclimatisation:	n/a

	Daphnia magna
Culture medium:	modified highhardness COMBO medium (Baer and Goulden 1998)
Temperature:	22 +/- 1°C
Photoperiod:	16:8 light:dark cycle
Light intensity:	n/a
pH:	n/a
Oxygen saturation:	n/a
Food and feeding regime:	n/a
Acclimatisation prior to testing:	n/a
Observations during acclimatisation:	n/a

B. Study design and methods

1. Test procedure

	Pseudokarchneriella subcapitata
Test system:	n/a
Test concentration(s):	40; 80; 120 and 160 µg/L
Control(s):	Yes
Number of replicates:	4 replicates
Test conditions:	n/a
Feeding:	n/a
Medium renewal:	n/a
Frequency of test item application:	n/a
Test duration:	72 h
Endpoints:	IC_{50} and IC_{10} (Inhibition concentration)
Statistics:	PROC PROBIT

	Daphnia magna
Test system:	n/a
Test concentration(s):	90; 130; 200; 300; 450; 670 and 1000 µg/L
Control(s):	yes
Number of replicates:	4 replicates
Test conditions:	n/a
Feeding:	daily
Medium renewal:	n/a
Frequency of test item application:	n/a
Test duration:	96 h
Endpoints:	LC_{50} and LC_{10}
Statistics:	PROC PROBIT

2. Measurements during the test

Water/medium parameters:	n/a

3. Sampling

Sampling frequency:	n/a
Transport/storage of samples:	n/a

4. Chemical analysis

Guideline/protocol:	n/a

Method:	n/a
Pre-treatment of samples:	n/a
Conduction:	n/a
Reference item:	n/a
Recovery:	n/a
Limit of detection:	n/a
Limit of quantification:	n/a

RESULTS

1. Validity criteria:

No validity criteria defined.

2. Biological findings:

Algae were exposed for 72 hours to trifloxystrobin, the IC_{50} was calculated to be 120 µg/L, the IC_{10} was calculated to be 5.7 µg/L (Table 1).

Table 1: Summary of median (IC_{50}) and tenth percentile (IC_{10}) inhibition concentrations (µg/l) of *Pseudokirchneriella subcapitata* after 72 h

Trifloxystrobin	Endpoint	
	IC_{50}	IC_{10}
	120 (80-190)	5.7 (3.4–8.3)

Toxicity endpoints were calculated based on nominal concentrations. Values in parenthesis correspond to the 95% confidence interval of each estimate

The median and 10th percentile mortality rates for each exposure period and fungicide are shown in Table 2. The LC_{50} after 48 h was calculated to be 740 µg/L after 96 h the LC_{50} was 530 µg/L.

Table 2: Summary of median (LC_{50}) and tenth percentile (LC_{10}) lethal concentrations (µg/L) of *Daphnia magna* exposed for 24, 48, 72 and 96 h

Trifloxystrobin	Exposure duration			
	24 h	48 h	72 h	96 h
LC_{50}	750 (700-810)	740 (640-890)	690 (610-810)	530 (470-610)
LC_{10}	610 (530-660)	380 (300–440)	360 (290–420)	290 (230-330)

Toxicity endpoints were calculated based on nominal concentrations. Values in parenthesis correspond to the 95% confidence interval of each estimate

RESULTS SUMMARY

Daphnia:
LC_{50} (48 h) = 740 µg/L
LC_{10} (48 h) = 380 µg/L
LC_{50} (96 h) = 530 µg/L
LC_{10} (96 h) = 290 µg/L

Comment by the Notifier

The publication is well documented study without analytics and is not used for risk assessment. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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 trifloxystrobin is not an insecticide and does not show an insecticidal mode of action. However, for information on studies already evaluated during the first EU review of trifloxystrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

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

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.2.5.1- 1: Long-term toxicity to *Daphnia magna* exposed to trifloxystrobin and its metabolite

Test substance	Test species	Endpoint	Reference
Trifloxystrobin	Invertebrate, chronic *Daphnia magna*	NOEC 0.00276 mg a.s./L	[redacted] et al. (1997) 1117-CG M-032097-01-1 KCA 8.2.5.1/01
CGA 321113	Invertebrate, chronic *Daphnia magna*	NOEC 4.2 mg/p.m./L	[redacted] (1998) 1117-CG M-056619-01-1 KCA 8.2.5.1/02

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

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

CA 8.2.5.3 Development and emergence in Chironomus species

No acute study on the development and emergence in Chironomus species was provided during the evaluation of the first EU review of this compound. However, the chronic toxicity was addressed. For information please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.2.5.3- 1: Long-term toxicity to *Chironomus riparius* exposed to trifloxystrobin and its metabolite

Test substance	Test species	Endpoint		Reference
Trifloxystrobin	Chironomid, chronic *Chironomus riparius*	NOEC	0.200 mg a.s./L	[redacted] (1998) 983812 M-033988-01-1 KCA 8.2.5.3/01
CGA 321113	Chironomid, chronic *Chironomus riparius*	NOEC	25 mg p.m./L	[redacted] (1998) 983811 M-033990-01-1 KCA 8.2.5.3/02

CA 8.2.5.4 Sediment dwelling organisms

See point 8.2.5.1. No additional studies were performed.

CA 8.2.6 Effects on algal growth

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

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.2.8- 1: Toxicity to algal species exposed to trifloxystrobin and its metabolite

Test substance	Test species	Endpoint		Reference
Trifloxystrobin	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	0.0053 mg a.s./L 0.016 mg a.s./L	[redacted] (1995) 943533 M-032098-01-1 KCA 8.2.6.1/01
CGA 357261	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	1.4 mg p.m./L 2.0 mg p.m./L	[redacted] (1997) 649315 M-032109-01-1 KCA 8.2.6.1/03
CGA 321113	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50} E_rC_{50}	> 100 mg p.m./L > 100 mg p.m./L	[redacted] (1996) 953570 M-032651-01-1 KCA 8.2.6.1/04
CGA 373466	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	> 100 mg p.m./L > 100 mg p.m./L	[redacted] (1997) 649372 M-032653-01-1 KCA 8.2.6.1/05
NOA 413161	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50} E_rC_{50}	> 100 mg p.m./L > 100 mg p.m./L	[redacted] (1998) G 528 17 M-033979-01-1 KCA 8.2.6.1 /07
NOA 413163	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50} E_rC_{50}	> 100 mg p.m./L > 100 mg p.m./L	[redacted] (1998) G 529 17 M-033983-01-1 KCA 8.2.6.1/08
CGA 107170	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50} E_rC_{50}	30.9 mg p.m./L 42.2 mg p.m./L	[redacted] (1997) 649258 M-032659-01-1 KCA 8.2.6.1/06

In order to complete the risk assessment on algal species, additional studies are provided for metabolites of trifloxystrobin in this Supplemental Dossier.

Table 8.2.8- 2: Additional studies on toxicity to algal species exposed to trifloxystrobin and its metabolite

Test substance	Test species	Endpoint	Reference
Trifloxystrobin	Algae, growth inhibition *Navicula pelliculosa* (freshwater diatom)	E_bC_{50} 0.0944 mg a.s./L E_rC_{50} > 1.0 mg a.s./L	[redacted] et al. (2004) M-060371-01-1 200976 KCA 8.2.6.2/02
	Algae, growth inhibition *Anabaena flos-aquae* (blue-green bacterium)	E_bC_{50} > 0.13 mg a.s./L E_rC_{50} > 0.13 mg a.s./L	[redacted] et al (2001) M-088531-01-1 110409 KCA 8.2.6.2/03
CGA 357262	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50} > 2.65 mg p.m./L	[redacted] (2012) EBTFL018 M-429959-01-1 KCA 8.2.6.1/11
CGA 357276	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50} > 5.88 mg p.m./L	[redacted] (2012) EBTFX196 M-434282-01-1 KCA 8.2.6.1/12
NOA 409480	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50} > 5.88 mg p.m./L	[redacted] (2013) EBTFL032 M-467271-01-1 KCA 8.2.6.1/13
2-Hydroxymethyl-benzonitrile[a]	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50} 33.2 mg p.m./L	[redacted] (2012) EBTFL008 M-441244-01-1 KCA 8.2.6.1/14

[a] 2-Hydroxymethylbenzonitrile is present in a tautomeric equilibrium with 2-Benzofuran-1(3H)-imine, for further details please refer to the study report (M-441244-01-1).

Study summaries are given below.

CA 8.2.6.1 Effects on growth of green algae

Metabolite CGA 357262

Report: **KCA 8.2.6.1/11; [redacted] (2012)**
Title: *Pseudokirchneriella subcapitata* growth inhibition test with BCS-BJ39463 – limit test
Rep. No: EBTFL018
Document No: M-429959-01-1
Guidelines: OECD Guideline 201 (2006)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:
The objective of this 72 hour growth inhibition test is, to verify the assumption that the test item will cause no adverse effects on the growth of the green alga *Pseudokirchneriella subcapitata*.

Materials and Methods:

Test material: BCS - BJ39463 (CGA 357262, metabolite of trifloxystrobin), analysed purity: 99.4 % w/w was tested, specified by origin batch no.: SES 10487-2-1, certificate no.: AZ 17373, customer order no.: TOX09326-00 and LIMS no.: 1114276.

Pseudokirchneriella subcapitata were exposed in a chronic multi-generation test for 72 hours under static exposure conditions to the geometric mean measured concentration of 2.65 mg BCS-pure metabolite (p.m.)/L in comparison to a water and a solvent control [100 µL DMF = Dimethylformamide (including the appropriate concentration of the test item) + 1100 mL nutrient medium was added to all concentration levels and the solvent control].

The test system consisted of six 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 ranged from 7.2 to 8.2 in the controls and the incubation temperature ranged from 21.5°C to 22.5°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7256 lux.

Quantitative amounts of BCS - BJ39463 (CGA 357262) were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: October 07 2011 to March 22 2012

Results:

Validity of the study:

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

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

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

Analytical results:

The analytical finding of test item in the treatment level on day 0 and 3 was 26.7 and 26.3% of nominal, respectively. The test was performed using a limit concentration of nominally 10.0 mg

p.m./L. The results of the accompanying chemical analysis revealed a geometric mean measured concentration of 2.65 mg p.m./L. This concentration represents the saturation concentration (water solubility of the test item under exposure conditions). All results are based on geometric mean measured test concentrations of the test item.

Effect of BCS-BJ39463 (CGA 357262) on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Geom. mean measured concentration [mg p.m./L]	Cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Control	751980	1.440	--
Solvent control	767460	1.447	--
Pooled controls	759720	1.443	--
2.65	598130	1.363	5.5

test initiation with 10,000 cells/mL

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

Conclusions:

A 72-hour growth inhibition test conducted with BCS- BJ39463 (CGA 357262, metabolite of trifloxystrobin) on algae (*P. subcapitata)* under static exposure conditions revealed the following results:

E_rC_{50} (0 - 72h) > 2.65 mg p.m./L (based on geometric mean measured concentration).

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Metabolite CGA 357276

Report: **KCA 8.2.6.1/12; [redacted] (2012)**
Title: *Pseudokirchneriella subcapitata*, growth inhibition test with BCS-AB39835
Rep. No: EBTFX196
Document No: M-434282-01-1
Guidelines: OECD Guideline 201 (2006)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:

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

Materials and Methods:

Test material: BCS-AB39835 (CGA 357276, metabolite of trifloxystrobin) analysed purity: 97.8% w/w was tested, specified by origin batch no.: BCOO 6204-3-3, certificate no.: AZ 16891 and LIMS no.: 1026832.

Pseudokirchneriella subcapitata were exposed in a chronic multi-generation test for 3 days under static exposure conditions to the geometric mean measured concentration of 0.381, 0.999, 3.03, 3.87 and 5.88 mg pure metabolite (p.m.)/L in comparison to a water and a solvent control [110 µL DMF = Dimethylformamide (including the appropriate concentration of the test item) + 1100 mL nutrient medium was added to all concentration levels and the solvent control]. The test system consisted of three replicate vessels per test level and six replicate vessels per control. The initial cell number was 10,000 cells/mL.

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

The pH values ranged from 7.8 to 8.3 in the controls and the incubation temperature ranged from 19.8°C to 23.6°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7767 lux.

Quantitative amounts of BCS-AB39835 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: April 13 2011 to May 03 2012

Results:

Validity of the study:

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

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

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

Analytical results:

The analytical findings of BCS-AB39835 (CGA 357276) in the treatment levels found on day 0 were 9% to 78% of nominal (average 43%). On day 3 analytical findings of 15% to 87% of nominal (average 56%) were found. The low recoveries were observed especially in the two highest test

concentrations as they obviously exceeded the water solubility of the test item under exposure conditions. Therefore all results are based on geometric mean measured test concentrations of the metabolite.

Biological results:

Effect of BCS-AB39835 (CGA 357276) on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Geom. mean measured concentration [mg p.m./L]	Cell number after 72 h (means) per mL	(0-72h) average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Control	739 000	1.434	--
Solvent control	762 000	1.442	--
Pooled controls	751 000	1.438	--
0.381	689 000	1.410	1.9
0.999	564 000	1.343	6.6
3.03	619 000	1.373	4.5
3.87	532 000	1.324	7.9
5.88	481 000	1.283	10.8

test initiation with 10,000 cells/mL

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

Conclusions:

The (0 - 72h)-E_rC_x for BCS-AB39835 (CGA 357276) is > 5.88 mg p.m./L and the (0 - 72h)-NOE_rC is 0.381 mg p.m./L.

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Metabolite NOA 409480

Report: **KCA 8.2.6.1/13; [redacted] (2013)**
Title: *Pseudokirchneriella subcapitata* - Growth inhibition test with BCS-CR74871
Rep. No: EBTFL032
Document No: M-467271-01-1
Guidelines: OECD Guideline 201 (2006)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:

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

Materials and Methods:

Test material: BCS-CR74871 (NOA 409480, metabolite of trifloxystrobin) analysed purity: 99.0 % w/w was tested, specified by origin batch no.: BCOO 6263-3-4, TOX-no.:09206-021 and LIMS no.: 1312765.

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multi-generation test for 3 days under static exposure conditions to the geometric mean measured concentration of 1.06, 2.02, 3.65, 10.5 and 100 mg pure metabolite (p.m.)/L equivalent to 0.960, 3.07, 9.80, 31.3 and 100 mg p.m./L in comparison to a control. Dimethylformamid (DMF) was used as solvent in the study. 100 µL DMF (including the appropriate concentration of the test item) / 1000 mL nutrient medium was added to all concentration levels and the solvent control.

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

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

The pH values ranged from 7.9 to 8.2 in the controls and the incubation temperature ranged from 22.4°C to 22.9°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6163 lux.

Quantitative amounts of BCS-CR74871 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: May 31 2013 to July 30 2013

Results:

Validity of the study:

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

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

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

Analytical results:

The analytical and biological findings demonstrate that the solubility of the test item exceed the highest test concentrations. The observed growth inhibition values for the highest four test item concentrations vary between 43.4 and 55.3 %. Based on the analytical findings it can be assumed that the water solubility of the test item under exposure conditions is between 2.5 and 7.2 mg/L. In the highest test item concentrations, higher values were determined at day 3 but undissolved test item at the surface of the test medium and precipitations were already observed. The water solubility for the test item in deionized water was determined to be 2.6 mg/L. Based on the biological findings, the measured test concentrations and the known water solubility of the test item it was decided to only use the three lowest concentrations for statistical evaluation. The results are given as geometric mean measured concentrations of the test item in the test medium.

Biological results:

Effect of BCS-CR74871 (NOA 409480) on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Geom. mean measured concentration [mg p.m./L]	Cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Pooled controls	934 000	1.512	--
1.06	643 000	1.387	8.2
2.02	127 000	0.845	44.3
3.65	131 000	0.856	43.4
10.5	121 000	0.830	45.1
10.0	76 000	0.676	55.3

test initiation with 10,000 cells/mL

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

Conclusions:

The (0 - 72h)-E_rC_{50} for BCS-CR74871 (NOA 409480) is > 3.75 mg p.m./L and the (0 - 72h)-NOE_rC is <1.06 mg p.m./L.

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Metabolites 2-Hydroxymethylbenzonitrile + 2-Benzofuran-1(3H)-imine (tautomeric mixture)

Report: **KCA 8.2.6.1/14; [redacted] (2012)**

Title: *Pseudokirchneriella subcapitata* growth inhibition test with BCS-AR14212 + BCS-CR34532
Rep. No: EBTFL008
Document No: M-441244-01-1
Guidelines: OECD Guideline 201 (2006)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:

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

Materials and Methods:

Test material: BCS-AR14212 + BCS-CR34532 (2-Hydroxymethylbenzonitrile + 2-Benzofuran-1(3H)-imine, metabolites of trifloxystrobin) technical, analysed content: BCS-AR14212: 31.8 % w/w and BCS-CR34532: 63.8 % w/w % was tested, specified by origin batch no.: BCOO 6206-4-2, certificate no.: AZ16949 and LIMS no.: 1029419.

Pseudokirchneriella subcapitata (freshwater microalgae; formerly known as *Selenastrum capricornutum*) were exposed in a chronic multi-generation test for 3 days under static exposure conditions to nominal concentrations of 0.960, 3.07, 9.80, 31.3 and 100 mg pure metabolite (p.m.)/L in comparison to a water and a solvent control [100 µL DMF = Dimethylformamide (including the appropriate concentration of the test item) / 1000 mL nutrient medium was added to all concentration levels and the solvent control].

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

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

The pH values ranged from 7.8 to 8.3 in the controls and the incubation temperature ranged from 19.8°C to 23.6°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7767 lux.

Quantitative amounts of BCS-AR 14212 were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: November 18 2011 to April 19 2012

Results:

Validity of the study:

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

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

Strain material of defined sensitivity was used, as shown by reference substance testing with 3,5-dichlorophenol or potassium dichromate. Reference tests are conducted event driven *(i.e. in case of*

receiving new strains, introduction of new test conditions, apparatus, etc.). These tests are documented and archived together with strain protocols.

Analytical results:

The analytical findings of BCS-AR 14212 in the treatment levels found on day 0 were 92 % to 103 % of nominal. In the lowest test concentration only 67 % of nominal were found. On day 3 analytical findings of 10 % of nominal or lower were found. The low analytical recovery at this concentration has no impact on the outcome of the study as the NOEC is above this concentration. Given that the toxicity cannot be attributed to any of the compounds but to the metabolite mixture as a whole, all results are based on nominal test concentrations of the test item.

Biological results:

Effect of BCS-AR 14212 on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Geom. mean measured concentration [mg p.m./L]	Cell number after 72 h (means) per mL	(0-72h) average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Control	739 000	1.434	--
Solvent control	762 000	1.442	--
Pooled controls	751 000	1.438	--
0.960	851 000	1.481	-3.0
3.07	680 000	1.406	2.2
9.80	441 000	1.259	12.4
31.3	55 000	0.569	60.4
100	38 000	0.440	69.4

test initiation with 10,000 cells/mL

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

Conclusions:

The (0 - 72h)-E_rC_{50} for BCS-AR14212 + BCS-CR34532 (tech.) is 33.2 mg test item/L (95 % CI: 25.6 – 43.7 mg test item/L), the (0 - 72h)-E_rC_{10} is 5.53 mg test item/L (95 % CI: 2.56 – 8.28 mg test item/L) and the (0- 72h) - NOEC is 3.07 mg test item/L.

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Results literature review

Report: KCA 8.2.6.1/15 [redacted]; [redacted]; [redacted]; [redacted] (2009)
Title: Toxicity of soybean rust fungicides to freshwater algae and Daphnia magna.
Source: Ecotoxicology, Volume 18, Issue 4, p. 440-446
DOI No: DOI 10.1007/s10646-009-0298-1
Document No: M-459634-01-1
Guidelines: none
GLP: No
Classification: b) supplementary information (EFSA Journal 2011;9(2):2092)

RESULTS SUMMARY

Algae:
IC_{50} (72 h) = 120 µg/L
IC_{10} (72 h) = 5.7 µ/L

For summary details please refer to KCA 8.2.4.1/21

Comment by the Notifier

The publication is well documented study without analytics and is not used for risk assessment. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CA 8.2.6.2 Effects on growth of an additional algal species

Trifloxystrobin

Report:	**KCA 8.2.6.2/02; [redacted], [redacted], [redacted] (2004)**
Title:	Toxicity of Trifloxystrobin Technical to the Freshwater Diatom *Navicula pelliculosa*.
Rep. No:	200976
Document No:	M-060371-01-1
Guidelines:	FIFRA Guideline 123-2 OPPTS Guideline 850.5400 OECD Guideline 201
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The objective of this study was to determine the toxicity of trifloxystrobin to the freshwater diatom *(Navicula pelliculosa)* during a 96-hour exposure period.

Materials and Methods:

Test material: Trifloxystrobin (K-973), specified by reference no.: FL-950834, CAS no.: 141517-21-7.
Navicula pelliculosa were exposed in a chronic multi-generation test for 96 hours under static exposure conditions to nominal concentration of 1, 10, 100 and 1000 µg a.s./L in comparison to a water and a solvent control (Dimethylformamide)
The test system consisted of two replicate vessels per test level and control. The initial cell number was 10,000 cells/mL.
The response parameters used in this study were cell density (standing crop), cumulative biomass, and growth rate. The variable used to calculate the response parameters was cell density based on daily cell counts.
Inhibition values were calculated for each treatment group as the percent reduction in cell density, cumulative biomass and growth rate relative to the pooled control replicates.

The pH values ranged from 7.3 to 8.5 for all test levels during the exposure period. Temperature ranged from 24.3°C to 25.5°C over the whole period of testing at light intensity of 4300 lux.

Dates of experimental work: February 10 2003 to February 14 2003

Results:

Validity of the study:

Validity Criteria:	**Obtained in this study:**
Increase of biomass:	Biomass increased in the control by more than 16-fold within the evaluation period.
Sectional control rates:	Mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, day 2-3 and day 3-4 in the control did not exceed 35%
Control replicate rates:	Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%

Analytical results:

The measured concentrations of trifloxystrobin on Day 0 were 1.60, 11.7, 86, and 828 µg a.s./L for the 1, 10, 100, and 1000 µg/L nominal concentrations, respectively. The 1 µg a.s./L test concentration was below the limit of quantitation and therefore the measured concentration is not reliable. The Day 0 measured concentrations represented approximately 83 to 117% (excluding the 1 µg a.s./L test concentration) of the nominal test concentrations. The measured concentrations of trifloxystrobin on Day 4 were 4.36, 45.3, and 704 µg a.s./L for the 10, 100 and 1000 µg/L nominal concentrations, respectively. This represents a Day 4 measured concentration range of approximately 44 to 70% as compared to nominal concentrations. Due to Day 0 results, there was no analysis done on the 1 µg a.s./L test concentration from Day 4. No undissolved test substance was visually observed in the test vessels throughout the test period. Since the test material was not stable in the test system, and the 1 µg a.s./L test concentration was below the limit of quantitation, all subsequent observations will refer to nominal concentrations of the test solutions.

Effect of trifloxystrobin on freshwater diatom (*Navicula pelliculosa*) in a 96 h growth inhibition test

Nominal concentration [µg a.s./L]	(0-72h)-average specific growth rates [$days^{-1}$]	Inhibition of average specific growth rate (72h) [%]	(0-96h)-average specific growth rates [$days^{-1}$]	Inhibition of average specific growth rate (96h) [%]
Control	0.072671	-	0.054746	-
Solvent control	0.072083	-	0.053881	-
1.0	0.069409*	4	0.054751	-1
10.0	0.064974*	10	0.053208	2
100	0.059680*	18	0.054900	-1
1000	0.027097*	63	0.050743*	7

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

No physical abnormalities were observed in the controls or treatment groups during the study.

Conclusions:

Based on 96-hour regression calculations, cumulative biomass is the most sensitive endpoint to exposure to trifloxystrobin. The 96-hour EC_{50} and EC_{25} values for cumulative biomass were 94.4 µg a.s./L and 6.6 µg a.s./L, respectively. The 96-hour EC_{50} value for growth rate was >100 µg a.s./L. The LOEC for the study was 10.0 µg a.s./L and the NOEC was 1 µg a.s./L based on 96-hour cumulative biomass. The 96-hour toxic threshold effect concentration (TEC- the geometric mean of the NOEC and LOEC) is 3.2 µg a.s./L.

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Report:	**KCA 8.2.6.2/03; [redacted], [redacted], [redacted] (2001)**
Title:	Trifloxystrobin (CGA-279,202): A 96-Hour Toxicity Test with the Freshwater Alga *(Anabaena flos-aquae)*
Rep. No:	110409
Document No:	M-088531-01-1
Guidelines:	U.S. EPA OPPTS Number 850.5400 OECD Guideline 201 EU Directive 92/69/EEC, Method C.3
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The objective of this study was to determine the toxicity of trifloxystrobin to the freshwater alga *(Anabaena flos-aqtae)* during a 96-hour exposure period.

Materials and Methods:

Test material: Trifloxystrobin (K-962), analysed purity: 97.3 % w/w was tested, specified by reference no.: S96-1885, CAS no.: 141517-21-7.

Anabaena flos-aquae were exposed in a chronic multi-generation test for 96 hours under static exposure conditions to day 0 measured concentration of 0.0057, 0.012, 0.031, 0.061 and 0.13 mg a.s./L in comparison to a water and a solvent control (Dimethylformamide, the solvent concentration in the treatment and solvent control groups was 0.1 mL/L).

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

Inhibition values were calculated for each treatment group as the percent reduction in cell density, area under the growth curve and growth rate relative to the pooled control replicates.

The pH values ranged from 7.1 to 7.2 at test initiation and ranged from 7.7 to 7.8 at test termination. Temperature ranged from 23.0°C to 24.2°C over the whole period of testing at light intensity ranging from 1940 to 2320 lux.

Dates of experimental work: October 19 2001 to October 24 2001

Results:

Validity of the study:

Validity Criteria:	Obtained in this study:
Increase of biomass:	Biomass increased in the control by more than 16-fold within the evaluation period.
Sectional control rates:	Mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, day 2-3 and day 3-4 in the control did not exceed 35%
Control replicate rates:	Percent coefficient of variation of the average growth rate in each control replicate did not exceed 7%.

Analytical results:

Nominal concentrations selected for use in this study were 0.01, 0.02, 0.04, 0.08 and 0.16 mg a.s./L. Samples collected at the beginning of the test had measured concentrations of 0.0057, 0.012, 0.031, 0.061 and 0.13 mg a.s./L, representing 57, 59, 79, 76 and 84% of nominal concentrations, respectively. The recoveries in the two lowest test concentrations fell below 70% of nominal concentrations. However, the low recoveries at those concentrations are not critical due to the lack of effects in the higher test concentrations which had recoveries of >70%. Samples collected at test termination ranged from 64 to 69%. Due to the decline in the test substance concentration during the test, the results of the study were based on Day 0 measured concentrations.

Effect of trifloxystrobin on freshwater algae (*Anabaena flos-aquae*) in a 96 h growth inhibition test

Day 0 measured concentration [mg a.s./L]	(0-72h)-average specific growth rates [days^{-1}]	Inhibition of average specific growth rate (72h) [%]	(0-96h)-average specific growth rates [days^{-1}]	Inhibition of average specific growth rate (96h) [%]
Control	0.0437	-	0.0473	-
Solvent control	0.0412	-	0.0489	-
Pooled controls	0.0425	-	0.0481	-
0.0057	0.0412	2.8	0.0468	2.5
0.012	0.0432	-1.7	0.0489	-1.8
0.031	0.0445	-5.0	0.0461	4.0
0.061	0.0421	0.66	0.0490	-2.0
0.13	0.0457	-7.8	0.0484	-0.68

After 72 and 96 hours of exposure, there were no apparent treatment-related effects upon growth at any of the concentrations tested. After 96 hours of exposure, there were no noticeable changes in cell shape, size or color in any of the treatment and solvent control group when compared to the negative control replicates. In addition, there were no evidence of aggregations or flocculation of cells, nor were there evidence of algal cells adhering to the test chambers in any of the control or treatment groups during the test or at test termination.

Conclusions:

The 72 and 96-hour EC_{50}, E_bC_{50} and E_rC_{50} values, based on cell density, area under the growth curve and growth rate, respectively, for *Anabaena flos-aquae* exposed to trifloxystrobin were

>0.13 mg a.s./L, the highest measured concentration tested. The 72 and 96-hour NOAEC for each growth parameter was 0.13 mg a.s./L.

CA 8.2.7 Effects on aquatic macrophytes

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

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.2.7- 1: Toxicity to aquatic macrophytes exposed to trifloxystrobin and its metabolite

Test substance	Test species	Endpoint	Reference
Trifloxystrobin	Aquatic plants, growth *Lemna gibba*	$EC_{50\ (frond\ number)}$ > 1.93 mg a.s./L	[redacted] et al. (1996) 671-CG M-032662-01-1 KCA 8.2.7/01

CA 8.2.8 Further testing on aquatic organisms

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

Additional studies and statements are submitted within this Supplemental Dossier for renewal of approval of trifloxystrobin. Summaries are given below.

Table 8.2.8- 1: Additional studies on other aquatic species exposed to trifloxystrobin

Test species	Test system	Endpoint		Reference
Trifloxystrobin	Aquatic vertebrate, acute *Xenopus laevis*	LC_{50}	0.0386 mg a.s./L	[redacted] et al. (2011) EBTFY003 M-358069-01-1 KCA 8.2.8/13
	Lentic freshwater community- mesocosm (WG 50)	NOEAEC NOEC* LOEC	4 x 0.0120 mg a.s./L 4 x 0.0037 mg a.s./L 4 x 0.0067 mg a.s./L	[redacted] et al. (2002) HBF/BT 04 M-067201-01-1 KCA 8.2.8/09
	Expert statement EAC of trifloxystrobin	EAC	0.0067 mg a.s./L	[redacted] & [redacted] (2002) M-067239-01-1 KCA 8.2.8/14 and [redacted] (2002) M-076994-01-1 KCA 8.2.8/15
	Fish, acute *Gasterosteus aculeatus* three-spined stickleback	LC_{50}	0.057 mg a.s./L	[redacted] (2001) DOM 21026 M-050563-01-1 KCA 8.2.8/16
	Analytical report to M-030536-01-1 (microcosm)			[redacted] (1997) 43274 M-049272-01-1 KCA 8.2.8/17

*The outdoor experimental pond study can be used for the ecological risk assessment of the test compound to invertebrates, algae and macrophytes. Effect class 2 (slight effects) was observed only in the endpoint categories 'Micro-Crustacea' and 'Phytoplankton'. The responses in 'Micro-Crustacea' concerned a slight reduction in population densities of *Daphnia longispina*, while the responses in Phytoplankton concerned a limited increase in one algal species (most probably an indirect effect). In all other organisms, no effects occurred. Thus the mesocosm NOEC is used to derive the RAC of 3.7 µg/L.

Report: **KCA 8.2.8/13; [redacted], C.S., [redacted], [redacted], C.V.; 2011**

Title: Acute Toxicity of Trifloxystrobin Technical to *Xenopus laevis* Under Flow-through Conditions

Report No.: EBTFY003

Document No.: M-358069-01-1

Guidelines: No formal international guideline exists for this test protocol. Methodologies from USEPA, OPPTS Guideline 850.1075 (1996), USEPA-FIFRA, 40 CFR, Part 158, Guideline No. 72-1 (1982) and OECD Guideline 203 (2004) were considered in the development of this protocol

Deviations: None

GLP: Yes (certified laboratory)

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

Materials and Methods:
Test item: trifloxystrobin, batch No.: TR605092, purity 99.5%.
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), 9.38 (8.39), 18.8 (15.7), 37.5 (27.9), 75.0 (53.5) and 150 (118) µg a.s./L.
There were three replicates of 10 tadpoles in the control and each toxicant level.
Water temperature was 22.0 – 22.4 °C and pH 8.1 to 8.2 during the test, the photoperiod was 16 hours of light and 8 hours dark. Light intensity was 632 to 995 lux and the dissolved oxygen range was 7.4 to 8.0 mg/L.
Survival and sublethal behavioral effects of tadpoles were recorded after 4, 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: September 18 to September 20, 2009

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	7.4 to 8.0 mg/L
pH value during the test	constant	8.1 – 8.2

Analytical results:
Mean measured recoveries ranged from 71 to 89% of nominal values. Results are based on mean measured test concentrations.

Biological results:

Mean Measured Concentration (µg a.s./L)	Hour 4		24 Hour		48 Hour	
	Dead	Obs	Dead	Obs	Dead	Obs
Control	0	30 N	0	30 N	0	30 N
Solvent Control	0	30 N	0	30 N	0	30 N
8.39	0	30 N	0	30 N	0	30 N
15.7	0	30 N	0	30 N	0	30 N
27.9	0	30 N	0	30 N	0	30 N
53.5	20	8 AS,Q; 2 OB,Q	30	---	---	---
118	30	---	---	---	---	---

Obs = Observations (number of individuals observed plus observation)
Q = Quiescent, AS = At Surface
Dead = Cumulative number of dead
OB = On Bottom, N = Normal
--- = No observations taken
Note: There were 30 organisms present in each test concentration at the start of the test

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

Test substance	**Trifloxystrobin**
Test object	***Xenopus laevis***
Exposure	**48-hour, flow-through**
LC_{50} 48 hours (95% C.I.)	38.6 (27.9 and 53.5) µg a.s./L
LOEC	53.5 µg a.s./L
NOEC	29.7 µg a.s./L

Conclusion:
Based on the results presented above, the 48h-LC_{50} is determined to be 38.6 µg a.s./L.

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Report: **KCA 8.2.8/14 [redacted] I. & [redacted] A.; 2002**
Title: Refined Risk Assessment on the Effects of Trifloxystrobin on the Aquatic Freshwater Community
Rep. No: Not given
Document No: M-067239-01-1
Guidelines: Expert evaluation based on HARAP and CLASSIC
Deviations: not applicable
GLP: not applicable

Report:	**KCA 8.2.8/15; [redacted], T.C.M., (2002)**
Title:	Assessment of the aquatic risks of the fungicide trifloxystrobin on basis of an experimental pond study and laboratory tests with aquatic species
Rep. No:	HBF/BT 04
Document No:	M-076994-01-1
Guidelines:	Expert evaluation based on HARAP and CLASSIC
Deviations:	not applicable
GLP:	not applicable (statement)

Conclusion:

The RAC for trifloxystrobin can be established at 0.0037 mg/L based on:

• short environmental persistence of trifloxystrobin (DT50 < 2 days), combined with negligible toxicity of the degradation products of trifloxystrobin (c.f. Table 10.2.i), leaving high potential for recovery already in the short-term and no chronic concern
• rather small species sensitivity differences demonstrated within the group of fish or aquatic invertebrates and reduced need for an interspecies extrapolation factor
• small acute-to-chronic ratio with reduced extrapolation factor to the no effect concentration range
• reduced risk to aquatic invertebrates and algae demonstrated in outdoor mesocosm study (KIIIA1 10.2.3/01) under realistic exposure and effect conditions, resulting in no need for lab-to field extrapolation factor for aquatic invertebrates & algae
• reduced risk to fish demonstrated in an indoor higher tier early life stage study with the most sensitive life stage of the most sensitive fish species and under realistic exposure conditions (KIIIA1 10.2.5.2/01) and no need for lab-to field extrapolation factor for fish
• consistence of HC5 values with results of higher tier studies (KIIIA1 10.2.3/02 & 03) and thus confirmation of the NOEC of 0.0037 mg/L from higher tier studies.

Summary:

The outdoor experimental pond study can be used for the ecological risk assessment of the test compound to invertebrates, algae and macrophytes. Effect class 2 (slight effects) was observed only in the endpoint categories 'Micro-Crustacea' and 'Phytoplankton'. The responses in 'Micro-Crustacea' concerned a slight reduction in population densities of *Daphnia longispina*, while the responses in Phytoplankton concerned a limited increase in one algal species (most probably an indirect effect). In all other organisms, no effects occurred. Thus the mesocosm NOEC is used to derive the RAC of 3.7 µg/L.

• To address risks of trifloxystrobin in freshwater ecosystems of the agricultural landscape the EAC could also be set at 6.7 µg a.s./L., since in the experimental ponds four times treated with this concentration only a few populations (*Daphnia longispina, Calanoidae, Cryptomonas, Navicula*) showed a possible treatment related response, and all these responses were confined in magnitude and duration.

• The calculated HC_5 values on basis of acute static tests with invertebrates (HC_5 = 8.3 µg a.s./L) or invertebrates and algae (HC_5 = 4.7 µg a.s./L) are in line with the EAC of 6.7 µg a.s./L derived from the experimental pond study.

• Of the several taxa of fish species tested in the laboratory, Rainbow trout is the most sensitive to trifloxystrobin. In addition, the acute toxicity tests with Rainbow trout and the long-term indoor microcosm study with a sensitive stage of Rainbow trout indicate that a repeated application of trifloxystrobin does not result in a lower toxicity value. Simulating more or less realistic field conditions, a long-term NOEC of 25.3 µg a.s./L was observed for the sensitive early life stage of Rainbow trout in indoor microcosms three times treated with the formulated product. Consequently, an adequate assessment of risks of trifloxystrobin to fish on basis of static 96-h laboratory tests with fish seems possible.

• Using LC_{50} and NOEC values of 96-h static tests with eight species of fish, a HC_5 of 11.0 µg a.s./L on basis of LC_{50} data, and an HC_5 of 7.1 µg a.s./L on basis of NOEC data can be calculated.

• When adopting the RAC of 3.7 µg a.s./L derived from the experimental pond study, effects of repeated application of the fungicide on invertebrates and algae of freshwater ecosystems in the agricultural landscape will be negligible.

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Report:	**KCA 8.2.8/16; [redacted]; 2001**
Title:	Orientierende Fischtoxizitaet von Flint WG 50 am Stichling (*G. aculeatus*)
Report No.:	DOM 21026
Document No.:	M-050563-01-1
Guidelines:	Largely following OECD Guideline No. 203 (1992)
Deviations:	Orientating non-GLP study, no analytics
GLP:	No

Objective:

An orientating non-GLP test with three concentrations (0.02, 0.04 and 0.08 µg a.s./L) was performed in order to determine the concentration which kills 50 percent of the fish (96h-LC_{50})

Materials and Methods:

Test item: Flint (Trifloxystrobin) WG50 (Tox No 5619-00)

Test organism: Three-spined stickleback (*Gasterosteus aculeatus*).

Ten fish per concentration (six in the highest concentration) were exposed for 96 h under static test conditions to nominal concentrations (expressed as a.s.) of 0.02, 0.04 and 0.08 µg a.s./L against a control (dilution water).

During the test, fish were examined daily for mortalities and signs of poisoning. Within the study the pH-value, the oxygen saturation level, and the temperature were measured with commercial measurement devices, daily. Dissolved oxygen concentrations was > 60%, the pH values ranged from 7.1 to 7.4 and the water temperature was within 12±1°C over the whole testing period. The photoperiod was 16 hours of light and 8 hours dark.

Fish were inspected daily for the number of deaths, toxic symptoms or abnormalities. The mortality (%) after 24, 48, 72 and 96 hours of exposure was calculated in each treatment group. The concentration of the test substance was not measured.

The endpoints were expressed in terms of nominal concentrations.

Dates of experimental work: March 29, 2001 to April 1, 2001

Results:

Validity criteria:

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

All validity criteria for the study were met.

Biological results:

All six fish in the highest test concentration died after 72h, whereas in the other concentrations and control no fish died or showed any symptoms within the 96h test period.

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

LC_{50} values for rainbow trout exposed to Flint WG50 based on nominal concentrations

Test substance:	Trifloxystrobin WG50
Test object:	Three-spined stickleback (*Gasterosteus aculeatus*)
Exposure:	96 hours, static test design (three concentrations + control))
LC_{50} 96 h (95% C.I.):	**0.057 mg a.s./L**

Conclusions:

The LC_{50} (96h) of **Flint WG50** to Three-spined stickleback (*Gasterosteus aculeatus*) in a static 96-hour-test was determined to be 0.057 mg a.s./L.

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Report:	**KCA 8.2.8/17; [redacted]; 1997**
Title:	Assessment Of The Potential Biological Effects Of CGA-279202 Exposures On Aquatic Ecosystems As Measured In An Outdoor Fiberglass Tank System
Report No.:	43274
Document No.:	M-049272-01-1
Guidelines:	EPA Guideline No. 72-7(a)
Deviations:	Protocol section 4.4.1 (Application Methodology) does not indicate how samples taken were to be preserved. Samples were shipped to ABC on dry ice. The application solutions were stored frozen at -20 °C resulting in absorbed phase samples that were frozen, while the dissolved phase samples (50 methanol: 50 water) remained a liquid. Protocol section 4.5.1 (Collection Of Water Residue For Analysis) states that water samples are to be received frozen at the analytical laboratory. A few water samples were received thawed but cold, or partially frozen Impact on this study would be minor as the samples were cold or only partially thawed when received.
GLP:	Yes

Water and hydrosoil samples were collected to verify application loading and determine dissipation rates of CGA-279202. Water samples were collected before hydrosoil samples to prevent contamination of water samples with sediment stirred up by hydrosoil sample collection.
Two 500-mL water samples were collected from composite samples taken from each control tank. Spiking solution was added to each sample in the field by dispensing the contents of a premeasured vial into the sample container. The vial was also added to the sample.
Six sediment cores were collected (two from each control tank). The cores were spiked in the field by adding 500 µL of spiking solution using a microsyringe.

Extraction of analytes from the water matrix is accomplished by three liquid-liquid partitions against hexane. The combined hexane extracts are reduced to dryness by vacuum rotary evaporation. The dried extract is then dissolved by separate additions of 1 mL of acetonitrile and 1 mL of 2% acetic acid (aqueous) [total final volume is 2.0 mL]. Samples are ready for injection onto the HPLC system for quantitation of CGA-279202 and CGA-321113.
Extraction of analytes from the hydrosoil matrix is accomplished by shaking for two, 10 minute periods with 90% acetonitrile (ACN): 10% water. Solids are separated from the liquid extract by centrifugation. The extraction solvent is reduced to approximately 3-5 mL using vacuum rotary evaporation. The extract is then partitioned three times against hexane. The combined hexane extracts are reduced to dryness by vacuum rotary evaporation. The extract is then dissolved by separate additions of 1.0 mL of acetonitrile and 1.0 mL of 2% acetic acid (aqueous). Samples are ready for injection onto the HPLC system for quantitation of CGA-279202 and CGA-321113.
HPLC analysis for water and hydrosoil was performed on a column switching UV system. The limit of detection (LOD) and limit of quantitation (LOQ) for the two compounds in water were 0.05 ppb and 0.10 ppb, respectively. The initial validated LOD and LOQ for CGA-279202 and CGA-321113 in hydrosoil were 0.5 and 1.0 ppb, respectively. Seasonal biological growth produced chromatographic interferences that forced an increase of the LOD and LOQ values for analysis of CGA-279202 in hydrosoil without the silica column cleanup and for the analysis of CGA-321113 in water. The LOQ for CGA-279202 in hydrosoil increased to 10 ppb while the LOQ for CGA-321113 in water increased to 2.5 ppb.

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Results from literature review

Report: **KCA 8.2.8/19; [redacted] (2012)**

Title:	Acute toxicity of three strobilurin fungicide formulations and their active ingredients to tadpoles
Source:	Ecotoxicology (2012) 21:1458–1464
DOI No:	10.1007/s10646-012-0899-y
Document No:	M-464220-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

This study reports the acute toxicity of the active ingredients and formulation of the fungicide Stratego, using *Bufo cognatus* tadpoles exposed to four concentrations and a control. The fungicide, including AIs and formulation, demonstrates toxicity to tadpoles, with Stratego causing 100 % mortality at the highest concentrations (160 and 500µg/L). Overall, toxicity was comparable between AIs and formulation for all concentrations. Results suggest the AIs are responsible for most mortality for Stratego.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Stratego Fungicide
Active substance(s):	Trifloxystrobin (+ propiconazole)
Chemical state and description:	Not reported
Source of test item:	EPA Reg. No. 264-779, Bayer Crop Science
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Vehicle/solvent:	Formulation solutions were created by diluting formulations with deionized water
Method of preparation:	Fungicides were applied to experimental aquaria by adding 0.5 ml of the appropriate solution. Acetone (0.5 ml) was also added to formulation treatments and the control.

3. Test organism(s)

Species:	*Bufo cognatus*
Common name:	Great Plains toads
Source of test species:	captured in central Oklahoma in May and June 2011 during and after rain events and housed in our in-house animal facility

4. Culture conditions of test organism(s)

Culture medium:	Tanks consisted of standard 9.5 L glass aquaria and contained 6 L dechlorinated water. All tanks were washed 29 with acetone and rinsed 39 with water prior to the start of the experiment.

Temperature:	25 ± 2 °C
Photoperiod:	Constant at a 13:11 h light dark cycle
Light intensity:	Not reported
pH:	Not reported
Oxygen saturation:	above 5.4 mg/L
Food and feeding regime:	fed a mix of commercial rabbit food and TetraMin® (Tetra)
Acclimatisation prior to testing:	Seven haphazardly selected tadpoles from each pair were placed into each aquarium (21 tadpoles per tank) and allowed to acclimate for 24 h prior to the beginning of the toxicity test.
Observations during acclimatisation:	Not reported

B. Study design and methods

1. Test procedure

Test system:	Tadpoles were obtained from three adult toad pairings induced to breed by injecting luteinizing hormone-releasing hormone analog (LHRHa) at 20 µg LHRHa/10 g body mass into the dorsal lymph sacs
Test concentration(s):	Environmental concentration: 74µg/L; Test chamber concentrations: 500, 160, 50 and 15 µg/L
Control(s):	Water control
Number of replicates:	replicated 39 (n = 3; 75 experimental units)
Test conditions:	38 L of declorinated water with a piece of nylon mesh to aid in oviposition. Adults were removed after oviposition and aeration added to the aquaria. Tadpoles were fed a mix of commercial rabbit food and TetraMin® (Tetra). Tadpoles were 6 days old
Feeding:	fed a mix of commercial rabbit food and TetraMin® (Tetra)
Medium renewal:	None
Frequency of test item application:	Once at test start
Test duration:	96 h
Endpoints:	Mortality was defined as the failure to move after gentle probing with a glass rod
Statistics:	ANOVA and Tukey's multiple range test

2. Measurements during the test

Water/medium parameters:	Not reported

3. Sampling

Sampling frequency:	checked every 2 h for the first 12 h and then every 12 h through 96 h
Transport/storage of samples:	Tadpoles surviving to the end of the study were euthanized using 0.5% tricaine methanesulfonate (MS-222).

4. Chemical analysis

Guideline/protocol:	protocols approved by Oklahoma State University Institutional Animal Care and Use Committee
Method:	Analysis was performed using gas chromatography/mass spectrometry (GC/MS)
Pre-treatment of samples:	A 100 ml sample was taken from two of the three replicates (n = 2) for the concentrations analyzed and passed through a 1,000 mg C8 AccuBond® SPE cartridge (Agilent Technologies, Santa Clara, CA, USA). Cartridges were conditioned with methanol and distilled water and samples extracted at a rate of ~3.5 ml/minute.

Conduction:	quantitation ions: trifloxystrobin 116
Reference item:	Analytical standard [redacted] (Pestanal ®) AIs (pyraclostrobin, propiconazole, trifloxystrobin, and azoxystrobin) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved using HPLC [redacted] acetone to produce the same concentrations as the formulations
Recovery:	Not reported
Limit of detection:	Not reported
Limit of quantification:	Not reported

RESULTS

1. Biological findings:

Trifloxystrobin, in the lowest concentration treatment, declined the most in the test media with 96 h concentrations at 6–10 % of the initial concentration. Because most mortality occurred within 12–24 h, actual concentrations likely deviated little from initial target concentrations.

Control mortality was\2 % for the entire experiment. For all treatments with 100 % mortality, death occurred within the first 12 h, with over 95 % of the remaining mortalities occurring within the first 24 h. Stratego® resulted in 100 % mortality at 160 and 500 µg/L but minimal mortality at all other concentrations.

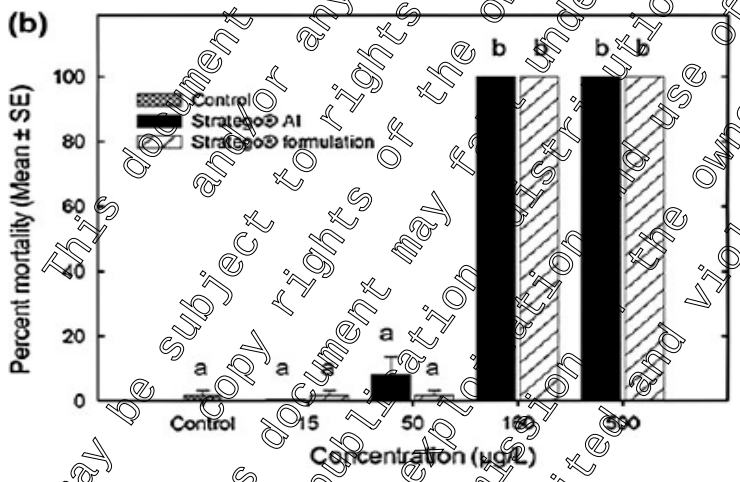

Figure 1: Mean (±SE) percent mortality of *B. cognatus* tadpoles exposed to formulations and the AIs of Stratego at four concentrations plus a control. Stratego contain two AIs. Control animals were exposed to solvent carrier used for AI treatments (acetone). Each treatment consisted of three replicates (n = 3). Lower case letters above bars indicate significant differences between the concentrations and/or chemical treatment (P < 0.05)

Stratego formulation and AIs treatments resulted in significant mortality ($P \leq 0.0012$), although there were no differences between formulation and AI at any concentration.

The 72h-LC_{50}-value was 104.1 µg/L for the Stratego formulation and 100.3 for the Stratego active ingredients trifloxystrobin and propiconazole.

Table 1: Estimates of median lethal concentrations (72 h- LC_{50}) of fungicide formulations and AI following exposure to *B. cognatus* tadpoles

		Average % recovery (standard deviation)			
Fungicide chemical	**72h-LC_{50} [µg/L]**	**High (3h)**	**High (96h)**	**Low (3h)**	**Low (96h)**
Stratego AIs	100.3				
Trifloxystrobin		75 (4)	-	71 (4)	6(1)
Stratego formulation	104.1				
Trifloxystrobin		68 (4)	-	68 (1)	10 (4)

RESULTS SUMMARY

The fungicide, including AIs and formulation, demonstrates toxicity to tadpoles, with Stratego causing 100 % mortality at the highest concentrations (160 and 500µg/L). At lower concentrations, mortality was significantly lower. The 72h-LC_{50}-value was 104.1 µg/L for the Stratego formulation and 100.3 µg/L for the Stratego active ingredients trifloxystrobin and propiconazole.

Effects on tadpoles

Comment by the Notifier

The effect of the fungicide formulation Stratego ® containing trifloxystrobin and propinconazole on tadpoles of the Great Plains toad (*Bufo cognatus*) has been published by [redacted] et al. (2010), see KCA 8.1.4/01, and [redacted] et al. (2012), see KCA 8.2.8/18. Concentrations of 740 µg trifloxystrobin/L plus 740 µg propinconazole/L caused 100% mortality of the tadpoles after 72 hours, whereas the mortality levels at 74 plus 74 µg/L and 7.4 plus 7.4 µg/L did not significantly differ from the control mortality. [redacted] et al. (2012) reported 72-h LC_{50}-levels of 100.3 µg/L and 104.1 µg/L for Stratego® and the mixture of trifloxystrobin plus propinconazole, respectively. Although no results have been obtained with trifloxystrobin alone, it can be concluded, that *Bufo cognatus*-tadpoles are not more sensitive to trifloxystrobin than fish (acute LC_{50}-figures range from 15 to 520 µg/L).

The effects on *Bufo cognatus*-tadpoles are not used in the risk assessment for the following reasons:

1. The results indicate, that the tadpoles are not more sensitive than fish
2. The experiments have been conducted with mixtures of trifloxystrobin and propinconazole or a formulation containing these two active ingredients.

The acute risk assessment for fish covers the potential risk to larval stages of amphibians as well. The papers by [redacted] et al. (2010), see KCA 8.1.4/01, and [redacted] et al. (2012), see KCA 8.2.8/19, are presented here as supplemental information.

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

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Report:	**KIIA 8.2.8/20; [redacted], C.M.; [redacted], P.M.; [redacted], R.C.; [redacted], A.M.; [redacted], M.C.; [redacted], A. (2012)**
Title:	Toxicity of the fungicide trifloxystrobin on tadpoles and its effect on fish-tadpole interaction.
Source:	Chemosphere, Volume 87, Issue 11, p. 1348-1354
DOI No:	doi:10.1016/j.chemosphere.2012.02.026
Document No:	M-459339-01-1
Guidelines:	None
GLP:	No
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

Contamination of aquatic systems is a major environmental stress that can interfere with predator–prey interactions, altering prey or predator behavior differentially. Toxicity parameters of the fungicide trifloxystrobin (TFS) were determined and its effects on predation rate, using a fish predator *Synbranchus marmoratus*) and four anuran tadpole species as prey (*Rhinella arenarum, Physalaemus santafecinus, Leptodactylus latrans*, and *Elachistocleis bicolor*) were examined. TFS was not equally toxic to the four tadpole species, *E. bicolor* being the most sensitive species, followed by *P. santafecinus*, *R. arenarum*, and *L. latrans*. Predation rates were evaluated using different treatments that combined predator and prey exposed or not to this fungicide. TFS would alter the outcome of eel–tadpole interaction by reducing prey movements; thus, prey detection would decrease and therefore tadpole survival would increase. In addition, eels preyed selectively upon non-exposed tadpoles avoiding the exposed ones almost all throughout the period evaluated.

Predation rate differed among prey species; such differences were not due to TFS exposure, but to interspecific differences in behavior. The mechanism that would explain TFS-induced reduction in predation rates remains unclear; however, what is clear is that sublethal TFS concentrations have the potential to alter prey behavior, thereby indirectly altering predator–prey interactions. In addition, it was considered that predator–prey relationships are measurable responses of toxicant exposure and provide ecological insight into how contaminants modify predator–prey interactions.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Flint (=50 WG (Wettable Granular) formulation (commercial [redacted] 50% a.i. of trifloxystrobin)
Active substance(s):	(E,E)-methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl] phenyl}-acetic acid methyl ester (50% a.i. of trifloxystrobin)
Chemical state and description:	Not reported
Source of test item:	Bayer CropScience A.G., Argentina
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Vehicle/solvent:	Wettable granular

Source of vehicle/solvent:	Bayer Crop Science AG
Concentration of vehicle/solvent:	10mg/L
Method of preparation:	Prepared by appropriate dilution of the stock solution
Evidence of unsolved material:	none

3. Test organism(s)

Species:	*Synbranchus marmoratus*
Common name:	Eel
Source of test species:	floodplain of Parana River ([redacted] Province, Argentina; [redacted])
Species:	*Rhinella arenarum* (Bufonidae), *Physalaemus santafecinus* (Leiuperidae), *Leptodactylus latrans* (Leptodactylidae), and *Elachistocleis bicolor* (Microhylidae)
Common name:	tadpole
Source of test species:	semipermanent pond at the University Ecological Reserve in [redacted] city ([redacted] Province, Argentina, [redacted])

4. Culture conditions of test organism(s)

Culture medium:	Dechlorinated tap water
Temperature:	22 ± 2°C)
Photoperiod:	12 h light:12 h dark
Light intensity:	Not reported
pH:	7.4 ± 0.05
Oxygen saturation:	6.5 ± 1.5 mg/L
Food and feeding regime:	fed on lettuce at the beginning of the experiment
Acclimatisation prior to testing:	Not reported
Observations during acclimatisation:	Not reported

B. Study design and methods

1. Test procedure

Acute toxicity test

Test system	Static
Test concentration(s):	Eight different concentrations ranged from 0.077 to 0.35 mg a.i./L
Control(s):	One negative control
Number of replicates:	3 replicates
Test conditions:	1 L of test solution at 25 +/- 1 °C and 12 h light:12 h dark
Feeding:	Lettuce at the beginning of the test
Medium renewal:	None
Frequency of test item application:	Not reported
Test duration:	48 h
Endpoints:	LC_{50}, LOEC; NOEC
Statistics:	Trimmed Spearman Karber method

Exposure phase

Test system:	6 h before the start of the testing phase
Test concentration(s):	LOEC previously calculated in toxicity tests

Control(s):	Yes, subsample (n = 24 eels and n = 120 tadpoles of each species) was assigned to the 'water exposure' treatment. In the 'TFS exposure' treatment.
Number of replicates:	No replicates, subsample of eels (n = 24) and tadpoles (n = 120 of each species) were randomly assigned to the 'TFS exposure' treatment.
Test conditions:	1 L of test solution at 25 +/- 1 °C and 12 h light:12 h dark
Feeding:	none
Medium renewal:	n/a
Frequency of test item application:	n/a
Test duration:	6 h before the start of the testing phase
Endpoints:	n/a
Statistics:	ANOVA
	Testing phase: Predator-prey experiment
Test system:	predation rate of eels (E) on tadpoles (T) exposed to TFS (+) and not exposed TFS (-) using four treatments: (1) neither eels nor tadpoles were exposed (E_, T_), (2) both eels and tadpoles were exposed (E+, T+), and either tadpoles (3) or eels (4) were exposed (E_, T+ and E+, T_, respectively).
Test concentration(s):	n/a
Control(s):	n/a
Number of replicates:	replicates
Test conditions:	experiments were conducted in a temperature-controlled room, with light/dark cycles that reflected natural day length
Feeding:	n/a
Medium renewal:	n/a
Frequency of test item application:	n/a
Test duration:	24 h
Endpoints:	instantaneous mortality rate of prey
Statistics:	ANOVA; Dunnett's test; Dunnett's and Tukey's HSD tests; Student's t-test; Kolmogrov-Smirnov and Levene tests

RESULTS

1. Validity criteria:

No validity criteria defined.

2. Biological findings:

In toxicity tests, mortality of tadpoles occurred within the first 24 h of exposure. LC_{50} values at 24 h ranged from 0.1 to 0.26 mg/L, and analysis of variance on LC_{50} values of TFS tadpoles showed significant variations among species (Table 1).

Table 1: Summary of median lethal concentrations (LC_{50}), lowest-observed-effect concentrations (LOEC), and no-observed-effect concentrations (NOEC) (mg/L) of TFS on anuran tadpoles after 24-h exposure.

Species	LC_{50}	NOEC	LOEC
Rhinella arenarum	0.22 (0.19–0.25)[ac]	0.096	0.125
Physalaemus santafecinus	0.14 (0.12–0.16)[ab]	0.096	0.125
Elachistocleis bicolor	0.10 (0.09–0.11)[b]	0.077	0.096
Leptodactylus latrans	0.26 (0.23–0.28)[c]	0.180	0.230

Toxicity endpoints were calculated based on nominal concentrations. Values in parenthesis correspond to the 95% confidence interval of each estimate. Different letters (a, b, c) indicate significant differences in LC_{50} among species (Kruskall–Wallis ANOVA with post-hoc Dunnett's test; $p < 0.05$).

Exposure phase

No mortality occurred in tadpoles or eels during 6-h exposure to LOEC of TFS. No signs of reduced swimming performance or altered behavior were observed in tadpoles or eels after 6-h exposure.

At each of these times, predation rates were highest in the control treatment (E-, T-) and lowest in the treatment in which tadpoles and eels were simultaneously exposed to TFS (E+, T+). Fig. 1 shows the effect, pooled on all species, of sublethal TFS exposure on predation rates.

Non-exposed tadpoles (T-) of all species were captured at a higher rate than exposed ones (T+) at 1, 6 and 18 h, whereas at 24 h no differences in predation rates were found between T+ and T-. Similarly, the same trend was observed for eels exposed (E+) and not exposed (E-), where E- consumed more tadpoles of all species than E+ at 1, 6 and 18 h, whereas at 24 h no differences in predation rates were found between E+ and E-.

RESULTS SUMMARY

In the acute toxicity test significant variations among species occurred. LC_{50} values at 24 h ranged from 0.1 to 0.26 mg/L. No mortality occurred during the exposure phase of 6 h. At each of these times, predation rates were highest in the control treatment (E-, T-) and lowest in the treatment in which tadpoles and eels were simultaneously exposed to TFS (E+, T+). Non-exposed tadpoles of all species were captured at a higher rate than exposed ones. Similarly, the same trend was observed for eels exposed and not exposed, where not exposed eels consumed more tadpoles of all species than exposed eels.

Comment by the Notifier

The publication is well documented study without analytics and is not used for risk assessment. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

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

The following study, which was evaluated during the first EU review (SANCO/4339/2000-Final) is considered, amongst other studies, in the risk assessment:

Table 8.3.1- 1: Acute toxicity to honey bees exposed to trifloxystrobin

Test substance	Test species	Endpoint	Reference
Trifloxystrobin	Honey bee, acute *Apis melifera*	oral 48 h LD_{50} > 200 µg a.s./bee contact 48 h LD_{50} > 200 µg a.s./bee	[redacted] (1995) 95 10 48 023 M-032668-01-1 KCA 8.3.1.1.1/01

Additional studies on bees have been performed and are submitted within this Supplemental Dossier for renewal of approval of trifloxystrobin. A further laboratory study on acute oral and contact toxicity to honey bees has been performed with technical trifloxystrobin according to current guidelines and requirements.

In addition, a chronic 10 day adult feeding limit test was conducted with Trifloxystrobin WG 50. Moreover, in order to investigate the intrinsic properties of trifloxystrobin on immature honey bee live stages, a honey bee brood feeding study has been performed with Trifloxystrobin WG 50.

The respective study summaries are presented below.

Table 8.3.1- 2: Additional studies on honey bees exposed to trifloxystrobin

Test substance	Test species	Endpoint	Reference
Trifloxystrobin, tech.	Honey bee, acute *Apis melifera*	oral 48 h LC_{50} > 110 µg a.s./bee contact 48 h LC_{50} > 100 µg a.s./bee	[redacted] (2012) 67571035 M-431911-01-1 KCA 8.3.1.1.1/04
Trifloxystrobin WG 50	Honey bee,10 d chronic adult feeding study *Apis melifera*	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted] (2013) S13-00149 M-468755-01-1 KCA 8.3.1.2/01
Trifloxystrobin WG 50	Honey bee brood feeding (Oomen *et al.*, 1992) *Apis melifera*	No adverse effects on bee colonies or bee brood development	[redacted] (2012) 64821031 M-438966-01-1 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: **KCA 8.3.1.1.1/04; [redacted] (2012)**

Title:	Effects of trifloxystrobin tech. (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory
Report No:	67571035
Document No:	M-431911-01-1
Guidelines:	OECD Guideline 213 and 214 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objective:

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

Materials and Methods:

Test item: Trifloxystrobin tech. (Origin Batch No.: EDEL006101, Customer Order No.: TOX 09277-00, Specification No.: 102000007792, LIMS No.: 1037837; Article No.: 05579724, Purity: 99.1% w/w analytical)
Test organism: Honey bee (*Apis mellifera* L.), female worker bees, obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.
Under laboratory conditions, *Apis mellifera* (50 worker bees per 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. per bee by topical application (contact limit test) and to a single dose of 110.0 µg a.s. per bee by feeding (oral limit test; value based on the actual intake of the test item).

Oral toxicity study

Appropriate amounts of trifloxystrobin tech. dilutions in acetone were mixed with syrup (ready-to-use syrup, sugar component: 50% sucrose, 31% glucose, 19% fructose) in order to achieve the required test concentrations in a final dilution of 50% syrup solution (50% syrup, 40% water and 5% acetone (w/w)). For the solvent control and the reference item, a final dilution of 50 % syrup solution (45% water, 50% syrup and 5% acetone or water (w/w)) was used whereas the water control consisted of a 50 % aqueous syrup solution (50 % water and 50 % syrup (w/w)).
The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 2 hours 5 minutes for the test item treatments). After a maximum of 2 hours 5 minutes, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food.
The mean target dose levels (e.g. 100 µg a.s./bee nominal) would have been obtained if 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.
The test was conducted in darkness, temperature was 25°C and humidity between 59 and 86%.

Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing. Results are based on measured concentrations of the a.s. per bee.

Contact toxicity study

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

For the control, one 5 µL droplet of tap water containing 0.5% Adhäsit[1] and pure acetone, respectively, was used. The reference item was also applied in 5 µL tap water (dimethoate made up in acetone).

A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.

The test was conducted in darkness, temperature was 25°C and humidity between 59 and 86%. Biological observations, including mortality and behavioural changes were recorded at 4, 24 and 48 hours after application. Results are based on nominal concentrations of the product per bee.

Results:

The results can be considered as valid, as all validity criteria of the test were met: control mortality is < 10% in the oral and in the contact test, LD_{50} (24 h) of the toxic standard in the oral test equals 0.11 µg a.s./bee, the LD_{50} (24 h) of the toxic standard in the contact test equals 0.17 µg/bee.

A summary of effects of the test item on mortality and behavioural abnormalities of the bees is given below for both tests:

Table: 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	2.0	0.0	2.0	0.0	2.0	0.0
solvent	0.0	0.0	0.0	0.0	0.0	0.0
reference item						
0.30	10.0	26.0	98.0	2.0	100.0	0.0
0.20	4.0	16.0	70.0	12.0	88.0	0.0
0.15	0.0	0.0	20.0	2.0	36.0	0.0
0.10	0.0	0.0	8.0	0.0	12.0	0.0

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

[1] The Adhäsit was used to improve the adhesion of the droplet on the bee body. Adhäsit is non-toxic to honey bees.

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

consumed 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 110.0	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						
0.21	44.0	24.0	98.0	2.0	100.0	0.0
0.14	8.0	26.0	82.0	2.0	86.0	0.0
0.08	0.0	14.0	6.0	0.0	6.0	0.0
0.06	0.0	0.0	2.0	0.0	4.0	0.0

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

Observations:

Contact toxicity test:

At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg a.s./bee. In the water control group 2% mortality, and in the solvent control group no mortality occured, respectively. No induced behavioural effects were observed at any time.

Oral toxicity test:

In the oral toxicity test, the maximum nominal test level of trifloxystrobin tech. (i.e. 100 µg a.s./bee) corresponded to an actual intake of 110.0 µg a.s./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, respectively.

Conclusion:

Toxicity to Honey Bees; laboratory tests

Test Item	Trifloxystrobin tech.	
Test object	*Apis mellifera*	
Application rate (µg a.s./bee)	100.0	110.0
Exposure	contact (solution in acetone)	oral (sugar/acetone solution)
LD_{50} µg product/bee	> 100.0	> 110.0

The toxicity of trifloxystrobin tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees.
The LD_{50} (48 h) value was > 100.0 µg a.s./bee in the contact toxicity test.
The LD_{50} (48 h) value was > 110.0 µg a.s./bee in the oral toxicity test.

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 Trifloxystrobin WG 50 as technical trifloxystrobin is only very slightly soluble in water.

Report:	**KCA 8.3.1.2/01; [redacted] (2013)**
Title:	Trifloxystrobin WG 50 W - Assessment of Chronic Effects to the Honeybee, *Apis mellifera* L., in a 10 Days Continuous Laboratory Feeding Limit Test
Report No:	S13-00149
Document No:	M-468755-01-1
Guidelines:	No agreed and ring tested guideline available
Deviations:	Not applicable
GLP:	Yes (certified laboratory)

Objective:
To investigate the potential chronic effects of trifloxystrobin on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory and to investigate whether the LC_{50}-/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 Trifloxystrobin WG 50 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 Trifloxystrobin WG 50 W was not statistically significantly different when compared to the control group. The cumulative control mortality was 0.0 %, as determined at the final evaluation after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg Trifloxystrobin WG 50 W was 1.0 % at the final assessment. At 120 mg a.s./kg Trifloxystrobin WG 50 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 Trifloxystrobin WG 50 W at the treatment level of 120 mg a.s./kg was 49.44 µg a.s./bee, the corresponding average daily dose was therefore 4.9 µg a.s./bee.

The overall mean daily consumption of the application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different (lower) when compared to the untreated control group (41.2 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 Trifloxystrobin WG 50 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:	**KCA 8.3.1.3/01; [redacted] S., 2012**
Title:	Study on the Effects of Trifloxystrobin WG 50 W on Honey Bee Brood (*Apis mellifera* L.) - Brood feeding test -
Report No:	64821031
Document No:	M-438966-01-1
Guidelines:	Oomen *et al.* (1992)
Deviations:	None
GLP:	Yes (certified laboratory)

Objective:

The purpose of this study was to investigate the effect of the test item Trifloxystrobin WG 50 W to honey bee brood when exposed by oral ingestion.

Materials and Methods:

Trifloxystrobin WG 50 W: trifloxystrobin (CGA 279202): 49.8 % w/w (analytical); Batch ID.: EDFL011509; Sample Description: TOX 09344-00; Material No.: 05584493; Specification No.: 102000007798 – 02.

Trifloxystrobin WG 50 W mixed in 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 0.151 g Trifloxystrobin WG 50 W (= 0.75 g trifloxystrobin) in 1 L sugar syrup. Also bee brood development (eggs, young and old larvae) was recorded at test initiation and after 6, 10, 16 and 22 days. As control pure sugar syrup (30% sucrose, 31% glucose, 39% fructose) was used. 3.0 g/L syrup Insegar (25% fenoxycarb, 0.75 g fenoxycarb/L) was used as reference substance.

Bee colonies were free flying in natural field conditions, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops of flowering weeds in the surrounding area.

Dates of experimental work: July 01, 2011 to July 27, 2011

Results:

Effect of Trifloxystrobin WG 50 W on honey bees (*Apis mellifera*) in a bee brood study

Test item Test object Exposure		Trifloxystrobin WG 50 W Honey bees (*Apis mellifera L.*), complete colonies *via* treated sugar solution		
		Control	Test item	Reference item
Termination rate [%]	eggs	15.6	40.4 n.s.	99.8*
	young larvae	32.4	24.9 n.s.	99.9*
	old larvae	7.9	3.6 n.s.	74.8*
Mean brood termination rate over all stages [%] [1]		18.6	23.0 n.s.	91.5*
Mean mortality of worker bees/colony/day during [2]	pre-application phase	2.6	2.7 n.s.	3.0 n.s.
	during entire post application phase	14.2	6.9 n.s.	22.1 n.s.
Mean mortality of worker pupae/colony/day during [3]	pre-application phase	0.0	0.0 n.s.	0.0 n.s.
	during entire post application phase	0.5	0.6 n.s.	1.0 n.s.

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 per day and colony found in dead bee traps
Statistics: n.s. = not statistically significant compared to the control;* = statistically significant compared to the control; n.d. = not determined; Student t-test or Mann-Whitney U-test, $\alpha = 0.05$, 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 Trifloxystrobin WG 50 W 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 studies already evaluated during the first EU review of this compound, please refer to corresponding section in the Monograph and in the Baseline Dossier provided by Bayer CropScience.
Studies on non-target arthropods have been performed with the representative formulation Trifloxystrobin WG 50 and additional formulations needed for the risk assessment. A list of these studies is presented in MCP; Annex point 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Please refer to point 8.3.2.

CA 8.3.2.2 Effects on Typhlodromus pyri

In the first Annex I listing process non-target arthropod data for two formulations of trifloxystrobin have been submitted and evaluated. The formulation FFS EC 125 (Twist) is no longer supported, but the new available non-target arthropod data for this formulation are provided as supportive information in this Supplemental Dossier.

Table 8.3.2.2- 1: Additional studies on Non-target arthropods for trifloxystrobin

Test species, Dossier-file-No., reference	Tested Formulation, study type, exposure		Ecotoxicological Endpoint	
Trifloxystrobin EC 125				
Typhlodromus pyri M-078388-01-1 Rep.No: B105TPE [redacted], 2003 KCA 8.3.2.2/06	TFS EC 125 Extended lab., exposure on detached cowpea leaves		Corr. Mortality [%]	Effect on Reproduction [%]
	4.7	g a.s./ha	1	15
	22.4	g a.s./ha	2	-13 [A]
	106	g a.s./ha	35	19
	250	g a.s./ha	15	10
	500	g a.s./ha	36	27

A: A negative value indicates a higher reproduction rate in the treatment than in the control.

Report: **KCA 8.3.2.2/06; [redacted], 2003**

Title: An extended laboratory dose-response study to evaluate the effects of Trifloxystrobin EC 125 on survival and reproduction of the predaceous mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) on cowpea leaves

Report No: B105TPE

Document No: M-078388-01-1

Guidelines: [redacted] et al. (2000)
Candolfi et al. (2001)

Deviations: None

GLP: Yes (certified laboratory)

Objective:

This extended laboratory study is designed to evaluate the effects of Trifloxystrobin EC 125, applied to the underside of detached cowpea leaves, on survival and reproduction of the predaceous mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae).

Materials and Methods:

Test item: Trifloxystrobin EC 125 (active ingredient CGA 279202, purity/content: 126.26 g/l, Sample no.: TOX06005-01, Art. no: 00-05584566, Batch no.: P002003) was tested.

The test item was applied to the underside of cowpea leaves at rates of 4.7, 22.4, 106, 250 and 500 g a.s./ha and the effects were compared to a water treated control. A toxic reference (a.s.: dimethoate) applied at 1920 mg test item/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Typhlodromus pyri Scheuten was exposed in groups of 10 per unit to dry residues within 1.5 hours after application. There were 10 units for the water control, 6 units for each Trifloxystrobin EC 125 treatment and 6 units for the toxic reference.

Mortality was assessed after a 7-day exposure period. The toxic reference treatment was stopped after mortality assessments.

All surviving individuals of the deionised water control group and all Trifloxystrobin EC 125 rates were transferred to untreated open glass arenas, because corrected mortality in these rates was <50%. Reproduction for these treatments was determined during 7-days in total (3 consecutive assessments at 2-3 day intervals).

Dates of experimental work: November 13 to November 27, 2002

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality in water control	≤ 20%	13%
Corrected mortality reference item	> 50%	81%
Mean reproduction in water control	≥ 4	6.4

All validity criteria for the study were met

Mortality and reproduction of predatory mites after exposure to Trifloxystrobin EC 125

Test item	**Trifloxystrobin EC 125**			
Test organism	***Typhlodromus pyri***			
Exposure	**7 days on the underside of cowpea leaves in glass/plexiglass mortality units**			
Nominal application volume	**200 L/ha**			
	Mortality after 7 days [%]		**Reproduction [eggs/female/7 days]**	
control	**13**		**6.4**	
Treatment [g a.s./ha]	**Corrected mortality after 7 days [%]**		**Reproduction in eggs/female/7 days (reduction relative to control in%)**	
4.7	2	P = 0.793	5.4 (15%)	P = 0.102
22.4	2	P = 0.805	7.3 (-13%)	P = 0.358
106	35	P = <0.001*	5.2 (19%)	P = 0.159
250	15	P = 0.064	5.8 (10%)	P = 0.545
500	36	P = <0.001*	4.7 (27%)	P = 0.047 *
Reference item (1920 mg dimethoate/ha)	81	P = <0.001*	Not assessed	
LR_{50}: > 500 g a.s./ha **ER_{50}: > 500 g a.s./ha**				

* Statistically significantly different from deionised water control. Statistical analysis: mortality data with Fisher's Exact Test and reproduction data with ANOVA/Fisher's Least Significant Difference Test

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

After 7 days of exposure to Trifloxystrobin EC 125 at rates equivalent to 106 and 500 g a.s./ha, survival of *Typhlodromus pyri* was statistically significantly reduced compared to the water control. Exposure to a rate equivalent to the 4.7, 22.4 and 250 g a.s./ha had no significant effect on survival.

Reproduction of *T. pyri* on untreated glass plates of Trifloxystrobin EC 125 at a rate equivalent to 500 g a.s./ha was statistically significantly reduced (27%) compared to reproduction in the water

control. Exposure to rates equivalent to 4.7, 22.4, 106 and 250 g a.s./ha had no significant effect on reproduction.

Conclusion:
The LR_{50} and ER_{50} were estimated to be > 500 g a.s./ha.

CA 8.4 Effects on non-target soil meso- and macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

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

In order to address new data requirements according to Regulation (EC) No 1107/2009, several additional studies on chronic exposure to earthworm have been performed and are submitted within the Baseline Dossier or this Supplemental Dossier:

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

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Trifloxystrobin (tech.)	*Eisenia fetida* reproduction 56 d, mixed	NOEC **NOEC$_{corr.}$**	7 mg a.s./kg dws **3.5 mg a.s./kg dws** [a]	[redacted] (2009) LRT-Rg-R-56/09 M-350077-01-1 KCA 8.4.1/03
CGA 357261	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) kra-Rg-R-111/11 M-428262-02-1 KCA 8.4.1/04
CGA 321113	*Eisenia fetida* reproduction 56 d, sprayed	NOEC	≥ 750 g/ha ≙ ≥ 2.32 mg/kg dws [b]	[redacted] (1999) 1047066.630 M-033997-01-1 KCA 8.4.1/01
CGA 321113	*Eisenia fetida* reproduction 56 d, mixed	NOEC **NOEC$_{corr}$**	≥100 mg/kg dws **≥50 mg/kg dws** [a]	[redacted] (2013) Kra/Rg-R-149/13 M-464328-01-1 KCA 8.4.1/05
CGA 373466	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2011) LRT-Rg-R-114/11 M-414741-01-1 KCA 8.4.1/06
CGA 381318[c]	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2013) Kra/Rg-R-150/13 M-466037-02-1 KCA 8.4.1/07
NOA 413161	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥91.8 mg/kg dws**	[redacted] (2011) LRT-Rg-R-116/11 M-416856-01-1 KCA 8.4.1/08
NOA 413163	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] & [redacted] (2012) EBTFN011 M-445494-01-1 KCA 8.4.1/09
CGA 357276	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**50 mg/kg dws**	[redacted] (2012) kra-Rg-R-115/12 M-437130-01-1 KCA 8.4.1/10
NOA 409480	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) kra-Rg-R-106/11 M-424075-01-1 KCA 8.4.1/11

dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected
Bold values: endpoints used for risk assessment
[a] adjusted by a factor of 2 to address the log $P_{ow} > 2$
[b] study was repeated with higher (100 mg/kg) concentration (see M-464328-01-1, KCA 8.4.1/05). The new value is used in the risk assessment.
[c] Test substance: CGA 381318, sodium salt

Report:	**KCA 8.4.1/03; [redacted], 2009**
Title:	Trifloxystrobin (technical): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5% peat
Report No:	EBTFL006
Document No:	M-350077-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of trifloxystrobin technical on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil with 5 different test concentrations.

Materials and Methods:

Test material: Trifloxystrobin (technical), Specification No.: 102000007792; Article No.: 05579724; Batch Code: AE C642802-01-03; Origin Batch No.: TR605092; Certificate-No.: AZ 15656; content of a.s. (analysed): 99.5% (w/w).

Adult earthworms (*Eisenia fetida*, about 6 months old, 8 × 10 animals for the control group and 4 × 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 4, 7, 12, 20 and 34 mg test item/kg soil dry weight.

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

Artificial soil composition was 73.82% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.18% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

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

Dates of experimental work: January 23 to March 26, 2009

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	280
Coefficient of variation of reproduction	≤ 30%	14.3%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25, 2.5 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from January to April 2008. No mortality of the adult earthworms was observed 28 days after application. No statistically significant different values for the biomass relative to the control were observed at the lowest test concentration of 1.25 mg a.s./kg dry weight artificial soil.
The change of body weight of the adult earthworms of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (results of a Dunnett´s multiple t-test, two sided, $\alpha = 0.05$).
The number of juveniles per test vessel of the test concentrations of 1.25, 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced to the control (results of a Williams multiple sequentlial t-test, one-sided smaller, $\alpha = 0.05$.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item	trifloxystrobin technical		
Test object	*Eisenia fetida*		
Exposure	Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>34	>34	>7
NOEC	≥34	≥34	≥12

trifloxystrobin technical [mg a.s. /kg dws]						
	Control	**4**	**7**	**12**	**20**	**34**
	Mortality of adult worms after 4 weeks					
Mortality [%]	0	2.5	0	0	0	0
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)					
Mean ± SD [%]	+16.8 ± 4.9	+22.4 ± 3.3	+23.9 ± 2.2	+22.4 ± 10.1	+21.3 ± 7.2	+21.7 ± 2.7
	Number of juveniles per surviving adult worm after 8 weeks					
Mean± SD	28.0 ± 4.0	26.3 ± 3.6	31.1 ± 1.8	24.3 ± 1.8	24.4 ± 2.5	24.2 ± 0.4
	Number of juveniles per replicate after 8 weeks					
Mean± SD	280.0±40.1	258.0± 46.2	311.0±18.1	242.8±18.4	244.3±25.3	241.8 ± 3.9
	Reproduction compared to control [%]					
% to control *	-	92.1 n.s.	111.1 n.s.	86.7 s.	87.2 s.	86.3 s.

* Statistical comparison of mean reproduction per test vessel:
Result of a Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$
n.s.: mean value not statistically significant different compared to the control ($p \geq 0.05$)
s.: mean value statistically significantly different compared to the control ($p < 0.05$)

No mortality of adult earthworms was observed after 28 days of exposure at the control group and at any test item concentration (just one worm died in one test vessel of the lowest concentration).
No statistically significant different values for the growth relative to the control were observed at all tested concentrations of 4, 7, 12, 20 and 34 mg test item/kg dry weight artificial soil.
Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 12, 20 and 34 mg test item/kg dry weight artificial soil.

Conclusions:

Overall, it is concluded, that the NOEC for this study is 7 mg trifloxystrobin/kg dry weight artificial soil. The overall LOEC is determined to be 12 mg trifloxystrobin/kg dry weight artificial soil.

NOEC related to reproduction: 7 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 12 mg test item/kg dry weight artificial soil

Metabolite CGA 357261

Report: **KCA 8.4.1/04; [redacted], M.-A., 2012**
Title: Trifloxistrobin-CGA357261 (AE 1393224): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Report No: kra-Rg-R-111/11
Document No: M-428262-02-1
Guidelines: OECD-Guideline No. 222 (2004)
ISO 11268-2 (1998)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of CGA357261 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil with 5 different test concentrations.

Materials and Methods:

Test material: Trifloxistrobin-CGA357261 (Batch code: AE 1393224-PU-01; Material: AE 1393224, pure substance; Origin Batch No.: SES-10350-10-1; purity 99.4% w/w).
Adult earthworms (*Eisenia fetida*, about 5 months old, 8 × 10 animals for the control group and 4 × 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 10, 17, 31, 56 and100 mg test item/kg soil dry weight.
Toxic standard: 1.25, 2.5, 5.0 mg Carbendazim (360 g a.s./L)/ kg dry weight soil.; control: quartz sand.
Artificial soil composition was 73.82% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.18% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to

8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.
The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Dates of experimental work: June 22 to August 29, 2011

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	190.8 (151 – 235)
Coefficient of variation of reproduction	≤ 30%	13.3%

All validity criteria for the study were met.

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from January 31, 2011 to April 05, 2011. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel of all test concentrations were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 1.66 mg a.s./kg dry weight with 95% confidence limits between 1.62 – 1.69 mg a.s./kg dry weight artifical soil.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	CGA357261 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

CGA357261 [mg test item /kg dws]						
	Control	**10**	**17**	**31**	**56**	**100**
	Mortality of adult worms after 4 weeks					
Mortality [%]	0	0	0	0	0	0
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)					
Mean ± SD [%]	+87.6 ± 10.48	+77.08 ± 7.12	+74.66 ± 7.31	+85.31 ± 4.32	+79.82 ± 7.58	+79.04 ± 5.76
	Number of juveniles per surviving adult worm after 8 weeks					
Mean± SD	19.1 ± 2.5	23.2 ± 7.8	18.8 ± 4.0	18.7 ± 1.3	18.5 ± 3.5	19.5 ± 2.8
	Number of juveniles per replicate after 8 weeks					
Mean± SD	190.8±25.4	231.8± 77.7	187.5±40.1	187.0±13.4	184.5±35.1	193.8 ± 28.0
	Reproduction compared to control [%]					
% to control	-	121.5	98.3	98.0	96.7	101.6

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Williams Multiple Sequential t-test, $p > 0.05$, one-sided smaller).

No statistically significant different value for the growth relative to the control was observed at the tested concentrations of 10, 17, 31, 56 and 100 mg test item/kg dry weight artificial soil.

No mortality of adult earthworms was observed after 28 days of exposure at the control group and at any test item concentration including the highest test concentration of 100 mg test item/kg dry weight artificial soil in this study.

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 100 mg test item/kg dry weight artificial soil.

Conclusions:

Overall, it is concluded, that the NOEC for this study is greater than or equal 100 mg Trifloxystrobin – CGA357261/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg Trifloxystrobin – CGA357261/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

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Metabolite CGA 321113

Report:	**KCA 8.4.1/05; [redacted], M.-A., 2013**
Title:	Trifloxystrobin-CGA 321113 (BCS-AL58660): Effects on survival, growth and reproduction of the earthworm *Eisenia fetida* tested in artificial soil
Report No:	kra/Rg-R-149/13
Document No:	M-464328-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of CGA 321113 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentrations.

Materials and Methods:

Test item: Trifloxystrobin-CGA 321113 (BCS-AL58660); (Customer Order No: Tox-No: 09586-00; Batch code: AE 1344138-01-02; Origin Batch No.: BCOQ 6132-3-9; Material: AE 1344138, technical; purity 98.4%w/w).

Adult earthworms (*Eisenia fetida*, about 11 months old, 8 × 10 animals for the control group and treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentration of 100 mg test item/kg soil dry weight.

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

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

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

Dates of experimental work: March 13 to May 21, 2013

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	232, 249, 246, 312, 293, 282, 252, 305
Coefficient of variation of reproduction	≤ 30%	11.2%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25, 2.5 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from September 21, 2012 to November 28, 2012 (Study No.: Rg-R-Ref 19/12; Report No. kra-Rg-R-Ref 19/12; NON-GLP). No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel in the two highest test concentrations of 2.5 and 5.0 mg a.s./kg dry weight artificial soil were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 3.54 mg a.s./kg dry weight. Confidence limits (95%) could not be calculated.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	CGA 321113 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

CGA 321113 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	0
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean ± SD [%] [a]	36.68 ± 11.68	42.48 ± 6.36
	Number of juveniles per replicate after 8 weeks	
Mean± SD [b]	271.4 ± 30.3	297.4 ± 57.8
	Reproduction compared to control [%]	
% to control		109.6

[a] No statistical significance compared to the control (Student-t-test, $p > 0.05$, two-sided)
[b] No statistical significance compared to the control (Student-t-test, $p > 0.05$, one-sided smaller)

No statistically significant different value for the growth relative to the control was observed at the tested concentration of 100 mg test item/kg dry weight artificial soil.
No mortality of adult earthworms was observed after 28 days of exposure at the control group and at the treatment group.

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 100 mg test item/kg dry weight artificial soil.

Conclusions:

Overall, it is concluded, that the NOEC for this study is greater than or equal 100 mg Trifloxystrobin – CGA 321113/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg Trifloxystrobin – CGA 321113/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

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Metabolite CGA 373466

Report:	**KCA 8.4.1/06; [redacted], Th., 2011**
Title:	Trifloxystrobin – CGA373466: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5% peat LIMIT - Test
Report No:	LRT-Rg-R-11411
Document No:	M-414741-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of CGA373466 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil at one test concentration (Limit test).

Materials and Methods:

Test material: Trifloxystrobin – CGA373466: (Origin Batch No.: M18457; Batch Code: AE 1344148 00 1B960001; Material No. AE 1344148; content of a.s. (analysed): 96.3% w/w).
Adult earthworms (*Eisenia fetida*, about 5 months old, 8 × 10 animals for the control group and 8 × 10 animals for the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentration of 100 mg test item/kg soil dry weight.
Toxic standard: 1.25, 2.5, 5.0 mg Carbendazim (360 g a.s./L)/ kg dry weight soil.; control: quartz sand.
Artificial soil composition was 73.83% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.17% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

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

Dates of experimental work: March 21 to May 20, 2011

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	241 (177 – 296)
Coefficient of variation of reproduction	≤ 30%	15.2%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from January 31, 2011 to April 05, 2011. No mortality of the adult earthworms was observed 28 days after application.
The change of body weight of the adult earthworms of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel of all test concentrations were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 1.66 mg a.s./kg dry weight with 95% confidence limits between 1.62 – 1.69 mg a.s./kg dry weight artifical soil.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	CGA373466 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

CGA373466 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	0
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean ± SD [%]	+44.6 ± 4.5	+41.1 ± 7.1
	Number of juveniles per surviving adult worm after 8 weeks	
Mean± SD	24.1 ± 3.7	24.9 ± 3.0
	Number of juveniles per replicate after 8 weeks	
Mean± SD	240.6 ± 36.6	248.6 ± 29.8
	Reproduction compared to control [%]	
% to control	-	103.3

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Student-t-test, $p > 0.05$, one-sided smaller)

During the first 28 days of exposure, no reduced food consumption of the adults could be observed.
No mortality of adult earthworms was observed after 28 days of exposure at the control group and at the test concentration 100 mg test item/kg dry weight artificial soil.
No statistically significant different value for the growth relative to the control was observed at the tested concentration 100 mg test item/kg dry weight artificial soil.
No statistically significant effect on the number of juveniles compared to the control group was recorded at 100 mg test item/kg soil d.w.

Conclusions:
Overall, it is concluded, that the NOEC for this study is greater than or equal 100 mg Trifloxystrobin – CGA373466/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg Trifloxystrobin – CGA373466/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

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Metabolite CGA 381318

Report:	**KCA 8.4.1/07; [redacted], M.A., 2013**
Title:	Trifloxystrobin – CGA 381318 (BCS-CU98569): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Report No:	kra/Rg-R-150/13
Document No:	M-466037-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of CGA 381318 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil at one test concentration (Limit test).

Materials and Methods:

Test material: Trifloxystrobin – CGA 381318, sodium salt (BCS-CU98569, chemical name: sodium (Z)-(methoxyimino)-(2-{1-(Z)-[3-(trifluoromethyl)phenyl]ethylideneaminooxy-methyl}phenyl)acetate), (Batch code: BCS-CU98569-PU01; Origin Batch No.: SES 11821-2-2; Material: BCS-CU98569, pure substance; purity 94.0%w/w).

Adult earthworms (*Eisenia fetida*, about 11 months old, 8 × 10 animals for the control group and treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentration of 100 mg test item/kg soil dry weight.

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

Artificial soil composition was 69% quartz sand, 20% kaolin clay, 10% sphagnum peat and 1% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

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

Dates of experimental work: March 13 to May 21, 2013

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	232, 249, 246, 312, 293, 282, 252, 305
Coefficient of variation of reproduction	≤ 30%	11.2%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25, 2.5 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from September 21 to November 28, 2012. No mortality of the adult earthworms was observed 28 days after application.
The change of body weight of the adult earthworms of the test concentrations of 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel of the two highest test concentrations of 2.5 and 5.0 mg a.s./kg dry weight artificial soil were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 3.54 mg a.s./kg dry weight. The 95% confidence limits could not be determined.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item	CGA 381318; sodium salt		
Test object	*Eisenia fetida*		
Exposure	Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

CGA 381318, sodium salt [mg test item /kg dws]		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	0
	Biomass change * (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean ± SD [%]	+36.68 ± 11.68	+46.32 ± 3.52
	Number of juveniles per replicate after 8 weeks **	
Mean± SD	371.4 ± 30.3	294.1 ± 31.9
	Reproduction compared to control [%]	
% to control		108.4

* no statistical significance compared to the control (Student t-test, two-sided, $\alpha = 0.05$)

** no statistical significance compared to the control (Student t-test, one-sided smaller, $\alpha = 0.05$)

After 28 days of exposure no worms died in the control group and no mortality was observed in the treatment group.
Statistically significant different values for the growth relative to the control were not observed.
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 100 mg pure metabolite/kg dry weight artificial soil.

Conclusions:
Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is ≥ 100 mg pure metabolite/kg dry weight artificial soil. Thus, the overall LOEC is determined to be >100 mg pure metabolite/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

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Metabolite NOA 413161

Report:	**KCA 8.4.1/08; [redacted], Th., 2011**
Title:	Trifloxystrobin – NOA 413161: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5% peat LIMIT - Test
Report No:	LRT-Rg-R-116/11
Document No:	M-416856-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of NOA 413161 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil at one test concentration (Limit test).

Materials and Methods:

Test material: Trifloxystrobin – NOA 413161; (Origin Batch No.: M19118; Batch Code: AE 1344143 00 1C92 0001; Material No. AE 1344143; content of a.s. (analysed): 91.8% w/w).

Adult earthworms (*Eisenia fetida*, about 5 months old, 8 × 10 animals for the control group and 8 × 10 animals for the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentration of 100 mg test item/kg soil dry weight.

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

Artificial soil composition was 73.82% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.18% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

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

Dates of experimental work: March 21 to May 20, 2011

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	241 (177 – 296)
Coefficient of variation of reproduction	≤ 30%	15.2%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from January 31, 2011 to April 05, 2011. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel of all test concentrations were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 1.66 mg a.s./kg dry weight with 95% confidence limits between 1.62 - 1.69 mg a.s./kg dry weight artifical soil.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	NOA 413161 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

NOA 413161 **[mg test item /kg dws]**		
Control		**100**
Mortality of adult worms after 4 weeks		
Mortality [%]	0	0
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)		
Mean ± SD [%]	+44.6 ± 4.5	+43.5 ± 5.5
Number of juveniles per surviving adult worm after 8 weeks		
Mean± SD	24.1 ± 3.7	21.9 ± 3.1
Number of juveniles per replicate after 8 weeks		
Mean± SD	240.6 ± 36.6	219.4 ± 30.9
Reproduction compared to control [%]		
% to control	-	91.2

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Student-t-test, $p > 0.05$, one-sided smaller)

During the first 28 days of exposure, no reduced food consumption of the adults could be observed.

No statistically significant different value for the growth relative to the control was observed at the tested concentration 100 mg test item/kg dry weight artificial soil.
No mortality of adult earthworms was observed after 28 days of exposure at the control group and at the test concentration 100 mg test item/kg dry weight artificial soil.
No statistically significant effect on the number of juveniles compared to the control group was recorded at 100 mg test item/kg soil d.w.

Conclusions:
Overall, it is concluded, that the NOEC for this study is greater than or equal 100 mg Trifloxystrobin – NOA 413161/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg Trifloxystrobin – NOA 413161/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

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<u>Metabolite NOA 413163</u>

Report:	**KCA 8.4.1/10; [redacted], Th., [redacted], 2012**
Title:	Trifloxystrobin-NOA413163 (BCS-AL58659): Reproduction toxicity to the earthworm *Eisenia fetida* in an artificial soil test
Report No:	EBTFN011
Document No:	M-445494-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:
The purpose of this study was to assess the sublethal effects of Trifloxystrobin-NOA413163 (BCS-AL58659, metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil (limit test).

Materials and Methods:
Test material: Trifloxystrobin-NOA413163 (BCS-AL58659); (Batch code: AE 1344149 00 1B98 0001; Origin Batch No. M18477; Material: AE 1344149; purity: 99.2%).
Adult earthworms (*Eisenia fetida*, more than 2 months old, 8 × 10 animals for the control group and 8 × 10 animals for the treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentration of 100 mg test item/kg soil dry weight.
Toxic standard: 0.32, 1.00 and 3.2 mg Carbendazim / kg dry weight soil; control: quartz sand.
Artificial soil composition was 69.6% quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.4% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour

light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.
The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Dates of experimental work: August 30 to October 25, 2012

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	409.0
Coefficient of variation of reproduction	≤ 30%	9.5%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal (active substance: Carbendazim) is routinely tested at concentrations of 0.32, 1.0 and 3.2 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from June 21 to August 16, 2012.
The $NOEC_{Reproduction}$ was determined as 0.32 mg/kg soil (dw) and accordingly the $LOEC_{Reproduction}$ was determined as 1.0 mg/kg soil (dw). These observed effects are within the range expected from the guideline (1-5 mg Carbendazim/kg soil (dw)) and hence acceptable sensitivity of the test system is assured.

Effects on mortality, growth and reproduction of the earthworms

Test item	NOA413163		
Test object	*Eisenia fetida*		
Exposure	Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

NOA413163 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	3.8
	Biomass change (fresh weight after 4 weeks relative to initial fresh weight)	
Mean [%]	135.9	138.2
	Number of juveniles per replicate after 8 weeks	
Mean± SD	409.0 ± 38.8	385.3 ± 58.5
	Reproduction compared to control [%]	
% to control		94.2

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Student-t-test, $p > 0.05$, one-sided smaller)

After 28 days of exposure no worms died in the control group and 3.8% mortality was observed at the test item concentration.
Statistical analysis showed no significant difference concerning biomass development of individual adults over 28 days (Student-t-test, 2-sided; $p \leq 0.05$) and concerning the number of juveniles after 56 days (Student-t test, 1-sided; $p \leq 0.05$) between the control and the only concentration of the test item tested.

Conclusions:
Overall, it is concluded that the NOEC for this study is greater than or equal 100 mg Trifloxystrobin – NOA413163/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg Trifloxystrobin – NOA413163/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

Metabolite CGA 357276

Report:	**KCA 8.4.1/10; [redacted], M.-A., 2012**
Title:	Trifloxystrobin-CGA357276 (BCS-AB39835): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Report No:	kra-Rg-R-115/12
Document No:	M-437130-01-1
Guidelines:	OECD-Guideline No. 222 (2004) ISO 11268-2 (1998)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the sublethal effects of CGA357276 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the 1st run and 5 different test concentrations in the 2nd run.

Materials and Methods:

Test material: Trifloxystrobin-CGA357276 (BCS-AB39835); (Batch code: BCS-AB39835-PU-01; Origin Batch No: BCOO 6204-3-3; Material: BCS-AB3983, pure substance; purity: 97.8%w/w).

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

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

Artificial soil composition was 73.82% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.18% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

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

Dates of work: 1st run: March 21 to May 20, 2011
2nd run: September 30, 2011 to November 11, 2011

Results:

Validity Criteria	Recommended	Obtained	
		1st run	2nd test
Adult mortality	≤ 10%	0%	1.25%
Number of juveniles per replicate	≥ 30	240.6	244.3
Coefficient of variation of reproduction	≤ 30%	15.2%	12.1%

All validity criteria for the study were met

To verify the sensitivity of the test system, the reference item Derosal flüssig (Carbendazim, 360 g/L) is routinely tested at concentrations of 1.25 and 5.0 mg product/kg soil dry weight.
In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from February 24 to May 02, 2012. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.
The number of juveniles per test vessel of all test concentrations were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 2.19 mg a.s./kg dry weight artificial soil. Confidence limits (95%) could not be calculated.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item	CGA357276		
Test object	*Eisenia fetida*		
Exposure	Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	90	50
NOEC	≥100	100	90

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

CGA357276 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	0
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean ± SD [%]	+44.6 ± 4.5	+36.2 ± 7.7
	Number of juveniles per surviving adult worm after 8 weeks	
Mean± SD	24.1 ± 3.7	20.8 ± 1.9
	Number of juveniles per replicate after 8 weeks	
Mean± SD	240.6±36.6	207.5±19.0
	Reproduction compared to control [%]	
% to control	-	86.2 *

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

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

CGA357276 [mg test item /kg dws]						
	Control	**9**	**16**	**28**	**50**	**90**
	Mortality of adult worms after 4 weeks					
Mortality [%]	1.25	0	0	0	0	0
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)					
Mean ± SD [%]	+65.48 ± 8.39	+65.40 ± 7.39	+58.14 ± 4.80	+72.52 ± 2.57	+65.33 ± 11.1	+63.35 ± 8.21
	Number of juveniles per surviving adult worm after 8 weeks					
Mean± SD	24.8 ± 3.3	23.6 ± 2.9	24.7 ± 1.1	23.8 ± 3.1	22.3 ± 2.3	20.4 ± 1.7
	Number of juveniles per replicate after 8 weeks					
Mean± SD	244.3±29.6	236.3± 28.7	247.0±10.6	237.8±31.0	223.3±23.4	203.8 * ± 16.8
	Reproduction compared to control [%]					
% to control	-	96.7	101.1	97.3	91.4	83.4

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

After 28 days of exposure, no mortality was observed at any test item concentration and at the control group of the 1st run. In the 2nd run just one worm died in the control group.

Statistically significant different values for the growth relative to the control were observed only at the 1st run (100 mg test item/kg dry weight soil). In all tested concentrations of the 2nd run, no statistically significant different values for the growth relative to the control were observed.
In the 1st run, statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 100 mg test item/kg dry wt. artificial soil. No statistically significant different values for the number of juveniles per test vessel relative to the control were observed in the 2nd run at the test concentrations of 9, 16, 28 and 50 mg test item/kg dry weight artificial soil. Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the highest test concentration of 90 mg test item/kg dry weight artificial soil.

Conclusions:
Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 50 mg CGA357276/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 90 mg CGA357276/kg dry weight artificial soil.
Therefore, based on the statistical significance:
NOEC related to reproduction: 50 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 90 mg test item/kg dry weight artificial soil

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Metabolite NOA 409480

Report: **KCA 8.4.1.11; [redacted], M.-A., 2012**
Title: Trifloxystrobin-NOA409480 (BCS-CR74871): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Report No.: kra-Rg-R-10611
Document No: M-424075-01-1
Guidelines: OECD-Guideline No. 222 (2004)
ISO 11268-2 (1998)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:
The purpose of this study was to assess the sublethal effects of NOA409480 (metabolite of trifloxystrobin) on reproduction, mortality and growth of the earthworm *Eisenia fetida* during an exposure in an artificial soil with two different test concentrations.

Materials and Methods:

Test material: Trifloxystrobin-NOA409480; (TOX No. 09206-00; Batch code: BCS-CR74871-01-01; Origin Batch No. BCOO 6263-3-4; Material: BCS-CR74871; purity: 98.7%-area).

Adult earthworms (*Eisenia fetida*, about 5 months old, 8 × 10 animals for the control group and 8 × 10 animals for the treatment group) were exposed in an artificial soil (with 5% peat content) to the nominal test concentration of 100 mg test item/kg soil dry weight.

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

Artificial soil composition was 73.82% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.18% $CaCO_3$. The vessels were kept in a temperature-controlled room at 20 ± 2 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period between approximately 400 – 800 Lux. Earthworms were fed with dried animal manure.

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

Dates of experimental work: May 31 to August 04, 2011

Results:

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0%
Number of juveniles per replicate	≥ 30	194.9 (160 – 245)
Coefficient of variation of reproduction	≤ 30%	14.0%

All validity criteria for the study were met.

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

In the most recent toxic standard study with the reference test item mixed into the artificial soil, was performed from January 31, 2011 to April 05, 2011. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentrations of 2.5 and 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control.

The number of juveniles per test vessel of all test concentrations were statistically significant reduced in comparison to the control. The EC_{50} for reproduction was calculated to be 1.66 mg a.s./kg dry weight with 95% confidence limits between 1.62 – 1.69 mg a.s./kg dry weight artifical soil.

The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality, growth and reproduction of the earthworms

Test item Test object Exposure	NOA409480 *Eisenia fetida* Artificial soil		
	Adult mortality	Biomass change	Reproduction
	[mg test item /kg dws]		
LOEC	>100	>100	>100
NOEC	≥100	≥100	≥100

NOA409480 **[mg test item /kg dws]**		
	Control	**100**
	Mortality of adult worms after 4 weeks	
Mortality [%]	0	1.3
	Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)	
Mean ± SD [%]	+53.9 ± 4.8	+52.2 ± 3.0
	Number of juveniles per surviving adult worm after 8 weeks	
Mean± SD	19.5 ± 2.7	18.6 ± 2.0
	Number of juveniles per replicate after 8 weeks	
Mean± SD	194.9 ± 27.4	183.6 ± 22.0
	Reproduction compared to control [%]	
% to control	-	94.2

No statistically significant differences between the control and test item were calculated for biomass and reproduction (Student-t-test, $p > 0.05$, one-sided smaller)

No statistically significant different value for the growth relative to the control was observed at the tested concentration 100 mg test item/kg dry weight artificial soil.

After 28 days of exposure no worms died in the control group and 1.3% mortality was observed at any test item concentration.

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 100 mg test item/kg dry weight artificial soil.

Conclusions:
Overall, it is concluded, that the NOEC for this study is greater than or equal 100 mg Trifloxystrobin – NOA409480/kg dry weight artificial soil. The overall LOEC is determined to be greater than 100 mg Trifloxystrobin – NOA409480/kg dry weight artificial soil.

Therefore, based on the statistical significance:
NOEC related to reproduction: ≥100 mg test item/kg dry weight artificial soil
LOEC related to reproduction: >100 mg test item/kg dry weight artificial soil

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

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

The following endpoints from studies evaluated during the first EU review (SANCO/4339/2000-Final) are used in the risk assessment:

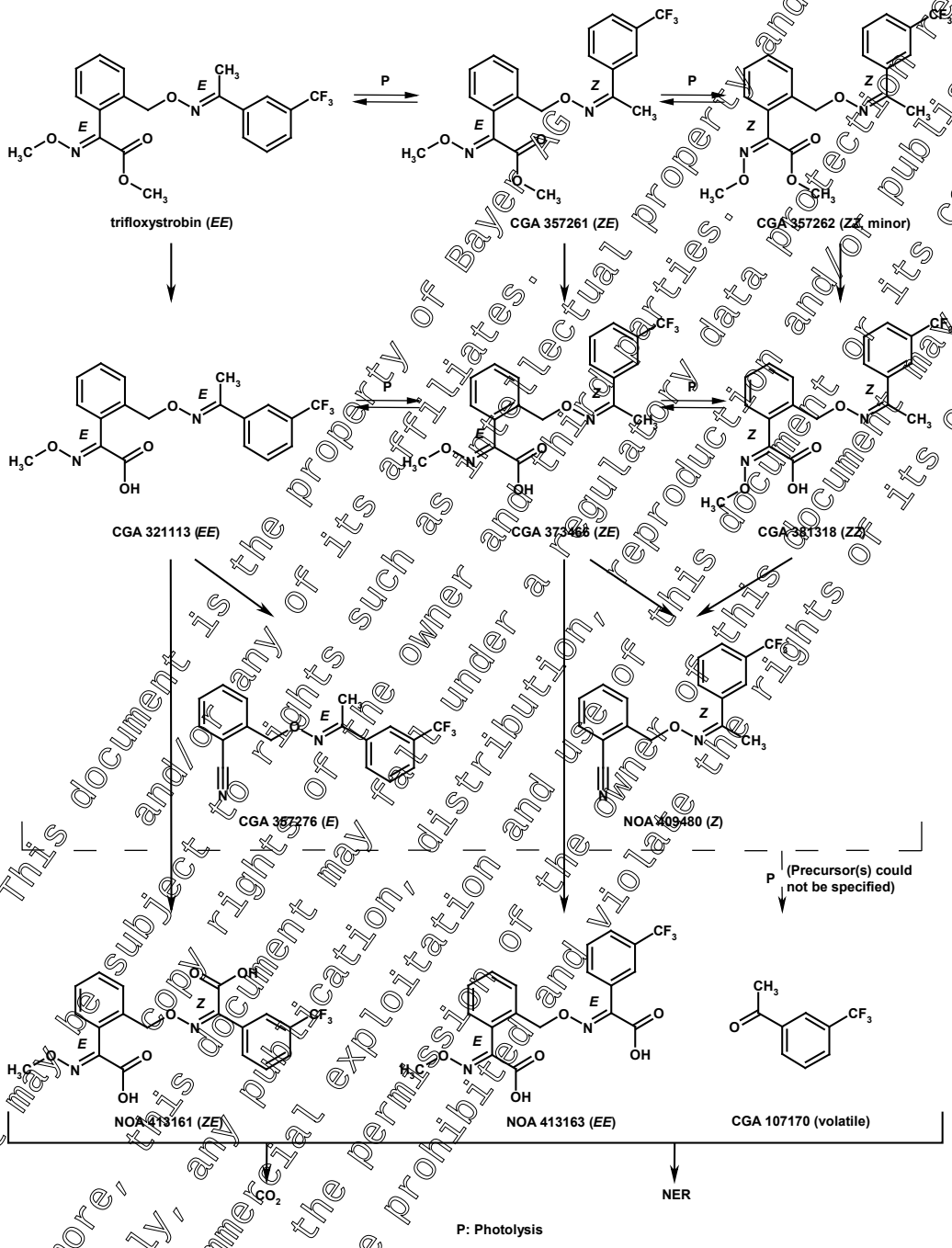
Table 8.4.2- 1: Effects on non-target soil meso- and macrofauna other than earthworms

Test item	Test species, test design	Ecotoxicological endpoint	Reference
CGA 321113	*Folsomia candida* reproduction 28 d, mixed	NOEC 316 mg/kg dws **$NOEC_{corr}$ 158 mg/kg dws**[a]	[redacted] & [redacted] (2002) M-030523-01-1 KCA 8.4.2.1 /01
NOA 413164	*Folsomia candida* reproduction 28 d, mixed	**NOEC 9.18 mg/kg dws**	[redacted] & [redacted] (2002) M-090863-02-1 KCA 8.4.2.1 /02

Bold values: endpoints used for risk assessment
[a] corrected by factor of 2 due to lipophilic substance (log P_{ow} >2);

Studies with springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) were performed with trifloxystrobin WG 50 formulation and most of the major soil degradation products. The corresponding summaries are provided below under point 8.4.2.1. For some of these metabolites studies with Folsomia and Hypoaspis are not considered necessary as justified in the text below.

Figure 8.4.2- 1: Proposed degradation pathway of trifloxystrobin in soil (major degradation products) (For further details see CA 7.1.1)



For the **metabolite CGA 381318 (*ZZ*)** no studies with Folsomia and Hypoaspis are considered necessary, since Folsomia and Hypoaspis studies, which have been performed with the structurally very similar parent compound trifloxystrobin, with the *EE*-isomer (CGA 321113) and the *ZE*-isomer (CGA 373466), did not show any toxicity to either Hypoaspis or Folsomia (all NOEC values > 100 mg metabolite/kg soil). Also the chronic earthworm study did not indicate any toxicity of this metabolite (NOEC value > 100 mg metabolite/kg soil). Furthermore, CGA 381318 has a maximum occurrence in

soil of only 6.2%. Therefore, the risk from the metabolite CGA 381318 to soil macro organisms is considered to be low.

Studies with Folsomia and Hypoaspis have been not conducted with the **metabolite NOA 409480 (*Z-isomer)*,** since the structural similar precursor metabolite CGA 373466 and the *E*-isomer (CGA 357276) of the metabolite NOA 409480 did not indicate to be toxic to these soil macro organisms, and also the chronic earthworm study did not indicate any toxicity of this metabolite (NOEC value > 100 mg metabolite/kg soil). Therefore, the risk from the metabolite NOA 409480 to soil macro organisms is considered to be low.

For **metabolite NOA 413163 (*EE)*** a study has been conducted with Folsomia. Testing Hypoaspis was not considered to be required since the precursor metabolite CGA 373466 did not show any toxicity to either Folsomia or Hypoaspis, and also the *ZE*-isomer (NOA 413161) of NOA 413163 showed no toxicity to Hypoaspis. Furthermore, the maximum occurrence of the metabolite NOA 413163 was only 6.0% and the metabolite showed a low toxicity to both Folsomia and earthworms (NOEC > 100 mg metabolite/kg soil). Therefore it can be concluded that the risk from the metabolite NOA 413163 to soil macro organisms is low.

The following studies are included in this Supplemental Dossier:

Table 8.4.2- 2: Ecotoxicological endpoints – Collembola and soil mites reproduction studies with active substance and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
TFS WG 50	*Folsomia candida* reproduction 28 d, mixed	NOEC **$NOEC_{corr.}$**	≥1000 mg prod./kg dws ≥498 mg a.s./kg dws **≥249 mg a.s./kg dws**[a]	[redacted] (2011) FRM-COLL-121/11 M-415346-01-1 KCA 8.4.2.1/03
CGA 357261	*Folsomia candida* reproduction 28 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) FRM-Coll-150/12 M-443697-01-1 KCA 8.4.2.1/05
CGA 373466	*Folsomia candida* reproduction 28 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) FRM-Coll-146/12 M-440109-01-1 KCA 8.4.2.1/08
NOA 413163	*Folsomia candida* reproduction 28 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] & [redacted] (2013) KBTFN012 M-444419-01-1 KCA 8.4.2.1/11
CGA 357276	*Folsomia candida* reproduction 28 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) FRM-Coll-145/12 M-441251-01-1 KCA 8.4.2.1/12
Soil mites, reproduction				
TFS WG 50	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC **$NOEC_{corr.}$**	≥1000 mg prod./kg dws ≥498 mg a.s./kg dws **≥249 mg a.s./kg dws**[a]	[redacted] (2012) KRA-HR-76/12 M-443226-01-1 KCA 8.4.2.1/04
CGA 357261	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) kra-HR-80/12 M-443311-01-1 KCA 8.4.2.1/06
CGA 321113	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) kra-HR-75/12 M-443145-01-1 KCA 8.4.2.1/07
CGA 373466	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) kra-HR-73/12 M-440955-01-1 KCA 8.4.2.1/09
NOA 413161	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2013) kra-HR-91/13 M-455220-01-1 KCA 8.4.2.1/10
CGA 357276	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2012) kra-HR-74/12 M-440367-01-1 KCA 8.4.2.1/13

dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected
Bold values: endpoints used for risk assessment
[a] corrected by factor of 2 due to lipophilic substance (log P_{ow} > 2)

CA 8.4.2.1 Species level testing

Report:	**KCA 8.4.2.1/03; [redacted], 2011**
Title:	Trifloxystrobin WG 50 W: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Report No:	FRM-Coll-121/11
Document No:	M-415346-01-1
Guidelines:	OECD-Guideline No. 232 (2009)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the effect of Trifloxystrobin WG 50 W on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Test material: Trifloxystrobin WG 50 W (analytical findings: 49.8% w/w Trifloxystrobin (CGA 279202), batch ID: EDFL011509, sample description: TOX 09344-00, specification no.: 102000007798-02, material no.: 05884493.

Ten collembola (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight (d.w.) containing 74.8% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.2% $CaCO_3$, at 20.0 ± 2°C and a photoperiod: light : dark = 16 h : 8 h (400-800 lux) and were fed weekly with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg soil d.w..

Dates of work: July 19 to August 31, 2011

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	5.0% after 4 weeks
Mean number of juveniles per replicate	≥ 100	1539.3
Coefficient of variation of reproduction	≤ 30%	7.6%

All validity criteria for the study were met

The most recent reference test (March 08, 2011) with the reference item 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. 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.

Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	Trifloxystrobin WG 50 W *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	[mg test item/kg dws]	
LOEC	>1000	>1000
LC_{50}/ EC_{50}	>1000	>1000
95% confidence limit	-	-
NOEC	≥1000	≥1000

Trifloxystrobin WG 50 W **[mg test item /kg dws]**						
	Control	**100**	**178**	**316**	**562**	**1000**
	Mortality of adult collembolans after 4 weeks					
Mortality [%]	5.0	7.5	10.0	10.0	7.5	7.5
	Mean number of juveniles after 4 weeks					
Mean	1539.3	1429.0	1414.8	1455.3	1483.3	1602.5
SD	117.0	80.0	69.1	209.1	95.1	43.3
	Reproduction compared to control [%]					
% to control	-	92.8 n.s.	91.9 n.s.	94.5 n.s.	96.4 n.s.	104.1 n.s.

n.s. statistically not significant (Bonferroni-U-test for non-homogeneous variances, one-sided smaller, $\alpha = 0.05$)
SD: standard deviation
Percent reproduction: (Rt/Rc) * 100%
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 7.5 to 10.0% parental mortality in the treatment groups. 5.0% parental mortality was observed in the control.
No statistically significant effect on parental mortality was found for the concentration tested.
Concerning the number of juveniles statistical analysis (Bonferroni-U test for non-homogeneous variances, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 1000 mg test item/kg artificial soil dry weight. 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.

Conclusions:

The test item Trifloxystrobin WG 50 showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil at any test concentration.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg d.w.

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Report:	**KCA 8.4.2.1/04; [redacted], M.A., 2012**
Title:	Trifloxystrobin WG 50 W: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	kra-HR-76/12
Document No:	M-443226-01-1
Guidelines:	OECD-Guideline 226 (2008)
Deviations:	None
GLP	Yes (certified laboratory)

Objective:

The purpose of the study was to assess the effects of Trifloxystrobin WG 50 W on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:

Test item: Trifloxystrobin WG 50 W (CGA 279202; Batch: EDFL011509; Sample description FAR01568-00; Material No.: 05584493; Specification No.:102000007798-02; Purity: 49.8%w/w).
Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control (water treated) and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight 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 (30 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): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground and 20% Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Dates of experimental work: July 23, 2012 to August 15, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	1.3%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	336.1
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	8.0%

All validity criteria for the study were met.

Effect of Trifloxystrobin WG 50 W on *Hypoaspis aculeifer* in a 14-day reproduction study

Test item Test object Exposure	**Trifloxystrobin WG 50 W *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	336.1 ± 26.8	-
100	2.5	358.0 ± 37.6	106.5 n.s.
178	2.5	356.0 ± 39.8	105.9 n.s.
316	2.5	332.5 ± 15.6	98.9 n.s.
562	2.5	334.8 ± 25.3	99.6 n.s.
1000	0.0	312.5 ± 34.6	93.0 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 1000
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 1000

n.s. = no statistically significant difference (Student-t-test, one-sided smaller, α = 0.05)

Mortality:

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

Reproduction:

Concerning the number of juveniles statistical analysis (Student-t-test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 1000 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.

Conclusion:

The $NOEC_{reproduction}$ is ≥ 1000 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-11/12, February 29, 2012) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were even after transformation not homogenous Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment procedure, $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 6.62 mg a.s./kg (95% confidence limits from 6.02 mg a.s./kg to 2469.54 mg a.s./kg) 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.

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Metabolite CGA 357261

Report:	**KCA 8.4.2.1/05; [redacted], 2012**
Title:	Trifloxystrobin-CGA 357261 (BCS-AR14200): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Report No:	FRM-Coll-150/12
Document No:	M-443697-01-1
Guidelines:	OECD-Guideline No. 232 (2009)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:
The purpose of this study was to assess the effect of Trifloxystrobin-CGA 357261 (BCS-AR14200, metabolite of trifloxystrobin) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test material: Trifloxystrobin-CGA 357261 (BCS-AR14200), analytical findings: 99.4% w/w AE 1393224, origin batch no.: SES 10350-10-1, certificate no.: AZ 17556, batch code: AE 1393224-PU-01, material: AE 1393224, pure substance.
Ten collembola (9-12 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 (d.w.) containing 75% quartz sand, 20% kaolin clay, 5% sphagnum peat and $CaCO_3$, at 20.0 ± 2°C and a photoperiod: light : dark = 16 h : 8 h (400-800 lux) and were fed weekly with granulated dry yeast.
Mortality and reproduction were determined after 28 days.
Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg soil d.w..

Dates of experimental work: September 10 to October 18, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	12.5% after 4 weeks
Mean number of juveniles per replicate	≥ 100	1189
Coefficient of variation of reproduction	≤ 30%	8.8%

All validity criteria for the study were met

The most recent reference test (May 25, 2012) with the reference item Boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95% confidence limits from 98 mg to 137 mg Boric acid/kg artificial soil dry weight) for reproduction. The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	CGA 357261 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	[mg test item/kg dws]	
LOEC	>100	>100
LC_{50}/ EC_{50}	>100	>100
95% confidence limit	-	-
NOEC	≥100	≥100

CGA 357261 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult collembolans after 4 weeks	
Mortality [%]	12.5	11.3
	Mean number of juveniles after 4 weeks	
Mean	1188.8	1224.1
SD	104.2	90.3
	Reproduction compared to control [%]	
% to control	-	103.0 n.s.

n.s. statistically not significant (Student's-t test, one-sided smaller, α = 0.05)
SD: standard deviation
Percent reproduction: (Rt/Rc) * 100%
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 12.5% parental mortality at the test concentration of 100 mg test item/kg d.w.. 11.3% parental mortality was observed in the control.
No statistically significant effect on parental mortality was found for the concentration tested.
Concerning the number of juveniles statistical analysis (Student's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Conclusions:

The test item Trifloxystrobin CGA 357261 (BCS-AR14200) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil at the test concentration of 100 mg test item/kg artificial soil.
Therefore the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg d.w.

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Report:	**KCA 8.4.2.1/06; [redacted], M.A., 2012**
Title:	Trifloxystrobin- CGA 357261 (BCS-AR14200): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	kra-HR-80/12
Document No:	M-443311-01-1
Guidelines:	OECD-Guideline 226 (2008)
Deviations:	Exposition to the test item: 15 days (instead of 14 days)
GLP	Yes (certified laboratory)

Objective:

The purpose of the study was to assess the effects of Trifloxystrobin-CGA 357261 (BCS-AR14200, metabolite of trifloxystrobin) 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: Trifloxystrobin-CGA 357261 (BCS-AR14200) (Certificate No.: AZ 17556; Batch-code: AE 1393224-PU-01; Origin Batch No. SES 10350-10-1; Purity AE 1393224: 99.4%w/w).
Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control (water treated) and 100 mg test item/ kg dry weight soil. 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 (30 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): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground and 20% Kaolin clay.
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: September 14, 2012 to October 11, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	8.8%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	357.1
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	16.6%

All validity criteria for the study were met.

Effect of trifloxystrobin- CGA 357261 on *Hypoaspis aculeifer* in a 15-day reproduction study

Test item **Test object** **Exposure**	**trifloxystrobin- CGA 357261 (BCS-AR14200)** ***Hypoaspis aculeifer*** **Artificial soil**		
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	8.8	357.1 ± 59.2	-
100	3.8	411.8 ± 37.4	115 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

n.s. = no statistically significant difference (Student-t-test, one-sided smaller, α = 0.05)

Mortality:

In the control group 8.8% 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 treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_{50} values could not be calculated and is considered to be > 100 mg test item/kg dry weight soil.

Conclusion:

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

Reference test:

The most recent non-GLP-test ([redacted] kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were even after transformation not homogenous Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment procedure, α = 0.05, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 6.62 mg a.s./kg (95% confidence limits from 6.02 mg a.s./kg to 2469.54 mg a.s./kg) 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.

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Metabolite CGA 321113

Report:	**KCA 8.4.2.1/07; [redacted], M.A., 2012**
Title:	Trifloxystrobin- CGA 321113 (BCS-AL58660): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	kra-HR-75/12
Document No:	M-443145-01-1
Guidelines:	OECD-Guideline 226 (2008)
Deviations:	None
GLP	Yes (certified laboratory)

Objective:

The purpose of the study was to assess the effects of Trifloxystrobin- CGA 321113 (BCS-AL58660, metabolite of trifloxystrobin) 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: Trifloxystrobin- CGA 321113 (BCS-AL58660) (TOX No. 09586-00; Batch-code: AE 1344138-01-02; Origin Batch No. BCOO 6132-3-9; Purity AE 1344138: 98.4% w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control (water treated) and 100 mg test item/ kg dry weight soil. 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 (30 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): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground and 20% Kaolin clay.

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 03, 2012 to August 27, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	1.3%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	346.8
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	7.0%

All validity criteria for the study were met.

Effect of trifloxystrobin- CGA 321113 on *Hypoaspis aculeifer* in a 14-day reproduction study

Test item **Test object** **Exposure**	**trifloxystrobin- CGA 321113 (BCS-AL58660)** ***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	346.8 ± 24.2	-
100	2.5	351.9 ± 29.2	101.54 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

n.s. = no statistically significant difference (Student-t-test, one-sided smaller, $\alpha = 0.05$)

<u>Mortality:</u>
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.

<u>Reproduction:</u>
Concerning the number of juveniles statistical analysis (Student-t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the concentration of 100 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_{50}-values could not be calculated and is considered to be > 100 mg test item/kg dry weight soil.

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

Reference test:
The most recent non-GLP-test ([redacted] [redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were even after transformation not homogenous Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment procedure, $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 6.62 mg a. s./kg (95% confidence limits from 6.02 mg a.s./kg to 2469.54 mg a.s./kg) 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.

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Metabolite CGA 373466

Report:	**KCA 8.4.2.1/08; [redacted], 2012**
Title:	Trifloxystrobin-CGA 373466 (BCA-AL58690): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Report No:	FRM-Coll-146/12
Document No:	M-440109-01-1
Guidelines:	OECD-Guideline No. 232 (2009)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:

The purpose of this study was to assess the effect of Trifloxystrobin-CGA 373466 (BCA-AL58690, metabolite of trifloxystrobin) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Test material: Trifloxystrobin-CGA 373466 (BCA-AL58690) analytical findings: 97.9% w/w
AE 1344148, origin batch no.: SES_11648-6-3, certificate no.: AZ 17621, batch code: AE 1344148-PU-01, LIMS no.: 1227123.

Ten collembola (9-12 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 soil dry weight (d.w.) containing 75% quartz sand, 20% kaolin clay, 5% sphagnum peat and $CaCO_3$, at 20.0 ± 2°C and a photoperiod: light : dark = 16 h : 8 h (400-800 lux) and were fed weekly with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg soil d.w..

Dates of experimental work: September 10 to October 18, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	12.5% after 4 weeks
Mean number of juveniles per replicate	≥ 100	1188.8
Coefficient of variation of reproduction	≤ 30%	8.8%

All validity criteria for the study were met

The most recent reference test (May 25, 2012) with the reference item Boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95% confidence limits from 98 mg to 137 mg Boric acid/kg artificial soil dry weight) for reproduction. The $NOEC_{reproduction}$ was calculated to be 67 mg

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

Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	CGA 373466 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	[mg test item/kg dws]	
LOEC	>100	>100
LC_{50}/ EC_{50}	>100	>100
95% confidence limit	-	-
NOEC	≥100	≥100

CGA 373466 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult collembolans after 4 weeks	
Mortality [%]	12.5	11.3
	Mean number of juveniles after 4 weeks	
Mean	1188.8	1120.0
SD	104.2	112.9
	Reproduction compared to control [%]	
% to control	-	94.2 n.s.

n.s. statistically not significant (Student's-t test, one-sided smaller, α = 0.05)
SD: standard deviation
Percent reproduction: (Rt/Rc) * 100%
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 11.3% parental mortality at the test concentration of 100 mg test item/kg d.w.. 12.5% parental mortality was observed in the control.
No statistically significant effect on parental mortality was found for the concentration tested.
Concerning the number of juveniles statistical analysis (Student's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Conclusions:

The test item Trifloxystrobin-CGA 373466 showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil at the test concentration of 100 mg test item/kg artificial soil.

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

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Report:	**KCA 8.4.2.1/09; [redacted], M.A., 2012**
Title:	Trifloxystrobin- CGA 373466 (BCS-AL58690); Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	kra-HR-73/12
Document No:	M-440955-01-1
Guidelines:	OECD-Guideline 226 (2008)
Deviations:	Exposition to the test item: 15 days (instead of 14 days)
GLP	Yes (certified laboratory)

Objective:

The purpose of the study was to assess the effects of Trifloxystrobin-CGA373466 (BCS-AL58690, metabolite of trifloxystrobin) 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: Trifloxystrobin- CGA 373466 (BCS-AL58690) (Certificate No.: AZ 17621; Batch-ID: AE 1344148-PU-01; Origin Batch No. SES 11648-6-3; Purity AE 1344148: 97.9%w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control (water treated) and 100 mg test item/ kg dry weight soil. 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 (30 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): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground and 20% Kaolin clay.

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: September 14, 2012 to October 11, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	8.8%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	357.1
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	16.6%

All validity criteria for the study were met.

Effect of trifloxystrobin- CGA373466 on *Hypoaspis aculeifer* in a 15-day reproduction study

Test item Test object Exposure	trifloxystrobin- CGA373466 (BCS-AL58690) *Hypoaspis aculeifer* Artificial soil		
mg test item/kg dry weight artificial soil	**Adult mortality (%)**	**Mean number of juveniles ± SD**	**Reproduction (% of control)**
Control	8.8	357.1 ± 59.2	-
100	5.0	360.9 ± 46.1	102.7 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

n.s. = no statistically significant difference (Student-t-test, one-sided smaller, α = 0.05)

Mortality:
In the control group 8.8% 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. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_{50}-values could not be calculated and is considered to be > 100 mg test item/kg dry weight soil.

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

Reference test:
The most recent non-GLP-test ([redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were even after transformation not homogenous Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment procedure, $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 6.62 mg a.s./kg (95% confidence limits from 6.02 mg a.s./kg to 2469.54 mg a.s./kg) 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.

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Metabolite NOA 413161

Report:	**KCA 8.4.2.1/10; [redacted], M.A., 2013**
Title:	Trifloxystrobin- NOA 413161 (BCS-AL58658): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No:	kra-HR-91/13
Document No:	M-455220-01-1
Guidelines:	OECD-Guideline 226 (2008)
Deviations:	None
GLP	Yes (certified laboratory)

Objective:

The purpose of the study was to assess the effects of Trifloxystrobin-NOA 413161 (BCS-AL58658, metabolite of trifloxystrobin) 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: Trifloxystrobin-NOA 413161 (BCS-AL58658) (analytical findings: 91.8% w/w AE 1344143; origin batch no.: M19118; batch code: AE 1344143 00 1C92 0001; certificate no.: AZ 17475).
Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for the treatment groups) were exposed to control and treatments. Concentrations of 10, 18, 32, 56 and 100 mg pure metabolite (corresponding to 11, 20, 35, 61 and 109 mg test item/kg) per artificial soil dry weight 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 (31 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): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground and 20% Kaolin clay.
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: April 09, 2013 to April 29, 2013

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	1.3%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	380.3
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	5.1%

All validity criteria for the study were met.

Effect of trifloxystrobin- NOA 413161 on *Hypoaspis aculeifer* in a 14-day reproduction study

Test item Test object Exposure	trifloxystrobin- NOA 413161 (BCS- AL58658) *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	380.3 ± 19.2	
10	2.5	376.8 ± 26.6	99.1 n.s.
18	2.5	386.3 ± 22.6	101.6 n.s.
32	0.0	393.5 ± 33.9	103.5 n.s.
56	2.5	388.3 ± 22.2	102.1 n.s.
100	0.0	398.3 ± 13.4	104.8 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

n.s. = no statistically significant difference (William´s-t-test, one-sided smaller, α = 0.05)

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

Reproduction:
Concerning the number of juveniles statistical analysis (William´s-t-test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg pure metabolite/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg pure metabolite/kg dry weight artificial soil.

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

metabolite/kg soil d.w.

Reference test:
The most recent non-GLP-test ([redacted] [redacted], kra/HR-O-12/13, April 08, 2013) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 4.32 mg a.s./kg (95% confidence limits from 4.31 mg a. s./kg to 4.32 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.
The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 5.67 mg a. s./kg (95% confidence limits from 5.58 mg a. s./kg to 5.79 mg a.s./kg) 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.

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<u>**Metabolite NOA 413163**</u>

Report:	**KCA 8.4.2.1/11; [redacted] Th., [redacted], 2013**
Title:	Trifloxystrobin-NOA413163 (BCS-AL58659): Acute and reproduction toxicity to the collembolan species *Folsomia candida* in artificial soil
Report No:	EBTFN012
Document No:	M-444419-01-1
Guidelines:	OECD-Guideline No. 232 (2009)
Deviations:	None
GLP:	Yes (certified laboratory)

Objectives:
The purpose of this study was to assess the effect of Trifloxystrobin-NOA413163 (BCS-AL58659, metabolite of trifloxystrobin) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test material: Trifloxystrobin-NOA413163 (BCS-AL58659), analytical findings: 99.2% w/w, origin batch no.: MI8477, certificate no.: AZ 17455, batch code: AE 1344149 00 1B98 0001, material: AE 1344149, pure substance.
Ten collembola (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control (quartz sand treated) and 100 mg test item/kg artificial soil dry weight (d.w.) containing 74% quartz sand, 20% kaolin clay, 5% sphagnum peat and < 1% $CaCO_3$, at 20.0 ± 2°C and a photoperiod: light : dark = 16 h : 8 h (400-800 lux) and were fed weekly with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Toxic standard: 5.6, 10.0, 17.8, 31.6, 100 and 178 mg boric acid/kg soil d.w..

Dates of work: August 29 to September 26, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	10% after 4 weeks
Mean number of juveniles per replicate	≥ 100	524.5
Coefficient of variation of reproduction	≤ 30%	17.0%

All validity criteria for the study were met

The most recent reference test (February to March, 2012) with the reference item Boric acid showed an EC_{50} of 50.9 mg test item/kg artificial soil dry weight (95% confidence limits from 27.2 mg to 98.4 mg Boric acid/kg artificial soil dry weight) for reproduction. This shows that the test organisms are sufficiently sensitive.

Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	NOA413163 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	[mg test item/kg dws]	
LOEC	>100	>100
LC_{50}/ EC_{50}	>100	>100
95% confidence limit	-	-
NOEC	≥100	≥100

NOA413163 [mg test item/kg dws]		
	Control	**100**
	Mortality of adult collembolans after 4 weeks	
Mortality [%]	10.0	12.5
	Mean number of juveniles after 4 weeks	
Mean	524.5	512.9
SD	89.0	48.3
	Reproduction compared to control [%]	
% to control	-	97.8 [n.s.]

[n.s.] statistically not significant (Student's-t test, one-sided smaller, $p \leq 0.05$)
SD: standard deviation
Percent reproduction: (Rt/Rc) * 100%

Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

10% mortality was observed at the control and 12.5% mortality at the tested limit concentration of 100 mg test item/kg soil dry weight (dw).
No statistically significant effect on parental mortality was found for the concentration tested.
Concerning the number of juveniles statistical analysis (Student's-t test, one-sided smaller, $p \leq 0.05$) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight.

Conclusions:
The test item Trifloxystrobin-NOA413163 (BCS-AL58659) showed no statistically significant adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil at the test concentration of 100 mg test item/kg artificial soil.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg d.w.

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Metabolite CGA 357276

Report: **KCA 8.4.2.1/12; [redacted], 2012**
Title: Trifloxystrobin-CGA 357276 (BCS-AB39835): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Report No: FRM-Coll-145/12
Document No: M-441251-01-1
Guidelines: OECD-Guideline No. 232 (2009)
Deviations: None
GLP: Yes (certified laboratory)

Objectives:
The purpose of this study was to assess the effect of Trifloxystrobin-CGA 357276 (BCS-AB39835, metabolite of trifloxystrobin) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:
Test material: Trifloxystrobin-CGA 357276 (BCS-AB39835): analytical findings: 97.8% w/w, origin batch no.: BOCOO 6204-3-3, batch code: BCS-AB39835-PU-01, Certificate no.:
AZ 16891.
Ten collembola (9-12 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 (d.w.) containing 75% quartz sand, 20% kaolin clay, 5% sphagnum peat and $CaCO_3$, at 20.0 ± 2°C and a photoperiod: light : dark = 16 h : 8 h (400-800 lux) and were fed weekly with granulated dry yeast.
Mortality and reproduction were determined after 28 days.
Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg soil d.w..

Dates of experimental work: July 20 to August 23, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	15.0% after 4 weeks
Mean number of juveniles per replicate	≥ 100	1048
Coefficient of variation of reproduction	≤ 30%	13.7%

All validity criteria for the study were met.

The most recent reference test (May 25, 2012) with the reference item Boric acid showed an EC_{50} of 116 mg test item/kg artificial soil dry weight (95% confidence limits from 98 mg to 137 mg Boric acid/kg artificial soil dry weight) for reproduction. The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Effects on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	CGA 357276 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	[mg test item/kg dws]	
LOEC	>100	>100
LC_{50}/ EC_{50}	>100	>100
95% confidence limit	-	-
NOEC	≥100	≥100

CGA 357276 [mg test item /kg dws]		
	Control	**100**
	Mortality of adult collembolans after 4 weeks	
Mortality [%]	15.0	7.5
	Mean number of juveniles after 4 weeks	
Mean	1048.0	1110.9
SD	143.7	77.2
	Reproduction compared to control [%]	
% to control	-	106.0 [n.s.]

[n.s.] statistically not significant (Student's-t test, one-sided smaller, α = 0.05)
SD: standard deviation
Percent reproduction: (Rt/Rc) * 100%
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 15.0% parental mortality at the test concentration of 100 mg test item/kg d.w.. 7.5% parental mortality was observed in the control.
No statistically significant effect on parental mortality was found for the concentration tested.
Concerning the number of juveniles statistical analysis (Student's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg artificial soil dry weight. An EC_{50} could not be calculated and is considered to be > 100 mg test item/kg artificial soil dry weight.

Conclusions:
The test item Trifloxystrobin CGA 357276 showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolans *Folsomia candida* in artificial soil at the test concentration of 100 mg test item/kg artificial soil.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg d.w.

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Report: **KCA 8.4.2.1/13; [redacted], M.A., 2012**
Title: Trifloxystrobin-CGA357276 (BCS-AB39835): Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No: kra-HR-74/12
Document No: M-440367-01-1
Guidelines: OECD-Guideline 226 (2008)
Deviations: None
GLP Yes (certified laboratory)

Objective:

The purpose of the study was to assess the effects of Trifloxystrobin-CGA357276 (BCS-AB39835, metabolite of trifloxystrobin) 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: Trifloxystrobin-CGA 357276 (BCS-AB39835) (Certificate No.: AZ 16891; Batch-ID: BCS-AB39835-PU-01; Origin Batch No. BCOO 6204-3-3; Purity BCS-AB39835: 97.8%w/w).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for each application rate) were exposed to control (water treated) and 100 mg test item/ kg dry weight soil. 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): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground and 20% Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Dates of experimental work: July 23, 2012 to August 14, 2012

Results:

Validity Criteria	Recommended	Obtained
Mean adult mortality	≤ 20%	1.3%
Mean number of juveniles per replicate (with 10 collembolan introduced)	≥ 50	336.1
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	8.0%

All validity criteria for the study were met.

Effect of trifloxystrobin-CGA 357276 on *Hypoaspis aculeifer* in a 14-day reproduction study

Test item **Test object** **Exposure**	**trifloxystrobin-CGA 357276 (BCS-AB39835)** ***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	336.1 ± 26.8	-
100	2.5	327.3 ± 34.6	97.4 n.s.
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			≥ 100
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)			> 100

n.s. = no statistically significant difference (Student-t-test, one-sided smaller, α = 0.05)

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. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg test item/kg dry weight artificial soil. EC_{50}-values could not be calculated and is considered to be > 100 mg test item/kg dry weight soil.

Conclusion:

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

Reference test:

The most recent non-GLP-test ([redacted], kra/HR-O-11/12, February 29, 2012) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 3.894 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were even after transformation not homogeneous Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment procedure, α = 0.05, one-sided smaller was used. Dimethoate EC 400E G showed a EC_{50} of 6.62 mg a. s./kg (95% confidence limits from 0.02 mg a.s./kg to 2469.54 mg a.s./kg) 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 this compound, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph.

The following endpoint from a study evaluated during the first EU review (SANCO/4339/2000-Final) is used in the risk assessment:

Table 8.5- 1: Effects on soil nitrogen transformation

Test substance	Test species	Endpoint		Reference
TFS WG 50	N-transformation 28d	no unacceptable effects	≥0.5 kg prod./ha **≥0.272 mg a.s./kg dws**[a]	[redacted] (1999) RF-D1.91/99 M-051718-01-1 KCP 10.5/01
Trifloxystrobin	N-transformation 28d	no unacceptable effects	**≥13.33 mg a.s./kg dws**	[redacted] (1998) 973591 M-034686-01-1 KCA 8.5/01
CGA 373466	N-transformation 28d	no unacceptable effects	**≥3.47 mg/kg dws**	[redacted] (2002) AJO/230902 M-070537-01-1 KCA 8.5/02
NOA 413161	N-transformation 28d	no unacceptable effects	**≥3.41 mg/kg dws**	[redacted] (2002) AJO/231102 M-071668-01-1 KCA 8.5/13

[a] 0.08 mg formulation containing 0.041 mg a.s. were sprayed onto 150 g soil resulting in 0.272 mg a.s./kg soil.
Values **in bold** are used in risk assessment

Studies on the influence on the nitrogen-transformation were performed for trifloxystrobin and for all metabolites with a maximum occurrence of ≥10% in soil. In no case a relevant influence on the nitrogen-transformation was found at the tested soil concentrations. Therefore, the risk from major soil metabolites with a maximum occurrence rate of lower than 10% (CGA 381318, CGA 357276, NOA 413163 and NOA 409480) to soil microorganisms is considered to be low since they would not indicate an unacceptable risk even if they would be 10 times more toxic as the parent compound trifloxystrobin. Therefore, no study on the nitrogen-transformation is considered necessary for these metabolites.

The following studies are included in this Supplemental Dossier:

Table 8.5- 2: Additional nitrogen transformation studies and endpoints for soil microbial activity

Test item	Test design	Ecotoxicological endpoint		Reference
CGA 357261	N-transformation 42 d	no unacceptable effects	**≥3.353 mg/kg dws**	[redacted] (2013) 13 10 48 093 N, M-464875-01-1 KCA 8.5/15
CGA 321113	N-transformation 28 d	no unacceptable effects	**≥3.261 mg/kg dws**	[redacted] (2013) 13 10 48 092 N, M-464870-01-1 KCA 8.5/16

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

Metabolite CGA 357261

Report: **KCA 8.5/15; [redacted], 2013**
Title: Trifloxystrobin-CGA 357261- (BCS-AR14200)- Effects on the activity of soil microflora (Nitrogen transformation test)
Report No: 13 10 48 093 N
Document No: M-464875-01-1
Guidelines: OECD-Guideline 216 (2000)
Deviations: None
GLP Yes (certified laboratory)

Objective:

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

Materials and Methods:

Test item: Trifloxystrobin-CGA 357261 (metabolite of trifloxystrobin) (BCS-code: BCS-AR14200, Batch code: AE 1393224-PU-01, Origin Batch No.: SES-10350-10-1, LIMS No.: 1124676, Certificate No.: AZ 17556, analysed purity: 99.4% w/w).

A loamy sand soil (DIN 4220) was exposed for 42 days to 0.335 and 3.353 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14, 28 and 42 days after treatment).

Dates of experimental work: June 12, 2013 to July 24, 2013

Results:

Validity Criteria	Recommended	Obtained
coefficients of variation in the control for NO_3-N	≤ 15%	1.9%

The validity criterion for the study was met.

Effects of CGA 357261 on non-target soil micro-organisms

Test item	**CGA 357261 (metabolite of trifloxystrobin)**				
Test object	Soil Micro-organisms Nitrogen-Transformation (silty sand soil)				
Time interval (days)	control	0.335 mg test item /kg soil dry weight equivalent to 0.252 kg test item/ha		3.353 mg test item /kg soil dry weight equivalent to 2.515 kg test item/ha	
	Nitrate-N	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	3.30 ± 0.02	3.75 ± 0.27	13.4 n.w.	4.81 ± 0.39	45.7 *w.
7 - 14	1.25 ± 0.10	1.37 ± 0.04	9.9 n.s.	1.77 ± 0.35	42.0 n.s.
14 – 28	0.74 ± 0.08	1.10 ± 0.14	49.2 *s.	1.10 ± 0.06	50.2 *s.
28 - 42	1.04 ± 0.08	0.83 ± 0.09	-20.6 *s.	0.96 ± 0.13	-7.3 n.s.

The calculations were performed with unrounded values

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

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

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, $p \leq 0.05$)

*w. = statistically significantly different to control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

The test item CGA 357261 caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.335 mg/kg dry soil at time interval 14-28 days after application.
However, no adverse effects of CGA 357261 on nitrogen transformation in soil could be observed at the tested concentration of 0.335 mg/kg dry soil, 42 days after application (time interval 28-42 days).
Temporary stimulations of the daily nitrate rate were also observed at 3.353 mg/kg dry soil beginning at time interval 7-14 until time interval 14-28 days after application. However, no adverse effects of CGA 357261 on nitrogen transformation in soil could be observed at the tested concentration of 3.353 mg/kg dry at the end of the test, 42 days after application (time interval 28-42 days).
Differences from the control of -20.6% (test concentration 0.335 mg/kg dry soil) and -7.3% (test concentration 3.353 mg/kg dry soil) were measured at the end of the 42-day incubation period (time interval 28-42).

Conclusion:
CGA 357261 (metabolite of trifloxystrobin) caused no adverse effects (difference to control < 25%) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of the 42-day incubation period. The study was performed in a field soil at concentrations up to 3.353 mg test item/kg soil dry weight.

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

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Metabolite CGA 321113

Report:	**KCA 8.5/16; [redacted], 2013**
Title:	Trifloxystrobin-CGA 321113- (BCS-AL58660): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	13 10 48 092 N
Document No:	M-464870-01-1
Guidelines:	OECD-Guideline 216 (2000)
Deviations:	None
GLP	Yes (certified laboratory)

Objective:

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

Materials and Methods:

Test item: Trifloxystrobin-CGA 321113 (metabolite of trifloxystrobin) (BCS-code: BCS-AL58660, Batch code: AE 1344138 00 1C93 0001, Origin Batch No.: M20619, LIMS No.: 1229383, Certificate No.: AZ 18276, analysed purity: 98.7% w/w).

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.326 and 3.261 mg test item/kg soil dry weight. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of experimental work: June 11, 2013 to July 09, 2013

Results:

Validity Criteria	Recommended	Obtained
coefficients of variation in the control for NO_3-N	≤ 15%	2.6%

The validity criterion for the study was met.

Effects of CGA 321113 on non-target soil micro-organisms

Test item	**CGA 321113 (metabolite of trifloxystrobin)**				
Test object	Soil Micro-organisms Nitrogen-Transformation (silty sand soil)				
Time interval (days)	control	0.326 mg test item /kg soil dry weight equivalent to 0.244 kg test item/ha		3.261 mg test item /kg soil dry weight equivalent to 2.446 kg test item/ha	
	Nitrate-N	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
0 - 7	3.60 ± 0.10	3.92 ± 0.29	9.0 n.s.	4.26 ± 0.28	18.4 *s.
7 - 14	1.46 ± 0.29	1.30 ± 0.35	-11.1 n.s.	1.30 ± 0.14	-11.1 n.s.
14 – 28	0.95 ± 0.11	0.97 ± 0.29	1.7 n.s.	1.02 ± 0.41	7.0 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, $p \leq 0.05$)

No adverse effects of CGA 321113 on nitrogen transformation in soil could be observed at both test concentrations (0.326 mg/kg dry soil and 3.261 mg/kg dry soil) during the 28-day experiment. Differences from the control of +1.7% (test concentration 0.326 mg/kg dry soil) and +7.0% (test concentration 3.261 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Conclusion:
CGA 321113 caused no adverse effects (difference to control < 25%) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 3.261 mg test item/kg soil dry weight.

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

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CA 8.6 Effects on terrestrial non-target higher plants

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

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 trifloxystrobin is not a herbicide, screening studies on terrestrial non-target plant are available (see MCP), no further data is considered necessary.

CA 8.6.2 Testing on non-target plants

Studies on non-target plants (seedling emergence and vegetative vigour) have been conducted with the representative formulation Trifloxystrobin WG 50 and are presented in MCP, Annex point 10.6.2.

In addition, four non-target terrestrial plant studies have been performed with soil metabolites of trifloxystrobin. Three of these are included in the Baseline Dossier. One study has not been submitted on EU level so far and is provided below for reasons of completeness. However, the study is not relevant for the non-target plant risk assessment which is based on results of studies with the formulation.

Table 8.6.2- 1: Additional studies for testing non-target plants

Test species	Test system	Endpoint	Reference
CGA 321113			
Terrestrial plants, 6 species	Vegetative vigour, Tier 2 dose-response, 21 days	ER_{50} >250 g p.m./ha	[redacted] & [redacted] (2002) 11112087 M-070976-01-1 KCA 8.6.2/07

Report:	**KCA 8.6.2/07; [redacted]., [redacted] M.; 2002**
Title:	CGA279202-CGA321113: Effects on Terrestrial (Non-Target) Plants - Vegetative Vigour Test
Report No:	11112087
Document No:	M-070976-01-1
Guidelines:	OECD Guideline for the Testing of Chemicals, Proposal for Updating Guideline 208, Draft Document July 2000
Deviations:	None
GLP:	Yes (certified laboratory)

Objective:

The purpose of this study was to determine the effects of multiple dosage levels of the test item on the vegetative vigour of 6 non-target plant species representing 6 plant families. Parameters measured include plant fresh weight, height and observed phytotoxicity.

Materials and Methods:

Test item: CGA279202 (Trifloxystrobin)-CGA321113 (purity 99 %); specification: batch no. M18778 = M17539 (formerly).

Six species of terrestrial non-target plants (2 monocots and 4 dicots) were sprayed at various application rates of 0 (control), 15.6, 31.25, 62.5, 125 and 250 g a.s./ha. The species tested were lettuce (*Lactuca sativa*), oilseed rape (*Brassica napus*), sugar beet (*Beta vulgaris*), soybean (*Glycine*

max), onion (*Allium cepa*) and oat (*Avena sativa*). The seeds were introduced manually into the soil. With respect to the different development of the species the sowing was done on different dates. At application the species had to be in 2 to 4 leaf stage and test duration was 21 days following application of the test substance.
The application of each spray solution was done by spraying two times 600 L/ha to reach the target amount of 1200 L/ha. Control pots were sprayed with deionized water. The fresh weight was determined at day 21. The plants of one pot represent one replicate. Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were assessed at day 7, 14 and 21 according to EPPO Standard 135. Number of living and dead plants were recorded at day 21. Weighing of dead plants was not necessary.
Pots were grown and maintained under glasshouse conditions with a temperature control set at 23 ± 4ºC during day and 18 ± 4ºC at night with a 16 h photoperiod.

Dates of experimental work: April 24 to May 15, 2002

Results:

CGA279202-CGA321113 was tested for effects on vegetative vigour of *Lactuca sativa*, *Brassica napus*, *Beta vulgaris*, *Glycine max*, *Allium cepa* and *Avena sativa*. Effects on fresh weight were not observed. Effects on height were only observed for the dicotyledoneae *Beta vulgaris*. Mortality was not observed during the study. Phytotoxic effects were not observed during the study.
The study is valid because control plants showed normal growth throughout the test and there was no mortality in the control.
The Day 21 No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), EC_{25} and EC_{50} values are summarised for each of the plant species in the following tables.

Summary of Effective Concentrations (based on fresh weight)

Vegetative vigour (based on fresh weight)						
Plant Species	EC_{25} [a] (g a.s./ha)	EC_{50} [a] (g a.s./ha)	Statistical analysis	NOEC (g a.s./ha)	LOEC (g a.s./ha)	Statistical analysis
Lettuce	> 250	> 250	2	≥ 250	> 250	3
Oil seed rape	> 250	> 250	1	≥ 250	> 250	3
Sugar beet	> 250	> 250	1	≥ 250	> 250	3
Soybean	> 250	> 250	1	≥ 250	> 250	3
Onion	> 250	> 250	1	≥ 250	> 250	3
Oat	> 250	> 250	1	≥ 250	> 250	3

Summary of Effective Concentrations (based on height)

Vegetative vigour (based on height)						
Plant Species	EC_{25} [a] (g a.s./ha)	EC_{50} [a] (g a.s./ha)	Statistical analysis	NOEC (g a.s./ha)	LOEC (g a.s./ha)	Statistical analysis
Lettuce	> 250	> 250	1	≥ 250	> 250	3
Oilseed rape	> 250	> 250	2	≥ 250	> 250	3
Sugar beet	> 250	> 250	2	15.6	31.3	3
Soybean	> 250	> 250	1	≥ 250	> 250	3
Onion	> 250	> 250	1	≥ 250	> 250	3
Oat	> 250	> 250	1	≥ 250	> 250	3

1 = multiple comparison Dunnett Test, a = 0.05
2 = multiple comparison Bonferroni U-Test, a = 0.05
3 = Probit Analysis
[a] upper and lower 95% C.I. could not be determined due to mathematical reasons

Conclusion:

In a vegetative vigour study with 6 non-target terrestrial plant species, 21 days after a post-emergent foliar application, phytotoxicity, EC_{50}, EC_{25} and NOEC values were determined for each species. All EC_{50} and EC_{25} values were > 250 g a.s./ha based on height and fresh weight. All NOECs were ≥ 250 g a.s./ha for both parameters except for sugar beet (plant height) with an NOEC of 15.6 g a.s./ha. No phytotoxic effects were observed during the study.

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

CA 8.9 Monitoring data

Please refer to MCA Section 7.5.