Bayer CropScience

Document Title

Summary of the ecotoxicological studies for Fluoxastrobin

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

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Bayer CropScience

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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

As published in Commission Directive 2008/44/EC of 04th April 2008 and with an Entry into Force (EIF) date of 01st August 2008, the fungicide Fluoxastrobin was first included in Annex I to Commission Directive 91/414/EEC.

Now, with the aim to achieve European Re-Approval under Regulation 1107/2009, Bayer CropScience (BCS) provides this '**Supplementary Dossier**'. It contains only new data which were not submitted at the time of the Annex I inclusion of fluoxastrobin under Commission Directive 91/414/EEC and which were therefore not evaluated during the first European review.

In addition to submitting the above mentioned Supplementary Dossier, all studies relied upon under 91/414 and contained in the Draft Assessment Report and its Addenda are – for the convenience of the reviewers – included in what BCS calls '**Baseline Dossier**' (Document K level only).

In order to ease the reviewers' orientation on 'old' studies in the Baseline Dossier versus 'new' studies in the Supplementary Dossier, BCS has decided to apply the following basic principles:

1. Conversion of the Document K part of the old EU dossier structure into the new structure (acc. to Commission Regulations 283/2013 and 284/2013 and linking the old studies to the new structure according to the cross-walk tables provided in Guidance Document SANCO/10181/2013 – rev. 2.1 of 13th May 2013).
2. On a case-by-case basis and where useful for the reader, old studies from the Baseline Dossier are occasionally summarised on the Document M level of the Supplementary Dossier; the text of those summaries is formatted in grey font colour.
3. For any referenced old study, its bibliographic information (e.g. author, year, document number) is formatted in grey font colour.
4. For any new study, its bibliographic information and its free flow summary text and table content is formatted in standard black font colour.

Where applicable, the above formatting rules above apply to all dossier elements (e.g. MCA, MCP, JCA etc.).

According to the guidance of EFSA on the "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" (EFSA Journal 2011; 9(2):2092), literature for the active substance and its metabolites needs to be presented, covering the last 10 years prior to the submission of this Annex I renewal dossier. In relation to this section 8 no adequate scientific peer-reviewed open literature was identified which would need to be reported. There were no findings in the scientific peer-reviewed open literature for the active substance fluoxastrobin and its metabolites which might have a possible impact on an end-point or the risk assessments.

For substance codes, synonyms and abbreviations please refer to '*Document N3 - 'Substances and metabolites: structure, codes, synonyms – Fluoxastrobin*'.

All new studies which will be used in the risk assessment are marked in the respective tables in bold.

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

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

Compartment	Residue Definition
Soil	fluoxastrobin (*E*- isomer), HEC 5725 -*Z*-isomer, HEC 5725-carboxylic acid (*M40*), HEC 5725-*E*-des-chlorophenyl (*M48-E*), 2-chlorophenol (*M82*)
Groundwater	fluoxastrobin (*E*-isomer), HEC 5725-*Z*-isomer, HEC 5725-carboxylic acid (*M40*), HEC 5725-*E*-des-chlorophenyl (M48-*E*), 2-chlorophenol (M82)
Surface water	fluoxastrobin (*E*- isomer), HEC 5725-*Z*-isomer, HEC 5725-carboxylic acid (*M40*), HEC 5725-*E*-des-chlorophenyl (*M48-E*)
Sediment	fluoxastrobin (*E*- isomer), HEC 5725-*Z*-isomer
Air	none

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

In addition, a list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound fluoxastrobin, is presented in Document N3 of this dossier.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

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

Table CA 8.1- 1: Endpoints used in risk assessment and additional studies for fluoxastrobin

Test substance	Test species	Endpoint	Reference
Fluoxastrobin	acute, oral *Colinus virginianus* (Bobwhite quail)	LD_{50} > 2000 mg a.s./kg bw $LD_{50\ extrapol}$ **≡ 3776 mg/kg bw[1)]**	[redacted]; 2003; M-024735-02-1
	5-dietary *Colinus virginianus* (Bobwhite quail)	LC_{50} > 5000 mg/kg diet LDD_{50} > 766 mg a.s./kg bw/d	[redacted]; 2003; M-054779-02-1
	5-dietary *Anas platyrhynchos* (Mallard duck)	LC_{50} > 5000 mg/kg diet LDD_{50} > 2294 mg a.s./kg bw/d	[redacted]; 2003; M-056071-02-1
	Reprod. 6 w dietary *Colinus virginianus* (Bobwhite quail)	NOEC 1000 mg/kg diet NOEL 76 mg a.s./kg bw/d	[redacted]M; 2001; M-037404-01-1
	Reprod. 6 w dietary *Anas platyrhynchos* (Mallard duck)	NOEC 461 mg/kg diet NOEL **51 mg a.s./kg bw/d**	[redacted]; 2003; M-087968-01-1

Bold letters – values considered relevant for risk assessment

1) LD_{50} extrapolated with EFSA GD factor 1.888 (10 birds, no mortality; EFSA GD Birds & Mammals (2009), Section 2.1.2, Tab. 1)

CA 8.1.1.1 Acute oral toxicity to birds

No additional studies were performed. Please refer to corresponding section in the Draft Assessment Report (DAR), addenda and to the studies in the baseline dossier provided by Bayer CropScience.
The following endpoint from a study evaluated during the first EU review (EFSA Scientific Report (2007)) is used for the risk assessment.

Table CA 8.1.1.1- 1: Avian acute oral toxicity data of fluoxastrobin

Test substance	Test species	Endpoint	Reference
Fluoxastrobin	acute, oral *Colinus virginianus* (Bobwhite quail)	LD_{50} > 2000 mg a.s./kg bw $LD_{50\ extrapol}$ **≡ 3776 mg/kg bw[1)]**	[redacted]; 2003; M-024735-02-1

Bold letters – value considered relevant for risk assessment

1) LD_{50} extrapolated with EFSA GD factor 1.888 (10 birds, no mortality; EFSA GD Birds & Mammals (2009), Section 2.1.2, Tab. 1)

CA 8.1.1.2 Short-term dietary toxicity to birds

No additional studies were performed. Please refer to corresponding section in the Draft Assessment Report (DAR), addenda and to the studies in the baseline dossier provided by Bayer CropScience. Details of the studies are provided in the following table.

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

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	5-dietary *Colinus virginianus* (Bobwhite quail)	LC_{50} LDD_{50}	> 5000 mg/kg diet > 966 mg a.s./kg bw/d	[redacted]; 2003; M-054779-02-1
	5-dietary *Anas platyrhynchos* (Mallard duck)	LC_{50} LDD_{50}	> 5000 mg/kg diet > 2194 mg a.s./kg bw/d	[redacted]; 2003; M-056071-02-1

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the Draft Assessment Report (DAR), addenda and to the studies in the baseline dossier provided by Bayer CropScience. Details of the studies already evaluated are provided in Table CA 8.1.1.3- 1.
In addition, a chronic toxicity study on mallard duck has been performed with the active substance fluoxastrobin. This study is summarized below.

Table CA 8.1.1.3- 1: Avian long-term toxicity of fluoxastrobin

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	Reprod. 6 w dietary *Colinus virginianus* (Bobwhite quail)	NOEC NOEL	1000 mg/kg diet 124 mg a.s./kg bw/d	[redacted]; 2001; M-037404-01-1
	Reprod. 6 w dietary *Anas platyrhynchos* (Mallard duck)	NOEC NOEL	501 mg/kg diet **42 mg a.s./kg bw/d**	[redacted]; [redacted]; 2003; M-087968-01-1

Bold letters – value considered relevant for risk assessment

CA 8.1.2 Effects on terrestrial vertebrates other than birds

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

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

Test substance	Test design	Ecotoxicological endpoint	Reference
Fluoxastrobin	acute, oral Rat	LD_{50} **> 2000 mg a.s./kg bw**	[redacted]; 1996; M-012717-01-1
	acute, oral Rat	LD_{50} > 2000 mg as/kg bw	[redacted]; 1998; M-012735-01-1
	Long-term (2-gen. repro. study) Rat	NOAEC 10000 mg a.s./kg diet NOAEL 742 (F) – 764 (M) mg a.s./kg bw/d	[redacted]; 2004; M-088589-02-1
	90-d study Rat	NOAEC 2000 mg a.s./kg diet (F) NOAEL **163** mg a.s./kg bw/d	[redacted]; 1998; M-012710-01-1

Bold letters – values considered relevant for risk assessment

CA 8.1.2.1 Acute oral toxicity to mammals

Please refer to the toxicology section in the Draft Assessment Report (DAR) and to the studies in the baseline dossier (CA 5) provided by Bayer CropScience. Details of the studies are provided in the following table.

Table CA 8.1.2.1- 1: Acute oral toxicity data for mammals exposed to fluoxastrobin and its metabolites

Test substance	Test design	Ecotoxicological endpoint	Reference
Fluoxastrobin	acute, oral Rat	LD_{50} **> 2000 mg as/kg bw**	[redacted]; 1996; M-012717-01-1
	acute, oral Rat	LD_{50} > 2000 mg as/kg bw	[redacted]; 1998; M-012735-01-1

Bold letters – value considered relevant for risk assessment

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

No additional studies were performed. Please refer to the corresponding section in the Draft Assessment Report (DAR) and to the studies in the baseline dossier provided by Bayer CropScience.
During the first EU review (EFSA Scientific Report (2007)) the NOAEL for reproductive performance of 10000 ppm (mg/kg bw/d) in the rat two generation reproduction study had been identified for use in the risk assessment under the previous EU guidance document on risk assessment for birds and wild mammals (SANCO/4145/2000; 2002).
However, more recent guidance from EFSA recommended to take into account also other endpoints from the toxicological data set generated in laboratory studies with rodents and rabbits.
Therefore a more detailed review of the data relevant for wild mammal reproductive risk assessment endpoint determination has been prepared by [redacted] ([redacted]; 2015; M-535973-01-1).

Based on this evaluation of the toxicological effect profile with relevance to wild mammal population level risk assessment, the relevant NOAEL for TER_{LT} calculation would best be selected as the dose level of 2000 ppm (corresponding to 163 mg/kg bw/d) for female rat in the 90-d study with dietary exposure.
Alternatively, the dose level of 100 mg/kg bw/d from the developmental toxicity studies in rat and in rabbit could be considered appropriate for use in the reproductive risk assessment, if an endpoint from a reproductive toxicity study is preferred.

Table CA 8.1.2.2- 1: Mammals long-term toxicity of fluoxastrobin

Test substance	Test design	Ecotoxicological endpoint		Reference
Fluoxastrobin	Long-term (2-gen. repro. study) Rat	NOAEC NOAEL	10000 mg a.s./kg diet 742 (F) – 764 (M) mg a.s./kg bw/d	[redacted]; [redacted]; 2004; M-088589-02-1
	90-d study Rat	NOAEC NOAEL	2000 mg a.s./kg diet (F) **163** mg a.s./kg bw/d	[redacted]; [redacted]; 1998; M-012710-01-1
	Expert evaluation	Wild mammal long-term risk assessment endpoint	**163** mg a.s./kg bw/d	[redacted]; 2015; M-535973-01-1 KCA 8.1.2.2

Bold letters – values considered relevant for risk assessment

Report: KCA 8.1.2.2/02 [redacted] T; 2015; M-535973-01-1
Title: Fluoxastrobin - Toxicity endpoint for the wild mammal reproductive risk assessment
Report No.: M-535973-01-1
Document No.: M-535973-01-1
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

In the scope of the first EU review of fluoxastrobin the agreed endpoint addressing the long-term risk for wild mammals has been based on results of a 2-generation reproduction study in rats where no adverse effects on the reproductive performance were detected at the high dose level (10000 ppm, equivalent to ~750 mg/kg bw/day). This value was agreed as the suitable endpoint for wild mammals despite the fact that at this dose level non-reproductive, but other adverse effects were seen in parent and offspring animals.

Since this last review of fluoxastrobin, EFSA has published guidance how to derive appropriate end-points for the wild mammal risk assessment. Available studies should be evaluated in an "integrative way" and the dose-effect relationship needs to be considered for the selection of the ecotoxicologically relevant NOAEL.

The current position paper analyses the crucial toxicological studies with regard to the relevance of findings for the wild mammal risk assessment. In essence the data as presented in the draft assessment report compiled by the Rapporteur Member State UK are considered for this. The objective is to propose an appropriate NOAEL that should be used for the wild mammal risk assessment.

An overview of the toxic effects seen with fluoxastrobin in the 90-day feeding, reproduction and developmental toxicity studies is provided in Table CA 8.1.2.2- 2 whereas Table CA 8.1.2.2- 3 provides an overview on the dose-effect relationship seen with fluoxastrobin in these studies by listing treatment related findings in a dose-dependent way. The data would indicate the following assessments:

• With fluoxastrobin no adverse effect on reproductive performance was seen in the rat and rabbit developmental toxicity study and in the rat reproduction toxicity study. Up to the highest dose level tested, the number of pups and their survival until weaning were similar to that of the untreated control group.

• Body weight gain of pups, however, was substantially reduced at 10000 ppm. During the lactation phase pups gained 25.6% less weight than those of the control group. This effect is considered to be of possible ecotoxicological relevance. The retarded development resulted in delayed sexual maturation of male pups.

Table CA 8.1.2.2- 2: Summary of subchronic, reproduction and developmental toxicity studies with fluoxastrobin

Study type species dose levels tested ppm / ***mg/kg bw/day***	**Overall NOAEL**	Findings at Lowest Effect Level	**Ecotox NOAEL**	Ecotox relevant findings
90 day feeding Wistar rat ♂: 0 – 125 – 1000 - 8000 ppm ***0 – 9 – 70 - 580*** *mg/kg* ♀: 0 – 250 – 2000 - 16000 ppm **0 – 22 – 163 - 1416** *mg/kg*	♂: 1000 ppm ♀: 2000 ppm	♂: bw ↓, liver parameter ↓ at 8000 ppm ♀: liver & red blood cell parameter ↓ at 16000 ppm	♂: 1000 ppm ♀: 16000 ppm	♂: bw ↓ at 8000 ppm ♀: no ecotox relevant findings
2-generation reproduction Wistar rat 0 – 100 – 1000 – 10000 ppm **♂: 0 – 7 – 74 – 764** mg/kg§ **♀: 0 – 8 – 87 – 871** mg/kg§	parental: 1000 ppm reprod.: 10000 ppm pups: 1000 ppm	bw ↓ at 10000 ppm no adverse findings pup weights ↓ at 10000 ppm	1000 ppm	bw of parents and pups ↓
developmental rat Wistar rat **0 – 100 – 300 – 1000** mg/kg	maternal: 1000 mg/kg developm.: 1000 mg/kg	no adverse findings	1000 mg/kg	no ecotox relevant findings
developmental rabbit Himalayan rabbit **0 – 25 – 100 – 400** mg/kg	maternal: 25 mg/kg developm.: 400 mg/kg	FC (↓) at 100 mg/kg no adverse findings	400 mg/kg	no ecotox relevant findings

§ premating phase; bw = body weights; FC = food consumption

• In the reproduction study moderately lower body weights were seen also in parent animals of the high dose level. At the end of the premating phase males were 6.6 % and females 4.2 % lighter than corresponding control group animals. At the end of the gestation phase high dose females were 8.4% lighter than control.

• Male rats showed substantially lower body weights at 8000 ppm in the 90-day feeding study. A marginal body weight difference at 1000 ppm is attributed to the lower start weight of this dose group.

• In the 90-day feeding study body weights were unaffected in females even at 16000 ppm. The slightly lower red blood cell parameter are of limited ecotoxicological relevance. This holds true for the increased liver weights which are to be seen as physiological adaptation of the organ to an increased metabolic burden and not as an adverse toxic effect.

• In females a clear NOAEL for lower body weights was established at 2000 ppm. The marginally lower liver parameter at this dose level are of no toxicological or ecotoxicological relevance.

Table CA 8.1.2.2- 3: Dose-effect relationship in subchronic, reproduction and developmental toxicity studies

study	ppm	[mg/kg bw/day] ♂ / ♀	findings
reproduction rat	100	7 / 8	NOAEL
90 day feeding rat ♂ / ♀	125 / 250	9 / 22	NOAEL
development rabbit		25	NOAEL
90 day feeding rat ♂	1000	70	males: $NOAEL_{ecotox}$; liver parameter ↓
reproduction rat	1000	74 / 87	$NOAEL_{parents}$, $NOAEL_{ecotox}$
development rabbit		100	FC (↓)
90 day feeding rat ♀	2000	**163**	females: $NOAEL_{ecotox}$; liver parameter ↓
development rabbit		400	$NOAEL_{ecotox}$; FC (↓), bw (↓)
90 day feeding rat ♂	8000	580	males: bw ↓ (-16%), FC ↓
reproduction rat	10000	764 / 871	parent animals: bw ↓ (-4 to -7%); liver weight ↑; pups: bw ↓ (-25%); delayed sexual maturation; thymus & spleen weight ↓; $NOAEL_{reproduction}$
development rat		1000	NOAEL
90 day feeding rat ♀	16000	1416	females: red blood cell parameter ↓; liver weight ↑

↓: decrease; (↓): slight decrease; ↑: increase; bw: body weight, FC: feed consumption

Conclusion
With fluoxastrobin no adverse effects on reproductive performance of rats were seen at 10000 ppm, equivalent to 764 / 871 mg/kg bw/day in males / females. Body weight development of pups, however, was substantially retarded at this dose level so that from an ecotoxicology perspective 10000 ppm are to be considered as a possible adverse effect level.

Up to the highest dose level tested in the rat and rabbit developmental toxicity studies no adverse findings were obtained that need to be considered for the selection of the appropriate NOAEL with relevance for wild mammals.

As for fluoxastrobin the most sensitive finding with ecotoxicological relevance was not related to direct reproductive toxicity, it is considered justified to set the wild mammal endpoint on basis of data from the 90-day feeding study in rats. At 2000 ppm no adverse findings with ecotoxicological relevance were obtained in female rats. Lower food consumption and retarded body weight development occurred in male rats only at a 3 x higher dose level (8000 ppm, equivalent to 580 mg/kg bw/day).

Proposed wild mammal endpoint: **$\text{NOAEL}_{\text{females, 90-day feeding}}$: 163 mg/kg bw/day** (2000 ppm)

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

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log $P_{OW} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation. As the log P_{OW} of the active substance fluoxastrobin and its metabolites is below the trigger, the potential for bioaccumulation is low and an evaluation of secondary poisoning is not required.

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

Information on effects of fluoxastrobin on terrestrial reptiles is not available. Data on amphibians is given under 8.2.8. Effects on birds and mammals are described in this MCA document and the risk is evaluated in the MCP documents.

CA 8.1.5 Endocrine disrupting properties

Wild Mammals

A detailed analysis of all apical toxicological studies (subchronic, chronic / oncogenicity, reproduction and developmental toxicity, see MCA 5) on fluoxastrobin revealed no endocrine disrupting effect. Therefore, based on a complete toxicological data set, there is no evidence for endocrine disrupting properties of fluoxastrobin in mammals.

Birds

The population relevant effects of fluoxastrobin on birds were studied in reproductive toxicity studies on Bobwhite quail and Mallard ducks. In the study on Bobwhite quails no statistically significant effects on adult birds, offspring or reproductive parameters were found at 1000 mg fluoxastrobin/kg diet, the highest dietary concentration tested. Mallard ducks tolerated a dietary concentration of 461 mg fluoxastrobin/kg diet without any effect on reproductive parameters or body weight. At the highest tested concentration of 1394 mg fluoxastrobin/kg diet terminal adult female body weights, number of eggs laid per hen, and eggs set per hen were statistically significant lower than in the control. There was no indication of an endocrine mediated effect in these studies.

As there have been established levels at which reproduction was not affected in two avian species, it is concluded that based on an appropriate risk assessment there are no population relevant adverse effects of fluoxastrobin.

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test protocol is available, this test was developed to evaluate potential effects on the thyroid system, and not to measure population relevant effects.

Therefore, no further studies can be suggested at this time for these groups of organisms.

Conclusion

Neither in mammals, nor birds, indications for endocrine activity were observed. Based on the absence of relevant effects it can be concluded that fluoxastrobin is not a (potential) endocrine disrupter.

Therefore, further special testing for endocrine disrupting properties is not warranted.

CA 8.2 Effects on aquatic organisms

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

For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the amendments to the Draft Assessment Report (DAR) and to the studies in the baseline dossier provided by Bayer CropScience. The chronic ecotoxicity of fluoxastrobin to aquatic invertebrates was also confirmed in two additional studies performed with the aquatic invertebrates *Neocaridina* and *Habrophlebia*.

The degradation pathways in soil and water and sediment are given in the two figures below. In this paragraph we specifically consider the approach to the risk assessment of the Z-isomer of fluoxastrobin, The chemical structure of fluoxastrobin contains an oxime ether moiety. Due to the substitution pattern of that double bond E- and Z-isomers exist. The common name fluoxastrobin denotes the *E*-isomer. The Z-isomer is known to be an impurity in technical fluoxastrobin (specification limit 2 mg/kg). The Z-isomer can be formed from the E-isomer by photolytic processes exclusively. The transformation will lead to an equilibrium state in which the E-isomer is the more stable and energetically preferred isomer (ratio in aqueous solution about 10:1 = E / Z). In the environment the Z-isomer shows very similar degradation behaviour and a better soil sorption than the E-isomer. Further, the Z-isomer shows a very similar toxicological profile. A study with *Daphnia magna* performed with an increased amount of Z-Isomer (isomer ratio (E/Z) = 65/35a demonstrated an at least comparable, potentially lower ecotoxicological profile than the parent E-isomer, demonstrating that there is no further risk for the aquatic compartment (please refer to CA 8.2.4.1 M-030533-01-1). Taking this information into account, both isomers can be evaluated as sum of E+Z-isomers, providing a conservative environmental risk assessments..

To complete the aquatic data package, information of relevant ecotoxicological endpoints is presented for the major soil metabolite 2-chlorophenol which can be transported from soil to surface water bodies via run-off and drainage events. For further details reference is made to Section 7: "Fate and behaviour in the environment". Summaries of the aquatic studies are provided in Table CA 8.2- 1.

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

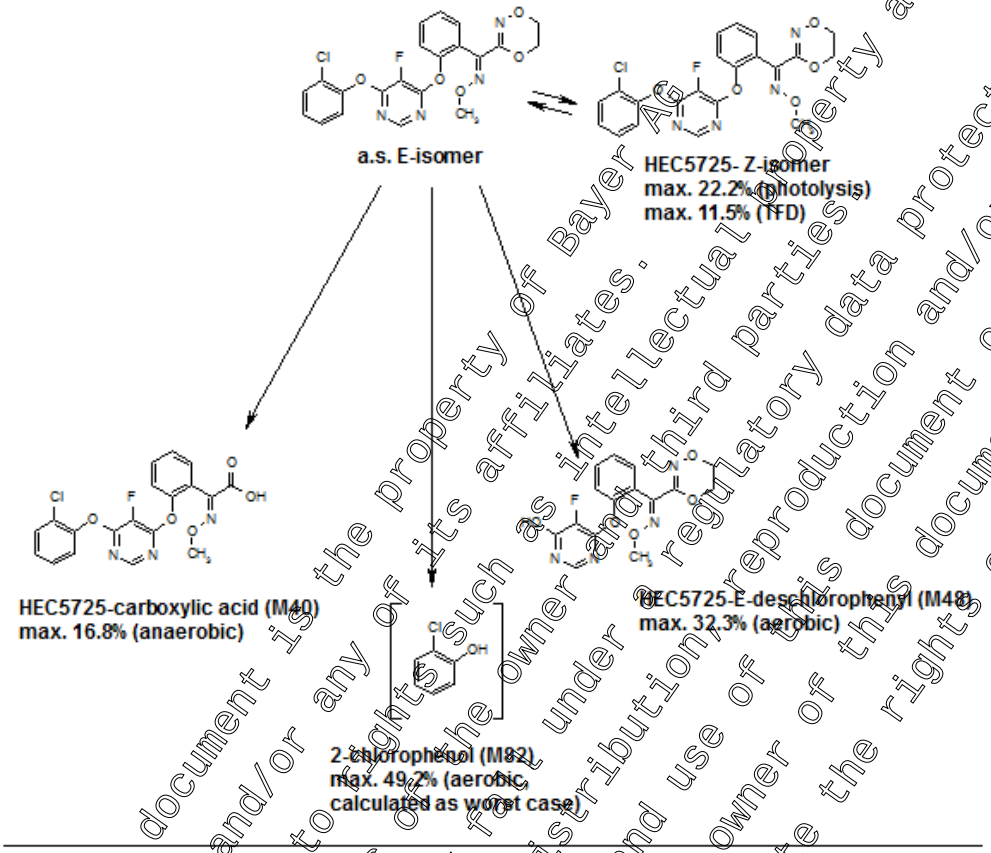


Figure 8.2-2: Proposed degradation pathway of fluoxastrobin in aqueous systems (major degradation products)

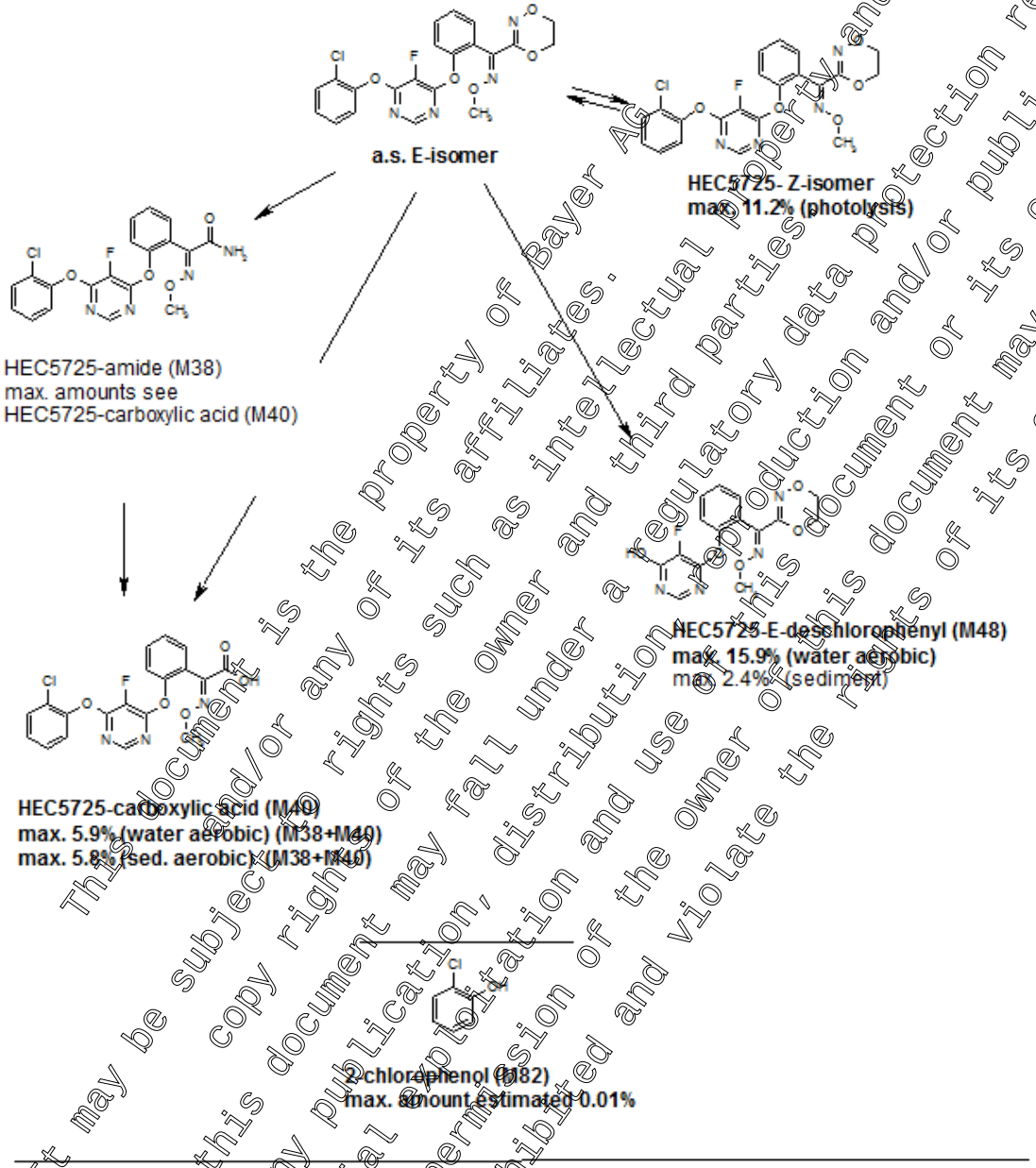


Table CA 8.2- 1: Aquatic toxicity data for fluoxastrobin and its metabolites

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50}**	**0.435 mg a.s./L**	[redacted]; 1999; M-016770-01-1
	Fish, acute *Lepomis macrochirus* (bluegill sunfish)	LC_{50}	0.97 mg a.s./L	[redacted]; 1999; M-018576-01-1
	Fish, acute *Cyprinus carpio* (common carp)	LC_{50}	0.57 mg a.s./L	[redacted]; 2000; M-031605-01-1
	Marine fish, acute *Cyprinodon variegatus* (sheepshead minnow)	LC_{50}	> 1.374 mg a.s./L	[redacted]; 2002; M-082981-01-1 KCA 8.2.1
	Fish, chronic *Oncorhynchus mykiss* (rainbow trout)	NOEC	**0.0286 mg a.s./L**	[redacted]; 2001; M-084463-01-1
	Fish, flow-through *Lepomis macrochirus* (bluegill sunfish)	$BCF_{parent\ (whole\ fish\ wet\ weight)}$ $BCF_{parent\ (whole\ fish\ wet\ weight)}$	1.2 13.3	[redacted]; 2001; M-080588-02-1
	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**9.48 mg a.s./L**	[redacted]; 1999; M-011257-01-1
	Invertebrate, acute *Daphnia magna* (cladoceran)	EC_{50}	0.94 mg a.s./L	[redacted]; 2002; M-030533-01-1
	Invertebrate, acute *Gammarus pulex* (amphipod)	EC_{50}	0.6 mg a.s./L	[redacted]; 2003; M-109491-01-2
	Acanthocyclops venustus (copepod)	EC_{50}	0.9 mg a.s./L	
	Oecetis sp. (mayfly)	EC_{50}	2.0 mg a.s./L	
	Daphnia gr. pulex (cladoceran)	EC_{50}	1.3 mg a.s./L	
	Asellus aquaticus (isopod)	EC_{50}	1.3 mg a.s./L	
	Chaoborus obscuripes (diptera)	EC_{50}	> 3.2 mg a.s./L	
	Simocephalus vetulus (cladoceran)	EC_{50}	> 3.2 mg a.s./L	
	Marine invertebrate, acute *Americamysis bahia* (*Mysidopsis bahia*, mysid shrimp)	LC_{50}	0.0604 mg a.s./L	[redacted]E; [redacted]; [redacted]; 2002; M-082793-01-1
	Invertebrate, acute geometric mean using 7 species	**EC_{50}**	**0.488 mg a.s./L[1)]**	See. MCA 8.2.4.2

Test substance	Test species	Endpoint		Reference
	Invertebrate, chronic *Daphnia magna* (cladoceran)	**NOEC**	**0.18 mg a.s./L**	[redacted]か; 2000; M-042059-01-1
	Invertebrate, chronic *Gammarus pulex* (amphipod) (conducted with EC 100 formulation)	NOEC	0.0316 mg a.s./L	[redacted]; 2003; M-110286-01-1
	Invertebrate, chronic *Habrophlebia lauta* (Mayfly)	NOEC	0.0422 mg a.s./L	[redacted]X; 2012; M-444119-01-1 KCA 8.2.5.2
	Invertebrate, chronic *Neocaridina heteropoda* (Freshwater shrimp)	NOEC	0.060 mg a.s./L	[redacted]; 2012; M-442121-01-1 KCA 8.2.5.2
	Marine invertebrate, chronic *Americamysis bahia* (*Mysidopsis bahia*, mysid shrimp)	$NOEC_{survival}$ $NOEC_{repro}$	0.0001 mg a.s./L 0.047 mg a.s./L	[redacted]; [redacted]; [redacted]; 2002; M-082820-01-1
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	**EC_{15}**	**2.3 mg a.s./L**	[redacted]; 2000; M-042042-01-1
	Pseudokirchneriella subcapitata (green algae)	E_bC_{50} **E_rC_{50}**	0.35 mg a.s./L **0.10 mg a.s./L**	[redacted]; 2000; M-033313-01-1
	Lemna gibba (Duckweed)	E_bC_{50} **E_rC_{50}**	> 6.0 mg a.s./L **> 6.0 mg a.s./L**	[redacted]; 2001; M-037727-01-1
	Lemna gibba (Duckweed)	E_bC_{50} E_rC_{50}	1.45 mg a.s./L 3.88 mg a.s./L	[redacted]; [redacted]; 2002; M-083021-01-1 KCA 8.2.7
HEC 5725-E-des-chlorophenyl	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50}**	**> 102 mg p.m./L**	[redacted]; 2000; M-033495-01-1
	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**> 100 mg p.m.//L**	[redacted]; 2000; M-038222-01-1
	Pseudokirchneriella subcapitata (green algae)	E_bC_{50} **E_rC_{50}**	> 100 mg p.m.//L **> 100 mg p.m.//L**	[redacted]み; 2000; M-025012-01-1
HEC 5725-carboxylic acid	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50}**	**> 95.7 mg p.m./L**	[redacted]; 2001; M-052093-01-2
	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**> 100 mg p.m./L**	[redacted]; [redacted]; 2001; M-030332-01-1
	Sediment dweller, chronic *Chironomus riparius* (chironomid)	**EC_{15}**	**98.5 mg p.m./L**	[redacted]; [redacted]; 2001; M-078605-01-1

Test substance	Test species	Endpoint		Reference
	Pseudokirchneriella subcapitata (*Selenastrum capricornutum*, green algae)	E_bC_{50} **E_rC_{50}**	115 mg /L **> 160 mg p.m./L**	[redacted]; [redacted] 2001; M-073836-01-1
2-chlorophenol	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50}**	**2.6 mg p.m./L**	[redacted].; 2006; M-277036-01-1 KCA 8.2.1
	Fish, chronic *Pimephales promelas* (fathead minnow)	**NOEC**	**4 mg p.m./L**	EFSA Scientific Report 102 (2007) [redacted].; 2006; M-277036-01-1
	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**7.4 mg /L**	[redacted].; 2006; M-277036-01-1 KCA 8.2.1
	Invertebrate, chronic *Daphnia magna* (cladoceran)	**NOEC**	**0.3 mg p.m./L**[2)]	EFSA Scientific Report 102 (2007) [redacted].; 2006; M-277036-01-1
	Pseudokirchneriella subcapitata (*Selenastrum capricornutum*, green algae)	**E_rC_{50}**	**70 mg p.m./L**	EFSA Scientific Report 102 (2007) [redacted].; 2006; M-277036-01-1

Bold letters – values considered relevant for risk assessment

1) When using the above acute invertebrate toxicity data (including Mysid, excluding the two "greater than" values), with the geomean approach according to the most recent aquatic guidance document (SANTE-2015-00080, 15 January 2015) a geometric mean value of 0.488 mg a.s./L can be calculated

2) In the statement on the exposure of aquatic organisms to 2-chlorophenol [redacted]; 2006; M-277036-01-1) a NOEC of 0.5 mg/L is presented as most sensitive chronic endpoint for Daphnia based on nominal concentrations applied during testing. According to the EFSA Scientific Report (2007) the minimum measured concentration of 0.3 mg/l must be considered as relevant endpoint.

CA 8.2.1 Acute toxicity to fish

For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR).

An additional study is available addressing the toxicity of the active substance fluoxastrobin on the marine fish *Cyprinodon variegatus* and is submitted within this Supplementary Dossier for renewal of approval of fluoxastrobin.

Information regarding the toxicity of the metabolite 2-chlorophenol to aquatic organisms was summarised in a statement by [redacted]F; 2006; M-277036-01-1 (including selection of five relevant chronic and acute endpoints). The information was already evaluated during the first EU review of fluoxastrobin and three of the respective endpoints were included in the EFSA list of relevant endpoints. For reasons of completeness and simplicity the information was summarised again to include the remaining two endpoints in the re-evaluation process. The summary for the aquatic compartment is presented under CA 8.2.1 as this is the first trophic level. Endpoints regarding toxicity of 2-chlorphenol on other aquatic organisms are also reported individually under the respective trophic level.

Details of all studies regarding acute endpoints on fish are provided in the following table.

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

Test substance	Test species	Endpoint	Reference
Fluoxastrobin	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50} 0.435 mg a.s./L**	[redacted]; 1999; M-016770-01-1
	Fish, acute *Lepomis macrochirus* (bluegill sunfish)	LC_{50} 0.97 mg a.s./L	[redacted]; 1999; M-018576-01-1
	Fish, acute *Cyprinus carpio* (common carp)	LC_{50} 0.37 mg a.s./L	[redacted]; 2000; M-031605-01-1
	Marine fish, acute *Cyprinodon variegatus* (sheepshead minnow)	LC_{50} > 1.374 mg a.s./L	[redacted]; 2002; M-082981-01-1 KCA 8.2.1
HEC 5725-E-des-chlorophenyl	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50} > 102 mg p.m./L**	[redacted]; 2000; M-033495-01-1
HEC 5725-carboxylic acid	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50} > 95.7 mg p.m./L**	[redacted]; 2001; M-052093-01-2
2-chlorophenol	Fish, acute *Oncorhynchus mykiss* (rainbow trout)	**LC_{50} 2.6 mg p.m./L**	[redacted]; 2006; M-277036-01-1 KCA 8.2.1

Bold letters – values considered relevant for risk assessment

Report: KCA 8.2.1/06 [redacted]; 2002; M-082981-01-1
Title: Acute toxicity of HEC 5725 (technical) to the sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No.: 110322
Document No.: M-082981-01-1
Guideline(s): USEPA, Pesticide Assessment Guidelines Subdivision E, FIFRA 72-3, Acute toxicity test for estuarine and marine organisms, October, 1982.
Guideline deviation(s): --
GLP/GEP: **yes**

Objective:

Aim of this study was to determine the acute toxicity of fluoxastrobin technical to the Sheepshead minnow (*Cyprinodon variegatus*). The primary measure for acute toxicity was mortality. Sublethal and behavioral effects were also assessed during the course of the study. Results of the test are expressed as a 96-hour median lethal concentration (LC_{50}).

Material and methods:

Test material: Fluoxastrobin (HEC 5725), technical; Batch No.: 898904001; Purity: 97.0%.
Sheepshead minnow (*Cyprinodon variegatus*) (mean length 21.2 mm, mean weight 0.31 g) were exposed to fluoxastrobin technical at nominal (mean measured) concentrations of 100 (80.7), 200 (169.5), 400 (356.0), 800 (684.0) and 1600 (1374) µg a.s./L (ppb), as well as a solvent control (< 10.0) and a control (< 10.0) under static conditions for 96 hours. One replicate of twenty fish each was used

at each test concentration. The test chambers were 38 liter glass aquaria with a dimension of 49.4 cm x 25.3 cm x 31.0 cm. The test temperature during the 96-hour exposure ranged from 21.6 to 23.3°C with a mean of 22.4°C as measured hourly by a data logger. A slight temperature deviation occurred from hours 35 to 40 in which the temperature exceed the desired ranged of 22.0 ± 1.0°C. The highest temperature recorded was 23.3°C which represents a deviation of only 0.3°C. It is very unlikely that this slight deviation had a negative impact on the study. Dissolved oxygen concentrations ranged from 6.0 to 7.7 mg/L representing 76.0 to 97.2 percent saturation, respectively, at 22°C. The pH values ranged from 7.2 to 7.7 and the salinity ranged from 16 to 17 ‰ (parts per thousand) throughout the test. The light cycle was programmed to produce an overall photoperiod of 16-hours light and 8-hours dark.
Daily observations were made for mortality and sublethal effects. Fish were not fed during the test.

Findings:

Analytical findings:
The mean measured concentrations during the test period ranged from 81 to 89 percent of the nominal concentration. The mean measured concentrations were 80.7, 169.5, 356.0, 684.0 and 1374 µg a.s./L for the nominal test levels of 100, 200, 400, 800 and 1600 µg a.s./L, respectively. No undissolved test substance was observed in the test chambers. All subsequent observations will refer to mean measured concentrations of the test solutions.

Biological findings:
No mortality, behavioral or sublethal effects were observed at any fluoxastrobin test level during the exposure period. Since no differences were observed between either of the control groups and any fluoxastrobin test level, no statistical comparisons could be made.

Table CA 8.2.1- 2: Cumulative mortality and behavioural observations of the Sheepshead minnows exposed to fluoxastrobin

Mean measured concentration [mg/L]	24 h		48 h		72 h		96 h	
	Dead	Observations	Dead	Observations	Dead	Observations	Dead	Observations
Control	0	20 N	0	20 N	0	20 N	0	20 N
Solvent control	0	20 N	0	20 N	0	20 N	0	20 N
80.7	0	20 N	0	20 N	0	20 N	0	20 N
169.5	0	20 N	0	20 N	0	20 N	0	20 N
356.0	0	20 N	0	20 N	0	20 N	0	20 N
684.0	0	20 N	0	20 N	0	20 N	0	20 N
1374	0	20 N	0	20 N	0	20 N	0	20 N

Key to Observations: N = Normal

Table CA 8.2.1- 3: Toxicity to Sheepshead minnow

Test substance	Fluoxastrobin technical
Test object	Sheepshead minnow
Exposure	96 hour, static
LC_{50} µg a.s./L	> 1374
Lowest observed effect concentration (LOEC) µg a.s./L	> 1374
No observed effect concentration (NOEC) µg a.s./L	1374

Conclusion:

The LC_{50} was determined to be > 1374 µg a.s./L.

Report:	KCA 8.2.1/07 [redacted],; 2006; M-277036-01-1
Title:	2-chlorophenol - Ecotoxicological risk assessment
Report No.:	M-277036-01-1
Document No.:	M-277036-01-1
Guideline(s):	Not applicable - Expert Statement
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective:

In the "Summary of the EFSA Scientific Report (2005) 47, 1-81, Conclusion on the peer review of fluoxastrobin (finalised: 10 August 2005)" EFSA asked for information regarding the toxicity of 2-chlorophenol to aquatic and soil organisms. In this statement suitable information on the exposure of aquatic organisms to 2-chlorophenol as a soil metabolite of fluoxastrobin after application of an application rate of annually 400 g fluoxastrobin per ha in cereals is provided and a risk assessment is performed.

Aquatic Risk Assessment

Toxicity data: Data on the acute and chronic toxicity to fish, daphnia and algae of 2-chlorophenol are available in open scientific literature. For this statement three documents were evaluated: Euro Chlor risk assessment for the marine environment, IUCLID Dataset, and the Bayer Chemicals Safety Data Sheet. All data presented in these three documents show a very consistent picture of the toxicity of 2-chlorophenol to aquatic organisms. Only those data which are considered to be the most reliable ones are considered in the risk assessment.

The endpoints used for the aquatic risk assessment (described in detail in the original report) are given in the table below.

Table CA 8.2- 1: Endpoints used for the aquatic risk assessment of 2-chlorophenol

Test substance	Test species	Endpoint used for the risk assessment [mg/L]		Reference
Acute				
2-chlorophenol	Fish, acute *Oncorhynchus mykiss* (Rainbow trout)	LC_{50} (96h)	2.6	[redacted],; 2006; M-277036-01-1
	Invertebrate, acute *Daphnia magna*	LC_{50} (48h)	7.4	[redacted],; 2006; M-277036-01-1
Chronic				
2-chlorophenol	Fish, chronic *Pimephales promelas* (Fathead minnow)	NOEC (> 4 weeks)	4	[redacted],; 2006; M-277036-01-1
	Invertebrate, chronic *Daphnia magna*	NOEC(21 d) (semi static)	0.3*	[redacted]S; 2006; M-277036-01-1
	Selenastrum capricornutum (green algae)	EC_{50} (96h)	70	[redacted],; 2006; M-277036-01-1

* A NOEC of 0.5 mg/L is used in the risk assessment of [redacted] (2006). According to the EFSA Scientific Report (2007) the minimum measured NOEC value of 0.3 mg/l is considered as relevant endpoint for the

renewal of approval of fluoxastrobin.

CA 8.2.2 Long-term and chronic toxicity to fish

For information on studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).
In an additional statement on the chronic risk of the metabolite 2-chlorophenol to the fish species *Pimephales promelas* (fathead minnow) a chronic endpoint is presented which is listed in the EFSA Scientific Report (2007). This endpoint is used for the risk assessment. Justification for providing this information again is given under CA 8.2.1.
Details of all studies are provided in the following table.

Table CA 8.2.2- 1: Chronic fish toxicity of fluoxastrobin and its metabolite 2-chlorophenol

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	Fish, chronic *Oncorhynchus mykiss* (rainbow trout)	NOEC	**0.0286 mg a.s./L**	[redacted].; 2001; M-084463-01-1
	Fish, flow-through *Lepomis macrochirus* (bluegill sunfish)	BCF_{parent} (whole fish wet weight) BCF_{parent} (whole fish wet weight)	18.2 13.3	[redacted]; [redacted]; 2001; M-080588-02-1
2-chlorophenol	Fish, chronic *Pimephales promelas* (fathead minnow)	**NOEC**	**4 mg p.m./L**	EFSA Scientific Report 102 (2007)) [redacted].; 2006; M-277036-01-1

Bold letters – values considered relevant for risk assessment

CA 8.2.2.1 Fish early life stage toxicity test

For the active substance fluoxastrobin no further studies are required. However, one additional study on the chronic risk of the metabolite 2-chlorophenol to the fish species *Pimephales promelas* (fathead minnow) was performed and is relevant to the assessment of the toxicity to fish. See point CA 8.2.2.

CA 8.2.2.2 Fish full life cycle test

No studies on fish full life cycle are available or required.

CA 8.2.2.3 Bioconcentration in fish

No additional studies were performed. Please refer to the corresponding section in the Draft Assessment Report (DAR) and to the studies in the baseline dossier provided by Bayer CropScience.

CA 8.2.3 Endocrine disrupting properties

Population relevant effects of fluoxastrobin on fish were studied in an early life-stage test (ELS, M-084463-01-1) with rainbow trout (O. mykiss) under continuous exposure, resulting in a NOEC of 28.6 µg/L. The NOEC was based on a transiently increased yolk-sac size of 10% of the fish fry (i.e. 6 out of 60) observed at the LOEC level (highest tested concentration) of 55.7 µg/L. At the end of the study

the number of fry with increased yolk-sac was comparable to control level and at the LOEC level there were no effects on other parameters like survival, growth (weight, length) or time to swim-up. The chronic fish NOEC of 28.6 µg/L is far above the regulatory acceptable concentration, which is driven by aquatic invertebrates.

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

CA 8.2.4 Acute toxicity to aquatic invertebrates

For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR).
Details of the studies are provided in the points below.

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

Additional information is available addressing the toxicity of the metabolite 2-chlorophenol on *Daphnia magna* and is submitted within this Supplementary Dossier for renewal of approval of fluoxastrobin. A summary as well as a justification for providing this information is given under MCA 8.2.1.

Details of all studies are provided in the following table.

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

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**0.48 mg a.s./L**	[redacted].; 1999; M-011257-01-1
	Invertebrate, acute *Daphnia magna* (cladoceran)	EC_{50}	0.94 mg a.s./L	[redacted]; [redacted]; 2002; M-030533-01-1
HEC 5725-E-des-chlorophenyl	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**> 100 mg p.m.//L**	[redacted].; 2000; M-038222-01-1
HEC 5725-carboxylic acid	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**> 100 mg p.m./L**	[redacted].; [redacted]; 2001; M-030332-01-1
2-chlorophenol	Invertebrate, acute *Daphnia magna* (cladoceran)	**EC_{50}**	**7.4 mg p.m./L**	[redacted]S; 2006; M-277036-01-1 KCA 8.2.1

Bold letters – values considered relevant for risk assessment

For a detailed summary of the statement please refer to CA 8.2.1 M-277036-01-1.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

In the course of the application, a screening study from [redacted] ([redacted].; 2003; M-087312-01-2) was mistakenly classified as relevant for this Supplementary Dossier for renewal of approval of fluoxastrobin. This non-GLP study was already evaluated during the first EU review and can be regarded as not relevant for the risk assessment and therefore, it is listed in the list of non-relevant studies (negative list). The study was performed again under GLP conditions to assess

the acute toxicity to aquatic invertebrates under GLP conditions. Please refer to corresponding sections in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR).

Details of all studies are provided in the following table.

Table CA 8.2.4.2- 1: Acute toxicity to aquatic invertebrates exposed to fluoxastrobin and its metabolites

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	Invertebrate, acute *Gammarus pulex* (amphipod)	EC_{50}	0.15 mg a.s./L	[redacted]; 2003; M-109491-01-2
	Acanthocyclops venustus (copepod)	EC_{50}	0.9 mg a.s./L	
	Cloeon dipterum (mayfly)	EC_{50}	1.0 mg a.s./L	
	Daphnia gr. galeata (cladoceran)	EC_{50}	1.3 mg a.s./L	
	Asellus aquaticus (isopod)	EC_{50}	1.3 mg a.s./L	
	Chaoborus obscuripes (diptera)	EC_{50}	> 3.2 mg a.s./L	
	Simocephalus vetulus (cladoceran)	EC_{50}	> 3.2 mg a.s./L	
	Marine invertebrate, acute *Americamysis bahia* (*Mysidopsis bahia*, mysid shrimp)	LC_{50}	0.0604 mg a.s./L	[redacted],; [redacted]; [redacted]; 2002; M-082793-01-1
	Invertebrate, acute geometric mean using 7 species	**EC_{50}**	**0.488 mg a.s./L***	-

Bold letters – values considered relevant for risk assessment
* When using the above acute invertebrate toxicity data (including Mysid, excluding the two "greater than" values), with the geomean approach according to the most recent aquatic guidance document (SANTE-2015-00080, 15 January 2015) a geometric mean value of 0.488 mg a.s./L can be calculated

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR).

Additional information is available addressing the toxicity of the metabolite 2-chlorophenol on *Daphnia magna* and is submitted within this Supplementary Dossier for renewal of approval of fluoxastrobin. A summary as well as a justification for providing this information is given under CA 8.2.1.
Details of all studies are provided in the following table.

Table CA 8.2.5.1- 1: Long-term toxicity to *Daphnia magna* exposed to fluoxastrobin and its metabolite 2-chlorophenol

Test substance	Test species	Endpoint	Reference
Fluoxastrobin	Invertebrate, chronic *Daphnia magna* (cladoceran)	**NOEC** **0.18 mg a.s./L**	[redacted], 2000, M-042059-01-1
2-chlorophenol	Invertebrate, chronic *Daphnia magna* (cladoceran)	**NOEC** **0.3 mg p.m./L**	EFSA Scientific Report 102 (2007); [redacted], 2006; M-277036-01-1

Bold letters – values considered relevant for risk assessment

Report: KCA 8.2.5.1/02[redacted], 2015; M-535147-01-1
Title: Statement on use of the time weighted average PEC values for fluoxastrobin based on chronic tests with aquatic invertebrates
Report No.: M-535147-01-1
Document No.: M-535147-01-1
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **no**

Objective:

In a pre-submission meeting with RMS UK it was agreed that a position paper would be developed to justify the use of PEC_{twa} in surface water risk assessment, in line with the EFSA guidance.
This position paper discusses the use of the PEC_{twa} for fluoxastrobin based on chronic aquatic invertebrate data.

Available chronic invertebrate data:

Table CA 8.2.5.1- 2: Existing chronic endpoints for aquatic invertebrate species based on mean measured or analytically confirmed constant concentrations

Test Organism	Test System	Substance	Endpoint [µg a.s./L]
Fluoxastrobin			
Chronic			
Daphnia magna	chronic, 21 d static renewal	a.s.	NOEC 180
Americamysis bahia	28 d, flow-through	a.s.	$NOEC_{Survival}$ 0.61 ($NOEC_{Reprod.}$ 4.7)
Neocaridina heteropoda (freshwater shrimp)	chronic, 21 d static renewal	a.s.	$NOEC_{Survival}$: 60
Habrophlebia lauta (mayfly larvae)	chronic 21 d static renewal	a.s.	$NOEC_{Survival\ (nom)}$: 64 $NOEC_{Survival\ (mm)}$: 42[1]
Gammarus pulex	28 d, static, water-sediment	EC 100	NOEC 31.6

[1] When used against a PEC_{twa}, the mean measured concentration should be used.

Requirements for using PEC_{twa}:

Chronic invertebrate data for Fluoxastrobin were checked against five conditions according to the most recent (2013) EFSA guidance, for usability of the PEC_{twa} refinement:

- *Maintenance of exposure in effect studies:*
  In all chronic invertebrate studies, exposure was maintained and analytically verified and in the one *Habrophlebia* (Mayfly larvae) study where recoveries were not within 80-120% of nominal, results were expressed as mean measured values.

- *Effects do not occur at specific sensitive life stages:*
  Reproductive parameters are unaffected in the study that yielded the lowest endpoint and drives the whole invertebrate assessment (marine Mysid shrimp study) and all developmental stages are included in this test. Effects very clearly are not due to developmental processes, but due to mortality after prolonged exposure.

- *Effects do not occur very early during the test:*
  After short term exposure, even up to 7 days (which is longer than the typical acute test) effect thresholds are clearly higher. Concentrations as high as 9 µg/L are needed to produce a shorter term effect.

- *Effects do not show latency:*
  There are no indications of latency or delayed effects.

- *Effects patterns show reciprocity: longer exposure leads to lower effect thresholds:*
  To further support this, we have re-analysed the toxicity test quantitatively (i.e. statistically) using, if possible, effect thresholds (NOECs or EC_{10}) and also EC_{20} values over exposure time. The relationship between time and effect threshold is visible not only in the test yielding the lowest endpoint that drives the risk assessment, but in all five chronic tests. As the NOEC by nature has to be one of the tested concentrations, and as it also depends on test power, this relationship is less clear, although in most tests NOECs do decline with increasing test power. For the EC_{10}, which (as a point estimate) is derived from the whole data set and can assume any value, the reciprocity is "smoother".

Conclusion:

All these conditions were shown to be fulfilled. Hence a 7d $PEC_{sw;twa}$ is considered appropriate as a risk assessment refinement.

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

For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR).
Three additional studies on the aquatic invertebrates *Habrophlebia lauta*, *Neocaridina heteropoda* and *Americamysis bahia* and one position paper are presented here, additional studies on Chironomus species are presented later.
Details of all studies are provided in the following table.

Table CA 8.2.5.2- 1: Long-term toxicity to additional aquatic invertebrates exposed to fluoxastrobin and its metabolites

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	Invertebrate, chronic *Habrophlebia lauta* (Mayfly)	NOEC	0.0422 mg a.s./L	[redacted].; 2012; M-444119-01-1
	Invertebrate, chronic *Neocaridina heteropoda* (Freshwater shrimp)	NOEC	0.060 mg a.s./L	[redacted].; 2012; M-442121-01-1
	Marine invertebrate, chronic *Americamysis bahia* (*Mysidopsis bahia*, mysid shrimp)	$NOEC_{survival}$ $NOEC_{repro}$	0.00061 mg a.s./L 0.0047 mg a.s./L	[redacted]; 2002; M-082820-01-1

Bold letters – values considered relevant for risk assessment

Report: KCA 8.2.5.2/02 [redacted].; 2012; M-444119-01-1
Title: Chronic bioassay with Habrophlebia lauta with fluoxastrobin (tech.)
Report No.: MEHEN001
Document No.: M-444119-01-1
Guideline(s): None Standardised Guideline
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

A chronic toxicity test was performed to identify possible effects of the test item on survival and growth development of *Habrophlebia lauta* over 21 days under static-renewal exposure, expressed as chronic NOEC for larval health and growth.

Material and methods:

Test item: Fluoxastrobin, technical; Batch code: AE 1228646-01-01; Specification No.: 102000008981; CAS No.: 361377-29-9; Origin Batch No.: PF90232555; LIMS No.: 1119470; Customer Order No.: TOX 09429-00; Purity (content): 98.8 % w/w.
Habrophlebia lauta (larvae, 10 animals per replicate, three replicates per study group), exposed in a static-renewal test system (weekly renewal interval) for 21 days to nominal concentrations of 0, 8, 16, 32, 64, 128, 256 and 512 µg a.s./L. There was also a control of the untreated test medium M4 only (six replicates). As endpoints, the rate of survivors and their body-length at the end of the study were recorded as database for NOEC / LOEC / MATC calculation. For verification of the actual test item concentrations during exposure, water-samples from start and end of 3 exposure-intervals were analysed.

Dates of experimental work: May 11, 2012 to August 20, 2012

Findings:

Validity criteria:
Although no validity criteria were available for this type of study, the mortality rate in the control revealed to be lower than the validity criteria for the chronic assay with Daphnia magna. In conclusion, the study performed with *Habrophlebia lauta* was proven to be valid.

Biological findings:

Table CA 8.2.5.2- 2: Biological Results (mean-values by study group)

Treatment [µg a.s./L] (nominally)	Endpoints			
	Body length on day 21 (mm)	Survival after 7 days (%)	Survival after 14 days (%)	Survival after 21 days (%)
Control	3.72	95.0	93.3	93.3
8	4.19	90.0	90.0	86.7
16	4.31	100.0	100.0	100.0
32	4.02	90.3	90.3	83.9
64	4.03	100.0	96.7	83.3
128	3.86	93.3	83.3	73.3
256	3.61	96.7	86.7	73.3
512	3.99	93.3	56.7	43.3

The biological endpoints revealed the following threshold concentrations (based on nominal test concentrations):

for survival of the test animals after 7, 14 and 21 days:

No observed effect concentration (NOEC)	day 7	≥ 512 µg a.s./L (nominal) ≥273 µg a.s./L (measured)
	day 14	256 µg a.s./L (nominal) 171 µg a.s./L (measured)
	day 21	64 µg a.s./L (nominal) 42.2 µg a.s./L (measured)
Lowest observed effect concentration (LOEC)	day 7	≥ 512 µg a.s./L (nominal) ≥273 µg a.s./L (measured)
	day 14	512 µg a.s./L (nominal) 273 µg a.s./L (measured)
	day 21	128 µg a.s./L (nominal) 82.0 µg a.s./L (measured)

for final body length of surviving test animals after 21 days:

No observed effect concentration (NOEC)	≥ 512 µg a.s./L (nominal) ≥ 273 µg a.s./L (measured)
Lowest observed effect concentration (LOEC)	≥ 512 µg a.s./L (nominal) ≥ 273 µg a.s./L (measured)

The above displayed threshold concentrations were obtained from statistical comparison to untreated water control ("negative control") by means of the Williams multiple sequential t-test procedure.
The accompanying chemical analysis of fluoxastrobin (tech.) in the freshly prepared test solutions at start of the chosen exposure intervals revealed recoveries between 42.8 % and 106.4 % (mean: 60.6 %) of the corresponding nominal concentrations.
The corresponding concentrations of the aged test solutions at the end of the exposure intervals ranged between 49.1 % and 122.1 % (mean: 71.9 %) of nominal.
All measured values for the untreated water control group were found to be below the lowest analytical-standard concentration during analysis of the test samples (< 0.5 µg a.s./L).

As the measured values were below 80 % of nominal, all results submitted by this report are related to the average of the measured concentrations of the active ingredient.

Conclusion:

The overall lowest chronic NOEC for 21 days of static renewal exposure of fluoxastrobin (tech.) to *Habrophlebia lauta*, expressed as measured test concentration, is 42.2 µg a.s./L (corresponding to 64 µg a.s./L nominal). This NOEC is based on a reduced survival at test termination. The corresponding LOEC is 82 µg a.s./L, expressed as measured concentration (corresponding to 128 µg a.s./L nominal). The "Maximum Acceptable Toxicant Concentration" (MATC), can be calculated as the geometric mean between NOEC and LOEC, and is 58.8 µg a.s./L (measured).

Report:	KCA 8.2.5.2/03 [redacted]Ü; 2012; M-442121-01-1
Title:	Chronic bioassay with Neocaridina heteropoda with fluoxastrobin (tech.)
Report No.:	MEHEN002
Document No.:	M-442121-01-1
Guideline(s):	None Standardised Guideline
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

A chronic toxicity test was performed to identify possible effects of fluoxastrobin technical on survival and growth development of *Neocaridina heteropoda* over 21 days under static-renewal exposure, expressed as chronic NOEC for larval health and growth.

Material and methods:

Test item: Fluoxastrobin, technical; Batch code: AE 1228646-01-01; Specification No.: 102000008981; CAS No.: 361377-29-9; Origin Batch No.: PF90232555; LIMS No.: 1119470; Customer Order No.: TOX 09429-00; Purity (content): 98.8 % w/w.
Neocaridina heteropoda (5 animals per vessel, 20 animals per treatment, 40 per control and solvent) were exposed in a static-renewal test system (medium renewal: day 7, 12, 16, 19) for 21 days to a untreated dilution water (blank) control, solvent control (DMF at a concentration equal to the concentration present in the highest test concentration of 240 µg a.s./L) and nominal concentrations of the test item of 7.5, 15, 30, 60, 120 and 240 µg a.s./L.
As endpoints, the rate of survivors and their body-length at the end of the study were recorded as database for NOEC / LOEC / MATC calculation. For verification of the actual test item concentrations during exposure, water-samples from start and end of 5 exposure-intervals were analyzed.

Dates of experimental work: June 27, 2012 to August 20, 2012

Findings:

Validity criteria:
Although no validity criteria were available for this type of study, the mortality rate in the control revealed to be lower than the validity criteria for the chronic assay with Daphnia magna. In conclusion, the study performed with *Neocaridina heteropoda* was proven to be valid.

Biological findings:

Table CA 8.2.5.2- 3: Biological Results (mean-values by study group)

Treatment [µg a.s./L] (nominally)	Endpoints			
	Body length on day 21 (mm)	% Survival		
		Survival 7d	Survival 14 d	Survival 21 d
Control	9.41	97.5	97.5	85
Solvent	9.75	90	85	80
7.5	9.92	100	95	95
15	9.49	100	90	90
30	9.70	95	85	65
60	9.44	95	85	65
120	9.87	65*)	25*)	10*)
240	no animal survived until day 21	35*)	0*)	0*)

*) Denotes statistically significant difference from pooled controls (verified by Williams Multiple Sequential t-test Procedure on a 5% level of significance one sided smaller probability).

The biological endpoints revealed the following threshold concentrations (based on nominal test concentrations):
for survival of the test animals:

No observed effect concentration	(NOEC)	60 µg a.s./L (nominal)
Lowest observed effect concentration	(LOEC)	120 µg a.s./L (nominal)

for final body length of surviving test animals:

No observed effect concentration	(NOEC)	≥ 120 µg a.s./L (nominal)
Lowest observed effect concentration	(LOEC)	≥ 120 µg a.s./L (nominal)

The accompanying chemical analysis of fluoxastrobin (tech.) in the freshly prepared test solutions at start of the chosen exposure intervals revealed recoveries between 90 % and 103 % (mean: 96.9 %) of the corresponding nominal concentrations.
The corresponding concentrations of the aged test solutions at the end of the exposure intervals ranged between 76 % and 102 % (mean: 92.4 %) of nominal.
All measured values for the untreated water control group were found to be below the lowest analytical standard concentration during analysis of the test samples (< 0.518 µg a.s./L).
One measured value was found to be below 80 % of nominal after the exposure period. For all other sampling days the measured values ranged within 80 and 120% of nominal. Thus all results submitted by this report are related to nominal test concentrations of the active ingredient.

Conclusion:

The overall lowest chronic NOEC for 21 days of static renewal exposure of fluoxastrobin (tech.) to *Neocaridina heteropoda* is 60 µg a.s./L (nominal). This NOEC is based on a reduced survival at test termination. The corresponding LOEC is 120 µg a.s./L (nominal).
From these data the "Maximum Acceptable Toxicant Concentration" (MATC) of 84.9 µg a.s./L can be calculated as the geometric mean between NOEC and LOEC (nominal).

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

No additional studies were performed. Please refer to the corresponding section in the Draft Assessment Report (DAR) and to the studies in the baseline dossier provided by Bayer CropScience.

CA 8.2.5.4 Sediment dwelling organisms

No additional studies are required. See point CA 8.2.5.1.

CA 8.2.6 Effects on algal growth

No additional studies are required. For studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR). Details of the studies are provided in the following table.

Table CA 8.2.6- 1: Toxicity to algal species exposed to fluoxastrobin and its metabolites

Test substance	Test species	Endpoint	Reference
Fluoxastrobin	*Pseudokirchneriella subcapitata* (green algae)	E_bC_{50} 0.75 mg a.s./L **E_rC_{50} 2.10 mg a.s./L**	[redacted]; 2000; M-033313-01-1
HEC 5725-E-des-chlorophenyl	*Pseudokirchneriella subcapitata* (green algae)	E_bC_{50} > 100 mg p.m./L **E_rC_{50} >100 mg p.m./L**	[redacted]; 2000; M-025012-01-1
HEC 5725-carboxylic acid	*Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*, green algae)	E_bC_{50} 150 mg p.m./L **E_rC_{50} >160 mg p.m./L**	[redacted]; [redacted]; 2001; M-073836-01-1
2-chlorophenol	*Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*, green algae)	**E_rC_{50} 70 mg p.m./L**	EFSA Scientific Report 102 (2007)) [redacted]; 2006; M-277036-01-1

Bold letters – values considered relevant for risk assessment

CA 8.2.6.1 Effects on growth of green algae

See point CA 8.2.6. No additional studies are required.

CA 8.2.6.2 Effects on growth of an additional algal species

See point CA 8.2.6. No additional studies are required.

CA 8.2.7 Effects on aquatic macrophytes

For the study already evaluated during the first EU review of fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Draft Assessment Report (DAR).
An additional study is available addressing the toxicity of the active substance to *Lemna gibba*.
Details of the studies are provided in the following table.

Table CA 8.2.7- 1: Toxicity to aquatic macrophytes exposed to fluoxastrobin and its metabolites

Test substance	Test species	Endpoint		Reference
Fluoxastrobin	*Lemna gibba* (duckweed)	E_bC_{50}	> 6.0 mg a.s./L	[redacted]; 2001; M-037727-01-1
		E_rC_{50}	**> 6.0 mg a.s./L**	
	Lemna gibba (duckweed)	E_bC_{50}	1.45 mg a.s./L	[redacted]; 2002; M-083021-01-1 KCA 8.2.7
		E_rC_{50}	3.88 mg a.s./L	

Bold letters – values considered relevant for risk assessment

Report: KCA 8.2.7/02[redacted]; 2002; M-083021-01-1
Title: Toxicity of HEC 5725 technical to duckweed (Lemna gibba G3)
Report No.: 200340
Document No.: M-083021-01-1
Guideline(s): USEPA, 1996. Series 850 - Ecological Effects Test Guidelines OPPTS Number: 850.4400: Aquatic Plant Toxicity Test Using Lemna spp., Tier I and II.
Guideline deviation(s): --
GLP/GEP: **yes**

Objective:

The primary objective of this growth study was to estimate the fifty percent effective concentration (EC_{50}) for fluoxastrobin technical. The concentration at which there is no observed effect (NOEC) was also determined. An effect is one that is a statistically significant (p _ 0.05) and a biologically relevant reduction from the control for the parameter being measured. The parameters measured in this study were standing crop growth rate and cumulative biomass (as area under the growth curve). The variable used to calculate these response parameters was frond number as determined by direct frond counts. Frond dry weight was also measured at test termination.

Material and methods:

Test item: Fluoxastrobin, technical; Batch number: 898904001; CAS (IE): 361377-29-9 non stereo: 193740-76-0; Purity: 96.9 %.
The duckweed *Lemna gibba* G3 was exposed for 7 days under static conditions. Nominal concentrations (mean measured of Day 0 and Day 7 solutions) were control (<0.015), solvent control (<0.015), 0.15 (0.16), 0.38 (0.39), 0.96 (1.03), 2.4 (2.38) and 6.0 (5.86) mg a.s./L. Three replicates with three Lemna plants for a total of 12 fronds were prepared for each concentration. Growth was determined by frond counts on days 0, 3, 5, and 7.

Dates of work: August 16, 2002 to August 23, 2002

Findings:

Analytical results:
The mean measured concentrations (mean of Day 0 and Day 7 new solution analyses) of fluoxastrobin technical were 0.16, 0.39, 1.03, 2.38, and 5.86 mg a.s./L for the 0.15, 0.38, 0.96, 2.4 and 6.0 mg a.s./L nominal concentrations, respectively. This represents 98 to 107% of the nominal test concentrations.

Biological findings:

Observations made on Day 0, 3, 5, and 7 showed no treatment related effects with regards to frond appearance.

Percent inhibition of frond counts ranged from 9-68% as compared to the pooled controls. Percent inhibition for cumulative biomass and growth rate ranged from 6 to 64% and 5 to 52%, respectively. Further statistical analysis was conducted to determine if the inhibition on growth was significant. Statistical analysis was performed for the endpoints of frond count (standing crop), growth rate, and cumulative biomass (area under the growth curve). No statistically significant differences were found between the control and solvent control groups for any of the parameters so the control groups were pooled for statistical comparisons. Statistical analysis of the data did not pass the criteria for normality and homogeneity of variance for the standing crop and growth rate endpoints. Therefore, nonparametric analyses were conducted for each. For cumulative biomass, the data passed the criteria for normality and homogeneity of variance so parametric analyses were conducted.

A significant effect ($p<0.05$) at all but the lowest test concentration (0.16 mg a.s./L) was found for frond count, growth rate and cumulative biomass as compared to the pooled controls. Therefore, the NOEC and LOEC for all three endpoints was determined to be 0.16 and 0.39 mg a.s./L, respectively. The EC_{25} and EC_{50} values for frond count were determined to be 0.22 and 1.18 mg a.s./L, respectively. For growth rate the EC_{25} was 0.54 mg a.s./L and the EC_{50} was 3.88 mg a.s./L. The EC_{25} and EC_{50} for cumulative biomass were 0.28 and 1.45, respectively. The Toxic Threshold Effect Concentration, TEC (Geometric mean of NOEC and LOEC) for frond number, the most sensitive endpoint, was 0.25 mg a.s./L. For frond dry weight, percent inhibition ranged from 5 to 37 %. No statistically significant differences were noted between the control and solvent control groups so the controls groups were pooled for comparisons. The data passed the criteria for normality and homogeneity of variance so parametric analyses were conducted. A significant effect at the 1.03 mg a.s./L test concentration was determined. Therefore, the NOEC and LOEC for the dry weight endpoint were 0.39 and 1.03, respectively. The EC_{25} was determined to be 1.79 mg a.s./L and the EC_{50} was >5.86 mg a.s./L.

Table CA 8.2.7- 2: Effects of fluoxastrobin technical on aquatic plants

Test substance	**Fluoxastrobin technical**
Test object	*Lemna gibba* G3
Exposure	7 d, static
7-day EC_{50} – standing crop	1.18 mg a.s./L
7-day EC_{50} – growth rate	3.88 mg a.s./L
7-day EC_{50} – cumulative biomass	1.45 mg a.s./L
7-day EC_{50} – frond dry weight	> 5.86 mg a.s./L
Lowest concentration with an effect (LOEC)	0.39 mg a.s./L
Highest concentration without toxic effect (NOEC)	0.16 mg a.s./L
Toxic threshold effect concentration, TEC (Geometric mean of NOEC and LOEC)	0.25 mg a.s./L

Conclusion:

The NOEC and LOEC in the 7-day exposure of *Lemna gibba* G3 to fluoxastrobin technical were 0.16 and 0.39 mg a.s./L, respectively. The EC_{50} was 1.18 mg a.s./L.

CA 8.2.8 Further testing on aquatic organisms

No further testing is required.

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 fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).

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

- Acute oral and contact toxicity of fluoxastrobin,
- Acute contact toxicity of fluoxastrobin to adult bumble bees under laboratory conditions,
- Chronic 10 day toxicity test with of Fluoxastrobin FS 480 on adult bees under laboratory conditions,
- Colony feeding study with Fluoxastrobin FS 480 according to [redacted] *et al.* 1992 (using a realistic worse case spray solution concentration and covering exposure for effects on brood (eggs, young and old larvae) and their development, nurse bee on-going behaviour in brood care and colony strength),
- Semi-field brood feeding study with Fluoxastrobin EC 100 following OECD guidance document 75 (using a more realistic spray scenario onto flowering *Phacelia tanacetifolia* at the maximum application rate for the approval renewal of fluoxastrobin and covering exposure for effects on brood (eggs) and their development and colony parameters).

These studies were not submitted during the first Annex I inclusion process and are submitted within this Supplementary Dossier for the Fluoxastrobin Annex I Renewal. The studies will be summarized below. A full list of the relevant ecotoxicological endpoints for fluoxastrobin and bees are presented in the following table.

Table CA 8.3- 1: EU evaluated and additional studies on bee toxicity of fluoxastrobin and fluoxastrobin formulations

Test substance	Test species	Test method	Endpoint	Reference
Fluoxastrobin	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h) (adults)	LD_{50} oral > 843 µg a.s./bee contact > 200 µg a.s./bee	[redacted].; 2000; M-026537-01-1
	Honey bee (*A. mellifera*)	Laboratory acute oral and contact (48 h) (adults)	LD_{50} oral **> 129.1 µg a.s./bee** contact **> 100 µg a.s./bee**	[redacted]F; 2014; M-503275-01-1 KCA 8.3.1.1.2
	Bumble bee (*B. terrestris*)	Laboratory acute contact (48 h) (adults)	LD_{50} contact > 100 µg a.s./bumble bee	[redacted].; 2014; M-512437-01-1 KCA 8.3.1.1.2
Fluoxastrobin FS 480	Honey bee (*A. mellifera*)	Laboratory chronic oral (10 d) (adults)	LC_{50} > 3333 mg a.s./kg LDD_{50} > 73.3 µg a.s./bee/day NOEC 1667 mg a.s./kg NOEDD 39.2 µg a.s./bee/day	[redacted].; 2015; M-534974-01-1 KCA 8.3.1.2
Fluoxastrobin FS 480	Honey bee (*A. mellifera*)	Bee brood feeding ([redacted] *et al.*)	No adverse effects on brood development and mortality after feeding honey bee colonies sugar syrup at 0.375 g a.s./L.	[redacted]; 2013; M-476181-01-1 KCA 8.3.1.3
Fluoxastrobin EC 100	Honey bee (*A. mellifera*)	Semi-field brood study (OECD 75)	No adverse effects on brood development, mortality, foraging activity, behaviour, colony condition and strength after application of 150 g a.s./ha onto flowering *Phacelia tanacetifolia*.	[redacted].; 2015; M-515147-01-1 KCA 8.3.1.3

a.s. = active substance
Bold letters – values considered relevant for risk assessment

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

A new study, not previously submitted providing information on the acute oral and contact toxicity of fluoxastrobin for bees is presented below.

Report: KCA 8.3.1.1.1/02 [redacted]P; 2014; M-503275-01-1
Title: Effects of fluoxastrobin tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.: 89471035
Document No.: M-503275-01-1
Guideline(s): OECD Guideline 213/214 for the Testing of Chemicals on Honeybee, Acute Oral/Contact Toxicity Test, adopted on 21st September 1998.
Guideline deviation(s): not specified
GLP/GEP: **yes**

Objective:

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

Material and methods:

Test item: Fluoxastrobin tech.; Origin Batch No.: HEC 21596-1-3; Customer Order No.: TOX 10661-00; Material: AE 1228646, technical substance; Specification No.: 102000008981, LIMS-No.: 1423843; Article No.: 05682541; Content: 96.4 % w/w (analytical).
Under laboratory conditions *Apis mellifera* 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees to a single dose of 129.1 µg a.s. per bee by feeding (oral limit test, value based on the actual intake of the test item). For the contact test a single 5 µL droplet of fluoxastrobin, dissolved in acetone, was placed on the dorsal bee thorax. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Adhäsit. For the controls, one 5 µL droplet of tap water containing 0.5 % Adhäsit and one 5 µL droplet of pure acetone were used. For the oral test the test item was diluted in acetone + DMSO and then applied in 50 % w/v sucrose solution, which was used as carrier (food). The reference item was diluted in tap water and applied in 50 % w/v sucrose solution. For the control pure 50 % w/v sucrose solution was offered to the bees and for the solvent control 50 % w/v sucrose solution with 5 % solvent (acetone + DMSO) was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 2 hours 10 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. The number of dead bees was determined after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours. Behavioural abnormalities (e.g. vomiting, apathy, intensive cleaning) were assessed after 4 (± 0.5 h) hours (first day), 24 and 48 (± 2 h) hours. Temperature during the test was 24 - 25 °C; relative humidity was 58 – 85 %. Bees were kept in darkness (except during observation).

Reference item: Perfekthion EC (BAS 152 11I); Batch No.: FRE-000926; Active ingredient: dimethoate; Content: 400.9 g/L (analytical); Density: 1.069 g/cm^3. Controls: Solvents and water.

Dates of work: July 21, 2014 to July 24, 2014

Findings:

Validity criteria:

Validity Criteria		Recommended	Obtained
Control Mortality		Contact Test	
	water control	< 10%	0.0 %
	acetone control	< 10%	0.0 %
		Oral Test	
	sugar control	< 10%	0.0 %
	acetone + DMSO/sugar control	< 10%	0.0 %
LD_{50} of Reference Item (24 h)		Contact Test	
		0.10 - 0.30 µg a.s./bee	0.26 µg a.s./bee
		Oral Test	
		0.10 - 0.35 µg a.s./bee	0.13 µg a.s./bee

The contact and oral test is considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Reference test:
The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.26 and 0.13 µg a.s./bee, respectively.

Biological findings:
Contact toxicity:
At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg a.s./bee. Also no mortality occurred in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (acetone) at the end of the contact test (after 48 hours), respectively. No behavioural abnormalities were observed during the entire contact test.

Oral toxicity:
In the oral toxicity test, the maximum nominal test level of fluoxastrobin tech. (i.e. 100 µg a.s./bee) corresponded to an actual intake of 129.1 µg a.s./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the water control group (50 % w/v sucrose solution = 500 g sucrose/L tap water) and in the solvent control group (50 % w/v sucrose solution containing 5% acetone + DMSO) at the end of the oral toxicity test (after 48 hours), respectively. No test item induced behavioural effects were observed at any time in the oral toxicity test.

Table CA 8.3.1.1.1- 1: Toxicity to honey bees; laboratory tests

Test Item	**Fluoxastrobin tech.**	
Test Object	***Apis mellifera***	
Exposure	**contact (solution in acetone)**	**oral (50 % w/v sucrose solution containing 5 % acetone + DMSO)**
Application rate µg a.s./bee	100.0	129.1
LD_{50} µg a.s./bee	> 100.0	> 129.1
LD_{20} µg a.s./bee	> 100.0	> 129.1
LD_{10} µg a.s./bee	> 100.0	> 129.1
NOED µg a.s./bee*	≥ 100.0	≥ 129.1

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

Conclusions:

The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 129.1 µg a.s./bee.

CA 8.3.1.1.2 Acute contact toxicity

New study, not previously submitted providing information on the acute oral and contact toxicity of fluoxastrobin to which bees could potentially be exposed.

Report:	KCA 8.3.1.1.2/02 [redacted],; 2014; M-503275-01-1
Title:	Effects of fluoxastrobin tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	89471035
Document No.:	M-503275-01-1
Guideline(s):	OECD Guideline 213/214 for the Testing of Chemicals on Honeybee, Acute Oral/Contact Toxicity Test, adopted on 21st September 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Results of the contact toxicity test with fluoxastrobin are summarized under point CA 8.3.1.1.1.

A new study on the acute contact toxicity of fluoxastrobin to a non-Apis species (*Bombus terrestris*) is presented below.

Report:	KCA 8.3.1.1.2/03 [redacted],; 2014; M-512437-01-1
Title:	Fluoxastrobin technical - Acute contact toxicity to the bumble bee, bombus terrestris L. under laboratory conditions - Final report
Report No.:	S14-00621
Document No.:	M-512437-01-1
Guideline(s):	no specific guidelines available, based on OEPP/EPPO 170 (4) (2010), OECD Guideline No. 214 (1998) and on the review article of [redacted] (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:

The objectives of this study were to determine possible effects of fluoxastrobin technical on the bumble bee, *Bombus terrestris* L. from contact exposure in order to determine the median lethal dose (LD_{50}) to *Bombus terrestris*, where possible.

Material and methods:

Test material: Fluoxastrobin technical- Short code: HEC5725; TOX No.: 10661-00; Specification No.: 102000008981, Batch code: AE 1228646-01-03; Content of a.s.: 97.3% fluoxastrobin (analysed).
The contact toxicity of fluoxastrobin technical to the bumble bee (*Bombus terrestris* L.) was determined in a limit test based on OEPP/EPPO 170 (4) (2010), the OECD Guideline No. 214 (1998) and the review article of [redacted] (2001).
In the laboratory, the bumble bees were exposed to 100 µg fluoxastrobin/bumble bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after application. The control groups were exposed for the same period of time under identical exposure conditions to acetone and mineral water. In the control and reference item groups, three replicate groups of 10 bumble bees each were tested. In the test item group, five replicate groups of 10 bumble bees each were tested. The test was carried out at the [redacted] testing facility in Spain ([redacted], Spain).

Dates of work: July 17, 2014 to July 18, 2014

Findings:

In the control groups, treated with acetone and mineral water, no mortality was observed during the 48 h test period.
In the test item treatment group, no mortality was observed at the dose level corresponding to 100 µg fluoxastrobin/bumble bee at the final assessment after 48 hours.
In the reference item group, mortality was ≥ 50 % at the end of the test. Thus, the test was considered to be valid.

Table CA 8.3.1.1.2- 1: LD_{50} values in the bumble bees contact toxicity test with fluoxastrobin technical

fluoxastrobin technical	Contact toxicity test [µg fluoxastrobin/ bumble bee]
LD_{50} (24 h)	> 100.0
LD_{50} (48 h)	> 100.0
NOED (48 h)	≥ 100.0

NOED = No Observed Effect Dose

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

Conclusion:

The 48 hour contact LD_{50} value for Fluoxastrobin technical was determined to be > 100 µg fluoxastrobin/bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Fluoxastrobin FS 480 as technical fluoxastrobin is only very slightly soluble in water.

Report: KCA 8.3.1.2/01 [redacted] V, 2015, M-534974-01-1
Title: Chronic oral toxicity test of fluoxastrobin FS 480 (480 g/L) on the honey bee (Apis mellifera L.) in the laboratory
Report No.: 87461136
Document No.: M-534974-01-1
Guideline(s): (GLP compliant study based on OECD 213 (1998) and CEB No.: 230 with modifications and current recommendations of the ring test group (2014))
Guideline deviation(s): not specified
GLP/GEP: **yes**

Objective:

The purpose of this study was to determine the chronic oral toxicity of Fluoxastrobin FS 480 (480 g/L) to the honeybee (*A. mellifera* L.) for a period of ten days.
Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and methods:

Test material: Fluoxastrobin FS 480 (480 g/L); Short code: FXA FS 480; Fluoxastrobin: 47.9 % w/w, 486.1 g/L; Batch ID.: 2013-001926; Sample description: TOX10076-00, Specification No.: 102000026368-N1; Certificate No.: 13004998; Density: 1.160 g/mL (23°C).

The chronic effects of the test item Fluoxastrobin FS 480 on the honey bee, *Apis mellifera* L. were assessed in a 10 days continuous oral feeding test in the laboratory (dose response test).
Under laboratory conditions 30 freshly emerged worker bees (*Apis mellifera* L.) per treatment level were exposed for 10 days to 5 concentrations (3333, 1667, 833, 417 and 208 mg a.s./kg food [ppm]) of the test item treated sugar solutions *ad libitum*. An untreated control (50 % w/v sucrose solution) and a reference item (1 mg dimethoate/kg feeding solution [ppm]) were included in this study. For each treatment, three replicates (with 10 bees per replicate unit) were used. Mortality and behavioural abnormalities were assessed every day throughout the 10 days exposure period.
The test conditions during the study were 33.0 - 34.0 °C temperature, 31 - 91% relative humidity (mean relative humidity: 74 %) and 24 h darkness.

Findings:

The test item was daily administered to the bees in a sugar solution at the following concentrations: 3333, 1667, 833, 417 and 208 mg a.s./kg sugar solution. These concentrations led to a daily mean dose of 73.3, 39.2, 21.6, 12.4 and 6.8 µg a.s./bee per day after 10 days. The nominal target dose levels of 100, 50.0, 25.0, 12.5 and 6.25 µg a.s./bee per day were not obtained.

Table CA 8.3.1.2- 1: Chronic oral toxicity of Fluoxastrobin FS 480 to young honey bees (laboratory test)

Test Object		*Apis mellifera carnica*	
Treatment Group	Concentration [mg a.s./kg]	Dose Level[1)] [µg a.s./bee/day]	Mortality at day 10[2)] [% Mean]
Fluoxastrobin FS 480 (480 g/L)	3333	73.3	36.7 (*)
Fluoxastrobin FS 480 (480 g/L)	1667	39.2	6.7 (n.s.)
Fluoxastrobin FS 480 (480 g/L)	833	21.6	3.3 (n.s.)
Fluoxastrobin FS 480 (480 g/L)	417	12.4	0.0 (n.s.)
Fluoxastrobin FS 480 (480 g/L)	208	6.8	3.3 (n.s.)
Water control	0.0	0.0	6.7
Reference Item	1.0	0.024	100.0
Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC**	**NOEDD**
> 3333 mg a.s./kg	**>73.3 µg a.s./bee/day**	**1667 mg a.s./kg**	**39.2 µg a.s./bee/day**

1) mean dose per bee per day; dose measured based on consumed feeding solution
2) Mortality at study termination 10 days after start of first feeding
Statistic:
<u>Mortality:</u> Fisher`s Exact Test, pairwise comparison, one-sided greater, α = 0.05
<u>NOEC/NOED</u>: was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).
n.s. = no statistically significant difference compared to the control, * = statistically significant difference compared to the control

Observations:

At test end, 10 days following start of exposure, 6.7 % mortality occurred in the untreated water control (50 % w/v sucrose solution). At 3333 mg a.s./kg (corresponding to 73.3 µg a.s./bee/day) 36.7 % mortality occurred. This effect was statistically significant (Fisher`s Exact Test, $\alpha = 0.05$).
In the test item treated groups at 1667, 833, 417 and 208 ppm mg a.s./kg sugar solution the mortality was less or equal to the control.
No test item related behavioural abnormalities occurred at any time of the test.
The reference item (dimethoate) at a concentration of 1 mg dimethoate/kg sugar solution corresponding to 0.024 µg a.s./bee per day caused 100 % mortality at day 7.

Analytical results:

The actual concentrations of Fluoxastrobin FS 480 in the feeding solutions were analysed in a separate study which is attached to this final report. The actual concentrations of the feeding solutions were in a range of 85% - 94%.

Conclusions:

The chronic toxicity of Fluoxastrobin FS 480 (480 g/L) was tested over 10 days.
The LC_{50} value (10 days) was > 3333 mg a.s./kg feeding solution.
The LDD_{50} value (10 days) was > 73.3 µg a.s./bee per day.
The NOEC and NOEDD values (10 days) were 1667 mg a.s./kg feeding solution and 39.2 µg a.s./bee per day, respectively.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Two new studies on the effect of fluoxastrobin to adult honey bees, the colony condition and the bee brood development are presented below. The studies were conducted with Fluoxastrobin FS 480 as technical fluoxastrobin is only very slightly soluble in water.

Report: KCA 8.3.1.3/01 [redacted]; 2013; M-476181-01-1
Title: Fluoxastrobin FS 480 (480 g/L): Effects on honey bee brood (Apis mellifera L.) - Brood feeding test - Short-code of test item: FLX FS 480
Report No.: 79021031
Document No.: M-476181-01-1
Guideline(s): [redacted] 1992: Method for honey bee brood feeding tests with insect growth-regulating insecticides, OEPP/EPPO Bulletin 22:613-616 (1992)
Guideline deviation(s): none
GLP/GEP: **no**

Objective:

The purpose of this study was to assess the effects of Fluoxastrobin FS 480 on brood development and mortality of honey bees. The colonies were freely flying with access to natural food sources, however, the study was conducted at a time without mass flowering plants/agricultural crops in the study region, so that the nectar flow of natural sources was low at the time of treatment administration.

Material and methods:

Test item: Fluoxastrobin FS 480; Short code: FLX FS 480; active ingredients (analysed content): 41.9 % w/w (486.1 g/L) fluoxastrobin (HEC 5725 E-ISO); Batch ID: 2013-001926; Sample description: TOX10076-00; Specification No.: 102000026368 - N1; Density: 1.160 g/mL (20 °C).

Test species: Honey bees (*Apis mellifera* L.); honey bee colonies were maintained according to normal beekeeping practice, containing two magazines with 11 combs, each. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply of nectar and pollen. The mean strength of the colonies per treatment group, two days before application, was similar and ranged between 13860 and 19305 adult bees. Colonies were free flying, with access to natural food sources, but due to the season, there were no main flowering, bee attractive crops or flowering weeds in the surrounding area
An untreated control and a toxic reference were included in the study. Three bee colonies were used per treatment group. The test item and reference item solutions were mixed with ready-to-use sugar syrup (Apiinvert) and applied to the bee colonies via a feeding trough, which was put directly into the colony on top of the second magazine. Pure sugar syrup (Apiinvert) was used for the controls. Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a period of 21 days following the application for each treatment group and colony. This was assessed one day before the application, by selecting one (or several) brood comb(s) from of each colony and by taking a digital photo of this (these) brood comb(s). After saving the photo-file on a computer, eggs, young- and old larvae were marked at this first Brood area Fixing Day (BFD0). For each subsequent brood assessment (BFDn), again, the same comb(s) was (were) selected from the respective colony and another digital photo was taken, in order to investigate the progress of brood development. Ontogenesis of the bee brood was observed for a period of 21 days after application (i.e. 22 days following BFD0). Mortality of adult bees and pupae was also assessed.

Endpoints:
Mortality of adult bees as well as pupae or larvae: between 3 days before to 21 days after application (= end of the trial);
Bee brood development (eggs, young- and old larvae): one day before (= BFD0) and 4 (= BFD 5), 8 (= BFD 9), 15 (= BFD 16), 21 (= BFD 22) days after the application.

Test concentrations:
<u>Control:</u> 1 L untreated commercial ready-to-use sugar syrup (Apiinvert, 30 % sucrose, 31 % glucose, 39 % fructose) per colony.
<u>Test Item:</u> 0.90 g test item (Fluoxastrobin FS 480) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to an active substance concentration of 0.375 g fluoxastrobin a.s./L.
<u>Reference Item:</u> 3.0 g reference item (Insegar; 25 % fenoxycarb) in 1 L com-mercial ready-to-use sugar syrup per colony, equivalent to a nominal active substance concentration of 0.75 g fenoxycarb a.s./L.

Test conditions: Natural field conditions. Temperature, relative humidity and rain were recorded during the experimental time.

Statistics: Statistical evaluation was done for mortality and the brood termination rates using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student`s t-test (pairwise). Software: ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Dates of work: June 24, 2013 to July 18, 2013

Findings:

<u>Validity criteria:</u>
The reference item treatment (Insegar, a.s. = fenoxycarb) resulted in a statistically significantly increase of unsuccessful eggs, young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Biological findings:

Table CA 8.3.1.3- 1: Effects of Fluoxastrobin FS 480 on honey bee brood

Test item	**Fluoxastrobin FS 480**		
Test species	**Honey bees (*Apis mellifera* L) (complete colonies)**		
Exposure	***via* treated sugar solution**		
Treatment	Untreated control	Fluoxastrobin FS 480	Reference item (Insegar, a.s. = fenoxycarb)
Rate per L sugar solution [product][1)]		0.90 g/L	3.0 g/L
Rate per L sugar solution [a.s.][1)]		0.375 g/L	0.75 g/L
Termination rate of the eggs [%][2)]	9.6 %	7.1 % (n.s.)	99.8 % (*)
Termination rate of the young larvae [%][2)]	24.4 %	9.1 % (n.s.)	99.8 % (*)
Termination rate of the old larvae [%][2)]	3.3 %	11.3 % (n.s.)	26.9 % (*)
Mean brood termination rate over all stages	12.3 %	9.2 % (n.s.)	75.6 % (*)
Mean mortality of worker bees/colony/day during pre-application phase[3)] during the entire post-application phase[3)]	 8.0 8.5	 17.4 (n.s.) 8.8 (n.s.)	 14.2 (n.s.) 18.7 (*)
Mean mortality of pupae/colony/day during pre-application phase[4)] during the entire post-application phase[4)]	 0.1 1.7	 0.7 (n.s.) 1.0 (n.s.)	 2.9 (n.s.) 0.8 (n.s.)
Mean number of Bees before application	16770	19305	13860

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

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

No effect on the development of eggs was observed after consumption of the test item treated sugar solution. The mean termination rate of eggs in the test item treatment group was lower with a mean of 7.1 % compared to 9.6 % in the control group. Accordingly, this was not statistically significant compared to the control group.
There was also no effect on the development of young larvae after consumption of the test item via treated sugar solution. The development success of the young larvae in the test item treatment group was better and resulted in a mean termination rate of 9.1 % compared to 24.4 % in the control group. This difference was not statistically significant compared to the control group.
Although the mean termination rate of old larvae was slightly higher in the test item treatment group (11.3 %) when compared to the values of the control group (3.3 %), there was no statistically significant difference.
Adult bee mortality in the test item treatment group was similar (mean of 8.8 dead bees per day) and thus not statistically significant different when compared to the control group (8.5 dead bees per day).
No effects of the test item on honey bee pupae and larvae were observed.
The reference item treatment (Insegar a.s. = fenoxycarb) resulted in a statistically significantly increase of unsuccessful eggs, young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Conclusion

Overall, it can be concluded according to the results of this study that the administration of Fluoxastrobin FS 480 fortified sugar syrup (375 ppm fluoxastrobin) to honey bee colonies does neither adversely affect honey bee colonies nor bee brood development.

Report:	KCA 8.3.1.3/02 [redacted]; 2015; M-515147-01-1
Title:	Assessment of side effects of fluoxastrobin EC 100 G on the honeybee (Apis mellifera L.) in the semi-field after one application on Phacelia tanacetifolia in Germany 2014
Report No.:	S14-00162
Document No.:	M-515147-01-1
Guideline(s):	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (PISTORIUS et al., 2012) OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

This study was designed to determine the potential effects of Fluoxastrobin EC 100 G on the honeybee (*Apis mellifera* L.) after one application on *Phacelia tanacetifolia* in Germany in a semi-field brood study following OECD guidance document No. 75 (2007), the current recommendations of the AG Bienenschutz ([redacted] *et al.*, 2012), and the OEPP/EPPO Guideline No. 170(4) (2010).
The evaluation of the treatment effects focused on mortality, flight intensity, behaviour, condition of the colonies, and development of the bee brood assessed in individually marked cells within a time period of approximately four weeks.

Material and Methods

Test Item: Fluoxastrobin EC 100 G; Sample description: Specification No.: 102000008126-01, TOX10110-00; Batch-ID: 2013-002009; content of a.i. (analysed): 101.0 g/L.

The aim of the study was to evaluate potential side effects of a spray application of Fluoxastrobin EC 100 G on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the OECD guidance document No. 75 (2007), with methodological improvements by the AG Bienenschutz ([redacted] et al., 2012). The crop used was full-flowering *Phacelia tanacetifolia*, the study was conducted in [redacted] in Baden-Württemberg, Germany.
The study included three treatment groups with four replicates (tunnels) each: one tap-water treated control group (C), one test-item group (T) and one reference item group (R).
Applications were made at full-flowering (BBCH 65) with honeybees actively foraging on the crop. The target application rate of the test item Fluoxastrobin EC 100 G was 150 g a.s./ha (actual rate applied 153 g a.s./ha). Tap water was applied in the control group and Insegar was applied at a target rate of 1200 g product/ha in the reference item group (corresponding to 300 g fenoxycarb/ha). The spray volume was 400 L/ha in all treatment groups.
The initial mean colony sizes per treatment group were in the range of 7829 to 9250 bees. The honeybees remained in the tunnels for 12 days and colonies were assessed once before set-up, twice during and four times after the end of the confined phase.
The following endpoints were assessed:
Total and mean number of dead bees on the linen sheets in tunnels, in the dead bee traps and in the dead bee bottoms before as well as after the start of exposure in T and the application in C and R, respectively.
Flight intensity (mean number of forager bees/m^2 *Phacelia tanacetifolia* before as well as after the start of exposure in T and the application in C and R, respectively.
Behaviour of the bees in the crop and around the hive.
Condition of the colonies (colony strength and area of the different brood stages and food storage per colony and assessment date).
Development of the bee brood assessed in individual brood cells. For this particular assessment, between 213 and 279 individually marked cells per colony were selected.

Dates of experimental work: July 04, 2014 to August 08, 2014

Findings:

Mortality:
Throughout the study (before and following exposure) mortality across all treatments was similar, indicating no effect of the test item. Some daily fluctuations occurred where mortality was higher in the test item colonies. However these were minor in nature and not clearly related to the treatment.
During the entire period after the application (0DAA to 28DAA), the average sum of dead pupae per colony recorded during the mortality assessments was 80.8, 63.0 and 569.0 for C, T, and R, respectively. Effects on pupae of the reference substance are a well-known effect.

Findings are summarised in the table below.

Table CA 8.3.1.3- 2: Summary of the effects on mortality of *Apis mellifera* L.

	Treatment group	Control (C)	Test item (T)	Reference Item (R)
Daily mean mortality (dead adults plus pupae/colony) ± STD	4DBA to 0DBA	210.4 ± 52.3	206.6 ± 63.8	184.6 ± 24.6
	0DAA	169.3 ± 62.5	169.0 ± 50.2	202.8 ± 36.2
	0DAA to 7DAA	94.6 ± 44.9	77.0 ± 12.0	97.0 ± 28.0
	0DAA to 28DAA	41.1 ± 14.2	36.4 ± 9.0	57.7* ± 4.8
Mean sum of dead pupae	4DBA to 0DBA	1.5 ± 1.2	1.4 ± 1.2	2.0 ± 2.1
	0DAA to 28DAA	80.8 ± 89.3	63.0 ± 59.1	569.0 ± 612.5

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

Flight intensity:
Foraging rates were similar across all treatments throughout the study before and following exposure up to the end of the confinement phase (7DAA).
During confinement of the colonies inside the tunnels until the day of the application (4DBA to 0DBA), the mean daily flight intensity was 5.1, 4.0 and 5.0 forager bees/m^2 in C, T and R, respectively. During the pre-application period the flight intensity was not statistically different between the treatment groups. (Tukey's test, two-sided, α = 0.05).
On the day of the application (0DAA), the mean daily flight intensity, assessed over a period of 6 hours, accounted to 18.0, 15.7 and 19.1 forager bees/m^2, for C, T, and R, respectively. Although statistically significant (Student's t-test, method pooled, one-sided, α = 0.05) the slight difference of flight activity in T compared to the control has no biological relevance and was on a normal level (higher than before application at 0DBA) in T throughout this day.
One day after the applications (1DAA), the mean flight intensity, assessed on three occasions during the day, was nearly on the same level in all treatment groups with 2.5, 2.2 and 2.6 forager bees/m2 in the C, T and R, respectively. No statistically significant differences to the control were observed in the test item group T and in the reference item group R, respectively (Student´s t-Test, method pooled, one-sided, α = 0.05).
Mean post-application flight intensity (0DAA to 7DAA) in C, T and R was 12.0 forager bees/m^2, 11.1 forager bees/m^2 and 10.9 forager bees/m^2. Statistically significant differences to the control were observed in T and R on 5DAA but these differences were only minor in nature, and flight intensity was on a normal level in all treatments (15.3 forager bees/m^2 in T and 14.8 forager bees/m^2 in R, compared to values of 18.1 forager bees/m2 in the control on 5DAA).
None of these slight differences is considered as biologically relevant or treatment-related.

Findings are summarised in the table below.

Table CA 8.3.1.3- 3: Summary of the effects on flight intensity of *Apis mellifera* L.

	Treatment group	Control (C)	Test item (T)	Reference Item (R)
Daily mean flight intensity (bees/m²) ± STD	4DBA to 0DBA	5.1 ± 0.6	4.0 ± 0.4	5.0 ± 0.9
	0DAA	18.0 ± 0.9	15.7* ± 0.7	19.1 ± 1.3
	0DAA to 7DAA	12.0 ± 0.9	11.1 ± 0.7	10.9 ± 0.8

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

Behaviour of the bees:
In the test item treatment group eleven cramping bees, three bees with locomotion problems and one trembling bee were observed on the day of application on 0DAA. On 2DAA twelve inactive bees were noticed in the replicates Ta, Tb and Td plus one cramping and one trembling bee in Ta. Furthermore one cramping bee (Tc) was observed on 3DAA and one bee with locomotion problems (Td) on 4DAA. Cramping bees were recorded on 5DAA (18 bees) and on 6DAA (5 bees). Clustering with about 30 bees was also recorded on 6DAA. On the last day of confinement (7DAA) ten cramping bee were noticed in the replicates Ta and Tc as well as one bee with locomotion problems (Td) and about 50 bees clustering in Td.
During most of the assessment times similar observations were also made in the control.
In the reference item group, behaviour such as cramping (28 bees), locomotion problems (7 bees), intensive cleaning (8 bees) and hanging bee (1 bee) were detected on 0DAA. On 2DAA twelve inactive bees were noticed plus four cramping bees and one trembling bee. Cramping bees were observed on 5DAA (25 bees), 6DAA (4 bees) and 7DAA (1 bee). Clustering behaviour on 6DAA with about 50 bees and on 7DAA with about 200 bees was noticed.
From day 8DAA some behaviour abnormalities still occurred in the test item but were less pronounced as in the control and are therefore not seen as an effect related to the test item.

Development of honey bee brood in individual cells:
In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+22) was acceptable at 29.99%.
In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were clearly lower than those observed in the control, indicating a strong adverse effect. The mean brood and compensation indices as well as the mean termination rates in R were statistically significantly different from the respective values in the control for all post treatment assessments (Student's t-Test, method pooled, one-sided, $\alpha = 0.05$). The mean termination rate at the end of the observation period (BFD+22) was 100.00 %, indicating that none of the initially marked eggs had completed its development.
In the test item treatment group T the brood development and mean termination rates were similar to the control. The mean brood and compensation indices as well as the mean termination rate in T on all BFD dates were not statistically significantly different from the respective values in the control (Student's t-Test, method pooled, one-sided, $\alpha = 0.05$). The mean termination rate at the end of the observation period (BFD+22) was acceptable at 35.53%.
Overall, the quantitative assessments of brood development in individually marked cells revealed that Fluoxastrobin EC 100 G, applied to full-flowering *Phacelia tanacetifolia* during daily honeybee flight at a rate of 150 g a.s./ha, did not cause any treatment-related adverse effect on honeybee brood development.

Findings are summarised in the table below.

Table CA 8.3.1.3- 4: Summary of the brood and compensation indices and termination rates

Treatment	Brood index / Compensation index at x days after brood area fixing day (BFD)					Termination rate (BFD+22)
	0	+5	+9	+15	+22	[%]
Control	**1.00 / 1.00**	**2.48 / 2.49**	**2.85 / 2.88**	**2.80 / 2.94**	**3.50 / 3.95**	**29.99**
STD	0.00 / 0.00	0.09 / 0.09	0.38 / 0.35	0.34 / 0.24	0.43 / 0.20	8.58
Test item T	**1.00 / 1.00**	**2.19 / 2.20**	**2.61 / 2.67**	**2.58 / 2.82**	**3.22 / 3.87**	**35.53**
STD	0.00 / 0.00	0.41 / 0.40	0.72 / 0.65	0.71 / 0.45	0.88 / 0.41	17.69
Reference item R	**1.00 / 1.00**	**0.00* / 0.04***	**0.00* / 0.15***	**0.00* / 0.66***	**0.00* / 1.61***	**100.00***
STD	0.00 / 0.00	0.00 / 0.01	0.00 / 0.12	0.00 / 1.03	0.00 / 1.37	0.00

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

Strength of the colonies:
The overall development of colony strength (mean number of bees per hive) of all treatment groups showed fluctuations in a typical and normal range. The colony strength values of the test item group were on approximately the same level or even higher during the entire study than the corresponding values of the control group. Therefore, no test-item related adverse effects on colony strength were observed.

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

Development of the food storage area:
The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed.
The majority of the colonies were well provided during the course of the study. Thus, no test-item related adverse effects on the development of the food storage area were observed.

Conclusions:

Fluoxastrobin EC 100 G was applied at a target rate corresponding to 150 g a.s./ha at full-flowering *Phacelia tanacetifolia* during honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated.
No test-item related adverse effects on mortality or flight intensity were observed.
The quantitative assessments of brood development in individually marked cells performed in this study revealed that Fluoxastrobin EC 100 G did not cause any treatment-related adverse effect on honeybee brood development.
The overall honeybee brood development in the test item treatment group T, measured as mean number of cells covered with the different types of brood per colony was not affected when compared to the control.

No test-item related adverse effects on colony strength or on the development of the food storage area were observed.
No test-item related effects on behaviour were observed.
Overall, Fluoxastrobin EC 100 G applied at 150 g a.s./ha to a flowering crop in presence of honey bees did not cause any effects on mortality, flight intensity, colony strength and 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 Draft Assessment Report (DAR) and in the Baseline Dossier provided by Bayer CropScience.
Studies on non-target arthropods have been performed with the representative formulations Fluoxastrobin + Prothioconazole EC 200 and Bixafen + Fluoxastrobin + Prothioconazole EC 190. A list of these studies is presented in the MCP documents; Annex point CP 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Please refer to point CA 8.3.2.

CA 8.3.2.2 Effects on Typhlodromus pyri

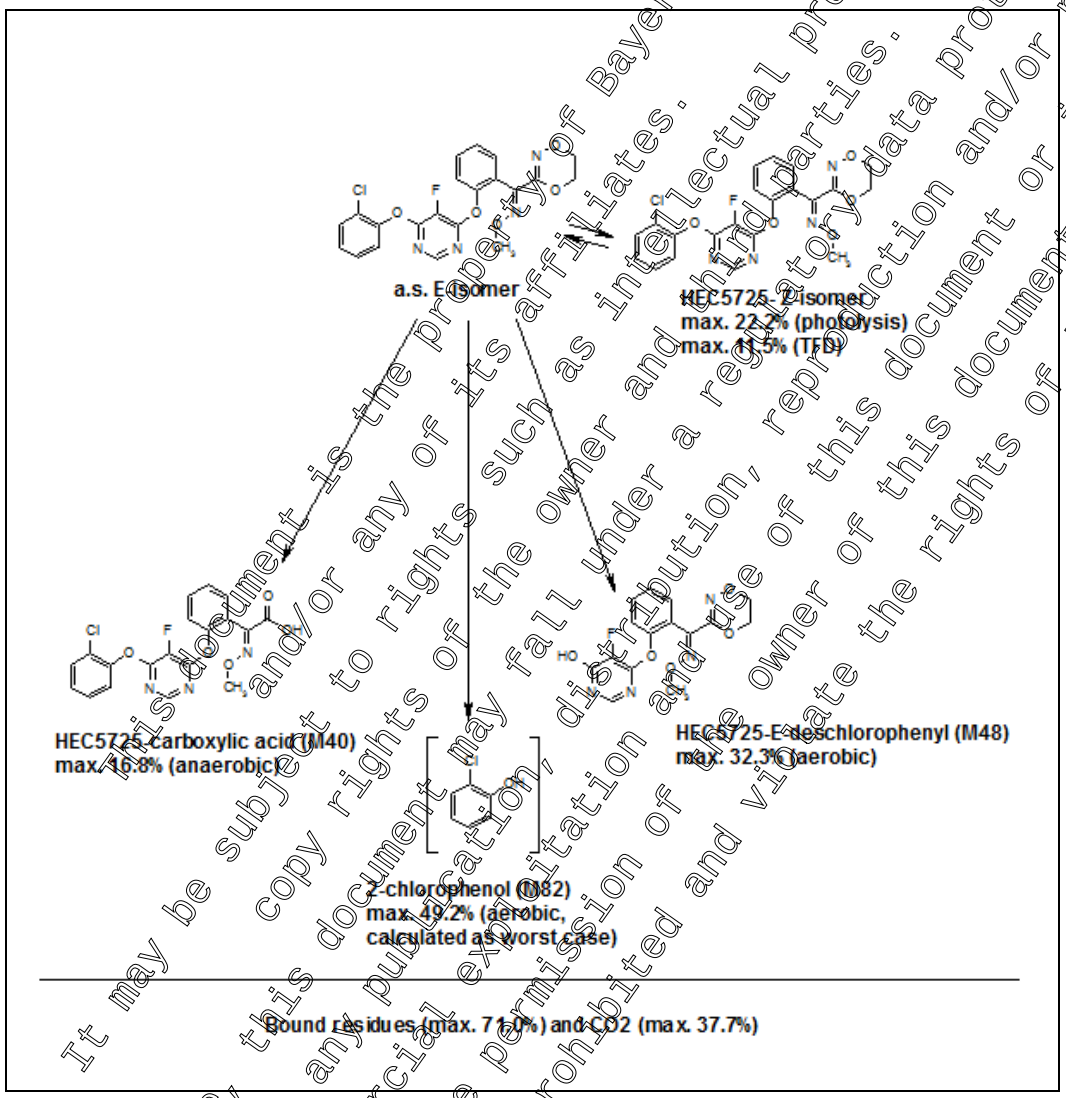
Please refer to point CA 8.3.2.

CA 8.4 Effects on non-target soil meso and macrofauna

Under Regulation (EC) No. 1107/2009 there are no data requirements for the acute toxicity to earthworms.

The degradation pathway in soil is given in the figure below. For further details refer to Section 7: "Fate and behaviour in the environment".

Figure 8.4-1: Proposed degradation pathway of fluoxastrobin in soil (major degradation products)



CA 8.4.1 Earthworm, sub-lethal effects

For information on studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).

Additional studies on earthworms were performed with the fluoxastrobin soil metabolites HEC 5725-E-des-chlorophenyl and HEC 5725-carboxylic acid and are submitted within this Supplementary Dossier.

No study on the chronic toxicity of the metabolite 2-chlorophenol to earthworms is available, but some information can be taken from the chronic earthworm study with the Fluoxastrobin EC 100 formulation. In this study the application of 1.0 kg a.s./ha fluoxastrobin had no influence on mortality, weight development, and reproduction of earthworms after 56 days. The NOEC (28 days) based on mortality and weight of adult earthworms is 1.0 kg a.s/ha. Additionally it is a NOEC and not an LC_{50}. Assuming that 2-chlorophenol is formed and reaches its maximum between about 15 to 23 days, the effects of this metabolite on mortality and weight of adult earthworms can be considered to be covered up to an application of 1.0 kg fluoxastrobin/ha.
Additionally, for the purpose of the earthworm risk assessment the conservative assumption has been made that the metabolite is 10 times more toxic than the parent a.s. (EFSA conclusion 102 (2007)).

Details of all studies regarding chronic endpoints on earthworms are provided in Table CA 8.4.1- 1.

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

Test substance	Test species	Endpoint		Reference
Fluoxastrobin[1)]	*Eisenia fetida* reproduction 56 d, mixed	NOEC NOEC NOEC	1000 g a.s./ha ≥4.32[2)] mg a.s./kg dws **≥2.16[2),3)] mg a.s./kg dws**	[redacted]; 2001; M-057395-01-1
HEC 5725-E-des-chlorophenyl	*Eisenia fetida* reproduction 56 d, mixed	NOEC	**≥ 1000 mg p.m./kg dws**	[redacted]K; 2002; M-058532-01-1
HEC 5725-carboxylic acid	*Eisenia fetida* reproduction 56 d, mixed	NOEC	**90 mg p.m./kg dws**	[redacted],; 2015; M-536000-01-1 KCA 8.4.1

a.s. = active substance, p.m. = pure metabolite, prod. = product, dws = dry weight soil
1) conducted with the formulation Fluoxastrobin EC 100
2) The endpoint of 1.33 mg as/kg dws listed in the EFSA Scientific Report 102 (2007) is based on the standard conversion. In the actual study the test material had been sprayed onto the soil, the recalculated endpoint according to the actual test conditions is calculated based on the actually applied test rate of 1090 g a.s./ha, test vessel surface of 198 cm^2 and test substrate of 500 g dws per test vessel
3) endpoint corrected by a factor of 2 due to high organic matter content of test soil and log Pow of >2
Bold letters – values considered relevant for risk assessment

Report: KCA 8.4.1/03 [redacted],; 2015; M-536000-01-1
Title: Fluoxastrobin-E-carboxylic acid (BCS-AR14771): Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil - Final report
Report No.: 101841022
Document No.: M-536000-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation (EC) No 1107/2009 (2009)
US EPA OCSPP Not Applicable
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of this study was to investigate the effects of Fluoxastrobin-E-carboxylic acid (BCS-AR14771) on the mortality, body weight, feeding activity and reproduction of the adult earthworm *Eisenia fetida*.

Material and methods:

Test substance: Fluoxastrobin-E-carboxylic acid (BCS-AR14771) ; Synonym: BCS-AR14771; Batch code: AE 1302955-01-02; Origin batch No.: KML 5767-2-5; LIMS No.: 1341147; Purity: AE 1302955: 88% w/w.

1st Experiment: Ten adult *Eisenia fetida* (with clitellum and weight range of 305 mg to 590 mg, 7 to 8 months old) per replicate (8 replicates per test item concentration) were exposed for 28 days to a single test concentration of 113.6 mg test item/kg soil dry weight (equivalent to 100 mg pure metabolite/kg soil dry weight) and to one untreated control. The pH was 6.1 to 6.2 at experimental start and 6.1 at experimental end; the water content at experimental start was 30.6 % to 31.3 % (54.6 % to 55.9 % of the maximum water holding capacity) and at experimental end 31.8 % to 32.7 % (56.8 % to 58.3 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux.
2nd Experiment: Ten adult *Eisenia fetida* (with clitellum and weight range of 302 mg to 599 mg, approximately 6 months old) per replicate (4 replicates per test item concentration and 8 replicates for the control) were exposed for 28 days to a the test concentrations of 11.36, 19.32, 34.09, 59.09 and 102.3 mg test item/kg soil dry weight (equivalent to 10, 17, 30, 52 and 90 mg pure metabolite/ kg soil dry weight) and to one untreated control. The pH was 5.5 to 5.9 at experimental start and 5.9 to 6.3 at experimental end; the water content at experimental start was 29.5 % to 30.5 % (53.7 % to 55.5 % of the maximum water holding capacity) and at experimental end 28.0 % to 32.7 % (50.9 % to 59.4 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux.
For the control of both experiments the same amount of quartz sand per g substrate as in the test item treated groups was added and moistened with deionised water. The effects of the reference item Carbendazim (499 g/kg nominal) were investigated in a separate study. The assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application). The test was performed according to the guideline ISO 11268-2 (2012) and the OECD Guideline 222 (2004).

Dates of experimental work: April 30, 2015 to October 02, 2015

Findings:

Validity criteria:

Table CA 8.4.1-2: Validity Criteria

	Required	Achieved	
		1st experiment	2nd experiment
Control Mortality:	≤ 10%	0%	0%
Control Reproduction (Juveniles per Container):	≥ 30	199 to 252	179 to 293
Coefficient of Variation of the Control Reproduction:	≤ 30%	8.7%	18.5%

All study validity criteria were met.

No mortality was observed in any treatment group, except for two dead worms (5%) at the test concentration of 52 mg pure metabolite/kg soil. This mortality was not statistically significantly different compared to the control (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$).
The body weight changes of the earthworms after 4 weeks exposure to Fluoxastrobin-E-carboxylic acid were not statistically significantly different compared to the control up to and including the highest test concentration of 100 mg pure metabolite/ kg soil dry weight (Student t-test, $\alpha = 0.05$, two-sided in the 1. experiment and Williams t-test, $\alpha = 0.05$, two-sided in the 2. experiment).

The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 90 mg pure metabolite/ kg soil dry weight (Williams t-test, α = 0.05, one-sided smaller). At the test concentration of 100 mg pure metabolite/ kg soil dry weight reproduction was slightly but statistically significantly reduced compared to the control (Student t-test, α = 0.05, one-sided smaller).
No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Table CA 8.4.1- 3: Effects of fluoxastrobin-E-carboxylic acid on earthworms (*Eisenia fetida)* in both experiments

1st experiment		
Treatment group	**Control**	**100**
Mortality (day 28) [%]	0	0
Statistical Significance	-	-
Body weight change (day 28) [%]	34.1	33.7
Statistical Significance [1)]	-	n.s.
Mean No. of juveniles (day 56)	229	210
Statistical Significance [1)]	-	*
Reproduction in [%] of control (day 56)	-	91.8

2nd experiment						
Treatment group	**Control**	**10**	**17**	**30**	**52**	**90**
Mortality (day 28) [%]	0	0	0	0	5.0	0
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	26.1	27.4	29.8	23.8	25.2	27.1
Statistical Significance [3)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	232	250	240	212	190	211
Statistical Significance [3)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 56)	-	108	103	91.5	82.1	91.0

Endpoints [mg pure metabolite/kg soil dry weight]	
NOEC (day 28 mortality and weight)	≥100
LOEC (day 28 mortality and weight)	>100
NOEC (day 56 reproduction)	90
LOEC (day 56 reproduction)	100

The results represent rounded values calculated on the exact raw data. The test item dosages are given as mg pure metabolite/kg artificial soil dry weight
- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Student t-test, α = 0.05, one-sided greater
[2)] Fisher's Exact Test, α = 0.05, one-sided greater
[3)] Williams t-test, α = 0.05, two-sided for weight changes and one-sided smaller for reproduction

Reference Item Test:
In the most recent test with the reference item carbendazim (performed under ibacon Study Number 91441022 from July 2014 to September 2014), there were statistically significant effects on reproduction at a concentration of 1.95 mg carbendazim/kg soil dry weight and higher, which is in line with the guideline OECD 222 (effects should be observed between 1 and 5 mg carbendazim/kg soil dry weight). The EC_{50} for reproduction was calculated as 1.87 mg carbendazim/kg soil dry weight.

Conclusions:

The No Observed Effect Concentration (NOEC) for mortality and growth of the earthworm *Eisenia fetida* was determined to be ≥100 mg pure metabolite/kg soil dry weight, *i.e.* the highest concentration tested.
The No Observed Effect Concentration (NOEC) for reproduction was determined to be 90 mg test item pure metabolite/kg soil dry weight and the Lowest Observed Effect Concentration (LOEC) for reproduction was determined to be 100 mg pure metabolite/kg soil dry weight.

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 fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).
Additional studies on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) were performed with the representative formulations and soil metabolites of fluoxastrobin and are submitted within this Supplementary Dossier:

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

Test substance	Test species	Endpoint	Reference
Collembola, reproduction			
Fluoxastrobin	*Folsomia candida* reproduction 28 d, mixed	$NOEC_{mort}$ 100 mg a.s./kg dws $NOEC_{repro}$ 10 mg a.s./kg dws **$NOEC_{corr}$ 5 mg a.s./kg dws[1)]**	[redacted]; 2001; M-081095-01-1
HEC 5725-E-des-chlorophenyl	*Folsomia candida* reproduction 28 d, mixed	**NOEC ≥ 100 mg p.m./kg dws**	[redacted]; 2001; M-033640-01-1
HEC 5725-carboxylic acid	*Folsomia candida* reproduction 28 d, mixed	**NOEC ≥ 100 mg p.m./kg dws**	[redacted]>; 2014; M-479456-01-1 KCA 8.4.2.1
2-Chlorophenol	*Folsomia candida* reproduction 28 d, mixed	$NOEC_{mort}$ 56 mg p.m./kg dws $NOEC_{repro}$ 10 mg p.m./kg dws **$NOEC_{corr}$ 5 mg p.m./kg dws[1)]**	[redacted],; 2013; M-472327-01-1 KCA 8.4.2.1
Soil mites, reproduction			
Fluoxastrobin	*Hypoaspis aculeifer* reproduction 22 d, mixed	**NOEC 10 mg p.m./kg dws[2)]**	[redacted]; 2002; M-039155-01-1
HEC 5725-E-des-chlorophenyl	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC ≥ 100 mg p.m./kg dws**	[redacted],; 2013; M-475673-01-1 KCA 8.4.2.1
HEC 5725-carboxylic acid	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC ≥ 100 mg p.m./kg dws**	[redacted],; 2014; M-484792-01-1 KCA 8.4.2.1
2-Chlorophenol	*Hypoaspis aculeifer* reproduction 14 d, mixed	$NOEC_{mort}$ ≥ 100 mg p.m./kg dws $NOEC_{repro}$ 56 mg p.m./kg dws **$NOEC_{corr}$ 28 mg p.m./kg dws[1)]**	[redacted]T; 2013; M-475688-01-1 KCA 8.4.2.1

a.s. = active substance, p.m. = pure metabolite, prod. = product, dws = dry weight soil
[1)] Corrected endpoint due to lipophilic substance (log Pow > 2)
[2)] Not corrected due to low organic matter content in test substrate LUFA 2.1
Bold letters – values considered relevant for risk assessment

CA 8.4.2.1 Species level testing

Report:	KCA 8.4.2.1/01 [redacted],; 2013; M-475673-01-1
Title:	Fluoxastrobin-deschlorophenyl (BCS-AO58740): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.:	13 10 48 191 S
Document No.:	M-475673-01-1
Guideline(s):	OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine potential effects of HEC 5725-deschlorophenyl (metabolite of fluoxastrobin) on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro-arthropods during a test period of 14 days. The test was performed as limit test according to the OECD guideline 226 (2008).

Material and Methods:

Test item: HEC 5725-deschlorophenyl; Substance code: AE 1302953; BCS-code: BCS-AO58740, Batch code: AE 1302953 00 1B98 0001, Origin Batch No.: HEC7155-4-1, CAS No.: 852429-78-8, LIMS No.: 1240600, Certificate No.: AZ 18440; analytical findings: 98.3 % w/w.

Ten adult, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for the treatment group) were exposed to control, toxic reference item and treatment. A single concentration of 100 mg test item/kg artificial soil dry weight was tested at 19.5 - 21.5 °C and a photoperiod: light : dark = 16 h : 8 h (527 lx). During the test, the *Hypoaspis aculeife*r were fed every 2 – 3 days with *Tyrophagus putrescentiae* (SCHRANK). The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.7 % industrial quartz sand, 5 % Sphagnum peat, dried and finely ground, 20 % Kaolin clay and 0.3 % $CaCO_3$. Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg artificial soil dry weight; control: quartz sand, solvent control: none.

Dates of experimental work: September 25, 2013 to October 29, 2013

Findings:

Validity criteria:

Validity criteria (control values)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	10.0 %
Mean number of juveniles per replicate	≥ 50	279.5
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	15.7 %

The validity criteria for the control group were accomplished.

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

Biological findings:
In the control group and in the test item treatment group a parental mortality of 10.0 % and 18.8 %, respectively, could be observed at the end of the 14-day exposure period.
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 279.5 in the control and 261.1 in the test item treatment group.

The test item caused no statistically significantly adverse effects on adult mortality (Chi² 2x2 test, α = 0.05, one-sided greater) and reproduction (Student t-test, α = 0.05, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight.

The results are summarised below.

Table CA 8.4.2.1- 1: Summary of the effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	HEC 5725-deschlorophenyl *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC_{10}	> 100	> 100
EC_{20}	> 100	> 100

Endpoint	HEC 5725-deschlorophenyl (mg metabolite/kg soil d.w.)	
	control	100
Mortality of soil mites after 14 days (%)	10.0	18.8
Mean number of juveniles after 14 days	279.5	261.1
CV %	15.7	8.8
Reproduction (% to control)	100	93

No statistically significant differences compared to the control were calculated (Chi² 2x2 Test for mortality, α = 0.05; Student t-test for reproduction, α = 0.05)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using non-rounded values
Percent reproduction: $[R_t / R_c] * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group

Conclusions:

The test item HEC 5725-deschlorophenyl showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight.

Report:	KCA 8.4.2.1/02[redacted]<; 2014; M-479456-01-1
Title:	Fluoxastrobin-carboxylic acid (BCS-AF84333): Effects on the reproduction of the collembolan Folsomia candida
Report No.:	14 10 48 098 S
Document No.:	M-479456-01-1
Guideline(s):	ISO 11267 (1999): Soil quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants. International Standard, First edition 1999-04-01. and OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009): Collembolan
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine potential effects of the metabolite Fluoxastrobin-carboxylic acid (BCS-AF84333) on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted. The test was performed as a limit test in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).

Material and methods:

Test item: Fluoxastrobin-carboxylic acid (BCS-AF84333, AE 1302955-01-02); metabolite of fluoxastrobin; Batch code: AE 1302955-01-02; Origin Batch No.: KML 5767-2-5; LIMS No.: 1330012; Customer order no.: FOX 09928-00; CAS No.: 852429-81-3; Analysed purity: 90.2% w/w.
10 *Collembola* (9-12 days old) per replicate (8 replicates for the control group and for the treatment group) were exposed to untreated control and 100 mg pure metabolite/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 18.6 – 21.8 °C and a photoperiod: light : dark = 16 h : 8 h (630 lx) and were fed weekly with granulated dry yeast.
Mortality and reproduction were determined after 28 days.

Toxic standard: 44 – 67 – 100 - 150 - 225 mg boric acid/kg soil dry weight; control: quartz sand, solvent control: none

Dates of work: January 09, 2014 to February 06, 2014

Findings:

Validity criteria:

Validity criteria (for control group)	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20%	2.5 %
Mean number of juveniles per replicate	≥ 100	663
Coefficient of variation (mean number of juveniles per replicate)	< 30%	12.1 %

The requirement of the ISO guideline concerning the precision of the counting method (average error <10 %) was fulfilled, the determined overall error of counting amounted to 3.4 %.

Reference test:
In the most recent study (BioChem project No. R 13 10 48 004 S, dated July 16, 2013) the EC_{50} was determined to be 108 mg a.s./kg soil dry weight. The LC_{50} was determined to be 192 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg a.s./kg soil dry weight, respectively.

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

Biological findings:
Mortality
2.5 % parental mortality was observed in the control. No statistically significant effect (Fisher`s Exact Binomial Test, α = 0.05, one-sided greater) on parental mortality was found for the concentration tested.

No effects on behaviour of the collembolans were observed during the test.

Reproduction
The mean number of juvenile springtails counted four weeks after introduction of the parental collembolans into the test vessels was 663 in the control and 686 at 100 mg pure metabolite/kg soil dry weight. No statistically significant effects (Student-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg pure metabolite/kg soil dry weight.

The no-observed-effect-concentration (NOEC) was determined to be ≥ 100 mg pure metabolite/kg soil dry weight.

Table CA 8.4.2.1- 2: Summary of the effects of fluoxastrobin-carboxylic acid on *Folsomia candida*

Test item **Test object** **Exposure**	**Fluoxastrobin-carboxylic acid (BCS-AF84333)** ***Folsomia candida*** **Artificial soil**			
mg pure metabolite/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles per test vessel ± standard deviation**	**Reproduction (% of control)**	**Significance (*)**
Control	2.5	663 ± 80	-	
100	2.5	686 ± 74	103	-
			Reproduction	
$NOEC_{reproduction}$ (mg pure metabolite/kg soil dry weight)			≥ 100	
$LOEC_{reproduction}$ (mg pure metabolite/kg soil dry weight)			> 100	

The calculations were performed with unrounded values
(*) = (Student-t-test one-sided-smaller, α = 0.05, + = significant, - = not significant)
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated groups
R_c = mean number of juveniles observed in the control group

Conclusion:

Fluoxastrobin-carboxylic acid (BCS-AF84333) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at 100 mg pure metabolite/kg soil dry weight.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg pure metabolite/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg pure metabolite/kg soil dry weight.

Report:	KCA 8.4.2.1/03 [redacted]; 2014; M-484792-01-1
Title:	Fluoxastrobin-carboxylic acid (BCS-AF84333): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.:	14 10 48 097 S
Document No.:	M-484792-01-1
Guideline(s):	OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine potential effects of the metabolite Fluoxastrobin-carboxylic acid (BCS-AF84333) on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro-arthropods during a test period of 14 days. The test was performed as limit test according to the OECD guideline 226 (2008).

Material and methods:

Test item: Fluoxastrobin-carboxylic acid (BCS-AF84333, AE 1302955-01-02); metabolite of fluoxastrobin; Batch code: AE 1302955-01-02; Origin Batch No.: KML 5767-2-5; LIMS No.: 1330012; Customer order no.: TOX 09928-00; CAS No.: 852429-81-3; Analysed purity: 90.2% w/w.
Ten adult soil mites (females) per replicate (8 replicates for the control group and for the treatment group) were exposed to untreated control and 100 mg pure metabolite/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.2 % $CaCO_3$, at 19.7 - 21.2 °C and a photoperiod: light : dark = 16 h : 8 h (517 lx) and were fed every 2 - 3 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg soil dry weight; control: untreated, solvent control: none.

Dates of work: February 14, 2014 to March 04, 2014

Findings:

Validity criteria:

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult female mortality	≤ 20%	5.0 %
Mean number of juveniles per replicate	≥ 50	244.6
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	11.9 %

The validity criteria for the control group were accomplished.

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

Biological findings:
Mortality
In the control group and in the test item treatment group a parental mortality of 5.0 % and 7.5 %, respectively, could be observed at the end of the 14-day exposure period.

The test item caused no statistically significantly adverse effects on adult mortality (Chi² 2x2 test, α = 0.05, one-sided greater) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg pure metabolite/kg soil dry weight.

Reproduction
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 244.6 in the control and 227.5 in the test item treatment group.
The test item caused no statistically significantly adverse effects on reproduction (Student t-test, α = 0.05, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg pure metabolite/kg soil dry weight.

Table CA 8.4.2.1- 3: Summary of the effects of fluoxastrobin-carboxylic acid on *Hypoaspis aculeifer*

Test item **Test object** **Exposure**			**Fluoxastrobin-carboxylic acid (BCS-AF84333)** ***Hypoaspis aculeifer*** **Artificial Soil**	
mg test item/kg dry weight artificial soil	**Mortality of soil mites after 14 days (%)**	**Mean number of juveniles after 14 days**	**CV (%)**	**Reproduction (% of control)**
Control	5.0	244.6	11.9	100
100	7.5	227.5	6.7	93
			Adult mortality	Reproduction
NOEC (mg pure metabolite/kg soil dry weight)			100	≥ 100
LOEC (mg pure metabolite/kg soil dry weight)			100	> 100
EC_{10} (mg pure metabolite/kg soil dry weight)			> 100	> 100
EC_{20} (mg pure metabolite/kg soil dry weight)			> 100	> 100

No statistically significant differences compared to control were calculated (Chi² 2x2 test for mortality, α = 0.05; Student t-test for reproduction; α = 0.05)
Calculations were done using non-rounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group

Conclusion:

The metabolite fluoxastrobin-carboxylic acid (BCS-AF84333) showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg pure metabolite/kg soil dry weight.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg pure metabolite/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg pure metabolite/kg soil dry weight.

Report: KCA 8.4.2.1/04 [redacted]; 2013; M-472327-01-1
Title: 2-chlorophenol: Effects on the reproduction of the collembolan Folsomia candida
Report No.: 13 10 48 187 S
Document No.: M-472327-01-1
Guideline(s): OECD 232 (2009): OECD Guideline for testing of chemicals No. 232 (adopted 7 September 2009): Collembolan reproduction test in soil; ISO 11267 (1999): Soil quality - Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of this study was to determine potential effects of different concentrations of 2-chlorophenol (metabolite of fluoxastrobin) on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted. The test was performed in accordance with the OECD Guideline 232 (2009) and the International Standard ISO 11267 (1999).

Material and Methods:

Test item: 2-chlorophenol; BCS-code: BCS-AA99770, Batch code: AE C505780-01-02, Customer Order No.: TOX 10013-01, Material No.: AE C505780, Origin Batch No.: GSE 2569-2-1, CAS No.: 97-57-8, LIMS No.: 1324971, analysed purity: 99.4 % w/w, water solubility: 28.5 g/L at 20 °C.

10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to reference item, untreated control and 10, 18, 32, 56, 100 mg test item/kg artificial soil dry weight containing 74.7 % industrial quartz sand, 20 % kaolin clay, 5 % sphagnum peat, dried and finely ground, and 0.3 % $CaCO_3$, at 18.0 – 21.8 °C and a photoperiod: light : dark = 16 h : 8 h (570 lx). During the test, the collembolans were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44 – 67 – 100 - 150 - 225 mg boric acid/kg artificial soil dry weight; control: deionised water, solvent control: none

Dates of work: September 05, 2013 to October 03, 2013

Findings:

Validity criteria:

Validity criteria	Recommended	Obtained
Mean adult mortality	≤ 20 %	5.0 %
Mean number of juveniles per test vessel	≥ 100	average of 624/vessel
Coefficient of variation for the mean number of juveniles	≤ 30 %	6.4 %

The requirement of the ISO guideline concerning the precision of the counting method (average error <10 %) was fulfilled, the determined overall error of counting amounted to 2.5 %.

Reference test:
To verify the sensitivity of the test system the reference item boric acid is routinely tested at concentrations of 44, 67, 100, 150 and 225 mg a.s./kg soil dry weight. The collembolans of the reference test were from the same source culture as those used in the definitive test.
In the most recent study (BioChem project No. R 13 10 48 004 S, dated July 16, 2013) the EC_{50} was determined to be 108 mg a.s./kg soil dry weight. The LC_{50} was determined to be 192 mg a.s./kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg a.s./kg soil dry weight, respectively.
The EC_{50} value for the reproduction was close to the value of 100 mg a.s./kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

Biological findings:
Mortality:
Mortality rates of 5.0 % - 95.0 % were recorded in the test item treatment groups. 5.0 % parental mortality was observed in the control. Statistically significant effects (Fisher`s Exact Binomial Test, $\alpha = 0.05$, one-sided greater) compared to the control were observed at a concentration of 100 mg test

item/kg artificial soil dry weight. No effects on behaviour of the collembolans were observed during the test.
The NOEC for the mortality of parental collembolans was determined to be 56 mg test item/kg artificial soil dry weight.

Reproduction:
The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 624 in the control and 633, 512, 439, 372 and 136 at concentrations of 10, 18, 32, 56 and 100 mg test item/kg artificial soil dry weight, respectively. Statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the control group were recorded at concentrations of 18, 32, 56 and 100 mg test item/kg artificial soil dry weight.
The no-observed-effect-concentration (NOEC) was determined to be 10 mg test item/kg artificial soil dry weight.

Table CA 8.4.2.1- 4: Summary of the effects of 2-chlorophenol on mortality of parental collembolans

Test item Test object Exposure	2-chlorophenol *Folsomia candida* Artificial soil			
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles per test vessel ± standard deviation	Reproduction (% of control)	Significance (*)
Control	5.0	624 ± 40	-	
10	5.0	633 ± 118	101	-
18	5.0	512 ± 61	82	+
32	5.0	439 ± 43	70	+
56	20.0	372 ± 27	60	+
100	95.0	136 ± 53	22	+
			Reproduction	
$NOEC_{reproduction}$ (mg test item/kg soil dry weight) $LOEC_{reproduction}$ (mg test item/kg soil dry weight)			10 18	
			Reproduction	
EC_{10} (mg test item/kg soil dry weight)[1)] 95 % confidence limits EC_{20} (mg test item/kg soil dry weight) 95 % confidence limits			16 (7 – 36) 25 (14 – 44)	

The calculations were performed with unrounded values
[1)] Probit analysis
(*) = (Williams-t-test one-sided-smaller, $\alpha = 0.05$, + = significant, - = not significant)
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated groups
R_c = mean number of juveniles observed in the control group

Conclusions:

2-chlorophenol showed statistically significantly adverse effects on adult mortality of the collembolans *Folsomia candida* in artificial soil at 100 mg test item/kg artificial soil dry weight. Statistically significant effects on reproduction of the collembolan *Folsomia candida* in artificial soil were observed at 18, 32, 56 and 100 mg test item/kg artificial soil dry weight. Therefore, the No-Observed-Effect-Concentration (NOEC) was determined to be 10 mg test item/kg artificial soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 18 mg test item/kg artificial soil dry weight.

Report:	KCA 8.4.2.1/05 [redacted],; 2013; M-475688-01-1
Title:	2-chlorophenol: Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.:	13 10 48 188 S
Document No.:	M-475688-01-1
Guideline(s):	OECD 226 (2008): Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine potential effects of 2-chlorophenol (metabolite of fluoxastrobin) on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro-arthropods during a test period of 14 days. The test was performed as limit test according to the OECD guideline 226 (2008).

Material and Methods:

Test item: 2-chlorophenol; BCS-code: BCS-AA99770, Batch code: AE C505780-01-02, Customer Order No.: TOX 10013-01, Material No.: AE C505780, Origin Batch No.: GSE 2569-2-1, CAS No.: 97-57-8, LIMS No.: 1324971, analysed purity: 99.4 % w/w, water solubility: 28.5 g/L at 20 °C.

Ten adult, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control, toxic reference item and 10, 18, 32, 56, 100 mg test item/kg artificial soil dry weight containing 74.7 % industrial quartz sand, 20 % kaolin clay, 5 % sphagnum peat, dried and finely ground, and 0.3 % $CaCO_3$, at 19.5 – 21.4 °C and a photoperiod: light : dark = 16 h : 8 h (544 lx). During the test, the soil mites were fed every 2-3 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 400): 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg dry weight artificial soil; control: deionised water, solvent control: none.

Dates of experimental work: October 18, 2013 to November 11, 2013

Findings:

Validity criteria:

Validity criteria (control values)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	1.3 %
Mean number of juveniles per replicate	≥ 50	319.5
Coefficient of variation (mean number of juveniles per replicate)	≤ 30 %	9.9 %

The validity criteria for the control group were accomplished.

Reference test:

To verify the sensitivity of the test system, the reference item Dimethoate EC 400 was tested at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry weight artificial soil.
In a separate study (BioChem project No. R 13 10 48 001 S, dated February 04, 2013), the EC_{50} (reproduction) of the reference item Dimethoate EC 400 was calculated to be 6.64 mg a.s./kg dry weight artificial soil. The results of the reference test demonstrate the sensitivity of the test system.

Biological findings:

In the control group a parental mortality of 1.3 % could be observed. The mortality in the test item treatment groups ranged between 0.0 and 5.0 %.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 319.5 in the control and 321.3, 323.0, 331.3, 331.3 and 214.0 at concentrations of 10, 18, 32, 56 and 100 mg test item/kg dry weight artificial soil, respectively.
2-chlorophenol caused no statistically significant mortality of adult mites (Fisher`s Exact Binomial Test, α = 0.05) at all tested concentrations. 2-chlorophenol caused no statistically significant effect on reproduction (Williams-t-test, α = 0.05) up to and including a test concentration of 56 mg test item/kg dry weight artificial soil. However, at a test concentration of 100 mg test item/kg dry weight artificial soil a statistically significant effect on reproduction could be observed. The results are summarised below.

Table CA 8.4.2.1- 5: Summary of the effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	2-chlorophenol *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	56
LOEC	> 100	100
EC_{10} (95 % confidence limits)	> 100	68.3 (53.8 – 86.7)
EC_{20} (95 % confidence limits)	> 100	83.4 (74.4 – 93.4)

Endpoint	**Treatment group (mg metabolite/kg soil d.w.)**					
	control	**10**	**18**	**32**	**56**	**100**
Mortality of soil mites after 14 days (%)	1.3	5.0	2.5	0.0	5.0	2.5
Mean number of juveniles after 14 days	319.5	321.3	323.0	331.3	331.3	214.0*
CV %	9.9	14.7	4.3	11.8	8.1	8.2
Reproduction (% to control)	**100**	**101**	**101**	**104**	**104**	**67**

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

Conclusions:

The test item 2-chlorophenol showed no statistically significantly adverse effects on adult mortality of the predatory mite *Hypoaspis aculeifer* in artificial soil at all tested concentrations.
Furthermore, the test item 2-chlorophenol showed no statistically significantly adverse effects on reproduction of *Hypoaspis aculeifer* up to and including a test concentration of 56 mg test item/kg dry weight artificial soil. However, at a test concentration of 100 mg test item/ kg dry weight artificial soil a statistically significant effect on reproduction could be observed.
Therefore, the No-Observed-Effect-Concentration (NOEC) and Lowest-Observed-Effect-Concentration (LOEC) for mortality were determined to be ≥ 100 mg and > 100 mg test item/kg dry weight artificial soil, respectively.
The No-Observed-Effect-Concentration and Lowest-Observed-Effect-Concentration for reproduction were determined to be 56 mg and 100 mg test item/kg dry weight artificial soil, respectively.

CA 8.5 Effects on nitrogen transformation

For information on studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).
Additional N-transformation studies were performed with the representative formulations and are submitted within this Supplementary Dossier:

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

Test substance	Test design	Endpoint	Reference
Fluoxastrobin	Study duration 28 d	no unacceptable effects **≥ 2.81 mg a.s./kg dws**	[redacted] 1999; M-024686-01-1
HEC 5725-E-des-chlorophenyl	Study duration 28 d	unacceptable effects **≥ 2.73 mg p.m./kg dws**	[redacted]; 2000; M-026016-01-1
HEC 5725-carboxylic acid	Study duration 28 d	unacceptable effects **≥ 1.27 mg p.m./kg dws**	[redacted]; 2001; M-033474-01-1

a.s. = active substance, p.m. = pure metabolite, prod. = product, dws = dry weight soil
Bold letters – values considered relevant for risk assessment

For the metabolite 2-chlorophenol, in the absence of nitrogen transformation data the conservative assumption has been made that the metabolite is 10 times more toxic than the parent a.s. (EFSA conclusion 102 (2007)).

CA 8.6 Effects on terrestrial non-target higher plants

For information on studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).
Studies on non-target plants (seedling emergence and vegetative vigour) were conducted with the representative formulations of fluoxastrobin and are presented in document MCP 10.6.2.

CA 8.6.1 Summary of screening data

Please see CA 8.6.

CA 8.6.2 Testing on non-target plants

Please see CA 8.6.

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

For information on studies already evaluated during the first EU review of fluoxastrobin, please refer to corresponding sections in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).

CA 8.8 Effects on biological methods for sewage treatment

For information on studies already evaluated during the first EU review of fluoxastrobin, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Draft Assessment Report (DAR).

CA 8.9 Monitoring data

No monitoring data are available or required.