

# REGISTRATION REPORT

## Part B

### Section 9

#### Ecotoxicology

Detailed summary of the risk assessment

Product code: GF-3308

Product name(s): not yet defined

Chemical active substance(s):

Fenpicoxamid (XDE-777), 50 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### CORE ASSESSMENT

(authorization)

Applicant: Dow AgroSciences

Submission date: May 2021

(updated in December 2021 and March 2022)

MS Finalisation date: April 2022 (initial Core Assessment)

October 2022 (final Core Assessment)

Version history

When	What
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May 2021	New submission of GF-3308 in the Central Zone.
December 2021	Austria removed from cMS, GAP table updated with 1 use = 1 crop + 1 disease
March 2022	Efate and ecotox updates requested by PL authorities
January 2022	Initial zRMS assessment. The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are <del>struck through and shaded for transparency</del> .
October 2022	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant <del>is struck through and shaded</del> .

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## 9 Ecotoxicology (KCP 10)

This document reviews the environmental toxicology studies and risk calculations for the plant protection product G-3308, a formulation containing fenpicoxamid (XDE-777) (50 g a.s./L).

### 9.1 Critical GAP and overall conclusions

**Table 9.1-1: Table of critical GAPs**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
UseNo. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI ***	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1-3, 7-9, 13	PL, AT, CZ, SK, RO	Winter cereals	F	Various diseases	Tractor mounted spray	BBCH 30- 69 (spring appn.)	1	-	a) 2 b) 2	a) 100 b) 100	100-300	F		A	A	N Scenario R4	R	A	A	A
																R Remaining scenarios				
4-6, 1012, 14		Spring cereals	F	Various diseases	Tractor mounted spray	BBCH 30- 69 (spring appn.)	1	-	a) 2 b) 2	a) 100 b) 100	100-300	F		A	A	N Scenario R4	R	A	A	A
																R Remaining scenarios				

\* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

\*\* F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

\*\*\* F: PHI is defined by the application stage at last treatment (time elapsing between last treatment and harvest of the crop).

(14) Remarks may include: Extent of use/economic importance/restrictions

Explanation for column 15 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

- Remarks** (1) Numeration necessary to allow references **table:** (2) Use official codes/nomenclatures of EU
- (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
  - (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
  - (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (*e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
  - (6) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench  
Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
  - (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
  - (8) The maximum number of application possible under practical conditions of use must be provided
  - (9) Minimum interval (in days) between applications of the same product.
  - (10) For specific uses other specifications might be possible, *e.g.*: g/m<sup>3</sup> in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
  - (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
  - (12) If water volume range depends on application equipments (*e.g.* ULVA or LVA) it should be mentioned under “application: method/kind”.
  - (13) PHI - minimum pre-harvest interval

### 9.1.1 Overall conclusions

#### zRMS comments:

Conclusions presented in points 9.1.1.1 to 9.1.1.7 below were checked by the zRMS and amended where necessary.

#### 9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from fenpicoxamid, relevant metabolites, and GF-3308. There is low risk to birds from drinking water or consuming contaminated prey items.

TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to mammals from fenpicoxamid, relevant metabolites, and GF-3308. There is low risk to mammals from drinking water or consuming contaminated prey items.

Avian and mammalian TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to reptiles and amphibians (via surrogacy) from fenpicoxamid, relevant metabolites, and GF-3308.

#### 9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Acceptable risk is demonstrated for fenpicoxamid, relevant metabolites, and GF-3308 in cereals at 1 x 2 L GF-3308/ha (equivalent to 100 g a.s./ha) with a:  $\square$  ~~10 m NSZ + 10 m VFS + 75% DRN~~; and

$\square$  ~~5 m NSZ + 10 VFS + 90% DRN~~.

Acceptable risk is demonstrated for fenpicoxamid, relevant metabolites, and GF-3308 in cereals at 1 x 2 L GF-3308/ha (equivalent to 100 g a.s./ha) with a:  $\square$  ~~10 m NSZ + 10 m VFS + 75% DRN~~; and  $\square$  ~~5 m NSZ + 10 VFS + 90% DRN~~.

#### zRMS comments:

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation GF-3308, which was performed in line with the EU agreed methodology.

*“The endpoint ErC50 is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”*

#### 9.1.1.3 Effects on bees (KCP 10.3.1)

The HQ values for fenpicoxamid, relevant metabolites, and GF-3308 in honey bee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honey bees is acceptable. Based on results of the tunnel study the risk to bees present in the field treated with GF-3308 at 2.0 L/ha could not be ruled out and following risk mitigation measures are proposed:

1. Do not apply when flowering weeds are present.

2. Do not apply when honeydew is present.
3. Do not use where bees are actively foraging.

The risk would be acceptable for bees present in adjacent crops or foraging on weeds in nonagricultural land, since the drift rate (2.77 g a.s./ha) is considerably lower than the lower tested rate of 65 g a.s./ha.

Concerned Member States must decide on applicability of the proposed RMM in their countries at the product authorisation.

~~The larval bee TER for GF 3308 exceeds the trigger of 1 using the EPPO risk assessment approach indicating that the proposed use poses an acceptable risk to bee larval development. Chronic risk to honey bee is also acceptable as the proposed uses of GF 3308 are on crops that are not attractive to bees therefore the exposure to applications on the treated crop will be negligible.~~

#### 9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

For fenpicoxamid, the tier 1 in- and off-field HQ values are below the Annex VI trigger of 2 for both indicator species, thus indicating that the active substance is of low risk to non-target arthropods at the maximum in-field application rate.

For GF-3308, the tier 2 in-field risk to soil-dwelling organisms is acceptable at the proposed GAP. Infield risk to foliar-dwelling organisms (*Coccinella* and *Aphidius*) is acceptable 0 and 13 days post application, respectively, when exposed to an exaggerated rate (i.e. 2 x 2 L GF-3308/L). Acceptable off-field risk is demonstrated for GF-3308 when used according to the proposed GAP.

#### 9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

TER<sub>LT</sub> values for fenpicoxamid, relevant metabolites, and GF-3308 are above the Annex VI trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP.

The maximum concentrations with less than 25% effects for the active ingredients, relevant metabolites, and formulation are greater than their respective PEC<sub>soil</sub>. There will be no adverse effects to soil microflora when used at the proposed GAP.

#### 9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

It can be concluded that the risk to non-target plants from the application of GF-3308 in cereals according to good agricultural practice is acceptable.

#### 9.1.1.7 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

The risk to other terrestrial vertebrate wildlife (birds, mammals, reptiles, and amphibians) are covered by the assessments conducted in Bobwhite quail, rats, and rabbits. No additional risk is anticipated.

### 9.1.2 Grouping of intended uses for risk assessment

GF-3308 is intended to be used as a post-emergence fungicide for the control of *Septoria* spp. and other diseases in winter and spring cereals. Therefore, no grouping for intended uses (i.e. risk envelope) is needed.

Birds, mammals, aquatics (active substance and major metabolite X642188), bees, non-target arthropods, and non-target plants are assessed at the maximum rate of 1 x 100 g a.s./ha, equivalent to 2 L GF-3308/ha.

Aquatics (minor metabolites), soil organisms, and micro-organisms are assessed at an exaggerated rate of 2 x 100 g a.s./ha, which is protective of the lower application rate of 1 x 100 g a.s./ha.

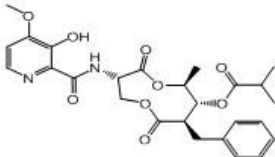
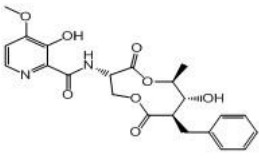
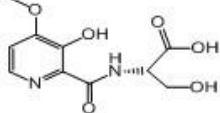
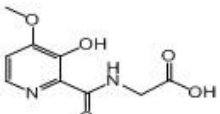
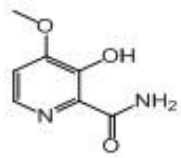
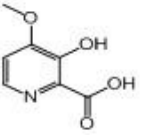
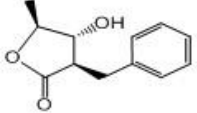
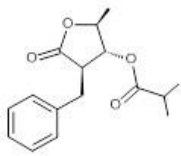
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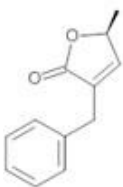
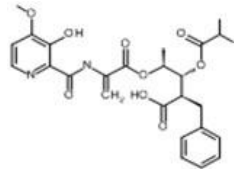
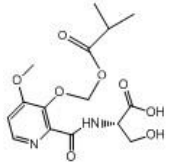
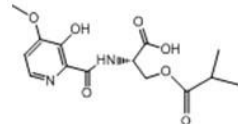
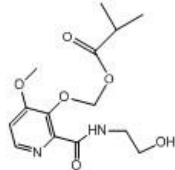
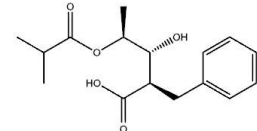
zRMS confirms that for birds, mammals, aquatic organisms and non-target terrestrial plants evaluation was performed with consideration of the use pattern intended in the Central Zone (i.e. single application to cereals at 100 g a.s./ha, BBCH 30-69). For soil organisms double application to cereals at 100 g a.s./ha (BBCH 30-69) was considered forming a risk envelope and representing worst case.

### 9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of GF-3308 is indicated in the table.

**Table 9.1-2 Major (>5% AR) metabolites of fencicoxamid relevant for exposure assessment (from Section 8.2)**

Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence (% AR) in compartments	Exposure assessment
X642188	514.2		Aerobic soil, 39.2% Water/sediment, 19.5%	PECsoil PECgw PECsw PECsed
X696872	444.2		Aerobic soil, 17.2%	PECsoil PECgw PECsw PECsed
X12264475	256.1		Anaerobic soil, 49.4% Water/sediment, 65.3%	PECsoil PECgw PECsw PECsed
X763024	226.1		Aerobic soil, 5.7%	PECsoil PECgw PECsw PECsed
X12313581	168.0		Field soil, 17.1% Aerobic mineralisation, 66.1% Water/sediment, 9.3%	PECsoil PECgw PECsw PECsed
X696476	169.0		Anaerobic soil, 46.9% Water/sediment, 67.1%	PECsoil PECgw PECsw PECsed
X11963422	206.1		Anaerobic soil, 80.3% Water/sediment, 45.0%	PECsoil PECgw PECsw PECsed
X12314005	276.3		Soil photolysis (irrad.), 5.4% Aq. photolysis (irrad.), 61.6% Water/sediment, 35.1%	PECsoil PECgw PECsw PECsed

X12019520	188.2		Soil photolysis (irrad.), 9.8% Aerobic mineralisation, 74.0% Water/sediment, 15.3%	PECsoil PECgw PECsw PECsed
X12255349	514.5		Soil photolysis (irrad.), 6.9%	PECsoil PECgw PECsw PECsed
Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence (% AR) in compartments	Exposure assessment
X12335723	356		Aq. photolysis (irrad.), 77.0% Water/sediment, 45.9%	PECsw PECsed
X12386481	326		Aerobic mineralisation, 69.5%	PECsw (water column metabolite only)
X12446477	312		Aq. photolysis (irrad.), 12.5%	PECsw (water column metabolite only)
X12433979	294		Hydrolysis (pH 9), 35.7%	PECsw (water column metabolite only)

#### Major fenpicoxamid (XDE-777) metabolites excluded from assessment

Per the EFSA Peer Review Report (EFSA, 2017) commenting tables, “metabolite X12386481 was found only in sterile hydrolytic degradation and dark control aqueous photochemical degradation, therefore it does not trigger the assessment and then it is not quoted in the list of metabolites included in FOCUS Surface water Step 1-2 calculation. However, worst case calculation done with the worst case default parameters were kept in the LoEP,” (pg 720). Therefore, “the aquatic risk assessment for this metabolite was removed since this metabolite is no longer a metabolite requiring further assessment for the aquatic compartment,” (pg 735).

The EFSA Peer Review Report (EFSA, 2017) commenting tables also state that the, “RMS to provide an amended LoEP removing metabolite X12442397/X12399889, which is considered an artefact formed in the photolysis dark control samples,” (pg 300).

#### **zRMS comments:**

Information regarding fenpicoxamid metabolites provided in Table 9.1-2 above is in line with EU agreed data reported in EFSA Journal 2018;16(1):5146.

With regard to information on consideration of metabolite X12386481 as not relevant for the aquatic risk assessment, it is not fully clear where the comments quoted by the Applicant were taken from - the zRMS checked the Reporting Table, Commenting Tables, Evaluation Table (all sections), text in the EFSA conclusion and the LoEP and in neither of documents mentioned the quoted text could be found. Issue of relevance of metabolite X12386481 for the risk assessment was also not discussed during the Pesticide Peer Review Meetings in area of efate and ecotoxicology. Comments of the RMS on draft EFSA conclusion suggest that the risk assessment for metabolite X12386481 has been removed from the LoEP, but without any justification, which was thus requested by the RMS. However, it seems that it has been not included by EFSA to the final version of the EFSA report. Nevertheless, metabolite X12386481 is not included in the definition of residues requiring further evaluation and no risk assessment for this compound is presented in EFSA Journal 2018;16(1):5146, although some toxicity endpoints were generated and are reported in the LoEP. Taking this into account, no specific risk assessment for metabolite X12386481 is deemed necessary.

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with fenpicoxamid and relevant metabolites. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Appendix 2 of this document (new studies).

Effects on birds of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds - fenpicoxamid**

Species	Substance	Exposure System	Results	Reference
Bobwhite quail ( <i>Colinus virginianus</i> )	Fenpicoxamid	Oral 1 d Acute	LD <sub>50</sub> >2000 mg/kg bw* <b>LD<sub>50</sub> = 3228 mg/kg bw ‡</b>	EFSA Journal 2018; 16(1):5146
Bobwhite quail ( <i>Colinus virginianus</i> )	Fenpicoxamid	Dietary Reproductive toxicity	NOAEL = 12.1 mg/kg bw/d	EFSA, 2018.

\*highest concentration or dose tested

‡ extrapolated endpoint in line with EFSA Risk Assessment for Birds and Mammals (2009) Where more than one endpoint is listed per study, the **bold** value is used for risk assessment.

**Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds - GF-3308**

Species	Substance	Exposure System	Results	Reference
Bobwhite quail ( <i>Colinus virginianus</i> )	GF-3308	Oral 1 d Acute	LD <sub>50</sub> >2000 mg/kg bw* <b>LD<sub>50</sub> = 3228 mg/kg bw ‡</b>	xxx/2016/DAS#160146

\* highest concentration or dose tested

‡ extrapolated endpoint in line with EFSA Risk Assessment for Birds and Mammals (2009); extrapolation factor of 1.614 applied Where more than one endpoint is listed per study, the **bold** value is used for risk assessment.

#### **zRMS comments:**

Avian toxicity data for fenpicoxamid are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146.

Study on acute on toxicity of GF-3308 to birds was evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.2-2 are confirmed to be correct. Since no



mortality was observed and 5 birds per dose were tested, the extrapolation factor of 1.614 is considered relevant, in line with indications of EFSA (2009).

### 9.2.1.1 Justification for new endpoints

Not applicable.

### 9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

#### 9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

**Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of fenpicoxamid in cereals**

<b>Intended use</b>	Cereals				
<b>Active substance</b>	Fenpicoxamid				
<b>Application rate (g a.s./ha)</b>	1 x 100				
<b>Acute toxicity (mg/kg bw)</b>	LD <sub>50</sub> = 3228 (extrapolated); >2000 mg/kg (actual)				
<b>TER criterion</b>	10				
<b>Crop scenario Growth stage</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>
Cereals, BBCH 30-69	Indicator species for screening (small omnivore)	158.8	1	15.9	203
<b>Reprod. toxicity (mg/kg bw/d)</b>	NOAEL = 12.1				
<b>TER criterion</b>	5				
<b>Crop scenario Growth stage</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>t</sub></b>
Cereals, BBCH 30-69	Indicator species for screening (small omnivore)	64.8	1 x 0.53	3.43	<b>3.52</b>
Cereals, BBCH 30-39	Small omnivorous bird “lark” Woodlark ( <i>Lullula arborea</i> )	5.4	1 x 0.53	0.286	42.3
Cereals, BBCH ≥ 40	Small omnivorous bird “lark” Woodlark ( <i>Lullula arborea</i> )	3.3	1 x 0.53	0.175	69.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Screening level TER<sub>A</sub> and Tier 1 TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from fenpicoxamid.**

Metabolite X12019520 was detected in grain at 5.5%, yet it was detected in the hen metabolism study at a lower percentage, 1.2%. An avian acute and chronic screening level risk assessment for the plant metabolite X12019520 not found at similar levels in the hen metabolism study and potentially formed in plant-derived food items is presented below. The DDD was determined using the following equation:

$$\text{DDD} = \text{parent app. rate (kg/ha)} * \% \text{ formation} * \text{SV} * \text{MAF}$$

A molecular weight conversion factor was not incorporated into the application rate because the endpoints shown are for the parent without a MW conversion. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X more toxic than the parent.

**Table 9.2-4: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of metabolite X12019520 in cereals**

Intended use	Cereals				
Metabolite	X12019520				
Application rate (g/ha)	1 x 5.5 g/ha <sup>1</sup>				
Acute toxicity (mg/kg bw)	LD <sub>50</sub> = 3228 (parent)/10 = 322.8				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals, BBCH 30-69	Indicator species for screening (small omnivore)	158.8	1	0.873	370
Reprod. toxicity (mg/kg bw/d)	NOAEL = 12.1 (parent)/10 = 1.21				
TER criterion	5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>
Cereals, BBCH 30-69	Indicator species for screening	64.8	1 x 0.53	0.189	6.41

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose

TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger. <sup>1</sup> 100 g a.s./ha x 5.5%

maximum formation = 5.5 g X12019520/ha

**Screening level TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from X12019520.**

**Table 9.2-5: First-tier assessment of the acute risk for birds due to the use of GF-3308 in cereals**

Intended use	Cereals				
Product	GF-3308				
Application rate (kg/ha)	1 x <del>2.02</del> <b>2.032</b> kg product/ha* or 0.1 kg a.s./ha				
Acute toxicity (mg/kg bw)	LD <sub>50</sub> = 3228 (extrapolated); >2000 mg/kg (actual)				
TER criterion	10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cereals, BBCH 30-69	Indicator species for screening	158.8	1	<b>320.8</b> <del>322.8</del>	<b>10.06</b> <del>10.0</del>

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose

TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger. \*Based on a formulation

density of **1.0108 g/mL** agreed in area of Section **1** ~~1.016 g/mL~~

**The screening level TER<sub>A</sub> value is equal to the Annex VI trigger value, therefore, there is acceptable acute risk to birds from GF-3308.**

#### **zRMS comments:**

The risk assessment for the active substance and metabolite X12019520 presented in Tables 9.2-3 and 9.2-4 above is agreed by the zRMS. The peak occurrence of X12019520 in cereals as reported in the LoEP has been considered in performed evaluation. No other fenpicoxamid metabolites were included in the risk assessment for birds presented in EFSA Journal 2018;16(1):5146.

Some minor corrections were made by the zRMS in the acute risk assessment for the formulated product, since slightly lower relative density has been agreed in area of Section 1.

Overall, based on the performed calculations, acceptable acute and long-term dietary risk to birds from fenpicoxamid, its relevant plant metabolites and the formulated product may be concluded following the intended Central Zone uses of GF-3308.

### 9.2.2.2 Higher-tier risk assessment

Not applicable.

### 9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

#### Leaf scenario

Since GF-3308 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

#### Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ( $K_{oc} < 500$  L/kg) or 3000 in the case of more sorptive substances ( $K_{oc} \geq 500$  L/kg).

With a  $K_{oc}$  of 5776 (geomean, EFSA, 2018), fenpicoxamid belongs to the group of more sorptive substances.

Application rate	1 x 100 g fenpicoxamid/ha		
Acute toxicity (mg/kg bw) =	3228	quotient =	0.03
Reprod. toxicity (mg/kg bw/d) =	12.1	quotient =	8.26

The ratio of effective application rate to the acute avian endpoint is below the trigger value of 3000 indicating the acute and chronic risk to birds from drinking water is acceptable.

**zRMS comments:**

The screening step of the drinking water risk assessment performed for fenpicoxamid in table above is agreed by the zRMS. Based on the performed calculations, acceptable risk to birds from the active substance present in drinking water may be concluded.

It is noted that the drinking water risk assessment should be also performed for the pertinent soil metabolites of the active substance. Since no calculations were performed by the Applicant, respective evaluation has been performed by the zRMS below. The metabolites pseudo-application rates were calculated with consideration of the molar ratio and peak occurrence in soil. In absence of the toxicity data for metabolites, 10 times toxicity of the parent was assumed (i.e. 322.8 and 1.21 mg/kg bw/d for acute and long-term risk, respectively). Please note that rounded values are reported in table below, but calculations were performed on unrounded values.

Substance	Molar mass [g/mol]	Molar ratio	Peak occurrence [%] <sup>1)</sup>	Application rate [g/ha]	Ratio for acute risk <sup>2)</sup>	Ratio for long-term risk <sup>3)</sup>	Trigger <sup>4)</sup>
Fenpicoxamid	614.6	parent	parent	100	see above	see above	see above
X642188	514.2	0.837	39.2	32.8	0.102	27.1	3000
X696872	444.2	0.723	17.2	12.4	0.039	10.3	3000
X12264475	256.1	0.417	49.4	20.6	0.064	17.0	50
X763024	226.1	0.368	5.7	2.1	0.006	1.7	50
X12313581	168.0	0.273	17.1	4.7	0.014	3.9	3000
X696476	169.0	0.275	46.9	12.9	0.040	10.7	3000
X11963422	206.1	0.335	80.3	26.9	0.083	22.3	50
X12314005	276.3	0.450	5.4	2.4	0.008	2.0	50
X12019520	188.2	0.306	9.8	3.0	0.009	2.5	50
X12255349	514.5	0.837	6.9	5.8	0.018	4.8	594

<sup>1)</sup> See EFSA Journal 2018;16(1):5146 or Table 8.2-1 in the Core Assessment, Part B, Section 8

<sup>2)</sup> Based on LD<sub>50</sub> of 322.8 mg/kg bw (parent endpoint divided by 10)

<sup>3)</sup> Based on NOEL of 1.21 mg/kg bw (parent endpoint divided by 10)

<sup>4)</sup> Determined based on geometric mean K<sub>foc</sub> reported in EFSA Journal 2018;16(1):5146

Ratios between the application rates and toxicity endpoints are below the respective triggers for all pertinent soil metabolites of fenpicoxamid demonstrating acceptable risk resulting from exposure of birds to these metabolites via drinking water in puddles.

#### 9.2.2.4 Effects of secondary poisoning

The LogK<sub>ow</sub> of fenpicoxamid amounts to 4.4, and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

##### Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil. EFSA 2009 allows for the peak PEC<sub>soil</sub> x 0.53 or the 21-d TWA PEC<sub>soil</sub> to be used for secondary poisoning; however for simplicity only the peak (initial) PEC<sub>soil</sub> was used and the TERs are well above the trigger values.

Note that a rate of 2 x 100 g fenpicoxamid/ha was modelled to estimate the PEC<sub>soil</sub> values, which is protective of the proposed GAP of 1 x 100 g fenpicoxamid/ha.

**Table 9.2-6: Assessment of the risk for earthworm-eating birds due to exposure to fenpicoxamid via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals**

Parameter	Fenpicoxamid	comments
PEC <sub>soil initial</sub> (mg/kg soil)	0.0533	Section 8.7 @ 2 x 100 g a.s./ha (risk envelope, covering 1x100 g a.s./ha, see Section 8 for details)
LogK <sub>ow</sub> / K <sub>ow</sub>	4.4/ 25119	
Koc	5776	EFSA, 2018 geomean
foc	0.02	Default
BCF <sub>worm</sub>	2.62	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC <sub>worm</sub>	0.139	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.146	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	12.1	
TER <sub>lt</sub>	83	

TER values shown in **bold** fall below the relevant trigger of 5.

LogK<sub>ow</sub> a synonym of LogP<sub>ow</sub>

X642188 exhibits measured values of LogK<sub>ow</sub> >3. While this metabolite was observed in the hen metabolism study and exhibited LogK<sub>ow</sub> value is less than that of the parent, a bioaccumulation and food chain transfer risk assessment is presented below. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X the parent.

**Table 9.2-7: Assessment of the risk for earthworm-eating birds due to exposure to X642188 via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals**

Parameter	X642188	comments
PEC <sub>soil initial</sub> (mg/kg soil)	0.0175	Section 8.7 @ 2 x 100 g a.s./ha (risk envelope, covering 1x100 g a.s./ha, see Section 8 for details)
LogK <sub>ow</sub> / K <sub>ow</sub>	3.58/ 3802	QSAR estimate
Koc	4518	EFSA, 2018 geomean
foc	0.02	Default
BCF <sub>worm</sub>	0.514	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC <sub>worm</sub>	0.0090	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0094	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	1.21	Parent/10
TER <sub>lt</sub>	128	

TER values shown in **bold** fall below the relevant trigger of 5.

LogK<sub>ow</sub> a synonym of LogP<sub>ow</sub>

The soil photolysis metabolite X12255349 was predicted to exhibit a LogK<sub>ow</sub> of 3.89 and was not observed in the hen metabolism study, thus a bioaccumulation and food chain transfer risk assessment for fish-eating and earthworm-eating birds was conducted. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X the parent.

**Table 9.2-8: Assessment of the risk for earthworm-eating birds due to exposure to X12255349 via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals**

Parameter	X12255349	comments
PEC <sub>soil</sub> initial (mg/kg soil)	0.0031	Section 8.7 @ 2 x 100 g a.s./ha (risk envelope, covering 1x100 g a.s./ha, see Section 8 for details)
LogK <sub>ow</sub> / K <sub>ow</sub>	3.89/ 7762.471	QSAR estimate
K <sub>oc</sub>	594	EFSA, 2018 geomean
f <sub>oc</sub>	0.02	Default
BCF <sub>worm</sub>	7.91	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
PEC <sub>worm</sub>	0.0245	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0258	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	1.21	Parent/10
TER <sub>It</sub>	47	

TER values shown in **bold** fall below the relevant trigger of 5.

LogK<sub>ow</sub> a synonym of LogP<sub>ow</sub>

### Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water. EFSA 2009 allows for the peak PEC<sub>sw</sub> x 0.53 or the 21-d TWA PEC<sub>sw</sub> to be used for secondary poisoning; however for simplicity the FOCUS Step 1 PEC<sub>sw</sub> was used and the TERs are well above the trigger values.

Note that a rate of 2 x 100 g fenpicoxamid/ha was modelled to estimate the peak PEC<sub>sw</sub> values, which is protective of the proposed GAP of 1 x 100 g fenpicoxamid/ha.

The zebrafish BCF study (Schlechtriem, 2014) found no detectable accumulation of fenpicoxamid, therefore the BCF<sub>ss</sub> was < 5 L/kg lipid uncorrected and < 2.6 L/kg 5% lipid corrected. However, due to deviations from the OECD TG 305 in the zebrafish BCF study, the DAR (United Kingdom, 2017) and subsequent EFSA Conclusion (2018) deferred to the QSAR BCF value of 18.36 L/kg for fenpicoxamid (Kramer, 2014), which is used in the risk assessment provided below.

**Table 9.2-9: Assessment of the risk for fish-eating birds due to exposure to fenpicoxamid via bioaccumulation in fish (secondary poisoning) for the intended use in cereals**

Parameter	Fenpicoxamid	comments
PEC <sub>sw</sub> (mg/L)	0.00475	Focus Step 1 PEC <sub>sw</sub> max, Section 8.9 @ 1 x 100 g a.s./ha
BCF <sub>fish</sub>	18.36 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.0872	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0139	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	12.1	
TER <sub>It</sub>	873	

TER values shown in **bold** fall below the relevant trigger of 5.

Per the EFSA Conclusion (2018), a fish BCF is not triggered as the metabolite X642188 is unstable in water. Therefore, the fish eating birds risk assessment is not relevant for X642188.

**Table 9.2-10: Assessment of the risk for fish-eating birds due to exposure to X12255349 via bioaccumulation in fish (secondary poisoning) for the intended use in cereals**

Parameter	X12255349	comments
PEC <sub>sw</sub> (mg/L)	0.00215	Focus Step 1 PEC <sub>sw</sub> max, Section 8.9 @ 2 x 100 g fenpicoxamid/ha (covering Step 1 PEC <sub>sw</sub> of 0.00108 mg/L from the intended use @ 1 x 100 g a.s./ha)
BCF <sub>fish</sub>	9.63 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.0207	PEC <sub>fish</sub> = PEC <sub>water</sub> × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.00329	DDD = PEC <sub>fish</sub> × 0.159
NOEL (mg/kg bw/d)	1.21	Parent/10
TER <sub>lt</sub>	368	

TER values shown in **bold** fall below the relevant trigger of 5.

**TER<sub>LT</sub> values for birds eating fish and earthworms are above the Annex VI trigger values, therefore, there is acceptable chronic risk to birds from fenpicoxamid and relevant metabolites.**

#### **zRMS comments:**

The evaluation of the risk of secondary poisoning for earthworm-eating birds is fully agreed by the zRMS.

Evaluation performed for fish-eating birds was amended accordingly with consideration of the surface water exposure agreed in area of Section 8. In line with information provided in EFSA Journal 2018;16(1):5146, no specific risk assessment was performed for metabolite X642188 since due to its instability in water, bioconcentration study in fish could not be performed.

According to conclusions taken during the EU review, no other compounds triggered the evaluation.

Overall, acceptable risk of secondary poisoning may be concluded for birds.

#### **9.2.2.5 Biomagnification in terrestrial food chains**

Not relevant.

#### **9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed**

Not relevant.

#### **9.2.4 Overall conclusions**

TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to birds from fenpicoxamid, relevant metabolites, and GF-3308 from the intended **Central Zone uses**. There is low risk to birds from drinking water or consuming contaminated prey items.

### 9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

#### 9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with fenpicoxamid and relevant metabolites. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Section 6 (Mammalian Toxicology) of this report (new studies).

Effects on mammals of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. However, the provision of further data on the GF-3308 formulation is not considered essential, because mixture calculations are provided below in order to generate a surrogate LD<sub>50</sub> for the assessment of acute risk to mammals.

**Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals – fenpicoxamid**

Species	Substance	Exposure System	Results	Reference
Rat	Fenpicoxamid	Oral 1 d Acute	LD <sub>50</sub> >2000 mg/kg bw*	EFSA, 2018
NZ White rabbit	Fenpicoxamid	Dietary Developmental toxicity (long-term)	NOAEL = 495 mg/kg bw/d*	EFSA, 2018

\*highest concentration or dose tested

#### **zRMS comments:**

Mammalian toxicity data for fenpicoxamid are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146.

No acute toxicity study was performed with the formulation, but GF-3308 is a solo formulation of fenpicoxamid and for this reason the risk assessment based on the active substance data is deemed sufficient.

#### 9.3.1.1 Justification for new endpoints

Not applicable.

#### 9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Bird and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

#### 9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

**Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of fenpicoxamid in cereals**

Intended use	Cereals
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Active substance		Fenpicoxamid				
Application rate (g/ha)		1 x 100				
Acute toxicity (mg/kg bw)		>2000				
TER criterion		10				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>	
Cereals, BBCH 30-69	Indicator species for screening	118.4	1	11.8	>169	
Reprod. toxicity (mg/kg bw/d)		495				
TER criterion		5				
Crop scenario Growth stage	Indicator/generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>	
Cereals, BBCH 30-69	Indicator species for screening	48.3	1 x 0.53	2.56	193	

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Screening level TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to mammals from fenpicoxamid.**

Metabolites X642188, X12264475, X12019520, and X12335723 were detected in both, the plant metabolism studies and the rat/goat metabolism studies, so it may be concluded that the risk of these metabolites is covered by the risk assessment on the parent fenpicoxamid. However, wheat metabolite X696476 was not detected in mammalian metabolism studies, so a mammalian acute and chronic screening level risk assessment was conducted. The maximum formation (%) was selected from the wheat, tomato, or cabbage metabolism studies, whichever had the highest percentage detected. The following equations were used to determine the DDD and TERs:

$$\text{DDD} = \text{parent App. rate} * \% \text{ formation} * \text{SV} * \text{MAF}$$

A molecular weight conversion factor was not incorporated into the application rate because the endpoints shown are for the parent without a MW conversion. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X more toxic than the parent.

**Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of metabolite X696476 in cereals**

<b>Intended use</b>		Cereals				
<b>Metabolite</b>		X696476				
<b>Application rate (g/ha)</b>		1 x 1.4 g/ha <sup>1</sup>				
<b>Acute toxicity (mg/kg bw)</b>		>2000 (parent)/10 = 200				
<b>TER criterion</b>		10				
<b>Crop scenario Growth stage</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>	
Cereals, BBCH 30-69	Indicator species for screening	118.4	1	0.166	>1207	
<b>Reprod. toxicity (mg/kg bw/d)</b>		495 (parent)/10 = 49.5				

<b>TER criterion</b>		5			
<b>Crop scenario Growth stage</b>	<b>Indicator/generic focal species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> TWA</b>	<b>× DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
Cereals, BBCH 30-69	Indicator species for screening	48.3	1 x 0.53	0.036	1381

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose

TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger. <sup>1</sup> 100 g a.s./ha x 1.4%

maximum formation = 1.4 g X696476/ha

**Screening level TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to mammals from metabolite X696476.**

#### **zRMS comments:**

The risk assessment for the active substance and metabolite X696476 presented in Tables 9.3-2 and 9.3-3 above is agreed by the zRMS. The peak occurrence of X696476 in cereals as reported in the LoEP has been considered in performed evaluation. No other fencicoxamid metabolites were included in the risk assessment for mammals presented in EFSA Journal 2018;16(1):5146. The Applicants' text regarding metabolites has been corrected in order to better reflect information presented in Vol. 3CP, B.9 ( December 2017).

Since GF-3308 is a solo formulation of fencicoxamid, performed evaluation covers also risk from the formulated product.

Overall, based on the performed calculations, acceptable acute and long-term dietary risk to mammals from fencicoxamid and its relevant plant metabolites may be concluded following the intended Central Zone uses of GF-3308.

### **9.3.2.2 Higher-tier risk assessment**

Not applicable.

### **9.3.2.3 Drinking water exposure**

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

#### **Puddle scenario**

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ( $K_{oc} < 500$  L/kg) or 3000 in the case of more sorptive substances ( $K_{oc} \geq 500$  L/kg).

With a  $K_{oc}$  of 5776 (geomean, EFSA, 2018), fencicoxamid belongs to the group of more sorptive substances.

Application rate	1 x 100 g fencicoxamid/ha		
Acute toxicity (mg/kg bw) =	>2000	quotient =	0.05
Reprod. toxicity (mg/kg bw/d) =	495	quotient =	0.20

The ratio of effective application rate to the acute mammalian endpoint is below the trigger value of 3000 indicating the acute and chronic risk to mammals from drinking water is acceptable.

**zRMS comments:**

The screening step of the drinking water risk assessment performed for fenpicoxamid in table above is agreed by the zRMS. Based on the performed calculations, acceptable risk to mammals from the active substance present in drinking water may be concluded.

It is noted that the drinking water risk assessment should be also performed for the pertinent soil metabolites of the active substance. Since no calculations were performed by the Applicant, respective evaluation has been performed by the zRMS below. The metabolites pseudo-application rates were calculated with consideration of the molar ratio and peak occurrence in soil. In absence of the toxicity data for metabolites, 10 times toxicity of the parent was assumed (i.e. 200 and 49.5 mg/kg bw/d for acute and long-term risk, respectively). Please note that rounded values are reported in table below, but calculations were performed on unrounded values.

Substance	Molar mass [g/mol]	Molar ratio	Peak occurrence [%] <sup>1)</sup>	Application rate [g/ha]	Ratio for acute risk <sup>2)</sup>	Ratio for long-term risk <sup>3)</sup>	Trigger <sup>4)</sup>
Fenpicoxamid	614.6	parent	parent	100	see above	see above	see above
X642188	514.2	0.837	39.2	32.8	0.164	0.663	3000
X696872	444.2	0.723	17.2	12.4	0.062	0.251	3000
X12264475	256.1	0.417	49.4	20.6	0.103	0.416	50
X763024	226.1	0.368	5.7	2.1	0.010	0.042	50
X12313581	168.0	0.273	17.1	4.7	0.023	0.094	3000
X696476	169.0	0.275	46.9	12.9	0.064	0.261	3000
X11963422	206.1	0.335	80.3	26.9	0.135	0.544	50
X12314005	276.3	0.450	5.4	2.4	0.012	0.049	50
X12019520	188.2	0.306	9.8	3.0	0.015	0.061	50
X12255349	514.5	0.837	6.9	5.8	0.029	0.117	594

<sup>1)</sup> See EFSA Journal 2018;16(1):5146 or Table 8.2-1 in the Core Assessment, Part B, Section 8

<sup>2)</sup> Based on LD<sub>50</sub> of 200 mg/kg bw (parent endpoint divided by 10)

<sup>3)</sup> Based on NOAEL of 49.5 mg/kg bw (parent endpoint divided by 10)

<sup>4)</sup> Determined based on geometric mean K<sub>foc</sub> reported in EFSA Journal 2018;16(1):5146

Ratios between the application rates and toxicity endpoints are below the respective triggers for all pertinent soil metabolites of fenpicoxamid demonstrating acceptable risk resulting from exposure of mammals to these metabolites via drinking water in puddles.

### 9.3.2.4 Effects of secondary poisoning

The LogK<sub>ow</sub> of fenpicoxamid amounts to 4.4, and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

#### Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil. EFSA 2009 allows for the peak PEC<sub>soil</sub> x 0.53 or the 21-d TWA PEC<sub>soil</sub> to be used for secondary poisoning; however for simplicity only the peak (initial) PEC<sub>soil</sub> was used and the TERs are well above the trigger values.

Note that a rate of 2 x 100 g fenpicoxamid/ha was modelled to estimate the  $PEC_{soil}$  values, which is protective of the proposed GAP of 1 x 100 g fenpicoxamid/ha.

**Table****9.3-4: Assessment of the risk for earthworm-eating mammals due to exposure to fenpicoxamid via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals**

Parameter	Fenpicoxamid	comments
PEC <sub>soil initial</sub> (mg/kg soil)	0.0533	Section 8.7 @ 2 x 100 g a.s./ha (risk envelope, covering 1x100 g a.s./ha, see Section 8 for details)
LogK <sub>ow</sub> / K <sub>ow</sub>	4.4/ 25119	
Koc	5776	EFSA, 2018 geomean
foc	0.02	Default
BCF <sub>worm</sub>	2.62	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + \mathbf{0.012} \times P_{ow}) / foc \times Koc$
PEC <sub>worm</sub>	0.139	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.179	DDD = PEC <sub>worm</sub> × 1.28
NOEL (mg/kg bw/d)	495	
TER <sub>lt</sub>	2773	

TER values shown in **bold** fall below the relevant trigger of 5.

LogK<sub>ow</sub> a synonym of LogP<sub>ow</sub>

X642188 exhibits measured values of LogK<sub>ow</sub> >3. While this metabolite was observed in the hen metabolism study and exhibited LogK<sub>ow</sub> value is less than that of the parent, a bioaccumulation and food chain transfer risk assessment is presented below. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X the parent.

**Table 9.3-5: Assessment of the risk for earthworm-eating mammals due to exposure to X642188 via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals**

Parameter	X642188	comments
PEC <sub>soil initial</sub> (mg/kg soil)	0.0175	Section 8.7 @ 2 x 100 g a.s./ha (risk envelope, covering 1x100 g a.s./ha, see Section 8 for details)
LogK <sub>ow</sub> / K <sub>ow</sub>	3.58/ 3802	QSAR estimate
Koc	4518	EFSA, 2018 geomean
foc	0.02	Default
BCF <sub>worm</sub>	0.514	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + \mathbf{0.012} \times P_{ow}) / foc \times Koc$
PEC <sub>worm</sub>	0.0090	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0115	DDD = PEC <sub>worm</sub> × 1.28
NOEL (mg/kg bw/d)	49.5	parent/10
TER <sub>lt</sub>	4298	

TER values shown in **bold** fall below the relevant trigger of 5.

LogK<sub>ow</sub> a synonym of LogP<sub>ow</sub>

The soil photolysis metabolite X12255349 was predicted to exhibit a LogK<sub>ow</sub> of 3.89 and was not observed in the mammalian metabolism studies, thus a bioaccumulation and food chain transfer risk assessment for fish-eating and earthworms-eating birds was conducted. As no toxicity data is available for the metabolite, the toxicity of the metabolite is assumed to be 10X more toxic than the parent.

**Table****9.3-6: Assessment of the risk for earthworm-eating mammals due to exposure to X12255349 via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals**

Parameter	X12255349	comments
PEC <sub>soil initial</sub> (mg/kg soil)	0.0031	Section 8.7 @ 2 x 100 g a.s./ha (risk envelope, covering 1x100 g a.s./ha, see Section 8 for details)
LogK <sub>ow</sub> / K <sub>ow</sub>	3.89/ 7762.471	QSAR estimate
Koc	594	EFSA, 2018 geomean
foc	0.02	Default
BCF <sub>worm</sub>	7.91	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC <sub>worm</sub>	0.0245	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.0314	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	49.5	parent/10
TER <sub>it</sub>	1577	

TER values shown in **bold** fall below the relevant trigger of 5.

LogK<sub>ow</sub> a synonym of LogP<sub>ow</sub>

**Risk assessment for fish-eating mammals via secondary poisoning**

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water. EFSA 2009 allows for the peak PEC<sub>sw</sub> x 0.53 or the 21-d TWA PEC<sub>sw</sub> to be used for secondary poisoning; however for simplicity the FOCUS Step 1 PEC<sub>sw</sub> was used and the TERs are well above the trigger values.

Note that a rate of 2 x 100 g fenpicoxamid/ha was modelled to estimate the peak PEC<sub>sw</sub> values, which is protective of the proposed GAP of 1 x 100 g fenpicoxamid/ha.

**Table 9.3-7: Assessment of the risk for fish-eating mammals due to exposure to fenpicoxamid via bioaccumulation in fish (secondary poisoning) for the intended use in cereals**

Parameter	Fenpicoxamid	comments
PEC <sub>sw</sub> (mg/L)	0.00475	Focus Step 1 PEC <sub>sw</sub> max, Section 8.9 @ <b>1</b> x 100 g a.s./ha
BCF <sub>fish</sub>	18.36 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.0872	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.0124	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	495	
TER <sub>it</sub>	39972	

TER values shown in **bold** fall below the relevant trigger of 5.

**Table****9.3-8: Assessment of the risk for fish-eating mammals due to exposure to X12255349 via**

Parameter	X12255349	comments
PEC <sub>sw</sub> (mg/L)	0.00215	Focus Step 1 PEC <sub>sw</sub> max, Section 8.9 @ 2 x 100 g fenpicoxamid/ha (covering Step 1 PEC <sub>sw</sub> of 0.00108 mg/L from the intended use @ 1 x 100 g a.s./ha)
BCF <sub>fish</sub>	9.63 L/kg	QSAR estimate
BMF	--	biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	0.0207	PEC <sub>fish</sub> = PEC <sub>water</sub> × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	0.00294	DDD = PEC <sub>fish</sub> × 0.142
NOEL (mg/kg bw/d)	49.5	parent/10
TER <sub>it</sub>	16837	

TER values shown in **bold** fall below the relevant trigger of 5.  
for the intended use in cereals

**bioaccumulation in fish (secondary poisoning)**

**TER<sub>LT</sub> values for mammals eating fish and earthworms are above the Annex VI trigger values, therefore, there is acceptable chronic risk to mammals from fenpicoxamid and relevant metabolites.**

**zRMS comments:**

The evaluation of the risk of secondary poisoning for earthworm-eating mammals is fully agreed by the zRMS.

Evaluation performed for fish-eating mammals was amended accordingly with consideration of the surface water exposure agreed in area of Section 8. In line with information provided in EFSA Journal 2018;16(1):5146, no specific risk assessment was performed for metabolite X642188 since due to its instability in water, bioconcentration study in fish could not be performed.

According to conclusions taken during the EU review, no other compounds triggered the evaluation.

Overall, acceptable risk of secondary poisoning may be concluded for mammals.

**9.3.2.5 Biomagnification in terrestrial food chains**

Not relevant.

**9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed**

Not relevant.

**9.3.4 Overall conclusions**

TER<sub>A</sub> and TER<sub>LT</sub> values are above the Annex VI trigger values, therefore, there is acceptable acute and chronic risk to mammals from fenpicoxamid, relevant metabolites, and GF-3308 from the intended

**Table**

**Central Zone uses.** There is low risk to mammals from drinking water or consuming contaminated prey items.



## 9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

Effects on terrestrial vertebrate wildlife (reptiles and amphibians) are covered by the effects studies conducted in bobwhite quail (*Colinus virginianus*, required by guideline to be phenotypically indistinguishable from wild-caught birds), laboratory-reared rats (*Rattus norvegicus*), mice (*Mus musculus*) and rabbits (*Oryctolagus cuniculus*).

Reptiles and terrestrial-phase amphibians can be covered by effects studies in birds and mammals because of their air-breathing life-style and evolutionary relationships that suggest physiological responses to toxicants may be covered directly, and more so, with the provision of the standard assessment factors of 10 and 5 applied to acute and long-term endpoints in the terrestrial risk assessment. Additional vertebrate studies on effects in wildlife are not justified when adequate data exists from standardized studies.

### **zRMS comments:**

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

## 9.5 Effects on aquatic organisms (KCP 10.2)

### 9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with fenpicoxamid and relevant metabolites. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below (9.5.1.1). When more than one endpoint exists for a taxa, the value in bold is used in the risk assessment.

**Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – fenpicoxamid and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Pimephales promelas</i> (fathead minnow)	Fenpicoxamid	96 h, f	<b>LC<sub>50</sub> = 1.79 µg a.s./L<sub>mm</sub></b>	EFSA, 2018
<i>Oncorhynchus mykiss</i> (trout)	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 2.2 µg a.s./L <sub>mm</sub>	EFSA, 2018
<i>Cyprinus carpio</i> (carp)	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 5.41 µg a.s./L <sub>gm</sub>	EFSA, 2018
<i>Lepomis macrochirus</i> (bluegill)	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 13.8 µg a.s./L <sub>mm</sub>	EFSA, 2018
<i>Danio rerio</i> (zebrafish)	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 104 µg a.s./L <sub>mm</sub>	EFSA, 2018
<i>O. mykiss</i>	GF-2925	96 h, ss	LC <sub>50</sub> = 9.15 µg prep/L <sub>gm</sub> (1.1 µg a.s./L)	EFSA, 2018
<i>O. mykiss</i>	GF-3308	96 h, f	LC <sub>50</sub> = 78 µg prep/L <sub>mm</sub> (3.8 µg a.s./L)	xxx/2016/DAS# 160101
Trout geomean for fenpicoxamid and GF-2925			LC <sub>50</sub> = 1.56 µg a.s./L	--
Acute geomean, 4 species (includes GF-2925)			LC <sub>50</sub> = 3.82 µg a.s./L	EFSA, 2018
Trout geomean for fenpicoxamid, GF-2925, and GF-3308			LC <sub>50</sub> = 2.10 µg a.s./L	See justification in Section 9.5.1.1
Acute geomean, 4 species (includes GF-2925 and GF-3308 trout)			<b>LC<sub>50</sub> = 4.1 µg a.s./L</b>	See justification in Section 9.5.1.1
<i>P. promelas</i>	Fenpicoxamid	32 d (ELS), f	NOEC = 0.37 µg a.s./L <sub>mm</sub> EC <sub>10</sub> (total length) = 0.11 µg a.s./L EC <sub>20</sub> = NA	EFSA, 2018
<i>Daphnia magna</i>	Fenpicoxamid	48 h, ss Rec. <LOQ-110%	EC <sub>50</sub> = 0.93 µg a.s./L <sub>gm</sub> **	EFSA, 2018
<i>D. magna</i>	Fenpicoxamid	21 d, ss	NOEC = 0.53 µg a.s./L <sub>twa</sub> Study invalidated; not considered a data gap	EFSA, 2018
<i>Pseudokirchneriella subcapitata</i>	Fenpicoxamid	72 h, s	<b>ErC<sub>50</sub> &gt;522 µg a.s./L<sub>gm</sub>****</b> <b>EyC<sub>50</sub> &gt;522 µg a.s./L<sub>gm</sub>****</b> <b>NOEC &gt;522 µg a.s./L<sub>gm</sub>****</b>	EFSA, 2018
<b>Fenpicoxamid metabolites</b>				

<i>O. mykiss</i>	X642188	96 h, f	LC <sub>50</sub> = 7.3 µg/L <sub>mm</sub>	EFSA, 2018
<i>D. magna</i>		48 h, ss (24 hr renewls) Rec. <MQL-168%	EC <sub>50</sub> = 1.3 µg/L <sub>gm</sub> Study invalidated; data gap	EFSA, 2018
<i>D. magna</i>		48 h, f	EC <sub>50</sub> = 0.79 µg/L <sub>mm</sub>	Goudie/2018/ DAS#180562

Species	Substance	Exposure System	Results	Reference
<i>Chironomus riparius</i>		28 d, s, sedimentspiked	overall NOEC = 1.9 mg/kg <sub>im</sub> survival EC <sub>10</sub> = 1.8 mg/kg NOEC = 0.63 <sub>wmm</sub> overall EC <sub>10</sub> = 0.58 mg/kg mg/kg, survival	Beasley/2018/DAS# 180563
<i>Lumbriculus variegatus</i>		28 d, s, sedimentspiked	NOEC = 34 mg/kg <sub>im</sub> NOEC = 14 mg/kg <sub>twmm</sub>	Dinehart/2019/DAS#180639
<i>P. subcapitata</i>		Parent/10	ErC <sub>50</sub> = 52.2 µg/L	EFSA, 2018
<i>O. mykiss</i>	X11963422	96 h, ss Rec. 92-103%	LC <sub>50</sub> >10000 µg/L <sub>nom</sub> * LC <sub>50</sub> >9800 µg/L <sub>mm</sub>	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 70-100%	EC <sub>50</sub> >9100 µg/L <sub>mm, gm</sub> *	EFSA, 2018
<i>P. subcapitata</i>		72 h, s	ErC <sub>50</sub> >9000 µg/L <sub>mm</sub> * EyC <sub>50</sub> >9000 µg/L <sub>mm</sub> NOEC >900 <sub>mm</sub> µg/L	EFSA, 2018
<i>O. mykiss</i>	X12264475	96 h, ss Rec. 78-106%	LC <sub>50</sub> >9980 µg/L <sub>gm</sub> *	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 96-108%	EC <sub>50</sub> >10000 µg/L <sub>nom</sub> *	EFSA, 2018
<i>P. subcapitata</i>		72 h, s	ErC <sub>50</sub> = 4440 µg/L <sub>m</sub> *** EyC <sub>50</sub> = 2750 µg/L <sub>gm</sub> *** NOEC = 243 µg/L <sub>gm</sub> ***	EFSA, 2018
<i>O. mykiss</i>	X12313581	96 h, ss Rec. 92-111%	LC <sub>50</sub> >10000 µg/L <sub>nom</sub> * LC <sub>50</sub> >9800 µg/L <sub>mm</sub>	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 92-114%	EC <sub>50</sub> >10000 µg/L <sub>nom</sub> * EC <sub>50</sub> >9800 µg/L <sub>mm</sub>	EFSA, 2018
Algae		QSAR	ErC <sub>50</sub> = 15000 µg/L	EFSA, 2018
<i>O. mykiss</i>	X696872	96 h, ss Rec. <MQL-102%	LC <sub>50</sub> >2000 µg/L <sub>gm</sub> *	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. <MQL-90%	EC <sub>50</sub> = 545 µg/L <sub>gm</sub> ***	EFSA, 2018
Algae		Parent/10	ErC <sub>50</sub> = 52.2 <del>522</del> µg/L	EFSA, 2018
<i>O. mykiss</i>	X696476	96 h, ss Rec. 89- 98%	LC <sub>50</sub> >10000 µg/L <sub>nom</sub> * LC <sub>50</sub> >9210 µg/L <sub>mm</sub>	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 79-109%	EC <sub>50</sub> >9500 µg/L <sub>gm</sub> *	EFSA, 2018
Algae		QSAR	ErC <sub>50</sub> = 350000 µg/L	EFSA, 2018

<i>O. mykiss</i>	X12314005	96 h, ss Rec. <MQL - 81%	LC <sub>50</sub> >1900 µg/L <sub>gm</sub> *	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. <MQL-83%	EC <sub>50</sub> >8500 µg/L <sub>gm</sub> *	EFSA, 2018
Algae		Parent/10	ErC <sub>50</sub> = 52.2 522 µg/L	EFSA, 2018
<i>D. magna</i>	X12386481	48 h, ss Rec. 64-85%	EC <sub>50</sub> >7800 µg/L <sub>mm, gm</sub> *	EFSA, 2018
Fish	X763024	QSAR	LC <sub>50</sub> = 568000 µg/L	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 82-91%	EC <sub>50</sub> >10000 µg/L <sub>nom</sub> *	EFSA, 2018
Algae		QSAR	ErC <sub>50</sub> = 275000 µg/L	EFSA, 2018
<del>Fish</del>	X12019520	<del>parent, GF-2925</del>	<del>LC<sub>50</sub> = 1.1 µg/L</del>	<del>EFSA, 2018</del>
<i>O. mykiss</i>		96 h, ss Rec. 94-103%	LC <sub>50</sub> >10000 µg/L <sub>nom</sub> * <del>LC<sub>50</sub></del> <del>&gt;9800 µg/L<sub>mm</sub></del>	Huges/2018/DAS# 180560
<i>D. magna</i>		48 h, ss	EC <sub>50</sub> >10000 µg/L <sub>mm, nom</sub> *	EFSA, 2018
<b>Species</b>	<b>Substance</b>	<b>Exposure System</b>	<b>Results</b>	<b>Reference</b>
		Rec. 95-111%		
Algae		Parent/10	ErC <sub>50</sub> = 52.2 522 µg/L	EFSA, 2018
Fish	X12335723	QSAR	LC <sub>50</sub> = 1.27 x 10 <sup>7</sup> µg/L	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. 67-102%	<del>EC<sub>50</sub> &gt;8600 µg/L<sub>gm</sub>*</del> EC <sub>50</sub> >8700 µg/L <sub>gm</sub> †	EFSA, 2018
<i>C. riparius</i>		28 d, s, sedimentspiked	overall NOEC = 6.8 mg/kg <sub>im</sub> * <del>&gt;6.8 mg/kg</del> EC <sub>10</sub> = NA mg/kg overall NOEC = 2.2 twmm mg/kg	Leak/2018/DAS# 180564
Algae		QSAR	ErC <sub>50</sub> = 1100000 µg/L	EFSA, 2018
<i>D. magna</i>	X12393285	48 h, ss Rec. 49-96%	EC <sub>50</sub> >7600 µg/L <sub>gm</sub> *	EFSA, 2018
<i>O. mykiss</i>	X12255349	96 h, ss Rec. 87-102%	LC <sub>50</sub> = 7100 µg/L <sub>nom</sub>	EFSA, 2018
<i>D. magna</i>		48 h, ss Rec. <MQL-100%	EC <sub>50</sub> = 11 µg/L <sub>gm</sub>	EFSA, 2018
<i>P. subcapitata</i>		72 h, s	ErC <sub>50</sub> >10000 µg/L <sub>nom</sub> * <del>ErC<sub>50</sub> &gt;8600 µg/L<sub>mm</sub></del> EyC <sub>50</sub> > 1000 nom NOEC = 1000 nom	EFSA, 2018
<del>Fish</del>	X12446477	<del>parent, GF-2925</del>	<del>LC<sub>50</sub> = 1.1 µg/L</del>	<del>EFSA, 2018</del>
<i>O. mykiss</i>		96 h, ss Rec. 92-106%	LC <sub>50</sub> >10000 µg/L <sub>nom</sub> * <del>LC<sub>50</sub></del> <del>&gt;9600 µg/L<sub>mm</sub></del>	xxx/2018/DAS# 180561
<i>D. magna</i>		48 h, ss Rec. 86-110%	EC <sub>50</sub> = 1100 µg/L <sub>mm</sub>	EFSA, 2018
Algae		Parent/10	ErC <sub>50</sub> = 52.2 522 µg/L	EFSA, 2018
<del><i>D. magna</i></del>	<del>X12399889</del> (X12442397)	<del>48 h, ss Rec.</del> <del>79-104%</del>	<del>EC<sub>50</sub> &gt;9000 µg/L<sub>gm</sub>*</del>	<del>EFSA, 2017; artifact,</del> <del>remove from LoEP</del>
<i>D. magna</i>	X122442403	48 h, ss Rec.68-80%	EC <sub>50</sub> = 2400 µg/L <sub>gm</sub>	EFSA, 2018

Fish	X12433979	QSAR	LC <sub>50</sub> = 81990 µg/L	Blickley/2018/DAS# 180910
Invertebrate		QSAR	EC <sub>50</sub> = 48857 µg/L	
Algae		QSAR	ErC <sub>50</sub> = 44437 µg/L	
Higher-tier studies (micro- or mesocosm studies)				
<i>D. magna</i>	Fenpicoxamid	35 d, indoor population, static, single pulse	NOEC = 1.88 µg/L <sub>im</sub> EC <sub>10-juveniles</sub> = 0.770 µg/L <sub>im</sub> (reduction on day 7) EC <sub>10-neonates</sub> = 1.11 µg/L <sub>im</sub> (reduction on day 7)	Hicks/2017/DAS# 160125
Invertebrate mesocosm (outdoor)	GF-2925	133 d, static	<del>NOEAEC = 3.3 µg a.s./L</del> NOEC = 0.1 µg a.s./L <sub>im</sub>	EFSA, 2018

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations; gm: based on geometric mean measured concentrations; twmm: based on time-weighted mean measured concentrations

\* highest concentration tested

\*\*indicates uncertainty per the EFSA Conclusion (2018) related to the exposure achieved in the study; refer to the EFSA Peer Review Report (2017) for additional detail (Some uncertainties still stand for this endpoint since the measured concentrations in the test system were about 10% of the nominal)

\*\*\*Due to the uncertainties related to the exposure achieved in this study, this endpoint could be used as supportive information only, please refer to the experts' meeting discussion (Peer Review Expert meeting 165) \*\*\*\* A detailed assessment of the validity criteria was missing from the RAR

† The EFSA Conclusion LoEP (2018) and DAR (United Kingdom, 2017) incorrectly lists an EC<sub>50</sub> of >8700 µg/L for X12335723. The correct 48 hr EC<sub>50</sub> values are >8700 µg/L arithmetic mean measured and >8600 µg/L geomean; the geomean was used in the risk assessment as concentrations fell below 80% of nominal.

Where more than one endpoint is listed per study, the **bold** value is used for risk assessment.

**zRMS comments:**

Aquatic toxicity data for fenpicoxamid are in general line with EU agreed endpoints reported in EFSA Journal 2018;16(1):5146. Additional clarifications as given in the LoEP were added by the zRMS in Table 9.5-1 above. Information not reported in the LoEP has been struck through.

Calculation of the fish geomean  $LC_{50}$  is agreed by the zRMS. Since toxicity endpoint for GF-2925 was included in calculation of the EU agreed geometric mean and fenpicoxamid and both formulations (GF-2925 and GF3308) are of comparable acute toxicity to rainbow trout, calculation of geomean  $LC_{50}$  for rainbow trout from all 3 endpoints and its inclusion into the calculated overall geomean  $LC_{50}$  for fish is justified. It is noted that in case endpoint of 1.1  $\mu\text{g a.s./L}$  (from study with GF-2925) was excluded and geomean for *Oncorhynchus mykiss* was calculated from data for fenpicoxamid and GF-3308 only, the overall geometric mean  $LC_{50}$  would be higher (4.43  $\mu\text{g a.s./L}$ ). Taking this into account, consideration of the endpoint for GF-2925 leads to an endpoint representing worst case.

Following additional studies with the active compound and its metabolites were submitted by the Applicant in support of this evaluation:

- metabolite X12019520: acute toxicity to *Oncorhynchus mykiss*,
- metabolite X12335723: long-term toxicity to *Chironomus riparius*,
- metabolite X12446477: acute toxicity to *Oncorhynchus mykiss*,
- estimation of the acute toxicity of metabolite X12433979 to fish and *Daphnia magna* and chronic toxicity to algae,
- *Daphnia magna* population study with fenpicoxamid.

All studies with metabolites were submitted in order to fulfil data gaps indicated in the EFSA Journal 2018;16(1):5146 and enable finalisation of the risk assessment. They were evaluated by the zRMS and considered acceptable. Summaries of the studies may be found in Appendix 2 together with zRMS evaluation. Endpoints reported in Table 9.5-1 are confirmed.

It is noted that although a data gap for submission of study on toxicity of metabolite X12335723 was identified in EFSA Journal 2018;16(1):5146, it seems that this was a mistake, since during the water/sediment studies X12335723 was not detected in sediment and exposure of sediment dwellers to this compound may be thus excluded. Nevertheless, some of metabolites present in sediment are formed from this compound and the study may be used in order to demonstrate decreased toxicity to aquatic organisms from metabolites formed in a metabolic pathway including formation of X12335723.

The study on effects of fenpicoxamid on population of *Daphnia magna* was submitted together with similar study performed with formulation GF-3308 in order to demonstrate that the active substance is more toxic than GF-3308. It has to be noted that according to the EFSA aquatic guidance (2013), refined exposure laboratory studies with population of invertebrates are not recommended due to the rapid onset of recovery. The following is stated in the guidance:

*“Although refined exposure tests with standard test species that more or less resemble the design of tier 1 toxicity studies can be used for RAC derivation, the PPR Panel recommends not using refined exposure laboratory tests with populations of invertebrates (e.g. Daphnia) for this purpose when recovery is also considered. These population-level laboratory experiments with invertebrates are usually performed with individuals that differ in age and developmental state. As a result a rapid onset of recovery will occur after contamination under such test scenarios. Resources for surviving individuals will increase after contamination and will trigger an unrealistic strong recovery as no competitors are present (Knillmann et al., 2012b).”*

Nevertheless, as already mentioned above, results of this study were used to compare effects from the active compound and the formulation and obtained endpoints were not used directly in the risk assessment. Taking this into account, the study was evaluated by the zRMS for its relevance for such comparison and was considered to be sufficient for this purpose. Summary of the study may be found in Appendix 2 together with zRMS evaluation. Endpoint reported in Table 9.5-1 is confirmed. Additionally also  $EC_{10}$  values lower than the NOEC were added by the zRMS as being more sensitive and thus more relevant for the bridging purposes.

In addition to the above listed studies, the Applicant provided also study on effects of metabolite X642188 to *Lumbriculus variegatus*. However, the study was not evaluated by the zRMS since study on toxicity of this compound to *Chironomus riparius* was submitted and was deemed sufficient to address the data gap identified in EFSA Journal 2018;16(1):5146 for testing of sediment dwellers. Study on effects on second sediment dwelling species should be dealt with at the next renewal process of fenpicoxamid.

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GF-3308**

Species	Substance	Exposure System	Results	Reference
<i>O. mykiss</i>	GF-3308	96 h, f	LC <sub>50</sub> = 78 µg prep/L <sub>mm</sub> (3.8 µg a.s./L)	xxx/2016/DAS# 160101
<i>D. magna</i>	GF-3308	48 h, ss Rec. <MQL – 108%	EC <sub>50</sub> = 48 µg prep/L <sub>twgm</sub> (2.3 µg a.s./L)	xxx/2016/DAS# 160102
<i>P. subcapitata</i>	GF-3308	72 h, s	E <sub>r</sub> C <sub>50</sub> >30000 µg prep/L <sub>gm</sub> ( >1480 µg a.s./L) E <sub>y</sub> C <sub>50</sub> = 21000 µg prep/L (1030 µg a.s./L) NOE <sub>r</sub> C = 2400 µg product/L	Bergfield/2016/DAS# 160103
<b>Higher-tier studies (micro- or mesocosm studies)</b>				
<i>D. magna</i>	GF-3308	21d, indoor population, static	21-d NOEC = 550 µg prep/L <sub>im</sub> * (27 µg a.s./L) EC <sub>10-neonates</sub> = 39 µg prep/L <sub>im</sub> (1.9 µg a.s./L) (only on day 7, no effects at remaining samplings) EC <sub>10-juveniles</sub> = 370 µg prep/L <sub>im</sub> (18 µg a.s./L) (only on day 2, no effects at remaining samplings) LOEC >550 µg prep/L <sub>im</sub>	Hicks/2016/DAS#160126

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; gm: based on geometric mean measured concentrations; im: based on initial measured concentrations \* highest concentration tested

**GF-3308 endpoints demonstrate that the formulation is not more toxic than the active substance to aquatic organisms.**

#### **zRMS comments:**

Studies on acute toxicity of GF-3308 to fish and *Daphnia magna* and chronic toxicity to algae were evaluated by the zRMS and considered acceptable. Summaries of the studies may be found in Appendix 2 together with zRMS evaluation. Endpoints reported in Table 9.5-2 are confirmed.

As in case of the study with fenpicoxamid, the *Daphnia magna* population study with GF-3308 was evaluated by the zRMS for its relevance for comparison with study performed with the active compound and was considered to be sufficient for this purpose. Summary of the study may be found in Appendix 2 together with zRMS evaluation. Endpoints reported in Table 9.5-2 are confirmed. Additional information was added by the zRMS for clarity.

### **9.5.1.1 Justification for new endpoints**

#### Data Gaps Identified in EFSA Conclusion (2018) (Section 7.1)

#### **1. PEC in surface water and sediment and a risk assessment for aquatic organisms for metabolite X12433979.**

This is a unique aqueous photolysis and hydrolysis metabolite formed at similar levels. The pH at which it is seen is similar to that of the aerobic mineralisation and water/sediment studies where it was not detected. For this reason it is still considered "not relevant" to the environment.

Furthermore, X12433979 is highly unstable during synthesis and began to decompose on standing and during chromatography. X12433979 could only be obtained in 85-90% purity, which allowed for structure identification but not further study (Cremer, 2015, SPS-14-6). Thus, we are not able to synthesize test material for an acute daphnid or fish study.

However, Tier 1 PEC<sub>sw/sed</sub> values are presented in Section 8.9 based upon generic assumptions for the metabolite. X12433979 does not contain the toxicophore and QSAR predicted fish 96 hr LC<sub>50</sub>, daphnid 48 hr EC<sub>50</sub>, and green algae 96 hr EC<sub>50</sub> values for X12433979 were 81.990, 48.857, and 44.437 mg/L, (Blickley, 2018), respectively, indicating low hazard to aquatic organisms. For the intended uses in cereals, calculated RAC/PEC ratios for X12433979 did not indicate an unacceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios (Table 9.5-26). Therefore, no further assessment is necessary.

#### **zRMS comments:**

The zRMS agrees that metabolite X12433979 was formed neither in aerobic mineralisation nor water/sediment study and was observed only in hydrolysis and photolysis studies. In general, reference to aerobic mineralisation study is not relevant, since this study included only 72 hours incubation time which is too short for formation of X12433979, observed at 14-32 and 19 days in hydrolysis and photolysis studies, respectively. The water/sediment study was performed for much longer period of time (106 days) and X12433979 was not detected at any of the sampling dates. It may be argued that it was not formed since water/sediment study is performed under continuous darkness so the conditions were not favourable for this compound to be formed. It should be, however, noted during the photolysis study X12433979 was formed at >10% AR also in dark control samples (at 19 d), so it is not formed exclusively in the presence of light. In opinion of the zRMS in case of compounds that are formed in the dark, the water/sediment study is most relevant to identify compounds relevant for the aquatic risk assessment purposes, since hydrolysis is also covered in these tests. Photolysis studies are relevant to detect compounds formed exclusively in presence of light, since they will not be formed in the water/sediment studies. Since obviously X12433979 may be formed in the dark and was not detected in the water/sediment studies, in opinion of the zRMS it is not relevant for the aquatic risk assessment.

Nevertheless, in order to fulfil the data gap indicated in EFSA Journal 2018;16(1):5146 and due to difficulties with synthesis of X12433979, the Applicant provided the QSAR estimation of the acute endpoints for fish and *Daphnia magna* and chronic endpoint for algae. The calculations were agreed by the zRMS and are considered sufficient to address the risk from compound that in general should not be considered relevant.

## **2. A detailed assessment of the validity criteria for the studies on algae.**

All submitted algae studies met OECD 201 (freshwater alga and cyanobacteria, growth inhibition test) validity criteria, of which there are three: (1) The biomass in the control cultures should have increase exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day; (2) The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, and 2-3, for 72 hour tests), in control cultures must not exceed 35%; and (3) the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. A detailed assessment of the validity criteria for fenpicoxamid, four metabolites, and three relevant formulations is presented in the table below.



**Table 9.5-3: Assessment of algal growth inhibition validity criteria according to OECD 201 for fenpicoxamid, metabolites, and relevant formulations**

Molecule	Study name	DAS ID	Cell count <u>should</u> increase at least 16X at 72 hrs	Mean CV for section-by-section specific growth rates in the control cultures <u>must</u> not exceed 35%.	CV of average specific growth rates during the whole test period in replicate controls <u>must</u> not exceed 7%.	VC met?
Fenpicoxamid	XDE-777: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	120383	After 72 hours of exposure, mean cell density in the control was $84.2 \times 10^4$ cells/mL, or 168 times the initial nominal cell density. The mean cell density in the vehicle control was $84.5 \times 10^4$ cells/mL, or 169 times the initial nominal cell density.	The mean CV in growth rate between adjacent time periods was 13% for the control replicates	The CV of average specific growth rates during the whole test period in control replicates was 1% (pooled controls). CVs were 0% for negative control and vehicle control.	YES
X642188	X642188 Metabolite: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	120380	After 72 hours of exposure, mean cell density in the control was $61.4 \times 10^4$ cells/mL, or 123 times the initial nominal cell density. The mean cell density in the vehicle control was $61.2 \times 10^4$ cells/mL, or 122 times the initial nominal cell density	The mean CV in growth rate between adjacent time periods was 4% for the control replicates	The CV of average specific growth rates during the whole test period in control replicates was 0%.	YES
X11963422	X11963422 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	130385	After 72 hours of exposure, mean cell density in the control was $123 \times 10^4$ cells/mL, or 123 times the initial nominal cell density.	The mean CV in growth rate between adjacent time periods for the control replicates was 23% after 72 hours.	The CV of average specific growth rates in control replicates was 2% after 72 hours.	YES
X12264475	X12264475 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	130384	After 72 hours of exposure, mean cell density in the control was $132 \times 10^4$ cells/mL, or 136 times the initial nominal cell density	The mean CV in growth rate between adjacent time periods for the control replicates was 22% after 72 hours.	The CV of average specific growth rates in control replicates was 2% after 72 hours.	YES
X12255349	X12255349 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	141001	After 72 hours of exposure, mean cell density in the control was $132 \times 10^4$ cells/mL, or 275 times the initial nominal cell density	The mean CV in growth rate between adjacent time periods was 21% for the control replicates	The CV of average specific growth rates during the whole test period in control replicates was 1%.	YES

GF-2925	GF-2925: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	120376	After 72 hours of exposure, mean cell density in the control was $77.1 \times 10^4$ cells/mL, or 154 times the initial nominal cell density	The mean CV in growth rate between adjacent time periods was 7% for the control replicates.	The CV of average specific growth rates during the whole test period in control replicates was 1 %.	YES
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Molecule	Study name	DAS ID	Cell count <u>should</u> increase at least 16X at 72 hrs	Mean CV for section-by-section specific growth rates in the control cultures <u>must</u> not exceed 35%.	CV of average specific growth rates during the whole test period in replicate controls <u>must</u> not exceed 7%.	VC met?
GF-3308	GF-3308 Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	160103	After 72 hours of exposure, mean cell density in the control was $69.0 \times 10^4$ cells/mL, or 135 times the initial nominal cell density	The mean coefficient of variation in growth rate between adjacent time periods was 18% for the control replicates	The coefficient of variation of average specific growth rates during the whole test period in control replicates was 0%.	YES
GF-3307	GF-3307 Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata	140491	After 72 hours of exposure, mean cell density in the control was $55.2 \times 10^4$ cells/mL, or 110 times the initial nominal cell density	The mean coefficient of variation in growth rate between adjacent time periods was 24% for the control replicates	The coefficient of variation of average specific growth rates during the whole test period in control replicates was 1%.	YES

#### **zRMS comments:**

In general, validation of studies submitted in support of the EU evaluation of the given active compound should be done at the EU level. In case no respective information is provided in the study summaries or study reports, the RMS should request the Applicant to provide respective calculations, which should not be considered to be new data, since it concerns studies already provided for purposes of the active substance evaluation. In case for some reasons this is not possible, the check of validity criteria may be easily done by the RMS during evaluation of the studies based on the raw data available in the test reports.

In opinion of the zRMS it is not correct to shift the evaluation of the validity criteria of the EU studies to the zonal/national evaluators, as this would require re-evaluation of the studies itself, which should not be done at the zonal or national level, especially the endpoints are already reported in the LoEP.

Taking this into account, the validity criteria of the algae studies were not checked by the zRMS with consideration of the raw data from the study reports, but the information provided in Table 9.5-3 was simply noted as indicating that in fact, the validity criteria were met. The detailed evaluation should be performed at the EU level at next renewal. In addition to that it should be highlighted that algae are not driving the risk from either fenpicoxamid or its metabolites, so consideration of not fully checked endpoints for algae has no impact on the outcome of the risk assessment.

### 3. Further information to address the toxicity of the active substance when formulated with particular refer to aquatic invertebrates (chronic toxicity).

Fenpicoxamid breaks down rapidly in aquatic systems thus a chronic invertebrate study (OECD 211) on the active or formulation is not relevant.

A tier 1 OECD 211 chronic daphnid study with daily renewal of the test material was submitted during the annex I evaluation of fenpicoxamid (NOEC = 0.53 µg a.s./L<sub>tw</sub>); however, the study was rejected for failure to maintain concentrations between renewals.

During the acute daphnid testing of GF-3308 EC (4.8% fenpicoxamid), attempts were made to conduct the test under flow-through conditions as evidenced by the original protocol shown in Appendix C of the report (xxx, 2016; DAS# 160102). However, the high flow-rate required to maintain fenpicoxamid concentrations resulted in daphnid control mortality that was higher than the OECD 202 guideline allowed (i.e. ≤10%). Additional trials were undertaken to see how frequently the test material could be renewed while still keeping daphnid control mortality within acceptable limits. The result was that we could renew test material no more frequently than every 8 hours and that an interim analytical timepoint (we chose 2 hours) was needed because not all concentrations had measurable residues at the end of renewals.

Given this information, it is highly unlikely that a new tier 1 OECD 211 chronic daphnid study with the active substance or formulation could maintain concentrations even with 8 hour renewals over a 21 day exposure while still meeting the control validity criterion for mortality and reproduction. As such, we have utilized the GF-2925 invertebrate mesocosm endpoint to refine the acute (and chronic) toxicity to invertebrates.

#### GF-2925 Outdoor invertebrate mesocosm

In the EFSA Conclusion (EFSA, 2018), the fenpicoxamid risk assessment was refined with the NOEC from the formulation GF-2925 SC (12.1% fenpicoxamid) outdoor invertebrate mesocosm (NOEC = 0.1 µg/L<sub>im</sub>; AF = 3; RAC = 0.033 µg a.i./L).

The GF-2925 invertebrate mesocosm contained 180 identified taxa (52 phytoplankton, 33 zooplankton, 34 macro-invertebrates, 25 macrozoobenthos, and 36 emerging insects), had 2 applications of 130 g fenpicoxamid/ha with a 14 day interval, and was run for 133 day after the 1st application, which is significantly longer than the standard daphnia chronic toxicity test (i.e. 21 days).

#### **zRMS comments:**

The new *Daphnia magna* reproduction study with fenpicoxamid was deemed not necessary by the experts during the Pesticide Peer Review Meeting 165 due to difficulties with testing as described above and availability of the higher tier study. For further discussion on consideration of the endpoint from mesocosm study performed with GF-2925 in the risk assessment for GF-3308, please see commenting box below.

#### **Exposure for fenpicoxamid and metabolite X642188**

Water samples for quantifying fenpicoxamid (XDE-777) and the metabolite X642188 were taken from mesocosms at eight time points after the first application of the test item (from 10 min to 14 days) and at 12 time points (10 min to 112 days) after the second application. Sediment samples for the analytical quantification of fenpicoxamid and X642188 were taken from mesocosm ponds belonging to the two highest treatment levels on post-treatment days 2, 12, 16, 28, 49, 70, 91 and 112. Tables 9.5-4 and 9.5-5 show the results of the arithmetic mean measured concentration of fenpicoxamid and metabolite X642188 in the water (µg/L) and measured concentrations in sediment (µg/kg), respectively.

The dissipation of fenpicoxamid from the mesocosm water column was very fast as the concentrations of such active substance were fallen below the LOQ after four days at the highest test concentration. DT<sub>50</sub> values of fenpicoxamid were approximately 6 hours after the first application and ranged between 6 and 12 hours after the second application.

The soil metabolite X642188 was also built in the mesocosms but only found in the water samples of the two highest treatment levels within the first 24 hours after application with a maximum of 0.21 µg/L measured 10 min after application.

**Table 9.5-4: Mean measured concentrations of fenpicoxamid (XDE-777) and X642188 in water samples (µg/L)**

Time <sup>1</sup> (d)	Treatment level (µg/L) (nominal concentration)						
	0.1	0.3	1	3		10	
	XDE-777	XDE-777	XDE-777	XDE-777	X642188	XDE-777	X642188
<b>1st application of test item</b>							
0.007 (10 min)	0.10	0.33	1.05	3.25	0.07	9.31	0.21
0.042 (1h)	0.08	0.26	0.87	2.54	0.06	8.30	0.18
0.25 (6h)	0.05	0.12	0.36	1.08	< LOQ	3.92	0.10
1	< LOQ	< LOQ	0.10	0.31	< LOQ	1.21	< LOQ
2	< LOQ	< LOQ	< LOQ	0.06	< LOQ	0.18	< LOQ
4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
7	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
14	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
<b>2nd application of test item</b>							
14.007	0.10	0.33	1.16	3.27	0.00	11.70	0.21
14.042	0.13	0.51	1.04	2.91	0.00	9.97	0.21
14.25	0.07	0.16	0.58	2.08	0.01	7.12	0.16
15	< LOQ	0.05	0.16	0.63	0.05	3.00	0.08
16	< LOQ	< LOQ	< LOQ	0.16	< LOQ	0.97	< LOQ
18	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
21	nd	nd	nd	< LOQ	< LOQ	< LOQ	< LOQ

The exposure seen in water during the GF-2925 mesocosm for both parent and metabolite is consistent with the available Tier 1 acute invertebrate studies as well as the daphnid population studies. Given the rapid loss of fenpicoxamid and X642188 in the water column, direct effects (i.e. mortality) occur shortly after the applications as the molecules are not persistent in the water.

With regard to the sediment, the highest measured concentration of fenpicoxamid was 4.18 µg/kg analysed two days after the first application in the 10 µg/L treatment group (pond 5). This decreased to a concentration of 0.33 µg/kg by the end of the study (post-treatment day 112).

X642188 was also found in the sediment samples of the two highest treatments. The highest measured concentration of X642188 was 2.53 µg/kg dw analysed 16 days after the first application in one of the mesocosms treated with 10 µg/L and 1.99 µg/kg dw on day 28 in the other replicate. These concentrations decreased to a concentration of < LOQ (0.15 µg kg dw) and 0.35 µg/kg dw, respectively, by the end of the study (post-treatment day 112).

**Table 9.5-5: Mean measured concentrations of fenpicoxamid (XDE-777) and X642188 in sediment (µg/kg)**

Time <sup>1</sup> (d)	Treatment 3µg/L (nominal concentration)						Treatment 10 µg/L (nominal concentration)			
	Pond no. 3		Pond no. 11		Pond no. 16		Pond no. 5		Pond no. 13	
	XDE-777	X642188	XDE-777	X642188	XDE-777	X642188	XDE-777	X642188	XDE-777	X642188
2	< LOQ	< LOQ	0.96	0.38	0.40	0.16	4.18	1.08	0.95	0.26
12	< LOQ	< LOQ	< LOQ	< LOQ	0.40	0.39	1.49	1.43	0.92	0.99
16	0.48	0.36	1.07	0.58	0.87	0.45	3.52	1.78	2.58	2.53
28	0.22	0.39	< LOQ	< LOQ	0.47	0.42	1.42	1.99	1.53	1.89
49	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.36	0.51	0.41	0.59
70	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.47	0.54	< LOQ	< LOQ
91	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.38	0.37	< LOQ	< LOQ
112	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.33	0.35	< LOQ	< LOQ

LOQ sediment = 0.15 µg/kg<sup>1</sup> Time counted from the first test item application

In summary, this shows that while organisms residing in the water column were briefly exposed to fenpicoxamid and X642188, the sediment serves as a sink for the metabolite X642188. And yet sediment dwellers were not the most sensitive taxa identified in the GF-2925 mesocosm nor where they particularly sensitive in Tier 1 sediment-spiked chronic *Chironomus* and *Lumbriculus* studies (Beasley, 2018; Dinehart, 2019).

#### **zRMS comments:**

Based on the provided above results of chemical analyses it may be concluded that metabolite X642188 was formed during the mesocosm study performed with GF-2925. Concentration of the metabolite in the water column was low, but obviously it was rapidly partitioning to the sediment, where the sediment dwellers were exposed to both, active substance and X64218. Partitioning of X642188 to the sediment may be also expected based on strong sorption of this metabolite to soil with geometric mean K<sub>oc</sub> value of 4518 mL/g.

#### **Uncertainty**

GF-2925 was the representative formulation evaluated during Annex I inclusion of fenpicoxamid. An outdoor invertebrate mesocosm study is not available for GF-3308; however, use of the GF-2925 mesocosm endpoint is justifiable when considering that the Tier 1 acute daphnid studies indicate that **GF-2925 is more toxic to invertebrates than GF-3308** when the exposure regime is the same (Table 9.5-6). Where concentrations weren't maintained in the daily renewals until the end of the renewal period, the studies may be deemed unsuitable for risk assessment, but they can contribute to the overall evidence that use of the GF-2925 invertebrate mesocosm endpoint to refine the fenpicoxamid risk assessment is sufficiently conservative.

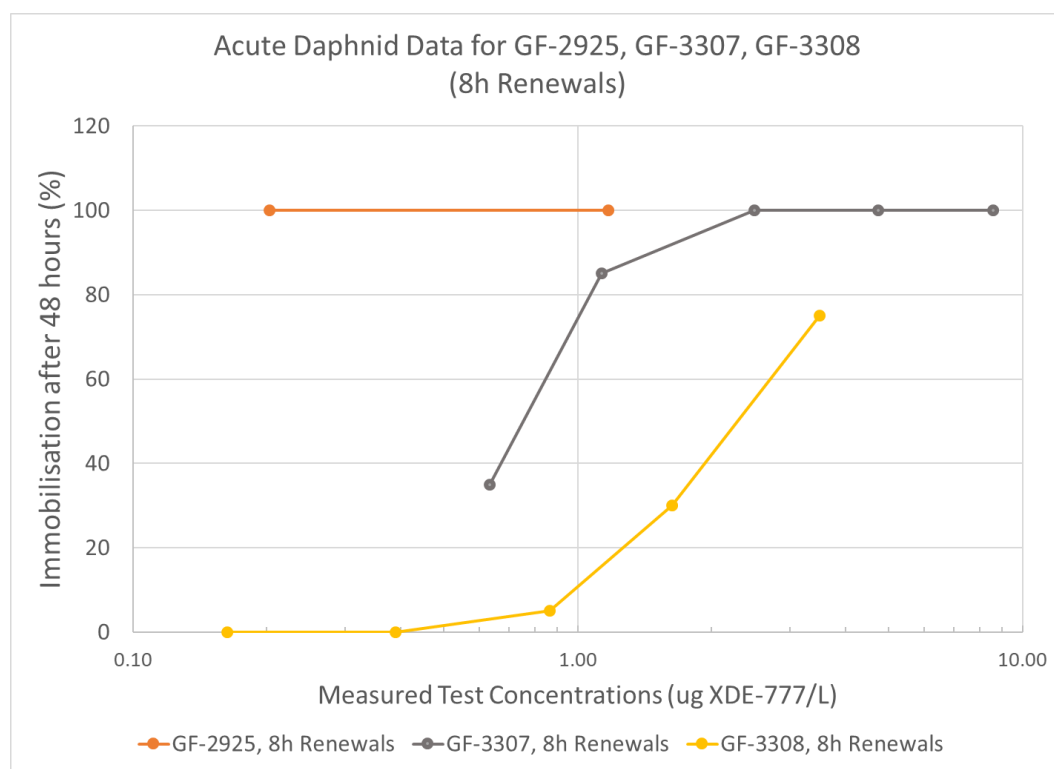
**Table 9.5-6: Comparison of acute daphnid endpoints – fenpicoxamid and formulations**

Species	Substance	Exposure System	Results	Reference
<b>Acute daphnid studies with daily renewals</b>				
<i>Daphnia magna</i>	Fenpicoxamid	48 h, ss (daily renewals) Rec. <LOQ - 110%	EC <sub>50</sub> = 0.93 µg a.s./L <sub>gm</sub> *	EFSA, 2018

<i>D. magna</i>	GF-2925	48 h, ss (daily renewals) Rec. <MQL - 184%	EC <sub>50</sub> = 0.068 µg GF-2925/L <sub>gm</sub> equivalent to <b>0.00823 µg fenpicoxamid/L</b> (12.1% a.s.)  EC <sub>50</sub> = 0.029 µg GF-2925/L <sub>gm</sub> , equivalent to <b>0.00363 µg fenpicoxamid/L</b> (12.5% a.s.)	Stadler/2013/ DAS# 120375;  Amended: Lamichhane/2014;  Revision 2: Goudie/ 2017  (both studies indicated as acceptable in Vol. 3CP B.9 of December 2017; reason for not reporting in the LoEP is unknown)
<i>D. magna</i>	GF-3307	48 h, ss (daily renewals) Rec. <MQL - 75% fenpicoxamid	EC <sub>50</sub> = 8.29 µg/L <sub>gm</sub> (0.40 µg fenpicoxamid/L based on 4.8% a.s.)	Hadsell/2014/DAS# 140489; amended Hoover/2018
<b>Acute daphnid studies with 8 hour renewals</b>				
<i>D. magna</i>	GF-2925	48 h, ss (8 hr renewals)	EC <sub>50</sub> < 1.65 µg prep/L <sub>twgm</sub> (0.203 µg fenpicoxamid/L based on 12.3% a.s.) (supportive information)	Goudie/2021/DAS# 202284
<i>D. magna</i>	GF-3307	48 h, ss (8 hr renewals) Rec. <LOQ - 98.9% fenpicoxamid	LC <sub>50</sub> = 15 µg prep/L <sub>twgm</sub> (0.71 µg fenpicoxamid /L based on 4.7% a.s.) (supportive information)	Goudie/2020/DAS# 191366
<i>D. magna</i>	GF-3308	48 h, ss (8 hr renewals) Rec. <MQL – 108%	EC <sub>50</sub> = 48 µg GF-3308/L <sub>twgm</sub> (2.3 µg fenpicoxamid/L based on 4.8% a.s.)	xxx/2016/DAS# 160102

\*Uncertainty per EFSA, 2018.

The new daphnid acute toxicity study with GF-2925 (DAS# 202284) shows that under equivalent testing conditions (8 hour renewals; time-weighted geometric mean measured concentrations, twgm) GF2925 SC is more toxic to *Daphnia* than GF-3307 EC (4.8% fenpicoxamid + 9.4% prothioconazole) and GF-3308 EC (4.8% fenpicoxamid). The lowest GF-2925 and GF-3307 concentrations that caused 100% immobility were 0.203 and 2.50 µg fenpicoxamid/L<sub>twgm</sub>, respectively - an order of magnitude difference, and GF-3308 did not achieve 100% immobility at concentrations up to and including 3.5 µg fenpicoxamid/L<sub>twgm</sub>. Therefore, **the Hommen et al. (2014) mesocosm endpoint can be used for refinement of the fenpicoxamid risk assessments in the GF-3308 registration application.**



**Figure 9.5-1: Acute daphnid immobilization (%) for GF-2925, GF-3307, and GF-3308 under 8 hour static renewal conditions. Concentrations are in µg fenpicoxamid/L, time-weighted geometric mean, and displayed on a log scale.**

Note that Figure 9.5-1 lists the immobilization after 48 hours (%) on the y-axis, however, only GF3307 and GF-3308 were conducted for 48 hours (6 total renewals). For GF-2925, 100% immobility was observed in the high and low concentrations at 8 and 32 hours, respectively, so per the protocol the study was terminated prematurely.

#### GF-3308 Daphnid population study

An indoor daphnid population study is available for GF-3308 (Hicks, 2016). In this study, juvenile (~4 days old) and adult daphnids (~10 days old) were acclimated for 7 days under laboratory test conditions and then exposed to a single pulse of GF-3308 at concentrations up to and including 550 µg prep/L<sub>initial</sub> measured (27 µg fenpicoxamid/L). Daphnid abundance per age class (neonates, juveniles, adults, and all daphnids) was monitored for 21 days post-exposure to examine if there were population level effects on the invertebrates due to GF-3308 exposure. There were no effects from GF-3308 on neonate, juvenile, adult, and total abundance on days 2, 7, 14, and 21 at the highest concentration tested (550 µg prep/L), thus the study was terminated.

An indoor daphnid population study is also available for fenpicoxamid (Hicks, 2017) under similar exposure conditions. The NOEC for Hicks (2017) was 1.88 µg fenpicoxamid/L<sub>im</sub>. While the daphnid population studies have not been used for risk assessment, **the daphnid population studies in conjunction with the acute invertebrate studies for the parent and formulation indicate that the formulated product GF-3308 is not more acutely or chronically toxic than the active substance** (Table 9.5-7).

**Table 9.5-7: Comparison of aquatic invertebrate endpoints – Fenpicoxamid and GF-3308**

Species	Substance	Exposure System	Results	Reference
Acute invertebrates				

<i>D. magna</i>	Fenpicoxamid	48 h, ss Rec. <LOQ-110%	EC <sub>50</sub> = 0.93 µg a.s./L <sub>gm</sub> **	EFSA, 2018
<i>D. magna</i>	GF-3308	48 h, ss Rec. <MQL – 108%	EC <sub>50</sub> = 48 µg prep/L <sub>twgm</sub> (2.3 µg a.s./L)	xxx/2016/DAS# 160102
<b>Chronic invertebrates</b>				
<i>D. magna</i>	Fenpicoxamid	35 d, indoor population, static, single pulse	NOEC = 1.88 µg/L <sub>im</sub> EC <sub>10-juveniles</sub> = 0.770 µg/L <sub>im</sub> (reduction on day 7) EC <sub>10-neonates</sub> = 1.11 µg/L <sub>im</sub> (reduction on day 7)	Hicks/2017/DAS# 160125
<i>D. magna</i>	GF-3308	21d, indoor population, static, single pulse	21-d NOEC = 550 µg prep/L <sub>im</sub> (27 µg a.s./L) EC <sub>10-neonates</sub> = 39 µg prep/L <sub>im</sub> (1.9 µg a.s./L) (only on day 7, no effects at remaining samplings) EC <sub>10-juveniles</sub> = 370 µg prep/L <sub>im</sub> (18 µg a.s./L) (only on day 2, no effects at remaining samplings) LOEC >550 µg prep/L <sub>im</sub>	Hicks/2016/DAS# 160126

s: static; ss: semi-static; im: based on initial measured concentrations; gm: based on geometric mean measured concentrations; twmm: based on time-weighted mean measured concentrations \*\*indicates uncertainty per the EFSA Conclusion (2018)

#### zRMS comments:

The available acute toxicity data derived in studies performed under the same conditions (8 hours renewals) clearly indicate that among three fenpicoxamid formulation (GF-2925, GF-3307 and GF-3308), GF-2925 is most toxic and produced 100% immobilisation of *Daphnia magna* at concentration of 0.203 µg a.s./L, while 100% immobilisation was never achieved in study with GF-3308 even at the maximum tested concentration corresponding to 3.5 µg a.s./L.

As may be seen from Table 9.5-6, formulation GF-2925 is also considerably more toxic than the active compound.

In order to demonstrate that presence of co-formulants does not increase toxicity of GF-3308 comparing to the active compound, two *Daphnia* population studies were performed in a microcosm tests under laboratory conditions. In general, refined exposure laboratory studies with population of invertebrates are not recommended by EFSA (2013) due to the rapid onset of recovery. The following is stated in the guidance:

*“Although refined exposure tests with standard test species that more or less resemble the design of tier 1 toxicity studies can be used for RAC derivation, the PPR Panel recommends not using refined exposure laboratory tests with populations of invertebrates (e.g. Daphnia) for this purpose when recovery is also considered. These population-level laboratory experiments with invertebrates are usually performed with individuals that differ in age and developmental state. As a result a rapid onset of recovery will occur after contamination under such test scenarios. Resources for surviving individuals will increase after contamination and will trigger an unrealistic strong recovery as no competitors are present (Knillmann et al., 2012b).”*

Nevertheless, both studies were performed for comparative purposes only and their results were not used in the risk assessment. Taking this into account, the zRMS evaluation was focused on purpose of these studies and they both were considered sufficient to serve as bridging data, especially in order to eliminate impact on the recovery the lowest endpoints calculated for particular observation intervals were considered, including day 2 or 7 endpoints, while recovery was unlikely after 2 days of exposure. Obtained results clearly showed that in the long-term the toxicity of GF-3308 is not increased due to presence of co-formulants:

- the lowest NOEC for GF-3308 (27 µg a.s./L) is 14 times higher comparing to lowest NOEC for fenpicoxamid (1.88 µg a.s./L),
- the lowest EC<sub>10-juvenile</sub> for GF-3308 (18 µg a.s./L) is 23 times higher comparing to lowest EC<sub>10-juvenile</sub> for fenpicoxamid (0.770 µg a.s./L),
- the lowest EC<sub>10-neonates</sub> for GF-3308 (1.9 µg a.s./L) is comparable level with the lowest EC<sub>10-neonates</sub> for

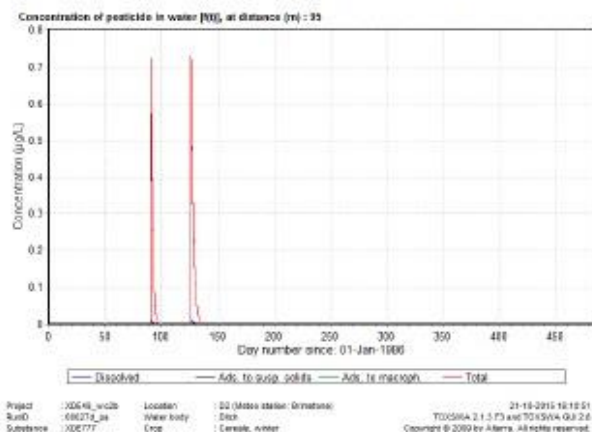


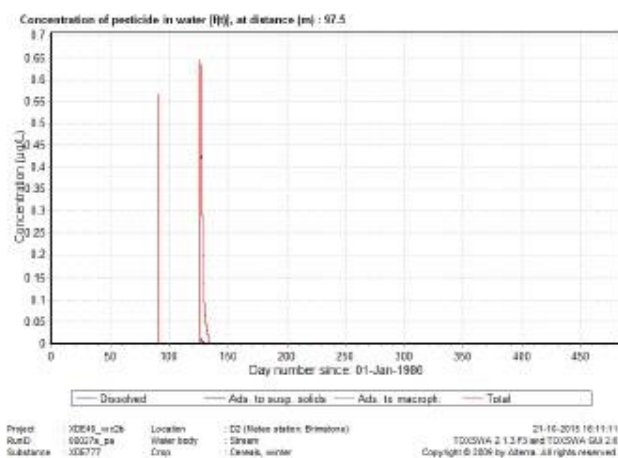
fenpicoxamid (1.11 µg a.s./L),

All available evidence shows that formulation GF-2925 is more toxic than the active compound and formulation GF-3308 is not more toxic than the active compound. Taking into account all available EU agreed and newly generated data, the zRMS is of the opinion that the NOEC of 0.1 µg a.s./L derived from the EU agreed mesocosm study performed with GF-2925 may be used to refine the risk for GF-3308, as it will still representing worst case. The Assessment Factor (AF) of 3 should be used, as agreed during the Pesticide Peer Review Meeting 165.

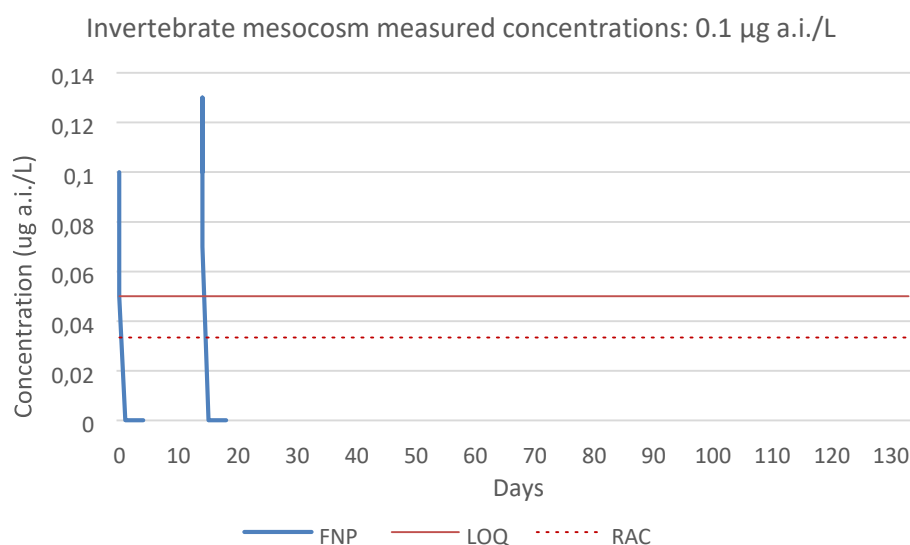
During the commenting period a concern was raised whether the exposure profile in the EU agreed mesocosm study covers the exposure profiles from the FOCUS modelling performed for uses of GF-3308. The Applicant was requested to provide such a comparison, however, possibly due to misunderstanding, the comparison of the FOCUS exposure profiles for GF-3308 with the RAC derived from the mesocosm study with GF-2925 was presented instead of the comparison with exposure profile in the study at concentration giving RAC. Nevertheless, even without the full comparison the RMS is still of the opinion that results of the mesocosm study with GF-2925 are relevant for purposes of refinement of the risk from application of GF-3308 similar application pattern of both formulations. The intended uses of GF-2925 included double spring application to winter and spring cereals at BBCH 25-69 with 14 days interval at 130 g a.s./ha/treatment. Formulation GF-3308 is also intended to be applied in spring to winter and spring cereals at BBCH 30-69, but there is only single application and the application rate is lower (100 g a.s./ha). As fenpicoxamid degrades in the water column within several hours, the exposure profiles predict one or two peaks (depending on number of applications) resulting from spray-drift with rapid decline afterwards. The example exposure profiles in scenario D2 taken from Vol. 3CP, B.9 for fenpicoxamid (December 2017) is presented below:

**Figure B.9.4.2.3. Selected graphical output D2 ditch\_2 applications to winter cereals**



**Figure B.9.4.2.4. Selected graphical output D2 stream\_2 applications to winter cereals**

The following exposure profile was observed in the study:



Measured fenpicoxamid concentrations for the 0.1 µg a.i./L treatment (blue line) and the RAC of 0.033 µg a.i./L from the assigned assessment factor of 3 (red dashed line).

Due to the same crops and the same growth stages, the only difference in the FOCUS exposure profiles following application of GF-3308 will be a single peak from single use instead of 2 peaks resulting from double application (as in case of GF -2925). Taking this into account, the exposure profile in mesocosm study is considered to represent worst case comparing to exposure profile s following application of GF -3308 and the derived endpoint may be thus used for purposes of the risk refinement.

#### 4. Further information to address to risk to aquatic organisms, in particular fish and aquatic invertebrates for Fenpicoxamid and metabolites X642188.

**Fenpicoxamid fish:** Valid acute and chronic fish studies (OECD 203 and 210, respectively) are available for fenpicoxamid and were accepted by the RMS (United Kingdom, 2017). Additional testing is not necessary as safe uses can be concluded for the proposed GAP for fish using all available data.

**Fenpicoxamid aquatic invertebrates:** Valid OECD tier 1 acute and chronic daphnid studies (OECD 202 and 211) were conducted in 2012 and submitted during the registration of the active; however, due to the

rapid degradation of the test material, concentrations were not adequately maintained between renewals (renewed daily) contrary to the recommendations in the EFSA Peer review meeting 133 (EFSA, 2015). Thus the acute *D. magna* study was accepted with uncertainty and the GF-2925 invertebrate mesocosm endpoint was used to refine the risk assessment. The chronic *D. magna* study was not accepted by the RMS, but it was not deemed a data gap as **exposure to the active is not relevant chronically**.

**X642188 fish:** Valid fish acute data (OECD 203) is available for X642188 (EFSA, 2018) and passes the RA at FOCUS Step 3 for the proposed GAP.

**X642188 aquatic invertebrates:** A valid OECD 202 daphnid acute study was conducted in 2013 and submitted during the registration of the active ( $EC_{50} = 1.3 \mu\text{g/L}_{\text{gm}}$ ); however, due to the rapid degradation of the test material, concentrations were not adequately maintained between renewals (renewed daily) contrary to the recommendations in the EFSA Peer review meeting 133 (EFSA, 2015). A new acute daphnid study has been conducted under flow-through conditions (48 hr  $EC_{50} = 0.79 \mu\text{g/L}_{\text{mm}}$ ) and is used in the risk assessment (see Section 9.5.2).

To address the risk to sediment dwellers, a sediment-spiked chironomid and *Lumbriculus* chronic studies were conducted according to OECD 218 and 225, respectively. X642188 is strongly bound to soil/sediment, as indicated by its high sorption constant (mean  $K_{oc}$  ca 4518). This means that in aquatic systems, sediment will be the likely sink for any remaining residues (as evidenced in the GF2925 invertebrate mesocosm study). However, theoretical concentrations in the sediment (PEC<sub>sed</sub>) from the FOCUS modelling, especially at Step 3, are low.

**Table 9.5-8: Step 1, 2 and 3 PEC<sub>sw</sub>/sed for X642188 on cereals (Central Zone) - GF-3308**

Compound	FOCUS scenario	Max. PEC <sub>sw</sub> (µg/L)	Max. PEC <sub>sed</sub> (µg/kg)
X642188	Step 1 – 1 x 100 g a.s./ha	2.48	<b>105.37</b>
	Step 2 – NZ – 1 x 100 g a.s./ha	0.30	<b>13.17</b>
	Step 3 – 1 x 100 g a.s./ha, winter cereals, R3 stream	0.04689	<b>0.4117</b>

The very low PEC<sub>sed</sub> values for X642188 (despite strong sorption) are due to its fast degradation in water/sediment systems ( $DT_{50}$  2.7 days for whole system; EFSA, 2018). Furthermore, the PEC<sub>sed</sub> values will be reduced further at Step 4 (not shown) due to the levels of mitigation required for the parent, fenpicoxamid. Despite this, there is potential for the release of small amounts sorbed X642188 with time (since sorption is not irreversible), therefore an OECD 218 sediment spiked chronic chironomid and *Lumbriculus* studies with X642188 have been conducted.

X642188 is of low toxicity to sediment-dwelling invertebrates. The X642188 *Chironomus riparius* NOEC and  $EC_{10}$  values were 0.63 and 0.58 mg/kg time-weighted mean measured (twmm), respectively (Beasley, 2018). For *Lumbriculus* the NOEC was 14 mg/kg<sub>twmm</sub>, indicating that chironomid is the more sensitive species.

**X12019520 fish:** A new acute trout study was conducted according to OECD 203 (xxx, 2018) and has a  $LC_{50} > 10000 \mu\text{g/L}$ . Given the low PEC<sub>sw</sub>-max, this metabolite passes the risk assessment at FOCUS Step 1 indicating low risk to aquatic organisms.

**X12019520 aquatic invertebrates:** An acute *Daphnia magna* endpoint is provided in the EFSA Conclusion (2018) for X12019520 ( $EC_{50} > 10000 \mu\text{g/L}$ ) indicating low hazard from the metabolite.

**X12446477 fish:** A new acute trout study was conducted according to OECD 203 (xxx, 2018) and has a  $LC_{50} > 10000 \mu\text{g/L}$ . Given the low PEC<sub>sw</sub>-max, this metabolite passes the risk assessment at FOCUS Step 1 indicating low risk to aquatic organisms.

**X12446477 aquatic invertebrates:** An acute *D. magna* endpoint is provided in the EFSA Conclusion (2018) for X12446477 ( $EC_{50} = 1100 \mu\text{g/L}$ ) indicating moderate hazard from the metabolite.

**zRMS comments:**

Additional data were submitted by the Applicant for some of fenpicoxamid relevant metabolites in order to fulfil the data gaps identified in EFSA Journal 2018;16(1):5146:

- metabolite X12019520: acute toxicity to *Oncorhynchus mykiss*,
- metabolite X12335723: long-term toxicity to *Chironomus riparius*,
- metabolite X12446477: acute toxicity to *Oncorhynchus mykiss*,
- estimation of the acute toxicity of metabolite X12433979 to fish and *Daphnia magna* and chronic toxicity to algae.

The above listed studies were evaluated by the zRMS and considered acceptable. Summaries of the studies may be found in Appendix 2 together with zRMS evaluation. Derived endpoints were used in the risk assessment.

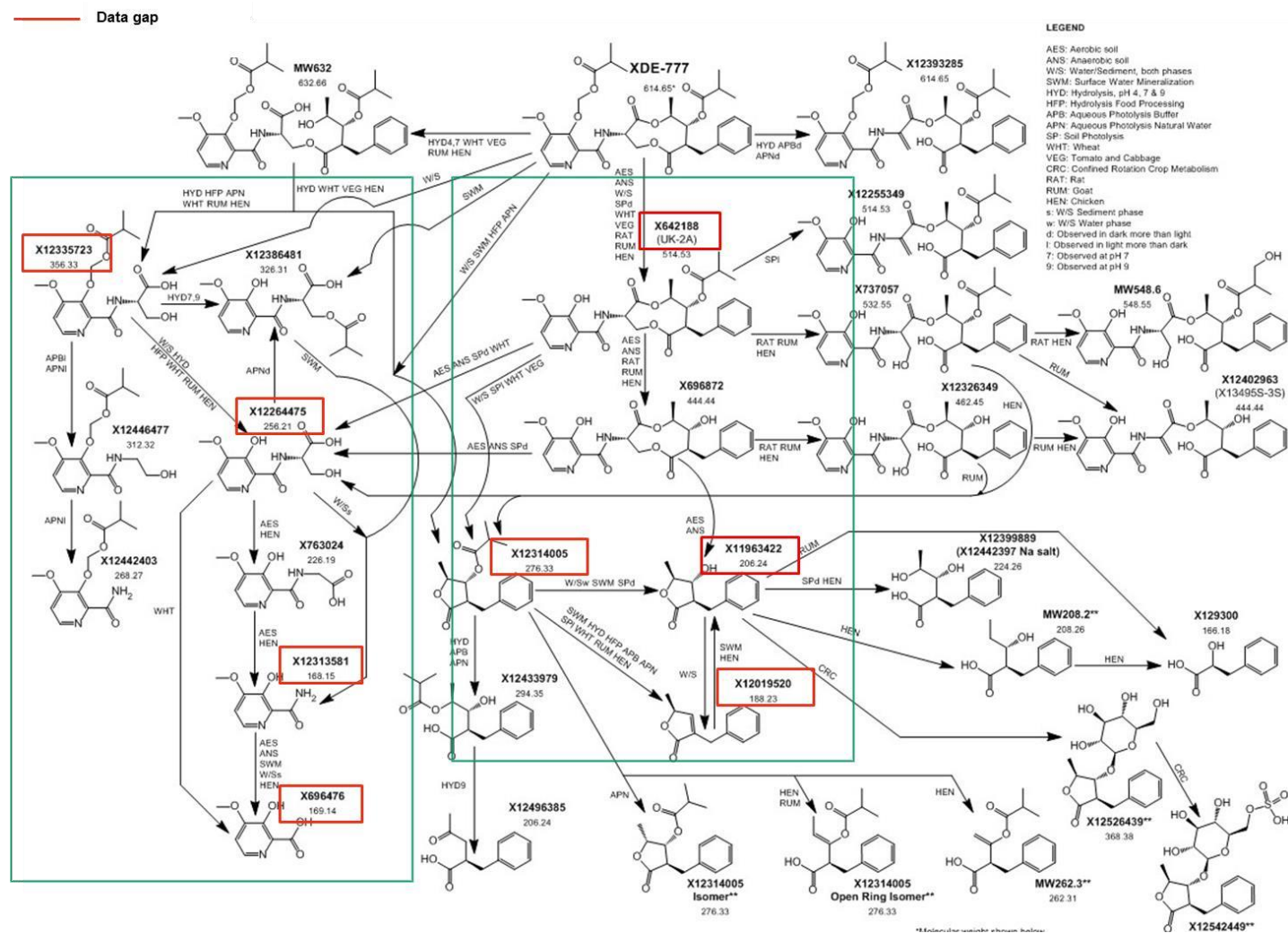
It is noted that although a data gap for submission of study on toxicity of metabolite X12335723 was identified in EFSA Journal 2018;16(1):5146, it seems that this was a mistake, since during the water/sediment studies X12335723 was not detected in sediment and exposure of sediment dwellers to this compound may be thus excluded. Nevertheless, some of metabolites present in sediment are formed from this compound and the study may be used in order to demonstrate decreased toxicity to aquatic organisms from metabolites formed in a metabolic pathway including formation of X12335723.

With regard to fenpicoxamid, sufficient data are available from the EU review, since acute toxicity study with *Daphnia magna* was accepted with some restrictions while the new chronic *Daphnia magna* study was deemed not necessary during the Pesticide Peer Review Meeting 165, since mesocosm study performed with the representative formulation (GF-2925) was available covering both, acute and chronic effects.

In addition to the above listed studies, the Applicant provided also study on effects of metabolite X642188 to *Lumbriculus variegatus*. However, the study was not evaluated by the zRMS since study on toxicity of this compound to *Chironomus riparius* was submitted and was deemed sufficient to address the data gap identified in EFSA Journal 2018;16(1):5146 for testing of sediment dwellers. Study on effects on second sediment dwelling species should be dealt with at the next renewal process of fenpicoxamid.

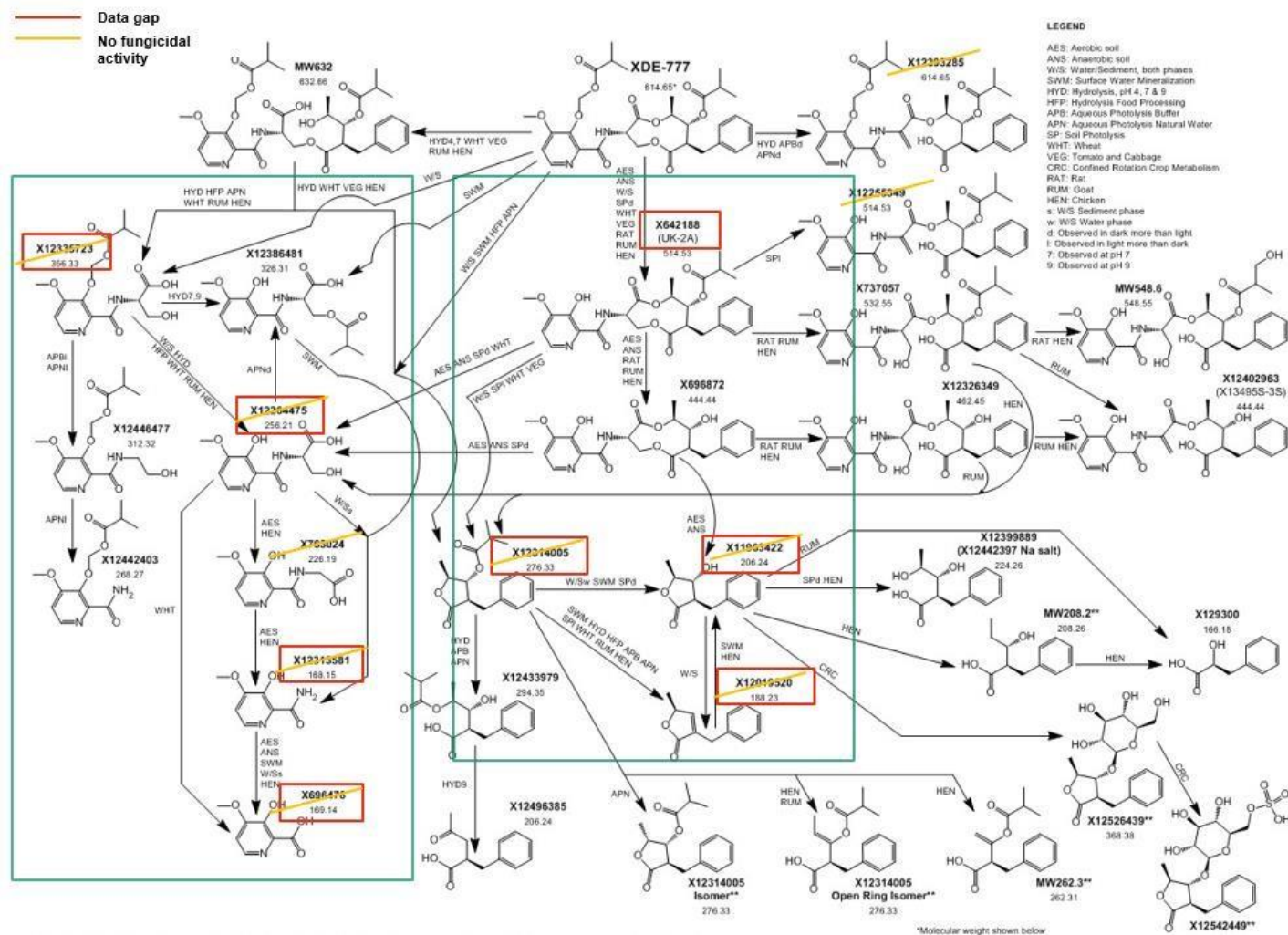
**5. Further information to address the risk to sediment dwellers for the metabolites X642188, X12264475, X12313581, X696476, X11963422, X12314005, X12019520 and X12335723.**

The metabolites in question are highlighted in the fenpicoxamid breakdown pathways diagram below (Figure 9.5-2) with boxes and can be divided into two pathways—the first stemming from X12335723 and the other from X642188.



**Figure 9.5-2. Global view of the metabolism of fenpicoxamid.**

Fenpicoxamid kills the fungal pathogen *Mycosphaerella graminicola* (formerly *Septoria tritici*) by entering fungal cells, being activated to the fungicidal toxicophore X642188, and binding to the Q<sub>i</sub> site of the cytochrome bc<sub>1</sub> complex within mitochondria, thereby inhibiting oxidative phosphorylation and the production of adenosine triphosphate (ATP), which is necessary for multiple aspects of cellular metabolism. Biological screening data confirm that fenpicoxamid and X642188 have the fungicidal toxicophore as they have protective and curative effect against SEPTTR, whereas X12264475, X11963422, X763024, X12393285, and X12255349 have no meaningful fungicidal activity (YAO/2014/DAI1370). Additional biological screening on metabolites X12313581, X696476, X12314005, X12019520, and X12335723 indicated no protective fungicidal activity (Mathieson, 2018) demonstrating the loss of the fungicidal toxicophore, which is assumed to be the same toxicophore in non-target species (Figure 9.5-3).



**Figure 9.5-3. Global view of the metabolism of fenpicoxamid with fungicidal activity screening data.**

As all eukaryotic organisms possess mitochondria, it is initially assumed that every eukaryotic species present in aquatic systems may be potentially sensitive to the mitochondrial inhibitory effects of fenpicoxamid and metabolite X642188. Acute daphnid data is available for fenpicoxamid and most of its metabolites and is overlaid on the fenpicoxamid breakdown pathway diagram below (Figure 9.5-4). While the  $EC_{50}$ s for fenpicoxamid and X642188 are around 1  $\mu\text{g/L}$ , confirming sensitivity to these two molecules, metabolites of concern that are downstream of fenpicoxamid and X642188 are 8500 to 10000 times less toxic supporting the notion that the toxicophore has been lost. Acute fish and algae endpoints are also shown on the breakdown pathway (Figure 9.5-5), where available, further confirming the loss of the toxicophore.



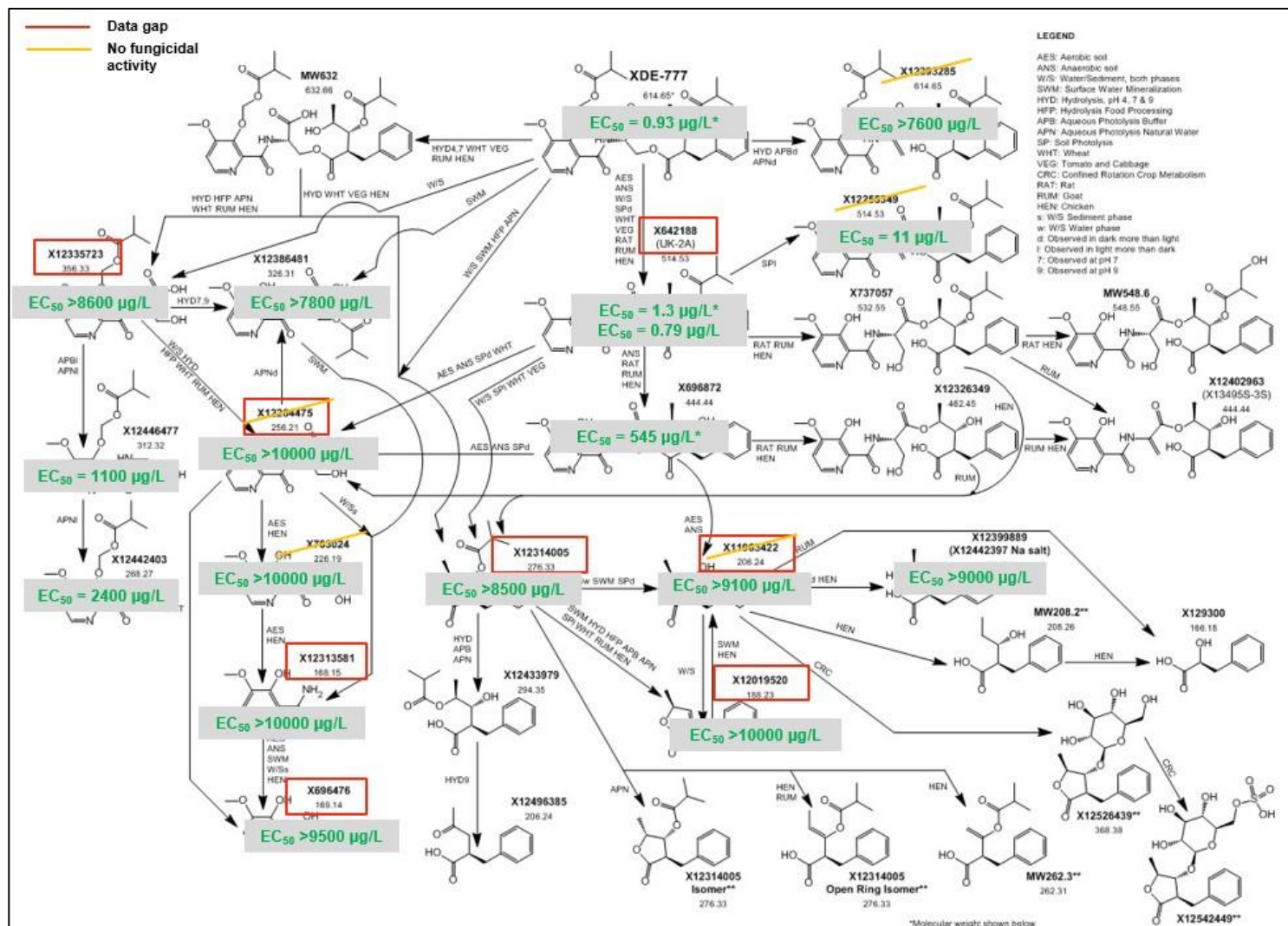
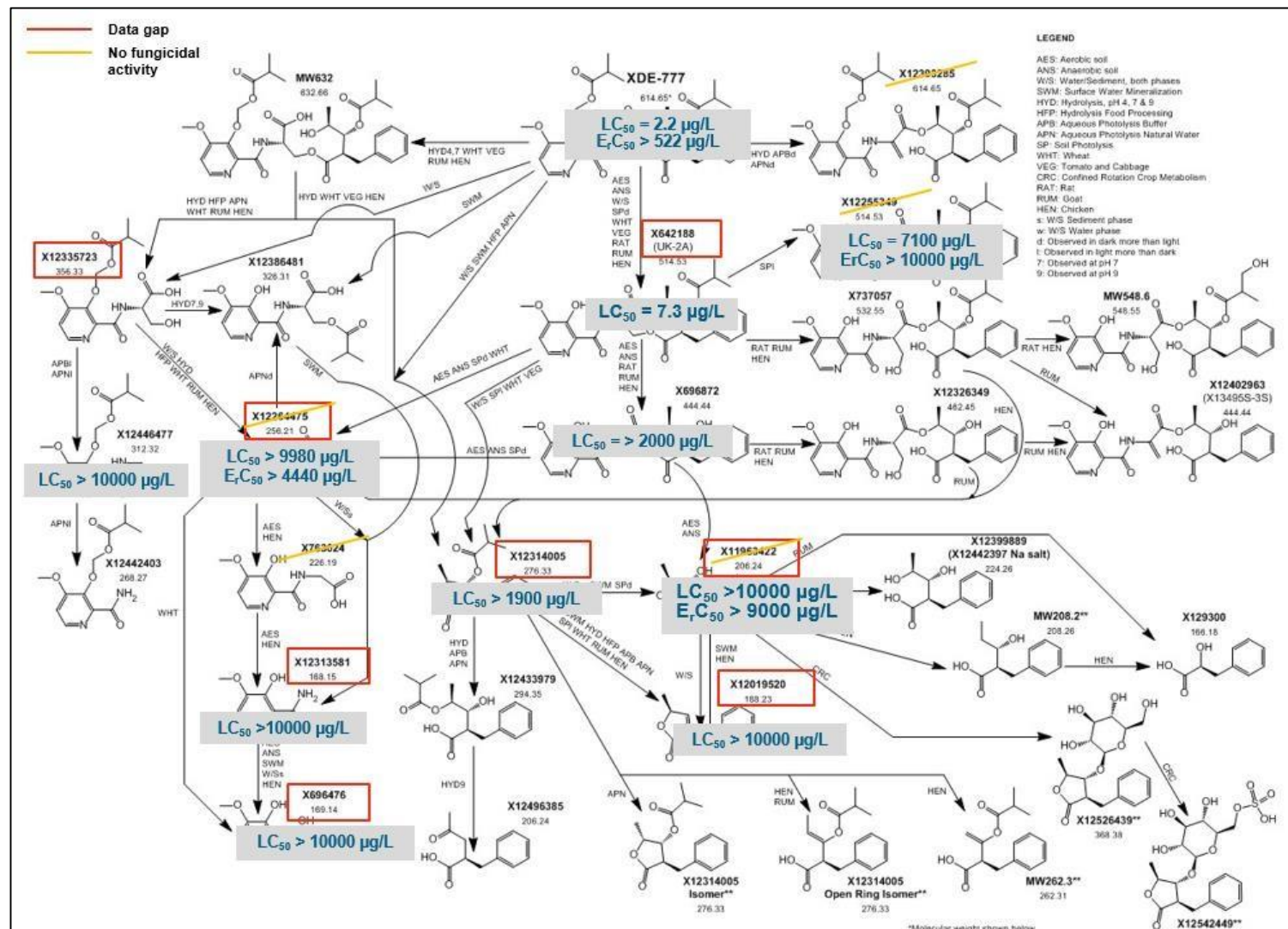


Figure 9.5-4. Global view of the metabolism of fenpicoxamid with acute daphnid endpoints.

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**Figure 9.5-5. Global view of the metabolism of fenpicoxamid with acute trout and algae endpoints.**

Additionally, metabolites X642188, X11963422, X12264475, X12314005, X12386481, X12019520 and X12335723 were analysed for and detected in the GF-2925 invertebrate mesocosm run at a GAP of 130 g a.s./ha with 2 applications at a 14 d interval. The potential chronic effects and risk of these metabolites were covered by the higher-tier study and the refined risk assessment on the parent. No further assessment is required to conclude that these metabolites present a low risk of chronic effects in the aquatic environment.

X642188 and X12335723 were tested in OECD 218 sediment spiked chronic chironomid test at concentrations up to 10 mg/kg (Beasley, 2018; Leak, 2018). Per the risk assessment for X642188 (Table 9.5-12), acceptable risk is demonstrated at the proposed GAP of 100 a.s./ha at FOCUS Step 1 when using the EC<sub>10</sub> value of 0.58 mg/kg<sub>twgm</sub>. As low risk is demonstrated for X642188, metabolites X11963422, X12314005, and X12019520 - which are further down the breakdown pathway - are also expected to be low risk.

The X12335723 sediment spiked chronic *C. riparius* NOEC was 2.2 mg/kg<sub>twgm</sub>, the highest concentration tested. Per the risk assessment for X12335723 (Table 9.5-23) acceptable risk is demonstrated at FOCUS Step 1. As low risk is demonstrated for X12335723, metabolites X12264475, X12313581, and X696476 - which are further down the breakdown pathway - are also expected to be low risk to sediment dwellers.

Considering all available data regarding the toxicophore, existing data from the GF-2925 mesocosm, and new data and the subsequent risk assessment from chronic chironomid testing of X642188 and X12335723, the risk of metabolites X12264475, X12313581, X696476, X11963422, X12314005, and X12019520 to sediment dwellers is also considered to be low. Therefore, no further testing is necessary.

#### **zRMS comments:**

The zRMS agrees with the Applicants' argumentation regarding toxicity of metabolites X642188, X12335723, X12264475, X12313581, X696476, X11963422, X12314005 and X12019520.

From the available data it is obvious that only metabolite X642188 shares toxicity of the parent due to presence of toxophore, while toxicity of remaining metabolites to the aquatic species is clearly lower, which is due to loss of the toxophore. This is confirmed in two tests screening for fungal activity of several fenpicoxamid metabolites, including X12335723, X12264475, X12313581, X696476, X11963422, X12314005 and X12019520. With exception of X642188, none of the tested metabolites showed antifungal activity. Details of these studies (evaluated and agreed by the zRMS efficacy expert) may be found in the Core Assessment, Part B, Section 3.

Decrease in toxicity of these metabolites is clearly presented on Figures 9.5-4 and 9.5-5 above.

Nevertheless, the Applicant performed two additional studies on long-term toxicity of metabolite X642188 and X12335723 to *Chironomus riparius*. For metabolite X642188 also study with *Lumbriculus variegatus* was performed, but was not evaluated by the zRMS since study performed with *C. riparius* is deemed sufficient to address the data gap identified in the EFSA conclusion. Derived endpoints were used in the risk assessment for tested metabolites.

Although increased toxicity of remaining metabolites to sediment dwellers is not expected, the zRMS decided to perform additional risk assessment for metabolites X12264475, X12313581, X696476, X12314005, X11963422 and X12019520 for precautionary reasons. Ten times toxicity of the precursor was assumed, i.e.:

- endpoint for X12335723 divided by 10 was considered for metabolites X12264475, X12313581 and X696476,
- endpoint for X642188 divided by 10 was considered for metabolites X12314005, X11963422 and X12019520.

Acute fish endpoint recalculation

Per the EFSA Conclusion (2018), the tier 1 acute rainbow trout (*O. mykiss*) LC<sub>50</sub> of 1.1 µg a.s./L (9.15 µg GF-2925/L) was selected for use in the risk assessment as the formulation was more toxic than the active substance (Table 9.5-9). However, the GF-3308 trout LC<sub>50</sub> value is not more toxic than the active ingredient. Therefore, we have used the lowest acute fenpicoxamid endpoint - *P. promelas* LC<sub>50</sub> = 1.79 µg/L - for the Tier 1 risk assessment, and have included the GF-3308 trout endpoint in the calculation of an acute trout geomean (2.1 µg a.s./L). The new acute fish geomean (4 species) for fenpicoxamid is 4.1 µg a.s./L and is used to refine the acute fish risk assessment.

**Table 9.5-9: Comparison of acute fish endpoints – fenpicoxamid, GF-2925, and GF-3308**

Species	Substance	Exposure System	Results	Reference
<b>Rainbow trout studies with active or monoformulations</b>				
<i>O. mykiss</i>	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 2.2 µg a.s./L <sub>mm</sub>	EFSA, 2018
<i>O. mykiss</i>	GF-2925	96 h, ss	LC <sub>50</sub> = 9.15 µg prep/L <sub>gm</sub> ( <b>1.1 µg a.s./L</b> )	EFSA, 2018
<i>O. mykiss</i>	GF-3308	96 h, f	LC <sub>50</sub> = 78 µg prep/L <sub>mm</sub> ( <b>3.8 µg a.s./L</b> )	xxx/2016/DAS# 160101
Trout <u>geomean</u> for fenpicoxamid, GF-2925, GF-3308			LC <sub>50</sub> = 2.1 µg a.s./L	--
<i>C. carpio</i>	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 5.41 µg a.s./L <sub>gm</sub>	EFSA, 2018
<i>P. promelas</i>	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 1.79 µg a.s./L <sub>mm</sub>	EFSA, 2018
<i>L. macrochirus</i>	Fenpicoxamid	96 h, f	LC <sub>50</sub> = 13.8 µg a.s./L <sub>mm</sub>	EFSA, 2018
New acute <u>geomean</u> , 4 species			<b>LC<sub>50</sub> = 4.1 µg a.s./L</b>	--

ss: semi-static; f: flow-through; mm: based on mean measured concentrations; gm: based on geometric mean measured concentrations

**zRMS comments:**

Calculation of the fish geomean LC<sub>50</sub> is agreed by the zRMS. Since toxicity endpoint for GF-2925 was included in calculation of the EU agreed geometric mean and fenpicoxamid and both formulations (GF-2925 and GF3308) are of comparable acute toxicity to rainbow trout, calculation of geomean LC<sub>50</sub> for rainbow trout from all 3 endpoints and its inclusion into the calculated overall geomean LC<sub>50</sub> for fish is justified. It is noted that in case endpoint of 1.1 µg a.s./L (from study with GF-2925) was excluded and geomean for *Oncorhynchus mykiss* was calculated from data for fenpicoxamid and GF-3308 only, the overall geometric mean LC<sub>50</sub> would be higher (4.43 µg a.s./L). Taking this into account, consideration of the endpoint for GF-2925 leads to an endpoint representing worst case.

**9.5.2 Risk assessment****zRMS comments:**

Please note that initially the RAC/PEC values were calculated by the Applicant in the risk assessment presented below with acceptable risk with ratio >1. However, according to EFSA (2013) PEC/RAC should be calculated with acceptable risk demonstrated when the ratio is <1.

In general, both methods will lead to the same outcome, but presentation of the results of calculations with acceptable risk for ratio  $>1$  is confusing, since in all current assessments acceptable risk is concluded with ratio  $<1$ , as PEC/RAC are considered.

In addition to that, the  $PEC_{SW/SED}$  values not agreed in area of Section 8 were used in the below calculations (see Core Assessment, Part B, Section 8 for details).

Taking all this into account, the applicant was requested to provide correctly performed calculations (i.e. PEC/RAC) based on the agreed exposure data.

New calculation were provided by the Applicant and for transparency reasons the initially performed risk assessment was not struck through (as usual) but was replaced with correctly performed risk assessment.

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2 and 3  $PEC_{SW}$  ( $PEC_{gl-max}$ ) for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. A PEC/RAC ratio (i.e. ETR) below 1 indicates acceptable risk. Where calculated PEC/RAC ratios do not indicate an acceptable risk in FOCUS Step 3 scenarios, risk assessments are presented using Step 4  $PEC_{SW}$  and the most sensitive species.

*Risk assessment for fenpicoxamid at 1 x 100 g a.s./ha*

In the following tables, the ratios between predicted environmental concentrations in surface water bodies ( $PEC_{SW}$ ,  $PEC_{SED}$ ) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

**Table 9.5-10: Aquatic organisms: acceptability of risk (PEC < RAC) for fenpicoxamid for each organism group based on FOCUS Step 1, 2 and 3 calculations for the use of GF-3308 in cereals\_100 g a.s./ha**

Group		Fish acute		Fish prolonged	Inverteb. acute	Inverteb. prolonged	Higher-tier info	Algae
Test species		<i>P. promelas</i> , XDE-777	4. species geomean	<i>P. promelas</i>	<i>D. magna</i>	<i>D. magna</i>	Invert. Mesocosm, GF-2925	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	LC <sub>50</sub>	EC <sub>10</sub>	EC <sub>50</sub>	NA	Class 1	ErC <sub>50</sub>
(µg/L)		1.79	4.1	0.91	0.93		0.1	>522
AF		100	100	10	100	10	3	10
RAC (µg/L)		0.0179	0.041	0.091	0.0093		0.033	52.2
FOCUS Scenario	PEC <sup>gl-max</sup> (µg/L)	ETR = PEC/RAC, ETR < 1 is acceptable risk						
Step 1 @ 1 x 100 g a.s./ha								
	4.75	265	116	52	511	NA	143	0.09
Step 2 @ 1 x 100 g a.s./ha								
N-Europe	0.92	51	22	10	99	NA	28	0.02
Step 3 - 1X winter cereals @ 100 g a.s./ha								
D3/ditch	0.6228	35	15	7	67	NA	19	NR
D4/pond	0.02118	1.18	0.52	0.23	2	NA	0.64	NR
D4/stream	0.46	26	11	5	49	NA	14	NR
D5/pond	0.02119	1.18	0.52	0.23	2	NA	0.64	NR
D5/stream	0.4969	28	12	5	53	NA	15	NR
R1/pond	0.02119	1.18	0.52	0.23	2	NA	0.64	NR
R1/stream	0.4098	23	10	5	44	NA	12	NR
R3/stream	0.5763	32	14	6	62	NA	17	NR
R4/stream	0.4116	23	10	5	44	NA	12	NR
Step 3 - 1X spring cereals @ 100 g a.s./ha								
D3/ditch	0.6235	35	15	7	67	NA	19	NR
D4/pond	0.0212	1.18	0.52	0.23	2	NA	0.64	NR
D4/stream	0.5093	28	12	6	55	NA	15	NR



D5/pond	0.0212	<b>1.18</b>	0.52	0.23	<b>2</b>	NA	0.64	NR
D5/stream	0.5232	<b>29</b>	<b>13</b>	<b>6</b>	<b>56</b>	NA	<b>16</b>	NR
R4/stream	0.4116	<b>23</b>	<b>10</b>	<b>5</b>	<b>44</b>	NA	<b>12</b>	NR

NA: Not Applicable; NR: Not required; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration PEC/RAC

= ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk; ratios shaded in green indicate acceptable risk

For the intended uses in cereals, calculated PEC/RAC ratios for fenpicoxamid did indicate an acceptable risk for algae at FOCUS Step 1.

For the intended uses in cereals, calculated PEC/RAC ratios for fenpicoxamid did not indicate an acceptable risk for fish and invertebrates in several FOCUS Step 1-3 scenarios. Acceptable risk for is demonstrated in the FOCUS Step 3 pond scenarios (using the fish geomean approach and the invertebrate mesocosm for refinement), therefore no further assessment is needed. The GF-2925 invertebrate mesocosm has the lowest RAC at 0.033 µg a.s./L (as characterised by NOEC endpoint of 0.1 µg/L in connection with an assessment factor of 3) and is used in the FOCUS Step 4 assessment below as the most sensitive species.

Note that the invertebrate mesocosm RAC (0.033 µg a.s./L) is lower than the acute fish geomean RAC (0.041 µg a.s./L), therefore any FOCUS Step 4 scenarios that demonstrate acceptable risk using the lower RAC would also be protective of the higher RAC for acute fish.

**Table 9.5-11: Aquatic organisms: PEC calculation and acceptability of risk (PEC < RAC) for fenpicoxamid based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation options for the use of GF-3308 in cereals\_100 g a.s./ha**

Intended use		Cereals						
Active substance		Fenpicoxamid						
Application rate (g/ha)		1 x 100						
Nozzle reduction	No-spray buffer (m)	<b>40</b>	<b>30</b>	<b>30</b>	<b>10</b>	<b>10</b>	<b>5</b>	<b>20</b>
	Vegetated filter strip (m)	<b>10</b>	<b>NA</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>20</b>
Step 4 - 1X winter cereals @ 100 g a.s./ha								
None	D3/ditch	0.02358	0.03116	0.03116				
50%					0.0443			
75%					0.02207			
90%						0.008781	0.01662	0.004542
None	D4/pond	0.005388	0.006651	0.006651				
50%					0.00654			
75%					0.003253			

90%						0.001291	0.001802	0.000859
None	D4/stream	0.02349	0.03103	0.03103				
50%					0.04413			
75%					0.02199			
90%						0.008746	0.01656	0.004524
None	D5/pond	0.005389	0.006652	0.006652				
50%					0.006541			
75%					0.003254			
90%						0.001292	0.001802	0.000859
None	D5/stream	0.02538	0.03353	0.03353				
50%					0.04767			
75%					0.02376			
90%						0.00945	0.01789	0.004889

None	R1/pond	0.005389	0.006652	0.006652				
50%					0.006541			
75%					0.003254			
90%						0.001292	0.001802	0.000859
None	R1/stream	0.02092	0.02764	0.02764				
50%					0.03931			
75%					0.01958			
90%						0.007789	0.01475	0.004029
None	R3/stream	0.02944	0.0389	0.0389				
50%					0.05531			
75%					0.02756			
90%						0.01097	0.02075	0.005674
None	R4/stream	0.02101	0.02776	0.02776				
50%					0.03948			
75%					0.01967			

90%						0.007823	0.01481	0.004047
<b>Step 4 - 1X spring cereals @ 100 g a.s./ha</b>								
None	D3/ditch	0.02361	0.0312	0.0312				
50%					0.04434			
75%					0.0221			
90%						0.00879	0.01664	0.004547
None	D4/pond	0.005391	0.006655	0.006655				
50%					0.006544			
75%					0.003255			
90%						0.001292	0.001803	0.000859
None	D4/stream	0.02602	0.03437	0.03437				
50%					0.04887			
75%					0.02435			
90%						0.009688	0.01834	0.005012
None	D5/pond	0.00539	0.006654	0.006654				
50%					0.006543			
75%					0.003255			
90%						0.001292	0.001802	0.000859
None	D5/stream	0.02672	0.03531	0.03531				
50%					0.0502			
75%					0.02502			
90%						0.009953	0.01884	0.005149
None	R4/stream	0.02101	0.03593	0.02776				
50%					0.03948			
75%					0.01967			
90%						0.01627	0.01627	0.008493
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	<b>40</b>	<b>30</b>	<b>30</b>	<b>10</b>	<b>10</b>	<b>5</b>	<b>20</b>

	Vegetated filter strip (m)	10	NA	10	10	10	10	20
RAC (µg/L)	0.033	ETR = PEC/RAC, ETR < 1 is acceptable risk						
Step 4 - 1X winter cereals @ 100 g a.s./ha								
None	D3/ditch	0.71	0.94	0.94				
50%					1.34			
75%					0.67			
90%						0.27	0.50	0.14
None	D4/pond	0.16	0.20	0.20				
50%					0.20			
75%					0.10			
90%						0.04	0.05	0.03
None	D4/stream	0.71	0.94	0.94				
50%					1.34			
75%					0.67			
90%						0.27	0.50	0.14
None	D5/pond	0.16	0.20	0.20				
50%					0.20			
75%					0.10			
90%						0.04	0.05	0.03
None	D5/stream	0.77	1.02	1.02				
50%					1.44			
75%					0.72			
90%						0.29	0.54	0.15
None	R1/pond	0.16	0.20	0.20				
50%					0.20			
75%					0.10			
90%						0.04	0.05	0.03

None	R1/stream	0.63	0.84	0.84				
50%					1.19			
75%					0.59			
90%						0.24	0.45	0.12
None	R3/stream	0.89	1.18	1.18				
50%					1.68			
75%					0.84			
90%						0.33	0.63	0.17
None	R4/stream	0.64	0.84	0.84				
50%					1.20			
75%					0.60			
90%						0.24	0.45	0.12
Step 4 - 1X spring cereals @ 100 g a.s./ha								
None	D3/ditch	0.72	0.95	0.95				
50%					1.34			
75%					0.67			
90%						0.27	0.50	0.14
None	D4/pond	0.16	0.20	0.20				
50%					0.20			
75%					0.10			
90%						0.04	0.05	0.03
None	D4/stream	0.79	1.04	1.04				
50%					1.48			
75%					0.74			
90%						0.29	0.56	0.15
None	D5/pond	0.16	0.20	0.20				
50%					0.20			
75%					0.10			
90%						0.04	0.05	0.03

None	D5/stream	0.81	<b>1.07</b>	<b>1.07</b>				
50%					<b>1.52</b>			
75%					0.76			
90%						0.30	0.57	0.16
None	R4/stream	0.64	<b>1.09</b>	0.84				
50%					<b>1.20</b>			
75%					0.60			
90%						0.49	0.49	0.26

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk; ratios shaded in green indicate acceptable risk

For aquatic invertebrates (and subsequently acute fish), acceptable risk is demonstrated in winter and spring cereals at 1 x 100 g a.s./L with:

- 40 m no spray zones (NSZ) + 10 m vegetated filter strips (VFS); □ 10 m NSZ + 10 m VFS + 75% drift reducing nozzles (DRN); and □ ~~≥5 m NSZ + 10 m VFS + 90% DRN.~~

**For all taxa, acceptable risk for fenpicoxamid is demonstrated for winter and spring cereals at 1 x 100 g a.s./ha with:**

- **40 m NSZ + 10 m VFS;**
- **10 m NSZ + 10 m VFS + 75% DRN; and**
- ~~5 m NSZ + 10 m VFS + 90% DRN.~~

**zRMS comments:**

The risk assessment for fenpicoxamid presented above is agreed by the zRMS. For justification of the selected endpoints, please refer to point 9.5.1 and 9.5.1.1 above.

Higher tier risk assessment based on RAC of 0.033 µg a.s./L derived from mesocosm study addresses also acute risk to aquatic invertebrates and is protective for fish with RAC of 0.041 and 0.091 µg a.s./L for acute and chronic exposure.

The risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

It is noted that during FOCUS Step 4 simulations the unsprayed buffer zone and vegetated filter strip are not summed up, but the vegetated filter strip is included in the unsprayed buffer zone, so the unsprayed buffer cannot be narrower than the VFS and mitigation measure with 5 m NSZ with 10 m VFS makes no sense and is thus struck through above.

Based on the performed calculations acceptable risk from fenpicoxamid following application of GF-3308 according to the Central Zone GAP may be concluded provided that:

- 40 m unsprayed buffer zone (including 10 m vegetated filter strip) to surface water bodies is respected, or
- 10 m vegetated filter strip to surface water bodies in combination with spray drift reduction by 75% are respected.

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

*Risk assessment for fenpicoxamid metabolite X642188 at 1 x 100 g a.s./ha*

Standard OECD tier 1 tests were conducted for X642188; however the acute invertebrate and algae studies were invalidated according to the EFSA Peer Review Report (2017) expert meeting notes and therefore do not appear in the EFSA Conclusion (2018). A new acute daphnid study was conducted under flow-through conditions (xxx, 2018) and used in the risk assessment below. For the algae endpoint, the metabolite was considered to be 10X more toxic than the parent.

Pursuant to Commission Regulation (EU) No. 283/2013, fenpicoxamid metabolite X642188 does not require testing for chronic toxicity in fish and aquatic invertebrates as it hydrolyses very rapidly (DT<sub>90</sub> of 0.73 days at pH 7 and 25°C; Austin/2013/DAS# 130663), therefore satisfying the condition that it exhibits a hydrolysis DT<sub>90</sub> of less than or equal to 24 hours; the chronic risk assessment for such metabolites may be considered to be satisfied as presenting a low risk without specific calculation of the long-term TER.



**Table 9.5-12: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X642188 for each organism group based on FOCUS Step 1, 2 and 3 calculations for the use of GF-3308 in cereals 100 g a.s./ha**

Group		Fish acute	Inverteb. acute	Algae		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>		<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>		EC <sub>10</sub>
(µg/L)		7.3	0.79	52.2		580 <del>±800</del>
AF		100	100	10		10
RAC (µg/L)		0.073	0.0079	5.22		58 <del>±80</del>
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk			PEC <sub>gl-max</sub> (µg/kg)	
Step 1 @ 1 x 100 g a.s./ha						
	2.48	34	314	0.48	105.37	1.82
Step 2 @ 1 x 100 g a.s./ha						
N-Europe	0.30	4	38	0.06	13.17	0.23
Step 3 - 1X winter cereals @ 100 g a.s./ha						
D3/ditch	0.001252	0.02	0.16	NR	0.1987	NR
D4/pond	0.002685	0.04	0.34	NR	0.06951	NR
D4/stream	0.000541	0.01	0.07	NR	0.005501	NR
D5/pond	0.002279	0.03	0.29	NR	0.06056	NR
D5/stream	0.00021	0.00	0.03	NR	0.006333	NR
R1/pond	0.006535	0.09	0.83	NR	0.0555	NR

R1/stream	0.03716	0.51	5	NR	0.273	NR
R3/stream	0.04689	0.64	6	NR	0.4117	NR
R4/stream	0.08179	1.12	10	NR	0.4101	NR
<b>Step 3 - 1X spring cereals @ 100 g a.s./ha</b>						
D3/ditch	0.001711	0.02	0.22	NR	0.2369	NR
D4/pond	0.001836	0.03	0.23	NR	0.04592	NR
D4/stream	0.000642	0.01	0.08	NR	0.01648	NR
D5/pond	0.002128	0.03	0.27	NR	0.0612	NR
D5/stream	0.000221	0.00	0.03	NR	0.009843	NR
R4/stream	0.05631	0.77	7	NR	1.033	NR

NA: Not Applicable; NR: Not required; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk; ratios shaded in green indicate acceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X642188 did indicate an acceptable risk for algae and sediment dwellers at FOCUS Step 1.

For the intended uses in cereals, calculated PEC/RAC ratios for X642188 did not indicate an acceptable risk for acute fish and invertebrates in some FOCUS Step 3 R-scenarios. Further PEC/RAC ratios (ETRs) were calculated for the failing R-scenarios based on FOCUS Step 4 PEC<sub>sw</sub> considering reduced exposure of surface water bodies.

**Table 9.5-13: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X642188 for acute fish based on FOCUS Step 4 calculations for the use of GF-3308 in cereals\_100 g a.s./ha**

<b>Intended use</b>		Cereals, for all failing Step 3 scenarios						
<b>Active substance</b>		X642188						
<b>Application rate (g/ha)</b>		1 x 100 g fenpicoxamid./ha						
<b>Nozzle reduction</b>	<b>No-spray buffer (m)</b>	<b>40</b>	<b>30</b>	<b>30</b>	<b>10</b>	<b>10</b>	<b>5</b>	<b>20</b>

	Vegetated filter strip (m)	10	NA	10	10	10	10	20
Step 4 - 1X winter cereals @ 100 g a.s./ha								
None	R1/stream	0.01687	0.03716	0.01687				
50%					0.01687			
75%					0.01687			
90%						0.01687	0.01687	0.008833
None	R3/stream	0.0214	0.04689	0.0214				
50%					0.0214			
75%					0.0214			
90%						0.0214	0.0214	0.01122
None	R4/stream	0.03719	0.08179	0.03719				
50%					0.03719			
75%					0.03719			
90%						0.03719	0.03719	0.01949
Step 4 - 1X spring cereals @ 100 g a.s./ha								
None	R4/stream	0.02101	0.03593	0.02776				
50%					0.03948			
75%					0.01967			
90%						0.01627	0.01627	0.008493
Nozzle reduction	No-spray buffer (m)	40	30	30	10	10	5	20
	Vegetated filter strip (m)	10	NA	10	10	10	10	20
RAC (µg/L)	0.0079	ETR = PEC/RAC, ETR < 1 is acceptable risk						
Step 4 - 1X winter cereals @ 100 g a.s./ha								
None	R1/stream	2	5	2				
50%					2			
75%					2			
90%						2	2	1.12

None	R3/stream	3	6	3				
50%					3			
75%					3			
90%						3	3	1.42
None	R4/stream	5	10	5				
50%					5			
75%					5			
90%						5	5	2
Step 4 - 1X spring cereals @ 100 g a.s./ha								
None	R4/stream	3	5	4				
50%					5			
75%					2			
90%						2	2	1.08

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; RAC/PEC = TER, ratios below the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in winter cereals, calculated RAC/PEC ratios for X642188 did not indicate an acceptable risk for acute invertebrates in the FOCUS Step 4 R stream scenarios using the OECD Tier 1 EC<sub>50</sub>.

#### **zRMS comments:**

The risk assessment for metabolite X642188 presented above is agreed by the zRMS. For justification of the selected endpoints, please refer to point 9.5.1 and 9.5.1.1 above.

Based on performed calculations acceptable risk could be concluded for algae and *Chironomus riparius* at Step 1-2 PEC<sub>SW/SED</sub>.

For fish acceptable risk could be concluded in all D scenarios and part R scenarios, while for *Daphnia magna* acceptable risk could be concluded in D scenarios but no acceptable risk was demonstrated in any of R scenario. Further calculations based on Step 4 PEC<sub>SW</sub> were performed with consideration of *Daphnia magna* RAC of 0.0079 µg pm/L, being protective for fish with RAC of 0.073 µg pm/L. No acceptable risk could be concluded with any of the risk mitigation measures considered. Further assessment is presented below.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

*Risk assessment for fenpicoxamid + metabolite X642188 at 100 g a.s./ha*

While acceptable risk to invertebrates has been demonstrated using the tier 1 RAC of 0.0079 µg X642188/L for the FOCUS Step 3 drainage scenarios, the run-off scenarios do not have a safe use. However, using the risk envelope approach, it is possible to use the GF-2925 invertebrate mesocosm endpoint (NOEC = 0.1 µg a.s./L; AF = 3; RAC = 0.033 µg a.s./L) to assess the risk of both parent and metabolite in the run-off scenarios as long as the maximum combined PEC is below the RAC. This approach is relevant given that:

- X642188 is a direct metabolite of fenpicoxamid (Figure 9.5-2);
- X642188 and fenpicoxamid are of comparable toxicity in aquatic invertebrates (acute daphnid EC<sub>50</sub> values of 0.79 µg X642188/L and 0.93 µg fenpicoxamid/L, respectively); and
- X642188 was detected in both water and sediment in the GF-2925 invertebrate mesocosm (Tables 9.5-4 and 9.5-5).

For this purpose, the FOCUS SwashProjects which produced the Steps 3 and 4 data previously shown above for application dates relevant to BBCH 30 according to AppDate 3.06 (June, 2019) were retrieved. All scenarios available for the crop were modelled for completeness, but only those relevant for the Central Zone are presented. A 30 day window was set in the model as relevant for a single application. The data from the run-off scenarios relevant for the Central Zone (R1, R3 and R4) were then used for an assessment where the hourly PEC<sub>sw</sub> values from TOXSWA for fenpicoxamid and X642188 from the full exposure profile were extracted and “summed” (i.e. fenpicoxamid PEC<sub>sw</sub> plus X642188 PEC<sub>sw</sub> as parent equivalent), and compared to the assumed “summed” RAC of 0.033 µg/L.

As noted in the B8 dRR, EPAT v1.2.0 was used to generate “seg1.con” or “seg20.con” text files for the pond or stream scenarios, respectively. This was done separately for fenpicoxamid and X642188, focusing on Step 4 with two levels of mitigation, i.e. 10 m NSZ and 75% DRN with a 10 m VFS, or 5 m NSZ and 90% DRN with a 10 m VFS (example screen shot below for fenpicoxamid and runs 76-79 which correspond in this analysis to R1 pond, R1 stream, R3 stream and R4 stream).

For the “summed” approach it was necessary to convert the X642188 PEC<sub>sw</sub> to a parent equivalent (x 614.2/514.2) which could be added to the parent PEC<sub>sw</sub>. The hourly “summed” PEC<sub>sw</sub> values were obtained for comparison to the invertebrate mesocosm RAC as shown below. ETRs less than 1 indicate acceptable risk.

**Table 9.5-14: Aquatic invertebrates: acceptability of risk (PEC < RAC) for fenpicoxamid + X642188 based on FOCUS Step 4 calculations for the use of GF-3308 in cereals\_1 x 100 g a.s./ha**

Group		Higher-tier info
Test species		Invert. Mesocosm, GF-2925
Endpoint		NOEC
(µg/L)		0.1
AF		3
RAC (µg/L)		0.033
FOCUS Scenario	combined PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk
Step 4 – 1X winter cereals, 10 m NSZ + 10 m VFS + 75% DRN		
R1/pond	0.00335	0.10
R1/stream	0.02177	0.66
R3/stream	0.0281	0.85

R4/stream	0.04655	<b>1.41</b>
<b>Step 4 – 1X spring cereals, 10 m NSZ + 10 m VFS + 75% DRN</b>		
R4/stream	0.03876	<b>1.17</b>
<del>Step 4 – 1X winter cereals, 5 m NSZ + 10 m VFS + 90% DRN</del>		
R1/pond	0.00282	0.09
R1/stream	0.02177	0.66
R3/stream	0.0281	0.85
R4/stream	0.04655	<b>1.41</b>
<del>Step 4 – 1X spring cereals, 5 m NSZ + 10 m VFS + 90% DRN</del>		
R4/stream	0.03876	<b>1.17</b>

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

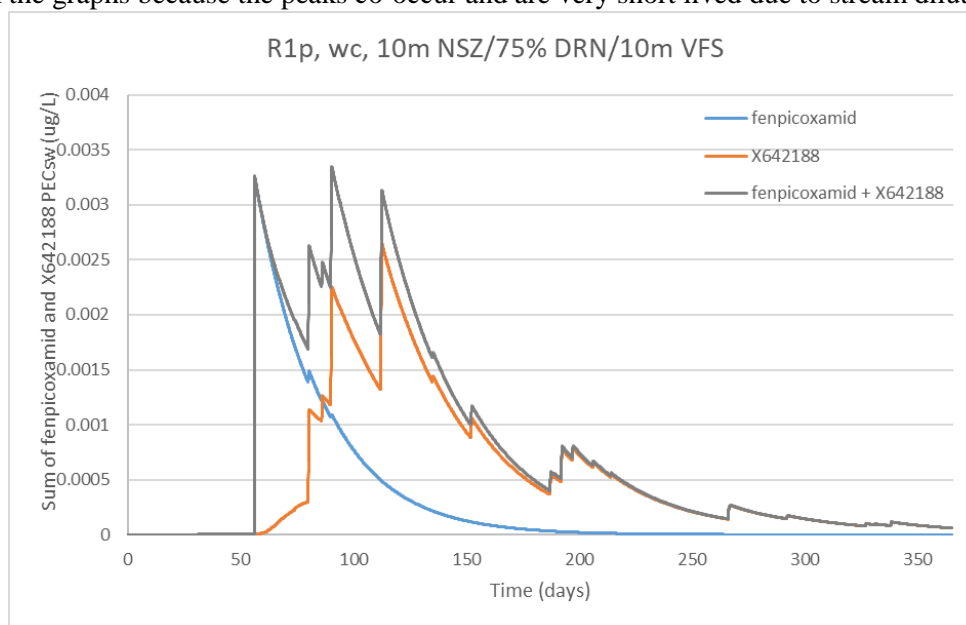
PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk; ratios shaded in green indicate acceptable risk

Whilst the “summed” R4 stream scenario PEC<sub>sw</sub> values exceeds the assumed RAC for both winter and spring cereals, this is of no consequence since R4 is only applicable to HU in the Central Zone, and this MS is not supported in the GAP table. There are no “summed” PEC<sub>sw</sub> values which exceed the assumed RAC of 0.033 µg/L for the R1 and R3 scenarios for the Central Zone MS (PL, CZ, RO and SK) relevant to this dRR.

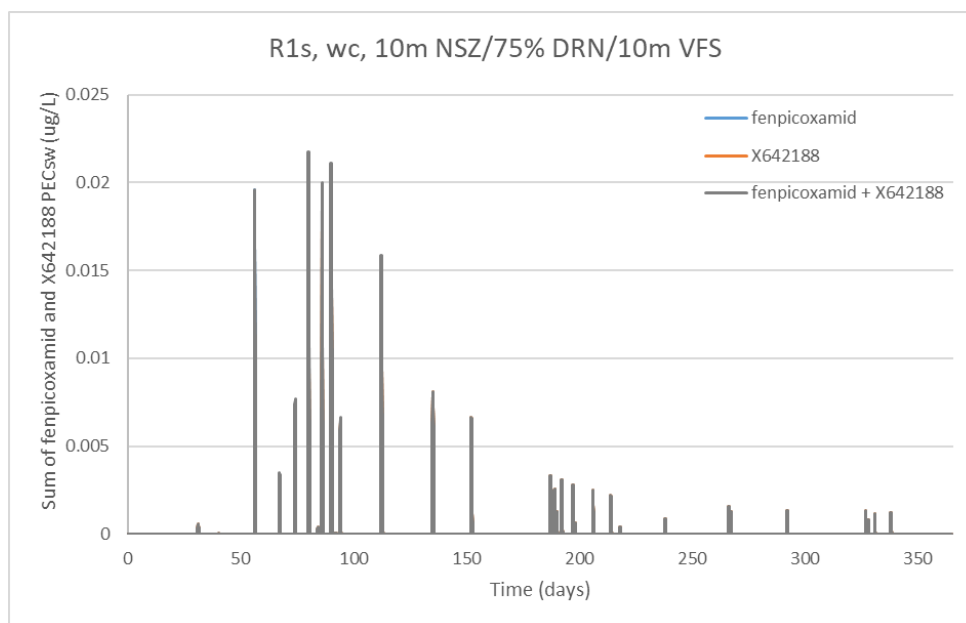
**Therefore, for aquatic invertebrates, acceptable risk for fenpicoxamid+X642188 using the ‘summed’ PEC<sub>sw</sub> values is demonstrated for winter cereals at 1 x 100 g a.s./ha with:**

- **10 m NSZ + 10 m VFS + 75% DRN; and** □
- ~~5 m NSZ + 10 m VFS + 90% DRN.~~

To illustrate the process and derivation of the “summed” PEC<sub>sw</sub> values further, graphs were generated of the fenpicoxamid (blue line) and X642188 (parent equivalent; orange line) concentrations and the “summed” total (grey line) against time (days), and examples for R1 pond and stream are presented as follows. Note that for the stream scenario, the fenpicoxamid and X642188 exposures cannot easily be seen from the graphs because the peaks co-occur and are very short lived due to stream dilution.



**Figure 9.5-6: EPAT profile of the FOCUS Step 4 R1 pond scenario at 1 x 100 g a.s./ha, winter cereals with mitigation measures including 10 m NSZ + 10 m VFS + 75% DRN**



**Figure 9.5-7: EPAT profile of the FOCUS Step 4 R1 stream scenario at 1 x 100 g a.s./ha, winter cereals with mitigation measures including 10 m NSZ + 10 m VFS + 75% DRN**

**zRMS comments:**

The zRMS agrees with the Applicant that the fenpicoxamid endpoint from the mesocosm study performed with GF-2925 may be used to address the risk from metabolite X642188 for the following reasons:

- the metabolite is formed directly from the active compound,
- it has the same toxophore responsible for the comparable fungicidal activity of both compounds which was demonstrated in the studies screening for the activity of fenpicoxamid and its metabolites against fungi (evaluated in area of Section 3),
- available data demonstrate comparable toxicity of both compounds,
- X642188 was formed in both, water column and sediment, during the mesocosm study, so the tested species were exposed to both compounds combined.

Since the route of migration of fenpicoxamid and X642188 to surface water bodies in R scenarios is different,

the exposure considered in the risk assessment should include both, parent and metabolite. Respective exposure data were obtained by extracting the maximum hourly  $PEC_{SW}$  values for the parent and metabolite in the R1, R3 and R4 scenarios from the EPAT analysis and summing them up using the Excel spreadsheet. The metabolite  $PEC_{SW}$  were converted into the parent equivalents using the molar ratio. From the summed  $PEC_{SW}$  values the maximum was found and used in the calculations presented in Table 9.5-14 above.

It is noted that during FOCUS Step 4 simulations the unsprayed buffer zone and vegetated filter strip are not summed up, but the vegetated filter strip is included in the unsprayed buffer zone, so the unsprayed buffer cannot be narrower than the VFS and mitigation measure with 5 m NSZ with 10 m VFS makes no sense and is thus struck through in evaluation above.

Based on the performed calculations, acceptable risk from combined exposure of aquatic invertebrates to fenpicoxamid and metabolite X642188 could be concluded from application of GF-3308 according the Central Zone GAP in scenarios R1 and R3 provided that 10 meters vegetated filter strip to surface water bodies is respected in combination with 75% drift reduction using appropriate drift reducing techniques.

The risk in scenario R4 remains unresolved and further assessment will be required in Member States which consider this scenario relevant.

Concerned Member States must decide on applicability of indicated risk mitigation measures in their countries at the product authorisation.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

*Risk assessment for fenpicoxamid relevant metabolites – Other metabolites at 1 x 100 g a.s./ha*

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC<sub>SW</sub>, PEC<sub>SED</sub>) and RAC for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

**Table 9.5-15: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X11963422 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae	Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>	EC <sub>10</sub> for X642188 / 10
(µg/L)		>9800	>9100	>9000	58 µg a.s./kg dws
AF		100	100	10	10
RAC (µg/L)		98	91	900	5.8
FOCUS Scenario	PEC <sup>SW</sup> gl-max (µg/L) / PEC <sup>SED</sup> (µg/kg dws)	ETR = PEC/RAC ETR < 1 is acceptable risk			
Step 1					
	12.71 / 10.91	<0.13	<0.14	<0.01	1.88
Step 2					
N-Europe	1.64 / 1.40	<0.02	<0.02	<0.00	0.24

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X11963422 did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.



Table

**9.5-16: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12264475 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae	Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>	NOEC for X12335723 / 10
(µg/L)		>9980	>10000	4440	220 µg a.s./kg dws
AF		100	100	10	10
RAC (µg/L)		99.8	100	444	22.0
FOCUS Scenario	PEC <sup>SW</sup> gl-max (µg/L) / PEC <sup>SED</sup> (µg/kg dws)	ETR = PEC/RAC ETR < 1 is acceptable risk			
Step 1					
	11.48 / 35.42	<0.12	<0.11	0.03	1.61
Step 2					
N-Europe	1.40 / 4.30	<0.01	<0.01	0.00	0.20

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X12264475 did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**Table 9.5-17: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12313581 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae	Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>	NOEC for X12335723 / 10
(µg/L)		>10000	>10000	15000	220 µg a.s./kg dws
AF		100	100	10	10
RAC (µg/L)		100	100	1500	22.0
FOCUS Scenario	PEC <sub>SW gl-max</sub> (µg/L) / PEC <sub>SED</sub> (µg/kg dws )	ETR = PEC/RAC ETR < 1 is acceptable risk			
Step 1					
	1.3 / 3.59	<0.01	<0.01	0.00	0.39
Step 2					
N-Europe	0.18 / 1.16	<0.00	<0.00	0.00	0.05

**Table**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X12313581 did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**9.5-18: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X696872 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	Parent / 10
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg/L)		>2000	545	52.2 <del>52.2</del>
AF		100	100	10
RAC (µg/L)		20	5.45	5.22 <del>52.2</del>
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk		
Step 1				
	2.19	<0.11	0.40	0.42 <del>0.04</del>
Step 2				
N-Europe	0.32 <del>2.19</del>	<0.02	0.06	0.06 <del>0.006</del>

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X696872 did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**Table 9.5-19: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X696476 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae	Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>	NOEC for X12335723 / 10
(µg/L)		>10000	>9500	350000	220 µg a.s./kg dws
AF		100	100	10	10
RAC (µg/L)		100	95	35000	22.0
FOCUS Scenario	PEC <sub>SW gl-max</sub> (µg/L) / PEC <sub>SED</sub> (µg/kg dws)	ETR = PEC/RAC ETR < 1 is acceptable risk			

**Table**

Step 1					
	0.98 / 73.4 3	<0.01	<0.01	0.00	3.34
Step 2					
N-Europe	0.17 / 8.99	<0.00	<0.00	0.00	0.41

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X696476 did indicate an acceptable risk for all groups of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**9.5-20: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12314005 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae	Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	Parent / 10	<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>	EC <sub>10</sub> for X642188 / 10
(µg/L)		>1900	>8500	52.2 <del>52.2</del>	58 µg a.s./kg dws
AF		100	100	10	10
RAC (µg/L)		19	85	5.22 <del>52.2</del>	5.8
FOCUS Scenario	PEC <sub>SW gl-max</sub> (µg/L) / PEC <sub>SED</sub> (µg/kg dws)	ETR = PEC/RAC ETR < 1 is acceptable risk			
Step 1					
	5.39 / 6.19	<0.28	<0.06	1.03 <del>0.10</del>	1.07
Step 2					
N-Europe	0.33 / 0.39	<0.02	<0.00	0.06 <del>0.01</del>	0.07

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X12314005 did indicate an acceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**Table 9.5-21: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X763024 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae
Test species		QSAR	<i>D. magna</i>	QSAR
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg/L)		568000	>10000	275000
AF		100	100	10

**Table**

RAC (µg/L)		5680	100	27500
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk		
Step 1				
	0.46	0.00	<0.00	0.00
Step 2				
N-Europe	0.07	0.00	<0.00	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X763024 did indicate an acceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**9.5-22: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12019520 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae	Sediment dwellers
Test species		<i>O. mykiss</i>	<i>D. magna</i>	Parent / 10	<i>C. riparius</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>	EC <sub>10</sub> for X642188 / 10
(µg/L)		>10000	>10000	52.2 <del>52.2</del>	58 µg a.s./kg dws
AF		100	100	10	10
RAC (µg/L)		100	100	5.22 <del>52.2</del>	5.8
FOCUS Scenario	PEC <sub>SW gl-max</sub> (µg/L) / PEC <sub>SED</sub> (µg/kg dws)	ETR = PEC/RAC ETR < 1 is acceptable risk			
Step 1					
	2.39 / 1.60	<0.02	<0.02	0.46 <del>0.05</del>	0.28
Step 2					
N-Europe	0.19 / 0.12	<0.00	<0.00	0.04 <del>0.00</del>	0.07

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X12019520 did indicate an acceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**Table 9.5-23: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12335723 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae		Sediment dweller
Test species		QSAR	<i>D. magna</i>	QSAR		<i>C. riparius</i>

Table

Table						
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>		NOEC
(µg/L)		12700000	>8600	1100000		2200
AF		100	100	10		10
RAC (µg/L)		127000	86	110000		220
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk			PEC <sub>gl-max</sub> (µg/kg)	
Step 1						
	9.10	0.00	<0.11	0.00	0.09	0.00
Step 2						
N-Europe	0.73	0.00	<0.01	0.00	0.01	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration;

PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk

For the intended uses in cereals, calculated PEC/RAC ratios for X12335723 did indicate an acceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**9.5-24: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12255349 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub> /E <sub>y</sub> C <sub>50</sub>
(µg/L)		7100	11	>10000
AF		100	100	10
RAC (µg/L)		71	0.11	1000
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk		
Step 1				
	1.08	0.02	10	<0.00
Step 2				
N-Europe	0.08	0.00	0.73	<0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk.

For the intended uses in cereals, calculated PEC/RAC ratios for X12255349 did indicate an acceptable risk for any group of aquatic organisms in FOCUS Step 2 scenarios. Therefore, no further assessment is necessary.

**Table****Table 9.5-25: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12446477 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	'arent / 10
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg/L)		>10000	1100	52.2 <del>52.2</del>
AF		100	100	10
RAC (µg/L)		100	11	5.22 <del>52.2</del>
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk		
Step 1				
	2.17	<0.02	0.20	0.42 <del>0.04</del>
Step 2				
N-Europe	0.21	<0.00	0.02	0.04 <del>0.00</del>

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk

For the intended uses in cereals, calculated PEC/RAC ratios for X12446477 did indicate acceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

X12433979 is highly unstable thus synthesis of this metabolite for the purpose of Tier 1 OECD testing proved impossible. QSAR evaluation (Blickley, 2018) indicates the 96 hr fish LC<sub>50</sub>, daphnid 48 hr EC<sub>50</sub>, and green algae 96 hr EC<sub>50</sub> values are 81.990, 48.857, and 44.437 mg/l, respectively.

**9.5-26: Aquatic organisms: acceptability of risk (PEC < RAC) for metabolite X12433979 for each organism group based on FOCUS Step 1 and 2 calculations for the use of GF-3308 in cereals**

Group		Fish acute	Inverteb. acute	Algae
Test species		QSAR	QSAR	QSAR
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg/L)		81990	48857	44437
AF		100	100	10
RAC (µg/L)		819.9	488.57	4443.7
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk		
Step 1				

**Table**

	5.85	0.01	0.01	0.00
<b>Step 2</b>				
N-Europe	0.57	0.00	0.00	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC = ETR, ratios above the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk

For the intended uses in cereals, calculated PEC/RAC ratios for X12433979 did indicate an acceptable risk for any group of aquatic organisms in FOCUS Step 1 and 2 scenarios. Therefore, no further assessment is necessary.

**zRMS comments:**

The risk assessment for fenpicoxamid metabolites (with exception of metabolite X642188 for which separate risk assessment has been performed) presented by the Applicant above is in general agreed by the zRMS with some minor corrections resulting from different approach on selection of the relevant endpoints. For discussion on agreed values, please refer to points 9.5.1 and 9.5.1.1 of this report.

Overall, acceptable risk from fenpicoxamid metabolites other than X642188 could be concluded following the application of GF-3308 according to the Central Zone GAP with no need for risk mitigation measures.

In the course of the commenting period it was pointed out that in aquatic system metabolism studies no decline of two sediment metabolites (X12313581 and X696476) was observed indicating their high persistent and that the risk assessment performed with consideration of the maximum annual  $PEC_{SED}$  will not cover situation after multiple years of use of GF-3308. Therefore it was proposed that the  $PEC_{SED, ACCU}$  should be calculated or, as a simplified approach, the annual  $PEC_{SED}$  multiplied by 20 (to account for application over 20 years) should be used for calculation of ETR for these two compounds. After the commenting period the Applicant provided relevant Step 3 and Step 4 (if necessary) calculations together with maximum annual  $PEC_{SED}$  multiplied by 20. All respective information on the performed calculations may be found in the final Core Assessment, Part B, Section 8. In tables below the ETR values calculated for *Chironomus riparius* exposed to metabolites X12313581 and X696476 accumulated in sediment over 20 years are presented. As in Tables 9.5-17 and 9.5-17, the experimentally derived  $EC_{10}$  for X12335723 (precursor of X12313581 and X696476) divided by 10 was assumed.

Metabolite	X12313581		X696476	
Group	Sed. dwell. prolonged		Sed. dwell. prolonged	
Test species	<i>C. riparius</i>		<i>C. riparius</i>	
Endpoint	NOEC for X12335723 / 10		NOEC for X12335723 / 10	
(µg/kg dws)	220		220	
AF	10		10	
RAC (µg/L)	22		22	
FOCUS Scenar-				
io	PEC		PEC gl-max (µg/kg)	
	gl-max (µg/kg)	ETR		ETR
Step 1				
	171.80	7.81	1468.60	66.75
Step 2				
N-Europe	23.20	1.05	179.80	8.17
Step 3, winter cereals				
D3/ditch	0.59	0.03	1.42	0.06

D4/pond	2.55	0.12	1.04
D4/stream	0.73	0.03	0.03
D5/pond	2.38	0.11	1.24
D5/stream	0.42	0.02	0.04
R1/pond	2.04	0.09	3.58
R1/stream	2.47	0.11	35.40
R3/stream	3.28	0.15	93.82
R4/stream	2.68	0.12	121.32
<b>Step 3, spring cereals</b>			
D3/ditch	0.88	0.04	1.94
D4/pond	2.91	0.13	1.15
D4/stream	0.77	0.04	0.12
D5/pond	2.30	0.10	1.23
D5/stream	0.39	0.02	0.07
R4/stream	8.83	0.40	185.0



Based on Step 3 20 years PEC<sub>SED</sub>, acceptable risk to sediment dwellers may be concluded from metabolite X12313581 for both crops in all scenarios. For metabolite X696476 an acceptable risk may be concluded for both crops in D scenarios and additionally in scenario R1 pond in winter cereals. However, potentially unacceptable risk is indicated in scenarios R1, R3 and R4 (in stream) following application to winter cereals and in scenario R4 (stream) following application to spring cereals. Further calculations for this compound were thus performed using Step 4 accumulated PEC<sub>SED</sub>. Risk mitigation measures resulting with acceptable risk for the parent compound were assumed (i.e. 10 m vegetated filter strip combined with 75% drift reduction).

Metabolite	X696476	
Group	Sed. dwell. prolonged	
Test species	C. riparius	
Endpoint	NOEC for X12335723 / 10	
(µg/kg dws)	220	
AF	10	
RAC (µg/L)	22	
FOCUS Scenario	PEC <sup>gl-max</sup> (µg/kg)	ETR
Step 4, winter cereals (10 m VFS + 75% DRN)		
D3/ditch		
D4/pond		
D4/stream		
D5/pond		
D5/stream		
R1/pond		
R1/stream	5.50	0.25
R3/stream	14.28	0.65
R4/stream	18.50	0.84
Step 4, spring cereals (10 m VFS + 75% DRN)		
D3/ditch		
D4/pond		
D4/stream		
D5/pond		
D5/stream		

R4/stream	28.06	1.28
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In case of application in winter cereals, assumption of the RMM resulting with acceptable risk for fenpicoxamid enabled to reduce s could be concluded for sediment dwelling organisms.

In case of spring cereals, the ETR in scenario R4 was still slightly above the trigger despite the assumed mitigation measures. It sh toxicity of its precursor (X12335723), while available EU agreed toxicity data for both compounds do not indicate any increased tox

in a pathway from fenpicoxamid via X12335723 to X12313581 and X696476 (being the terminal metabolite in this pathway) indicate low toxicity and are at comparable level (see Figures 9.5-4 and 9.5-5 in this document). Furthermore, the toxophore is lost in all these compounds. Taking this into account, the zRMS approach to assume 10 times toxicity was extremely conservative as given comparable toxicity of all metabolites in this pathway, in the risk assessment performed for X12313581 and X696476 assumption of the toxicity of their precursor would be relevant, which would result with acceptable risk from metabolite X696476 already with accumulated PEC<sub>SED</sub> calculated at Step 2. Taking all this into account, in opinion of the zRMS no unacceptable risk is expected from exposure of sediment dwellers to X696476 from uses in spring cereals also in scenario R4.

Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations p

*Risk assessment for GF-3308 at 1 x 2 L/ha*

The formulation consists of active substance and co-formulants. It will not remain intact in aquatic systems after application due to breakdown of its individual components. Therefore, only an initial spray drift PEC<sub>sw</sub> for a single application was calculated since applications would not be cumulative, and time-aged values (actual and TWA) not appropriate.

The initial Step 3 PEC<sub>sw</sub> was calculated using the SWASH drift calculator for the ditch, pond and stream, in addition to Step 4 using increased NSZ and DRN as required for the active substance. The ratios between PECs in surface water bodies and RACs for aquatic organisms are given per intended use for each SWASH scenario and each organism group at an application rate of 2 L GF-3308/ha. ETRs less than 1 indicate acceptable risk.

**Table 9.5-27: Aquatic organisms: acceptability of risk (PEC < RAC) for each organism group based on SWASH Step 3 and 4 calculations for the use of GF-3308 in cereals\_2 L/ha (100 g a.s./ha)**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	ErC <sub>50</sub>
(µg/L)		78	48	30000
AF		100	100	10
RAC (µg/L)		0.78	0.48	3000
SWASH	PEC <sub>gl-max</sub> (µg/L)	ETR = PEC/RAC ETR < 1 is acceptable risk		
Step 3 - cereals, 1 x 2 L product/ha (100 g a.s./ha)				
Ditch	13.0549	17	27	0.00
Pond	0.4451	0.57	0.93	0.00
Stream	9.6883	12	20	0.00
Step 4 - 40 NSZ + std nozzle				
Ditch	0.501	0.64	1.04	0.00
Pond	0.1142	0.15	0.24	0.00
Stream	0.501	0.64	1.04	0.00
Step 4 - 30 m NSZ + std nozzle				
Ditch	0.6611	0.85	1.38	0.00
Pond	0.1408	0.18	0.29	0.00
Stream	0.6611	0.85	1.38	0.00
Step 4 - 10 m NSZ + 50% DRN				
Ditch	0.9384	1.20	1.96	0.00
Pond	0.1385	0.18	0.29	0.00

Stream	0.9384	<b>1.20</b>	<b>1.96</b>	0.00
<b>Step 4 - 10 m NSZ + 75% DRN</b>				
Ditch	0.4692	0.60	0.98	0.00
Pond	0.0692	0.09	0.14	0.00
Stream	0.4692	0.60	0.98	0.00
<b>Step 4 - 10 m NSZ + 90% DRN</b>				
Ditch	0.1877	0.24	0.39	0.00
Pond	0.0277	0.04	0.06	0.00
Stream	0.1877	0.24	0.39	0.00
<b>Step 4 - 5 m NSZ + 90% DRN</b>				
Ditch	0.0975	0.45	0.74	0.00
Pond	0.0185	0.05	0.08	0.00
Stream	0.0975	0.45	0.74	0.00
<b>Step 4 - 20 m NSZ + 90% DRN</b>				
Ditch	0.0975	0.13	0.20	0.00
Pond	0.0185	0.02	0.04	0.00
Stream	0.0975	0.13	0.20	0.00

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

RAC/PEC = TER, ratios below the relevant trigger of 1 are shown in **bold** and indicate unacceptable risk; ratios shaded in green indicate acceptable risk

**For the intended uses in cereals, calculated RAC/PEC ratios for GF-3308 did indicate an acceptable risk for all taxa at 2 L GF-3308/ha (100 g a.s./ha) with:**

- **10 m NSZ +  $\geq$ 75% DRN**
- **$\geq$ 5 m NSZ + 90% DRN**

**zRMS comments:**

Provided above risk assessment for the formulated product based on surface water exposure calculated using Spray Drift Calculator is agreed by the zRMS.

Acceptable risk could be concluded from the intended Central Zone uses of GF-3308 provided that 10 m unsprayed buffer zone to surface water bodies is combined with 75% drift reduction or 5 m unsprayed buffer zone is combined with 90% drift reduction.

It has to be, however, noted that PEC/RAC calculations based on the formulation exposure data derived with consideration of the drift calculator is not foreseen by EFSA (2013) and the formulation endpoints expressed in terms of the active substance (in case lower than these derived for the active ingredient) are rather compared with PEC<sub>SW</sub> obtained for the active compounds using FOCUS modelling.

### 9.5.3 Overall conclusions

Acceptable risk is demonstrated for fenpicoxamid, relevant metabolites, and GF-3308 in cereals at 1 x 2 L GF-3308/ha (equivalent to 100 g a.s./ha) with a: ~~□ 10 m NSZ + 10 m VFS + 75% DRN; and □ 5 m NSZ + 10 VFS + 90% DRN.~~

#### zRMS comments:

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation GF-3308, which was performed in line with the EU agreed methodology.

*“The endpoint  $ErC_{50}$  is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: “... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future”. Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone.”*

## 9.6 Effects on bees (KCP 10.3.1)

### 9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with fenpicoxamid and relevant metabolites. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees – fenpicoxamid and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Fenpicoxamid	Oral	LD <sub>50</sub> >303.0 µg/bee*	EFSA, 2018
<i>Apis mellifera</i>	Fenpicoxamid	Contact	LD <sub>50</sub> >202.4 µg/bee*	EFSA, 2018
<b>Fenpicoxamid metabolites</b>				
<i>Apis mellifera</i>	X642188	Oral	LD <sub>50</sub> >101.9 µg/bee*	EFSA, 2018
<i>Apis mellifera</i>	X696476	Oral	LD <sub>50</sub> >14.2 µg/bee*	EFSA, 2018
<i>Apis mellifera</i>	X12019520	Oral	LD <sub>50</sub> = 132.6 µg/bee	EFSA, 2018

\*highest dose tested

**Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees - GF-3308**

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	GF-3308	Oral	LD <sub>50</sub> >205.6 µg/bee*	Schmitzer/2016/DAS# 160184
<i>Apis mellifera</i>	GF-3308	Contact	LD <sub>50</sub> = 53.4 µg/bee	
<i>Apis mellifera</i>	GF-3308	Chronic, adult	LDD <sub>50</sub> = 0.71 µg a.s./bee/day NOEDD <sub>mortality</sub> = 0.49 µg a.s./bee/day (based on actual intake of a.s.)	Verge/2017/DAS# 160522

<i>Apis mellifera</i>	GF-3308	22 d, larval (OECD 239)	NOEC = 55.6 µg prep/kg-diet period (2.84 µg a.s./kg-diet, based on 5.1% fenpicoxamid) NOED <sub>emergence</sub> = 8.56 µg prep/larva (0.437 µg a.s./larva, based on 5.1% fenpicoxamid)	Verge/2020/2018/DAS# 190305
<b>Higher-tier studies (tunnel test, field studies)</b>				
<i>Apis mellifera</i>	GF-3308	Semi-field, OECD 75/EPPO 170	NOER <sub>adult mortality, larval/pupae mortality</sub> = 130 g a.s./ha*  Had effect on foraging activity on ODAA at T1 and T2  Had an effect on worker behavior  NOER <sub>colony size, brood cells</sub> = 65 g a.s./ha  NOAER <sub>nectar cells, pollen storage</sub> = 130 g a.s./ha  NOER <sub>brood index, compensation index, termination rate of 1st brood</sub> = 130 g a.s./ha	Kleinhenz/2017/DAS# 160515

\*highest dose, concentration, or rate tested

#### **zRMS comments:**

The bee toxicity data for fenpicoxamid presented in Table 9.6-1 are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146.

Studies on acute and chronic toxicity of GF-3308 to adult bees and bee larvae were evaluated and agreed by the zRMS. Study summaries together with their evaluation may be found in Appendix 2. Endpoints presented in Table 9.6-2 are confirmed.

Although formally not required, the tunnel study on effects of GF-3308 on the bee colony was also submitted by the Applicant. It was evaluated and agreed by the zRMS. The study summary with zRMS evaluation may be found in Appendix 2. Endpoints presented in Table 9.6-2 are confirmed.

The test item had significant effect on bee colony strength at application rate equivalent to 130 g a.s./ha. Since the target rate of GF-3308 (100 g a.s./ha) was not tested, the NOER for colony strength was set to 65 g a.s./ha, the second rate tested. Consequences for the outcome of the risk assessment are discussed in point 9.6.2 below.

#### **9.6.1.1 Justification for new endpoints**

Not applicable.

#### **9.6.2 Risk assessment**

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

##### **9.6.2.1 Hazard quotients for bees**

The results of the first-tier risk assessments are summarised in the following tables.

**Table 9.6-3: First-tier assessment of the risk for bees due to the use of fenpicoxamid in cereals**

<b>Intended use</b>		Cereals	
<b>Active substance</b>		Fenpicoxamid	
<b>Application rate (g/ha)</b>		1 x 100	
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	>303.3	100	< 0.33
Contact toxicity	>202.4		< 0.49
<b>Intended use</b>		Cereals	
<b>Metabolite</b>		X642188	
<b>Application rate (g/ha)</b>		1 x 83.7 g/ha <sup>1</sup>	
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	>101.9 µg/bee	83.7	< 0.82
<b>Intended use</b>		Cereals	
<b>Metabolite</b>		X696476	
<b>Application rate (g/ha)</b>		1 x 27.5 g/ha <sup>2</sup>	
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	>14.2 µg/bee	27.5	< 1.9
<b>Intended use</b>		Cereals	
<b>Metabolite</b>		X12019520	
<b>Application rate (g/ha)</b>		1 x 30.6 g/ha <sup>3</sup>	
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	132.6 µg/bee	30.6	0.23

Q<sub>HO</sub>, Q<sub>HC</sub>: Hazard quotients for oral and contact exposure. Q<sub>H</sub> values shown in **bold** breach the relevant trigger.

<sup>1</sup> 100 g a.s./ha x molecular wt. conversion of 0.837 = 83.7 g X642188/ha

<sup>2</sup> 100 g a.s./ha x molecular wt. conversion of 0.275 = 27.5 g X696476/ha

<sup>3</sup> 100 g a.s./ha x molecular wt. conversion of 0.306 = 30.6 g X12019520/ha

**Table 9.6-4: First-tier assessment of the risk for bees due to the use of GF-3308 in cereals**

<b>Intended use</b>		Cereals	
<b>Product</b>		GF-3308	
<b>Application rate (g/ha)</b>		1 x 2032 (= 1 x 2 L prep/ha)	
<b>Test design</b>	<b>LD<sub>50</sub> (lab.) (µg/bee)</b>	<b>Single application rate (g/ha)</b>	<b>Q<sub>HO</sub>, Q<sub>HC</sub> criterion: Q<sub>H</sub> ≤ 50</b>
Oral toxicity	>205.6	2032	< 9.9
Contact toxicity	53.4		38

Q<sub>HO</sub>, Q<sub>HC</sub>: Hazard quotients for oral and contact exposure. Q<sub>H</sub> values shown in **bold** breach the relevant trigger. \*Based on a formulation density of 1.016 g/mL

**The HQ values for fenpicoxamid, relevant metabolites, and GF-3308 in honeybee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honey bees is acceptable.**

#### **zRMS comments:**

The risk assessment presented in Tables 9.6-3 and 9.6-4 is agreed by the zRMS.

On the basis of calculated HQ values acceptable risk to bees may be concluded from all intended Central Zone uses of GF-3308.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Therefore risk assessment based on indications of EFSA (2013) must be performed at the national level by cMS that do require such evaluation.

Nevertheless, in support of evaluation of GF-3308 the Applicant submitted the tunnel study (Kleinhenz, 22017020, KCP 10.3.1.5/1) in which application rate corresponding to 65 g a.s./ha had no effect on any of the investigated parameters, with exception of slight and transient effects on foraging activity and bees behaviour during the exposure phase, which had, however, no impact on any of the colony parameters or bee mortality during the monitoring phase over the 2 brood cycles.

The higher tested rate (130 g a.s./ha) also had some slight and transient effects on bee foraging activity and behaviour, but had also considerable impact on the colony size at the monitoring ~~size~~. ~~site~~ The overwintering success of the treated colonies was not investigated, but with such reduced number of bees successful overwintering is doubtful. The target rate of GF-3308 (equivalent to 100 g a.s./ha) was not tested and for this reason it is not known if it would have any adverse and unacceptable effect on the bee colonies. Taking this into account with NOER of 65 g a.s./ha from the tunnel study the low risk to bees exposed in the treated field from the intended uses of GF-3308 was not demonstrated. Nevertheless, as the formulation is applied to bee nonattractive crop, in opinion of the zRMS the risk mitigation measures are sufficient to reduce the in-field risk to the acceptable level. The RMM proposed by the zRMS include the following statements:

1. Do not apply when flowering weeds are present.
2. Do not apply when honeydew is present.
3. Do not use where bees are actively foraging.

The risk would be acceptable for bees present in adjacent crops or foraging on weeds in non-agricultural land, since the drift rate (2.77 g a.s./ha) is considerably lower than the lower tested rate of 65 g a.s./ha.

Concerned Member States must decide on applicability of the proposed RMM in their countries at the product authorisation.

~~DAS recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document issued in 2013 hasn't been noted and is not a realistically feasible way forward. Therefore, the risk assessment below has been conducted following the EPPO 2010<sup>1</sup> scheme which provides a comparable level of protection to the EFSA approach and is based on the current scientific state of the art for bee pollinator risk assessment. The maximum application rate of GF-3308 is 2 L/ha (equivalent to 100 g a.s./ha).~~

#### ~~Risk Assessment for Larvae~~

~~Worst case data from Rortais *et al.*, 2005<sup>2</sup> as proposed in the EPPO scheme have been used to estimate the consumption by bee larvae.~~

~~Worker larvae consuming 59.4 mg sugar in 5 days Assuming 30% sugar content of nectar the worst case consumption with worker larvae is:~~

$$\text{59.4/0.30} = 198 \text{ mg nectar in 5 days.}$$

<sup>1</sup> EPPO (2010a). Side effects on honey bees. Bulletin OEPP/EPPO Bulletin 40: 313-319. EPPO (2010b). Environmental risk assessment scheme for plant protection products. Bulletin OEPP/EPPO Bulletin 40: 323-331.

<sup>2</sup> Rortais A, Arnold G, Halm M-P, Touffet-Briens F (2005) Modes of honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36: 71-83



In addition worker larvae are considered to consume 2 mg pollen during their development phase (EFSA 2013). Thus considering the mean RUD values for nectar and pollen in EFSA 2013 exposure can be estimated either for the whole development period of 5 days.

The maximum application rate of GF-3308 is 2 L/ha (equivalent to 100 g a.s./ha). The proposed crops on the label are spring and winter wheat, triticale and rye, spelt and durum wheat, and barley. None of these crops are considered to be attractive to foraging bees therefore the exposure to applications in the treated crop will be negligible.

Nectar concentration:  $0.100 \text{ kg a.s./ha} \times 2.9 \text{ mg/kg/kg/ha} = 0.29 \text{ mg/kg nectar}$

Pollen concentration:  $0.100 \text{ kg a.s./ha} \times 6.1 \text{ mg/kg/kg/ha} = 0.61 \text{ mg/kg pollen}$

Nectar dose over 5 days of consumption by larvae:  $0.29 \text{ mg/kg nectar} \times 198 \times 10^{-6} \text{ kg nectar/larvae} = 5.74 \times 10^{-5} \text{ mg GF-3308/larvae} = 0.0574 \text{ } \mu\text{g a.s./larvae}$ .

Pollen dose over 5 days of consumption by larvae:  $0.61 \text{ mg/kg pollen} \times 2 \times 10^{-6} \text{ kg pollen/larvae} = 1.22 \times 10^{-6} \text{ mg a.s./larvae} = 1.22 \times 10^{-3} \text{ } \mu\text{g a.s./larvae}$ .

Total dose over 5 days = Nectar dose + Pollen dose =  $0.0574 + 1.22 \times 10^{-3} \text{ } \mu\text{g a.s./larvae} = 0.0586 \text{ } \mu\text{g a.s./larvae}$

A larval study has been conducted with GF-3308; the GF-3308 larval NOED of  $0.437 \text{ } \mu\text{g a.s./larva}$  (Verge (2020) GF-3308: Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure); DAS Study ID-190305) can be used in the risk assessment.

TER = Toxicity/Exposure =  $0.437 \text{ } \mu\text{g GF-3307/larvae} / 0.0586 \text{ } \mu\text{g a.s./larvae} = 7.44$

The EPPO-2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees. **It is clear that with a TER value of 7.44 there is a safety margin, indicating that the proposed use of GF3308 poses an acceptable risk to bee larval development.**

#### Chronic Adult Honey Bee Risk Assessment

This is based upon the method of EPPO-2010 risk assessment for systemic substances which is cited in the regulation as a current risk assessment scheme. It uses NOEDD values for the endpoint so avoids the issues associated with the generation of LDD<sub>50</sub> values for substances of low toxicity, and calculates exposure in a similar way to EFSA 2013. The approach is also in line with other chronic risk assessments (e.g. birds and mammals). EPPO-2010 recommended the calculation of a TER using the following equation:

$$\text{TER} = \text{NOEDD}/\text{daily dose}$$

Where daily dose (DD) is based on the worst case a sugar need of 128 mg/bee/day (Rortais *et al.*, 2005) of a bee feeding exclusively from nectar containing 30% sugar using the following equation:

$$\text{Daily dose (} \mu\text{g a.i./bee)} = \text{A.R.} \times (0.128 \text{ g}/0.3) \times \text{RUD}$$

Where: ——— A.R. = application rate in kg a.i./ha

RUD = residue per unit dose from the EFSA bee guidance. Mean RUD<sub>nectar</sub> = 2.9 mg a.i./kg (foliar sprays).

$$\text{Daily dose} = 0.100 \text{ kg a.s./ha} \times 0.427 \times 2.9 = 0.124 \text{ } \mu\text{g a.s./bee}$$

This value can be compared to the GF-3308 adult NOED of  $0.49 \text{ } \mu\text{g a.s./bee/day}$  (Verge, 2017). (2017): GF-3308: A laboratory study to determine the chronic oral toxicity to the adult worker honey bee *Apis mellifera* L. (Hymenoptera: Apidae); DAS Study ID-160522).

$$\text{TER} = \text{NOEDD}/\text{daily dose} = 0.49 \text{ } \mu\text{g a.s./bee/day} / 0.124 \text{ } \mu\text{g a.s./bee} = 3.96$$

The EPPO-2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees. **With a TER value of 3.96 there is a safety margin, indicating that the proposed use of GF-3308 poses an**

~~acceptable chronic risk to adult honeybees. It should also be noted that the proposed uses of GF3308 are all on crops that are not attractive to bees and the exposure to applications on the treated crop will be negligible.~~

**zRMS comments:**

The chronic and larvae risk assessment was not evaluated by the zRMS as being not required according to SANCO/10329/2002 rev 2 final. Furthermore, the assessment was performed in line with the revised EPPO scheme of 2010, while in opinion of the zRMS in case the chronic and larvae risk assessment is performed, it should be conducted in line with EFSA (2013).

Nevertheless, the Applicant submitted a tunnel study performed on flowering *Phacelia tanacetifolia*, which addressed to chronic effects of GF-3308 on adult bees and larvae. As discussed in the commenting box above, the low risk to bees present in the field treated with GF-3308 at 100 g a.s./ha could not be concluded based on results of the study and following risk mitigation measures are proposed by the zRMS:

1. Do not apply when flowering weeds are present.
2. Do not apply when honeydew is present.
3. Do not use where bees are actively foraging.

The risk would be acceptable for bees present in adjacent crops or foraging on weeds in non-agricultural land, since the drift rate (2.77 g a.s./ha) is considerable lower than the lower tested rate of 65 g a.s./ha.

Concerned Member States must decide on applicability of the proposed RMM in their countries at the product authorisation.

### 9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

To further evaluate the risk of GF-3308 applications to foraging bees and development of brood, an OECD 75 tunnel study was conducted in Germany in 2016 (Kleinhenz, 2017). This study evaluated the potential effects of GF-3308 on the honeybees applied to flowering *Phacelia tanacetifolia* in Germany in a semi-field study with two brood cycles.

The study consisted of four treatment groups: two test item groups T1 and T2 (GF-3308), one toxic reference item group R (Insegar) and a water-treated control C, applied during daily bee flight at the beginning of full flowering of *Phacelia tanacetifolia* at BBCH 63-65. The application rates of GF3308 were 65 g a.s./ha in treatment group T1 and 130 g a.s./ha in the treatment group T2. Commercial bee colonies were placed in the tunnel tents at beginning of flowering (BBCH 63). The mortality, foraging activity, behaviour of the bees and condition of the colonies were examined before and after application. Photographic assessments of the brood development of single cells initially containing eggs, young larvae and old larvae were conducted over two brood cycles. The condition of the colonies was assessed before the application, over two brood cycles and until the start of overwintering of the colonies. Additionally the weight and malformations of pupae collected from combs was evaluated, and the level of infestation with *Varroa* mites was recorded after anti-*Varroa* treatment in autumn.

For biological assessments five replicates/tunnels in T1, T2, R and C were installed. Additionally, one extra replicate/tunnel was used for residue sampling in C, T1 and T2 (replicate s). Samples of forager bees (for preparation of pollen and nectar) were taken once before and three times after start of application in Cs, T1s and T2s for subsequent residue analysis.

A summary of the effects of GF-3308 on the brood development is presented in the table below.

**Table 9.6-5: Effects of GF-3308 on honey bee brood under semi-field conditions**

Treatment	Untreated control	GF-3308		Toxic standard
Rate <sup>1</sup>	-	65 g a.s./ha	130 g a.s./ha	1200 g/ha
Brood termination rate (1 <sup>st</sup> brood cycle)				
Eggs:# Young	16.99	12.99	21.41	53.56*
larvae:	32.90	23.63	35.28	26.92
Old larvae:	7.32	3.67	12.53	33.72*
Brood termination rate (2 <sup>nd</sup> brood cycle)				
Eggs:	26.50	26.18	28.13	28.60
Young larvae:	21.37	6.09	22.17	17.60
Old larvae:	6.29	4.71	7.27	7.71
Dead worker bees <sup>2</sup> Exposure:				
Monitoring:	99.2	82.4	94.6	70.9
	44.0	51.9	41.0	43.4
Dead pupae <sup>2</sup> Exposure:				
Monitoring:	0.5	1.3	1.2	0.6
	1.1	0.5	1.1	28.1*

<sup>1</sup> Delivered in 100 L/ha of water<sup>2</sup> Over the post-application period (exposure period in the tunnels 8 days (0DAA to 7DAA), further monitoring 30 days (8DAA to 37DAA); mean value per hive per day (5 replicates))# one replicate (Cd) excluded from evaluation of eggs during the 1<sup>st</sup> brood cycle (outlier) \* statistically significant (Student's t-test, method: pooled, one-sided, p≤0.05)

The results show there was no effect of the test item treatments T1 or T2 on the brood index, compensation index or termination rate of eggs, young larvae or old larvae during the 1<sup>st</sup> (1DBA to 20DAA) or 2<sup>nd</sup> (15DAA to 37DAA) brood cycle. Additionally, there was no effect on the mortality of adult worker bees, worker bee pupae or male adult bees and male pupae. Foraging activity in T1 and T2 decreased on the day of application (0DAA). There was no effect of the test item treatments T1 or T2 on the storage of nectar and pollen. There was no effect of the test item treatments T1 or T2 on the weight or malformations of pupae sampled from combs towards the end of the 1<sup>st</sup> brood cycle (16DAA). There was no effect of the test item treatment T1 on the colony size and total number of brood cells throughout the entire study period. Test item treatment T2 had an effect on the colony size and a slight effect on the total number of brood cells or certain brood stages (larvae) during the postexposure monitoring period. Therefore, although there were no effects on the brood development from both treatments, the NOER for this study is considered to be 65 g a.s./ha.

As the proposed use of GF-3308 is on cereals, exposure to foraging bees will be minimal. Exposure to direct overspray and to residues in cereal pollen is unlikely to occur. Exposure to bees foraging on flowering weeds in the crop is considered to be a minor route and the maximum rate encountered will be 65 g a.s./ha due to crop interception. Exposure to residues of GF-3308 from spray drift onto flowering plants in field margins or adjacent crops will be to a maximum rate of 2.77 g a.s./ha (2.77% drift from a cereal crop following a maximum application of 100 g a.s./ha), which is significantly below the tunnel study NOER of 65 g a.s./ha **indicating the proposed uses of GF-3308 pose an acceptable chronic risk to honeybee larvae and brood development.**

**zRMS comments:**

Results of the tunnel study by Kleinhenz (2017) were already discussed in the commenting box in point 9.6.2.1 above. Based on the study results, the low risk to bees present in the field treated with GF-3308 at 100 g a.s./ha could not be concluded and following risk mitigation measures are proposed by the zRMS:

1. Do not apply when flowering weeds are present.
2. Do not apply when honeydew is present.
3. Do not use where bees are actively foraging.

The risk would be acceptable for bees present in adjacent crops or foraging on weeds in non-agricultural land, since the drift rate (2.77 g a.s./ha) is considerable lower than the lower tested rate of 65 g a.s./ha.

Concerned Member States must decide on applicability of the proposed RMM in their countries at the product authorisation.

### **9.6.3 Effects on bumble bees**

Studies not required.

### **9.6.4 Effects on solitary bees**

Studies not required.

### **9.6.5 Overall conclusions**

The HQ values for fenpicoxamid, relevant metabolites, and GF-3308 in honey bee are below the Annex VI trigger of 50; therefore, the acute oral and contact risk to honey bees is acceptable. Based on results of the tunnel study the risk to bees present in the field treated with GF-3308 at 2.0 L/ha could not be ruled out and following risk mitigation measures are proposed:

4. Do not apply when flowering weeds are present.
5. Do not apply when honeydew is present.
6. Do not use where bees are actively foraging.

The risk would be acceptable for bees present in adjacent crops or foraging on weeds in nonagricultural land, since the drift rate (2.77 g a.s./ha) is considerable lower than the lower tested rate of 65 g a.s./ha.

Concerned Member States must decide on applicability of the proposed RMM in their countries at the product authorisation.

~~The larval bee TER for GF 3308 exceeds the trigger of 1 using the EPPO risk assessment approach indicating that the proposed use poses an acceptable risk to bee larval development. Chronic risk to honey bee is also acceptable as the proposed uses of GF 3308 are on crops that are not attractive to bees therefore the exposure to applications on the treated crop will be negligible.~~

## **9.7 Effects on arthropods other than bees (KCP 10.3.2)**

### **9.7.1 Toxicity data**

Studies on the toxicity to non-target arthropods have been carried out with fenpicoxamid. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods – fenpicoxamid**

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	Fenpicoxamid	Laboratory test glass plates (2D)	LR <sub>50</sub> >400 g/ha	EFSA, 2018
<i>Aphidius rhopalosiphi</i> (adults)	Fenpicoxamid	Laboratory test glass plates (2D)	LR <sub>50</sub> = 129 g/ha	EFSA, 2018

**Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods - GF-3308**

Species	Substance	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	GF-3308	Laboratory test glass plates (2D)	LR <sub>50</sub> = 306 mL/ha	Moll/2016/DAS# 160188
<i>Aphidius rhopalosiphi</i> (adults)	GF-3308	Laboratory test glass plates (2D)	LR <sub>50</sub> = 314 mL/ha	Moll/2016/DAS# 160185
<i>Chrysoperla carnea</i> (larvae)	GF-3308	Laboratory test glass plates (2D)	LR <sub>50</sub> >3400 mL/ha	Vaughan/2016/DAS# 160216
<i>Typhlodromus pyri</i> (protonymphs)	GF-3308	Extended laboratory test bean leaves (2D)	LR <sub>50</sub> >3400 mL/ha ER <sub>50</sub> >3400 mL/ha  Red. of fecundity: -40.3% at 544 mL/ha 44.2 % at 1360 mL/ha -12.0% at 3400 mL/ha	Moll/2016/DAS# 160189
<i>Aphidius rhopalosiphi</i> (adults)	GF-3308	Extended laboratory test barley plants (3D)	LR <sub>50</sub> = 1636 mL/ha ER <sub>50</sub> >1176 mL/ha  Red. of fecundity: 19.3 % at 407 mL/ha 22.8 % at 692 mL/ha 44.1% at 1176 mL/ha	Moll/2016/DAS# 160186
<i>Aleochara bilineata</i>	GF-3308	Extended laboratory test LUFA 2.1 soil (2D)	ER <sub>50</sub> >4000 mL/ha  No adverse effects on reproduction	Schmidt/2016/DAS# 160161
<i>Aleochara bilineata</i>	GF-3308	Extended laboratory test LUFA 2.1 soil (2D)	ER <sub>50</sub> > 4000 mL/ha  No adverse effects on reproduction	Tew/2020/DAS# 200611
<i>Coccinella septempunctata</i>	GF-3308	Extended laboratory test bean leaves (2D)	LR <sub>50</sub> >2000 mL/ha ER <sub>50</sub> = 939 mL/ha (fertility)  Red. of fecundity: 15.4% at 500 mL/ha 43.0% at 1000 mL/ha 43.0% at 2000 mL/ha	Schmidt/2016/DAS# 160162

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i> (adults)	GF-3308	Aged-residue test bean plants (3D), assay uses bean leaves	Mortality (corrected) at 2 L/ha x 2 applications with a 15 day interval: 90% at 0 DALT 46.2% at 13 DALT 7.5% at 27 DALT  Red. of parasitism rate: 33.8% at 13 DALT 29.9 % at 27 DALT	Moll/2016/DAS# 160187
<i>Coccinella septempunctata</i>	GF-3308	Aged-residue test bean plants (3D), assay uses bean leaves	Mortality (corrected) at 2 L/ha x 2 applications with a 14 day interval: -16.7% at 0 DALT 5.0% at 14 DALT  Effects on reproduction: 13.2% at 0 DALT 1% at 14 DALT	Vaughan/2018/DAS# 170779

**zRMS comments:**

The toxicity data for fenpicoxamid presented in Table 9-6.1 are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146. Please note that reported EU endpoints originate from studies performed with the active substance and not the representative formulation and this is why they are retained in the Core Assessment.

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). The endpoints reported in Table 9.6-2 are confirmed to be correct.

**9.7.1.1 Justification for new endpoints**

Not applicable.

**9.7.2 Risk assessment**

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

**9.7.2.1 Risk assessment for in-field exposure**

The results of the first- and higher-tier risk assessments for fenpicoxamid and GF-3308 are summarised in the following tables.

**Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of fenpicoxamid in cereals**

Intended use	Cereals
Active substance	Fenpicoxamid

Application rate (g/ha)		1 x 100	
MAF		1	
Test species Tier I	LR <sub>50</sub> (lab.) (g/ha)	PER <sub>in-field</sub> (g/ha)	HQ <sub>in-field</sub> criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	>400	100	< 0.25
<i>Aphidius rhopalosiphi</i>	129		0.78

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in **bold** breach the relevant trigger.

### Acceptable in-field risk is demonstrated for fenpicoxamid at the proposed GAP.

**Table 9.7-4: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GF-3308 in cereals**

Intended use		Cereals	
Product		GF-3308	
Application rate (ml/ha)		1 x 2000	
MAF		1	
Test species Tier I	LR <sub>50</sub> (lab.) (mL/ha)	PER <sub>in-field</sub> (mL/ha)	HQ <sub>in-field</sub> criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	306	2000	6.5
<i>Aphidius rhopalosiphi</i>	314		6.4
<i>Chrysoperla carnea</i>	>3400		<0.59
Test species Higher-tier	Rate with ≤ 50 % effect* (ml/ha)	PER <sub>in-field</sub> (ml/ha)	PER <sub>in-field</sub> below rate with ≤ 50 % effect?
<i>Typhlodromus pyri</i>	>3400 (mortality and fecundity)	2000	yes
<i>Aphidius rhopalosiphi</i>	1636 (mortality)		no
	1179 (fecundity)		no
<i>Coccinella septempunctata</i>	>2000 (mortality)		yes
	939 (fecundity )		no
<i>Aleochara bilineata</i>	>4000 (mortality and fecundity)	yes	
Test species Higher-tier	Rate with ≤ 50 % effect (ml/ha) at DALT	PER <sub>in-field</sub> (ml/ha)	PER <sub>in-field</sub> below rate with ≤ 50 % effect?
<i>Aphidius rhopalosiphi</i>	2000 ml/ha x 2 applications: at 13 DALT	2000	No effect (mortality and parasitism) >50% at the GAP at DALT 13.
<i>Coccinella septempunctata</i>	2000 ml/ha x 2 applications: at 0 DALT		No effect (mortality and repro) >50% at the GAP at 0 DALT.

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in **bold** breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

**In-field risk to foliar-dwelling organisms (*Coccinella* and *Aphidius*) is acceptable 0 and 13 days post application, respectively, when exposed to an exaggerated rate (i.e. 2 x 2 L GF-3308/L).**

**zRMS comments:**

The in-field risk assessment for non-target arthropods presented in Tables 9.6-3 and 9.6-4 above is agreed by the zRMS. Additional information has been added in the risk assessment based on results of the extended laboratory studies in Table 9.6-4 in order to indicate endpoints relevant for mortality and fecundity.

Evaluation based on results of laboratory and extended laboratory studies performed with GF-3308 demonstrated acceptable in-field risk to *T.pyri*, *C. carnea* and *A. bilineata* with no need for further refinement. The risk to *A. rhopalosiphi* and *C. septempunctata* was not acceptable and therefore aged residue studies were performed for these two species at exaggerated rate of 2x2.0 L product/ha (single application at 2.0 L/ha is intended in the Central Zone). In performed studies no effects >50% on *A. rhopalosiphi* and *C. septempunctata* were observed after 13 and 0 days of aging, respectively, which demonstrates that there is potential for re-colonisation within less than 1 year.

Overall, acceptable in-field risk to non-target arthropods from the intended Central Zone uses of GF-3308 may be concluded.

The risk assessment based on EU agreed endpoints for the active substance was retained for informative purposes only, since relevant evaluation was performed with endpoints derived from studies performed with GF3308, in line with data requirements.

### 9.7.2.2 Risk assessment for off-field exposure

The results of the first- and higher-tier risk assessments for fenpicoxamid and GF-3308 are summarised in the following tables.

**Table 9.7-5: First-tier assessment of the off-field risk for non-target arthropods due to the use of fenpicoxamid in cereals**

<b>Intended use</b>	Cereals					
<b>Active substance</b>	Fenpicoxamid					
<b>Application rate (g/ha)</b>	1 x 100					
<b>MAF</b>	1					
<b>vdf</b>	5(per recurring issues, PRAPER 185, 2019)					
<b>Test species</b>	<b>LR<sub>50</sub> (lab.)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub></b>	<b>CF</b>	<b>corr.PER<sub>off-field</sub></b>	<b>HQ<sub>off-field</sub></b>
<b>Tier I</b>	<b>(g/ha)</b>		<b>(g/ha)</b>		<b>(g/ha)</b>	<b>criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	>400	2.77%	0.554	10 (default)	5.54	< 0.014
<i>Aphidius rhopalosiphi</i>	129					0.043

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

**Acceptable off-field risk is demonstrated for fenpicoxamid at the proposed GAP.**

**Table 9.7-6: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GF-3308 in cereals**

<b>Intended use</b>	Cereals
<b>Product</b>	GF-3308
<b>Application rate (ml/ha)</b>	1 x 2000



<b>MAF</b>		1					
<b>vdf</b>		5 (per recurring issues, PRAPER 185, 2019)					
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (ml/ha)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (ml/ha)</b>	<b>CF</b>	<b>corr.PER<sub>off-field</sub> (ml/ha)</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>	
<i>Typhlodromus pyri</i>	306	2.77%	11.1	10 (default)	111	0.36	
<i>Aphidius rhopalosiphi</i>	314					0.35	
<i>Chrysoperla carnea</i>	>3400					<0.033	
<b>Test species Higher-tier</b>	<b>Rate with effect* (ml/ha)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (ml/ha)</b>	<b>CF</b>	<b>corr.PER<sub>off-field</sub> (ml/ha)</b>	<b>corr. PER<sub>off-field</sub> below rate with ≤ 50 % effect?</b>	
<i>Typhlodromus pyri</i>	>3400 (mortality and fecundity)	2.77%	11.1	5	55.4	yes	
<i>Aphidius rhopalosiphi</i>	1636 (mortality) 1179 (fecundity)		55.4 (no VDF applied, 3D study)		277	yes	
<i>Coccinella septempunctata</i>	>2000 (mortality) 939 (fecundity)		11.1		55.4	yes	
<i>Aleochara bilineata</i>	>4000 (mortality and fecundity)		11.1 55.4		55.4 27.7	yes	

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in **bold** breach the relevant trigger.

\* If an LR<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

### Acceptable off-field risk is demonstrated when GF-3308 is used according to the proposed GAP.

#### zRMS comments:

The off-field risk assessment based on results of Tier I studies is agreed by the zRMS.

Although acceptable risk could be concluded based on Tier I studies, the Applicant performed also evaluation with consideration of results of the extended laboratory tests. However, calculation of the PER<sub>off-field</sub> was not fully clear - for *T. pyri*, *A. rhopalosiphi* and *C. septempunctata* single PER<sub>off-field</sub> was calculated with consideration of VDF, although study with *A. rhopalosiphi* was performed in a 3D design, so no VDF should have been used. For *A. bilineata* the off-field exposure was further reduced using additional factor of 2, but it is not known on what basis. The exposure calculation was thus amended by the zRMS - for 2D studies (*T. pyri*, *C. septempunctata* and *A. bilineata*) VDF of 5 has been used, while for 3D study (*A. rhopalosiphi*) no VDF has been applied. Furthermore, additional information has been added to indicate endpoints relevant for mortality and fecundity.

In all calculations VDF of 5 has been used, as discussed during the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology in 2018. It should be noted that although consideration of VDF of 5 as an interim solution is indicated in the EFSA Supporting publication 2019:EN-1673, it is also emphasised that this interim solution should be reflected in the guidance document SANCO/10329/2002 rev 2 final and its implementation should be further considered, which, however, has not taken place yet. For this reason consideration of VDF of 5 may be considered as a recommendation, but not as a requirement. It should be noted that in the Central Zone guidance in area of ecotoxicology this issue is also addressed as a reference to EFSA Supporting publication

2019:EN-1673. Nevertheless, since acceptable risk could be concluded for the worst case VDF of 5 (for 2D studies), no additional calculations based on VDF of 10 were deemed necessary.

Overall, acceptable off-field risk to non-target arthropods may be concluded from the intended Central Zone uses of GF-3308 with no need for risk mitigation measures.

The risk assessment based on EU agreed endpoints for the active substance was retained for informative purposes only, since relevant evaluation was performed with endpoints derived from studies performed with GF3308, in line with data requirements.

### 9.7.2.3 Additional higher-tier risk assessment

Not relevant.

### 9.7.2.4 Risk mitigation measures

No risk mitigation needed for off-field exposure.

### 9.7.3 Overall conclusions

For fenpicoxamid, the tier 1 in- and off-field HQ values are below the Annex VI trigger of 2 for both indicator species, thus indicating that the active substance is of low risk to non-target arthropods at the maximum in-field application rate.

For GF-3308, acceptable in-field risk could be concluded for some species at tier 1 (*Chrysoperla carnea*) or tier 2 (*Typhlodromus pyri* and *Aleochara bilineata*). For *Coccinella septempunctata* and *Aphidius rhopalosiphii* acceptable in-field risk could be concluded based on results of the aged-residue studies performed at exaggerated rate of 2 x 2 L GF-3308/ha with effects <50% after 0 and 13 days of aging, respectively, demonstrating potential for re-colonisation within <1 year. ~~the tier-2 in-field risk to soil-dwelling organisms is acceptable at the proposed GAP. In field risk to foliar dwelling organisms (*Coccinella* and *Aphidius*) is acceptable 0 and 13 days post application, respectively, when exposed to an exaggerated rate (i.e. 2 x 2 L GF-3308/L).~~ Acceptable off-field risk is demonstrated for GF-3308 when used according to the proposed Central Zone GAP with no need for risk mitigation measures.

## 9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

### 9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with fenpicoxamid and relevant metabolites. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Appendix 2 of this document (new studies). Metabolites that do not have any study data are conservatively assumed to be ten times as toxic as the parent compound.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – fenpicoxamid and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	Fenpicoxamid	Mixed into substrate 56 d, chronic 54% peat content	NOEC = 19.85 mg/kg dw NOEC <sub>corr</sub> = 9.925 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	Fenpicoxamid	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 7.94 mg/kg dw NOEC <sub>corr</sub> = 3.97 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	Fenpicoxamid	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 39.7 mg/kg dw NOEC <sub>corr</sub> = 19.85 mg/kg dw*	EFSA, 2018
<b>Fenpicoxamid metabolites</b>				
<i>Eisenia fetida</i>	X642188	Mixed into substrate 56 d, chronic 54% peat content	NOEC = 5.6 mg/kg dw NOEC <sub>corr</sub> = 2.8 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X642188	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 10 mg/kg dw NOEC <sub>corr</sub> = 5 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X642188	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 20 mg/kg dw NOEC <sub>corr</sub> = 10 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X11963422	Mixed into substrate 56 d, chronic 54% peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Folsomia candida</i>	X11963422	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 5 mg/kg dw	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X11963422	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Eisenia fetida</i>	X12264475	Mixed into substrate 56 d, chronic 54% peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Folsomia candida</i>	X12264475	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X12264475	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Eisenia fetida</i>	X12313581	Est. Assumes 10X parent (including 2X Kow factor)	NOEC <sub>corr</sub> = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12313581		NOEC <sub>corr</sub> = 0.3975 mg/kg dw*	EFSA, 2018
Species	Substance	Exposure System	Results	Reference
<i>Hypoaspis aculeifer</i>	X12313581		NOEC <sub>corr</sub> = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X696872 <del>X69648</del> <sub>2</sub>	Est. Assumes 10X parent (including 2X Kow factor)	NOEC <sub>corr</sub> = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X696872 <del>X69648</del> <sub>2</sub>		NOEC <sub>corr</sub> = 0.3975 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X696872 <del>X6964872</del>		NOEC <sub>corr</sub> = 1.985 mg/kg dw*	EFSA, 2018

<i>Eisenia fetida</i>	X696476	Mixed into substrate 56 d, chronic 54% peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Folsomia candida</i>	X696476	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X696476	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 10 mg/kg dw	EFSA, 2018
<i>Eisenia fetida</i>	X12314005	Est. Assumes 10X parent (including 2X Kow factor)	NOEC <sub>corr</sub> = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12314005		NOEC <sub>corr</sub> = 0.3975 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X12314005		NOEC <sub>corr</sub> = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X763024	Est. Assumes 10X parent (including 2X Kow factor)	NOEC <sub>corr</sub> = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X763024		NOEC <sub>corr</sub> = 0.3975 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X763024		NOEC <sub>corr</sub> = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X12019520	Est. Assumes 10X parent (including 2X Kow factor).	NOEC <sub>corr</sub> = 0.99 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12019520		NOEC <sub>corr</sub> = 0.3975 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X12019520		NOEC <sub>corr</sub> = 1.985 mg/kg dw*	EFSA, 2018
<i>Eisenia fetida</i>	X12255349	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 10 mg/kg dw NOEC <sub>corr</sub> = 5 mg/kg dw*	EFSA, 2018
<i>Folsomia candida</i>	X12255349	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 10 mg/kg dw NOEC <sub>corr</sub> = 5 mg/kg dw*	EFSA, 2018
<i>Hypoaspis aculeifer</i>	X12255349	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 10 mg/kg dw NOEC <sub>corr</sub> = 5 mg/kg dw*	EFSA, 2018

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

**Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – GF-3308**

Species	Substance	Exposure System	Results	Reference
<i>Eisenia fetida</i>	GF-3308	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 972 mg/kg dw NOEC <sub>corr</sub> = 486 mg/kg dw* EC <sub>10</sub> not relevant (no dose response)	Ganßmann/2016/DAS# 160193
<i>Folsomia candida</i>	GF-3308	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 51.4 mg/kg dw NOEC <sub>corr</sub> = 25.7 mg/kg dw* EC <sub>10</sub> = 79.1 mg/kg dw EC <sub>10corr</sub> = 39.6 mg/kg dw	Ganßmann/2016/DAS# 160191
<i>Hypoaspis aculeifer</i>	GF-3308	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 300 mg/kg dw NOEC <sub>corr</sub> = 150 mg/kg dw* EC <sub>10</sub> not relevant (no dose response)	Ganßmann/2016/DAS# 160192

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

#### zRMS comments:

The toxicity data for fenpicoxamid and its relevant soil metabolites presented in Table 9.8-1 are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146 with some minor corrections introduced by the zRMS.

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). The endpoints reported in Table 9.8-2 are confirmed to be correct.

The risk assessment was based on the lower of NOEC and EC<sub>10</sub> value.

### 9.8.1.1 Justification for new endpoints

Not relevant.

### 9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

#### 9.8.2.1 First-tier risk assessment

The relevant PEC<sub>soil</sub> for risk assessments are taken from Section 8.7. Note that the PEC<sub>soil</sub> values are calculated for the active substance and metabolites at a rate of 2 x 100 g a.s./ha and are therefore protective of the lower proposed GAP of 1 x 100 g a.s./ha. The PEC<sub>soil</sub> value for GF-3308 is calculated at the proposed GAP of 1 x 2 L/ha.

**Table 9.8-3: PEC<sub>soil</sub> for fenpicoxamid, relevant metabolites, and GF-3308 in cereals**

Product/metabolite/active substance	Initial PEC <sub>soil</sub> (mg/kg dw)
Fenpicoxamid	0.0533
X642188	0.0175
X11963422	0.0144
X12264475	0.0110
X12313581	0.0025
X696872	0.0066
X696476	0.0068 (initial); 0.144 (accumulation)
X12314005	0.0013
X763024	0.0012
X12019520	0.0016
X12255349	0.0031
GF-3308	0.5419

Note: X696476 has a PEC accumulation value of 0.144 mg/kg.

The results of the first-tier risk assessment for fenpicoxamid and GF-3308 are summarised in the following table.

**Table 9.8-4: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of GF-3308 in cereals**

Intended use	Cereals
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Chronic effects on earthworms			
Product/active substance	NOEC or NOEC <sub>corr</sub> <sup>†</sup> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Fenpicoxamid	9.925	0.0533	186
X642188	2.8	0.0175	160
X11963422	10	0.0144	694
X12264475	10	0.011	909
X12313581	0.99	0.0025	396
X696872	0.99	0.0066	150
X696476	10	0.144 0.0068	69.4 1471
X12314005	0.99	0.0013	762
X763024	0.99	0.0012	825
X12019520	0.99	0.0016	619
X12255349	5	0.0031	1613
GF-3308	486	0.5419	897
Chronic effects on other soil macro- and mesofauna: <i>Folsomia candida</i>			
Product/active substance	NOEC or NOEC <sub>corr</sub> <sup>†</sup> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Fenpicoxamid	3.97	0.0533	74
X642188	5	0.0175	286
X11963422	5	0.0144	347
X12264475	10	0.011	909
X12313581	0.397	0.0025	159
X696872	0.397	0.0066	60
X696476	10	0.144 0.0068	69.4 1471
X12314005	0.397	0.0013	305
X763024	0.397	0.0012	331
X12019520	0.397	0.0016	248
X12255349	5	0.0031	1613
GF-3308	25.7	0.5419	47
Chronic effects on other soil macro- and mesofauna: <i>Hypoaspis aculeifer</i>			
Product/active substance	NOEC or NOEC <sub>corr</sub> <sup>†</sup> (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Fenpicoxamid	19.85	0.0533	372
X642188	10	0.0175	571
X11963422	10	0.0144	694
X12264475	10	0.011	909
X12313581	1.985	0.0025	794

X696872	1.985	0.0066	301
X696476	10	<b>0.144</b> 0.0068	<b>69.4</b> 1471
X12314005	1.985	0.0013	1527
X763024	1.985	0.0012	1654
X12019520	1.985	0.0016	1241
X12255349	5	0.0031	1613
GF-3308	150	0.5419	277

TER values shown in **bold** fall below the relevant trigger. †Corrected values (i.e. divided by 2) are shown for endpoints where the LogK<sub>ow</sub> is >2.

**TER<sub>LT</sub> values for fenpicoxamid, relevant metabolites, and GF-3308 are above the Annex VI trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed GAP.**

~~According to the assessment of environmental fate data, multi-annual accumulation in soil does not need to be considered for fenpicoxamid.~~

~~X646476 has the potential to accumulate in soil, therefore an additional assessment using the PEC<sub>acc</sub> is shown below.~~

~~Table 9.8-5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of GF-3308 in cereals\_X696476 using PEC<sub>soil-acc</sub>~~

Intended use	Cereals		
Metabolite	X646476		
Species	NOEC <sub>corr</sub> (mg/kg dw)	PEC <sub>soil-acc</sub> (mg/kg dw)	TER <sub>lt</sub> (criterion TER ≥ 5)
<i>Eisenia fetida</i>	10	0.144	69
<i>Folsomia candida</i>	10		69
<i>Hypoaspis aculeifer</i>	10		69

~~TER values shown in **bold** fall below the relevant trigger.~~

~~TER<sub>LT</sub> values for X696476 are above the Annex VI trigger value of 5 indicating there is acceptable chronic risk to earthworms, meso-, and macrofauna.~~

#### **zRMS comments:**

The soil exposure provided in Table 9.8-3 is confirmed to be in line with PEC<sub>SOIL</sub> values agreed by the zRMS in area of Section 8.

The risk assessment presented in Table 9.8-4 is in general agreed by the zRMS. For metabolite X696476 the PEC<sub>SOIL,ACCU</sub> should have been used already at this first stage of the assessment and for this reason respective corrections were made in Table 9.8-4, while Table 9.7-5 with separate risk assessment based on accumulated PEC<sub>SOIL</sub> has been struck through.

Overall, acceptable risk to soil macro- and meso-fauna may be concluded from the intended Central Zone uses of GF-3308.

### 9.8.2.2 Higher-tier risk assessment

Not applicable.

### 9.8.3 Overall conclusions

TER<sub>LT</sub> values for fenpicoxamid, relevant metabolites, and GF-3308 are above the Annex VI trigger value of 5 indicating ~~there is~~ acceptable chronic risk to earthworms, meso-, and macrofauna at the proposed **Central Zone** GAP.

## 9.9 Effects on soil microbial activity (KCP 10.5)

### 9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with fenpicoxamid and relevant metabolites. Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

**Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms – fenpicoxamid and relevant metabolites**

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Fenpicoxamid	28 d, aerobic soil type	< 25% effect on nitrate formation rate soil dw at 2.187 mg/kg + 8.50 %	EFSA, 2018
<del>C-mineralisation</del>	<del>Fenpicoxamid</del>	<del>28 d, aerobic soil type</del>	<del>CO<sub>2</sub> formation 2.187 mg/kg soil dw - 4.39 %</del>	<del>Not required</del>
<b>Fenpicoxamid metabolites</b>				
N-mineralisation	X642188	28 d, aerobic soil type	< 25% effect on nitrate formation rate soil dw at 0.438 mg/kg + 2.6 %	EFSA, 2018
<del>C-mineralisation</del>	<del>X642188</del>	<del>28 d, aerobic soil type</del>	<del>CO<sub>2</sub> formation 0.438 mg/kg soil dw + 1.10 %</del>	<del>Not required</del>
N-mineralisation	X11963422	28 d, aerobic soil type	< 25% effect on nitrate formation rate soil dw at 0.600 mg/kg - 3.6 %	EFSA, 2018
N-mineralisation	X12264475	28 d, aerobic soil type	< 25% effect on nitrate formation rate soil dw at 0.733 mg/kg + 5.04 %	EFSA, 2018
N-mineralisation	X12313581		Not available*	EFSA, 2018
N-mineralisation	X696872		Not available*	EFSA, 2018
N-mineralisation	X696476		< 25% effect on	EFSA, 2018



		28 d, aerobic soil type	formation rate nitrate at 0.533 mg/kg soil dw +1.92%	
N-mineralisation	X12314005		Not available*	EFSA, 2018
N-mineralisation	X763024		Not available*	EFSA, 2018
N-mineralisation	X12019520		Not available*	EFSA, 2018
N-mineralisation	X12255349	28 d, aerobic soil type	< 25% effect on nitrate formation rate at 1.467 mg/kg : dw +8.91%	EFSA, 2018

\*data not available for these metabolites; however, low PEC<sub>soil</sub> and the parent data suggested low risk could be concluded

**Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms – GF-3308**

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	GF-3308	28 d, aerobic soil type	< 25% effect on nitrate formation rate at 13.5 mg/kg soil dw 2.62%	Hammesfahr/2016/DAS# 160194
C-mineralisation	GF-3308	28 d, aerobic soil type	CO <sub>2</sub> -formation 13.5 mg/kg soil dw 2.11 %	

**zRMS comments:**

The toxicity data presented in Table 9.9-1 are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146 with some minor corrections introduced by the zRMS.

The study performed with the formulated product was evaluated and agreed by the zRMS (for details, please refer to respective point in Appendix 2). The endpoint reported in Table 9.9-2 is confirmed to be correct.

Information regarding effects on carbon mineralisation is no longer a data requirement and for that reason is struck through in Tables 9.9-1 and 9.9-2.

### 9.9.1.1 Justification for new endpoints

Not applicable.

### 9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC<sub>soil</sub> for risk assessments covering the proposed use pattern are taken from Section 8.7 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna). Note that the PEC<sub>soil</sub> values are calculated for the active substance and metabolites at a rate of 2 x 100 g a.s./ha and are therefore protective of the lower proposed GAP of 1 x 100 g a.s./ha. The PEC<sub>soil</sub> value for GF-3308 is calculated at the proposed GAP of 1 x 2 L/ha. The results of the risk assessment for fenpicoxamid, relevant metabolites and GF-3308 are summarised in the following table.

**Table 9.9-3: Assessment of the risk for effects on soil micro-organisms due to the use of GF3308 in cereals**

Intended use	Cereals		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Fenpicoxamid	2.187 (at 14-28 d)	0.0533	yes
X642188	0.438 (at 14-28 d)	0.0175	yes
X11963422	0.600 (at 14-28 d)	0.0144	yes
X12264475	0.733 (at 14-28 d)	0.0110	yes
X696476	0.533 (at 14-28 d)	0.144 0.0068	yes
X12255349	1.467 (at 14-28 d)	0.0031	yes
GF-3308	13.5 (at 14-28 d)	0.5419	yes
X12313581	0.219 *	0.0025	yes
X696872	0.219 *	0.0066	yes
X12314005	0.219 *	0.0013	yes
X763024	0.219 *	0.0012	yes
X12019520	0.219 *	0.0016	yes
C-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Fenpicoxamid	2.187 (at 28 d)	0.0533	yes
X642188	0.438 (at 28 d)	0.0175	yes
GF-3308	13.5 (at 28 d)	0.5419	yes

\* In absence of respective toxicity data, 10 times toxicity of the parent was assumed

**Acceptable risk is demonstrated when GF-3308 is used according to the proposed GAP.**

#### **zRMS comments:**

The risk assessment presented in Table 9.9-3 is in general agreed by the zRMS. For metabolite X696476 the PEC<sub>SOIL,ACCU</sub> should have been used and for this reason respective corrections were made in Table 9.9-3.

In absence of the EU agreed toxicity data for metabolites X12313581, X696872, X12314005, X763024 and X12019520, the risk assessment was performed by the zRMS assuming 10 times toxicity of the parent as a worst case.

The risk assessment based on endpoints derived from studies on effects on carbon mineralisation were struck through as being no longer a data requirement.

Overall, no unacceptable effects on soil microbial activity are expected from the intended Central Zone uses of GF-3308.

### 9.9.3 Overall conclusions

The maximum concentrations with less than 25% effects for fenpicoxamid, relevant metabolites, and formulation are greater than their respective  $PEC_{soil}$ . There will be no adverse effects to soil microflora when used at the proposed GAP.

## 9.10 Effects on non-target terrestrial plants (KCP 10.6)

### 9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with fenpicoxamid (as GF2925). Full details of these studies are provided in the respective EU DAR (United Kingdom, 2017) and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target terrestrial plants of GF-3308 were not evaluated as part of the EU assessment of fenpicoxamid. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

**Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants**

Species	Substance	Exposure System	Results	Reference
<b>Seedling emergence</b>				
<del>Sunflower</del>	<del>GF-2925</del> (fenpicoxamid)	<del>21 d</del> <del>Seedling emergence</del>	<del><math>ER_{50} &gt; 2 \text{ L prep/ha}</math> (<math>\leftrightarrow</math>) <math>260 \text{ g a.s./ha}</math></del>	<del>EFSA, 2018</del>
<i>Lolium perenne</i> , <i>Avena sativa</i> , <i>Allium cepa</i> , <i>Glycine max</i> , <i>Brassica napus</i> , <i>Beta vulgaris</i> , <i>Daucus carota</i> , <i>Cucumis sativa</i> , <i>Helianthus annuus</i> , <i>Lycopersicon esculentum</i>	GF-3308	21 d Seedling emergence	$ER_{50} > 4 \text{ L prep/ha}$ $ER_{50} > 200 \text{ g a.s./ha}$  NOER for emergence and survival = 200 g a.s./ha	Stromel et al./2017/DAS# 160373
<b>Vegetative vigour</b>				
<del>Oat</del>	<del>GF-2925</del> (fenpicoxamid)	<del>21 d</del> <del>Vegetative vigour</del>	<del><math>ER_{50} &gt; 2 \text{ L prep/ha}</math> (<math>\leftrightarrow</math>) <math>260 \text{ g a.s./ha}</math></del>	<del>EFSA, 2018</del>
<i>Lolium perenne</i> , <i>Avena sativa</i> , <i>Allium cepa</i> , <i>Glycine max</i> , <i>Brassica napus</i> , <i>Beta vulgaris</i> , <i>Daucus carota</i> , <i>Cucumis sativa</i> , <i>Helianthus annuus</i> , <i>Lycopersicon esculentum</i>	GF-3308	21 d Vegetative vigour	$ER_{50} > 4 \text{ L prep/ha}$ $ER_{50} > 200 \text{ g a.s./ha}$  NOER for survival = 200 g a.s./ha	Stromel et al./2016/DAS# 160372

#### zRMS comments:

The toxicity data presented in Table 9.10-1 are in line with EU agreed endpoints reported in EFSA Journal 2018; 16(1):5146.

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). The endpoints reported in Table 9.10-1 are confirmed to be correct.

### 9.10.1.1 Justification for new endpoints

Not applicable.

### 9.10.2 Risk assessment

#### 9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

#### 9.10.2.2 Tier-2 risk assessment (based on dose-response data)

Dose-response tests at rates up to 4 L/ha were conducted with GF-3308 and effects were below the critical threshold as defined by the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). **The test rates exceed the highest field application rate in use group cereals and are thus considered an indicator for an acceptable risk.**

**Table 9.10-2: Assessment of the risk for non-target plants due to the use of GF-3308 in cereals**

<b>Intended use</b>	Cereals			
<b>Active substance/product</b>	GF-3308			
<b>Application rate</b>	1 × 2 L/ha (100 g fenpicoxamid/ha)			
<b>MAF</b>	1			
<b>Test species</b>	<b>ER<sub>50</sub></b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (L/ha)</b>	<b>TER criterion: TER ≥ 5</b>
<i>Lolium perenne</i> , <i>Avena sativa</i> , <i>Allium cepa</i> , <i>Glycine max</i> , <i>Brassica napus</i> , <i>Beta vulgaris</i> , <i>Daucus carota</i> , <i>Cucumis sativa</i> , <i>Helianthus annuus</i> , <i>Lycopersicon esculentum</i>	ER <sub>50</sub> >4 L prep/ha (200 g a.s./ha)	2.77% <del>2.38%</del>	0.0554 <del>0.0809</del> (VV) <del>0.0904</del> (SE)	>72.2 >49 ≥44

MAF: Multiple application factor; PER: Predicted environmental rate

PER<sub>off-field</sub> = Application rate x MAF x drift factor

TER: toxicity to exposure ratio. TER values shown in **bold** fall below the relevant trigger.

**Acceptable risk to terrestrial non-target plants is demonstrated for GF-3308 without the need of any specific risk mitigations.**

#### **zRMS comments:**

Although in the risk assessment presented in Table 9.10-2 the exaggerated PER<sub>off-field</sub> was considered by the Applicant, it is not clear for what application rate it was calculated and why different exposure was assumed for vegetative vigour and seedling emergence. Possibly, double application with respective foliar and soil MAF were considered, but this is not clear from the presented information. Taking this into account, the risk assessment in Table 9.10-2 corrected by the zRMS in order to comply with the GAP for GF-3308 in the Central Zone. Drift rate relevant for single application was used (2.77%). The same exposure is relevant for vegetative vigour and seedling emergence.

Based on the corrected calculations, acceptable risk to non-target terrestrial plants may be concluded from the intended Central Zone uses of GF-3308 with no need for risk mitigation measures.

#### **9.10.2.3 Higher-tier risk assessment**

Not relevant.

#### **9.10.2.4 Risk mitigation measures**

No risk mitigation needed.

#### **9.10.3 Overall conclusions**

It can be concluded that the risk to non-target plants from the application of GF-3308 in cereals according to good agricultural practice in the Central Zone is acceptable with no need for risk mitigation measures.

**9.11                      Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)**

No data available.

**9.12                      Monitoring data (KCP 10.8)**

No data available.

## 9.13 Classification and Labelling

The overall classification related to ecotoxicology is H410; see Part C for full explanation.

### **zRMS comments:**


Endpoints from studies on acute toxicity of GF-3308 to fish and *Daphnia magna* are <1.0 mg product/L and on this basis the formulation should be classified for aquatic hazard as Acute 1 with hazard statement H400.

No long-term studies were performed with the formulated product and no classification of fenpicoxamid is available in Regulation (EC) No 1272/2008. Nevertheless, a proposal is given in EFSA Journal 2018;16(1):5146 to classify fenpicoxamid as H410 with M factor of 100. The zRMS is of the opinion that indications from the available toxicity studies with fenpicoxamid should not be ignored as they indicate high long-term toxicity of fenpicoxamid to both, fish and aquatic invertebrates.

In absence of measured chronic formulation data, the summation method is considered by the zRMS with consideration of proposal given in the EFSA conclusion (2018).

Concentration of fenpicoxamid in GF-3308 of 4.92% (based on pure active ingredient) multiplied by M factor of 100 gives 492%, i.e. >25% which is the basis to classify the formulation for the long-term aquatic hazard as Chronic 1 with hazard statement H410.

Following classification and labelling are considered relevant for GF-3308:

<b>Hazard pictograms:</b>	GHS09 
<b>Signal word:</b>	Warning
<b>Hazard statement(s):</b>	H410 - Very toxic to aquatic life with long lasting effects
<b>Precautionary statement(s):</b>	P391: Collect spillage P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

The Applicant is kindly reminded that justification of CLP classification for aquatic hazard should be presented in the Core Assessment, Part B, Section 9 and not in Part C.

zRMS version

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## 9.14

## References



EFSA (European Food Safety Authority), 2015. Technical report: Outcome of the pesticide peer review meeting on general recurring issues in ecotoxicology (PPRM 133). EFSA Journal 2015: EN924.

EFSA, 2017. Peer review report on XDE-777 (NAS): Comments on the assessment report, reporting table, pesticide peer review meeting report. December 2017.

EFSA, 2018. Conclusion on the Peer Review of the Pesticide Risk Assessment of the Active Substance Fenpicoxamid (XDE-777). EFSA Journal 2018;16(1):5146.

EFSA, 2019. Outcome of the Pesticide Peer Review Meeting on General Recurring Issues in Ecotoxicology guidance EFSA Journal 2019: EN-1673.

United Kingdom, 2017. Draft Assessment Report (DAR) on the active substance fenpicoxamid prepared by the rapporteur Member State UK in the framework of Regulation EC/1107/2009, December 2017

## Appendix 1 Lists of data considered in support of the evaluation

### List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1.1/1	xxx.	2016	GF-3308: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure DAS# 160146 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.1/1	Bergfield, A.	2016	GF-3308: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata DAS# 160103 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/2	xxx	2016a	GF-3308: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-Through Test Conditions DAS# 160101 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.1/3	Goudie, O.	2016b	GF-3308: Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Static Renewal Test Conditions DAS# 160102 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/4	Goudie, O.J.	2018	X1642188 (a metabolite of XDE-777): Acute Toxicity Test to Cladoceran, Daphnia magna, Determined Under Flow-Through Test Conditions DAS# 180562 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 10.2.1/5	Goudie, O.J	2020	GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (Daphnia magna) DAS Report No. 191366 Eurofins EAG Agrosience, LLC, Easton, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/6	Goudie, O.J.	2021	GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran (Daphnia magna) DAS Report No. 202284 Eurofins EAG Agrosience, LLC, Easton, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/8	xxx	2018a	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS# 180560 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.1/9	xxx	2018b	X12446477 (metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS# 180561 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.2/1	Beasley, J.	2018	X1642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment DAS# 180563 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.2/3	Leak, T.	2018	X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment DAS# 180564 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.3/1	Blickley, T.M., Kramer, V.J.	2018	X12433979 (a metabolite of XDE-777): Prediction of Octanol-Water Partition Coefficient and Aquatic Toxicity using Computerized Quantitative Structure-Activity Relationships DAS# 180910 Dow AgroSciences, LLC, Zionsville, Indiana, USA GLP/GEP (Y/N): No Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.3/2	Hicks, S.	2016	GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS# 160126 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.3/3	Hicks, S.	2017	XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS# 160125 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.3/4	Mathieson, T.	2018	Efficacy of XDE-777 metabolites to Septoria tritici on wheat DAS# NA Dow AgroSciences, LLC, Zionsville, Indiana, USA GLP/GEP (Y/N): No Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.3/5	Yao, C.	2014	<i>Septoria tritici</i> Biological Screening Report for Five Metabolites of XDE-777 DAS# DAI 1370 Dow AgroSciences, LLC, Zionsville, Indiana, USA GLP/GEP (Y/N): No Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.1.1/1	Schmitzer, S.	2016	GF-3308: Acute contact and oral effects on honeybees ( <i>Apis mellifera</i> L.) in the laboratory DAS# 160184 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.2/1	Verge, E.	2020	GF-3308 - Honey Bee ( <i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure) DAS# 190305 Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.1.2/2	Verge, E.	2017	GF-3308 - Assessment of Effects on the Adult Honey Bee, <i>Apis mellifera</i> L., in a 10 Day Chronic Feeding Test under Laboratory Conditions DAS# 160522 Eurofins Agrosience Services EcoChem / Eurofins Agrosience Services Ecotox GmbH GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.1.5/1	Kleinhenz, M.	2017	GF-3308 (XDE-777): Brood Development of the Honey Bee ( <i>Apis mellifera</i> L.) in a Semi-Field Tunnel Study in <i>Phacelia tanacetifolia</i> in Germany 2016 DAS# 160515 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	N	DAS/Corteva Agriscience
KCP 10.3.2.1/1	Moll, M.	2016a	GF-3308: Effects on the Parasitoid <i>Aphidius rhopalosiphii</i> in the Laboratory (Tier I) - Dose Response Test DAS# 160185 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.2.1/2	Moll, M.	2016b	GF-3308: Effects on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory (Tier I) - Dose Response Test DAS# 160188 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.2.1/3	Vaughan, R.	2016	GF-3308: A laboratory test to evaluate the effects of fresh residues on the green lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae) DAS# 160216 Mambo-Tox Ltd. Southampton SO16 7NP, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2/1	Moll, M.	2016c	GF-3308: Effects on the Parasitoid <i>Aphidius rhopalosiphii</i> , Extended Laboratory Study (Tier II) - Dose Response Test DAS# 160186 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.2.2/2	Moll, M.	2016d	GF-3308: Effects on the Predatory Mite <i>Typhlodromus pyri</i> , Extended Laboratory Study (Tier II) - Dose Response Test DAS# 160189 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.2.2/3	Schmidt, T.	2016a	GF-3308: Effects on mortality and reproduction to <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae) under extended Laboratory Conditions DAS# 160162 Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.2.2/4	Schmidt, T.	2016b	GF-3308: Toxicity to the Parasitoid Rove Beetle <i>Aleochara bilineata</i> (Coleoptera: Staphylinidae) under Extended Laboratory DAS# 160161 Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.2.2/5	Tew, G.	2020	GF-3308: A Rate-Response Extended Laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, <i>Aleochara bilineata</i> (Coleoptera, Staphylinidae) DAS#200611 Mambo Tox, A Division of Cawood Scientific Ltd., Southampton, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

KCP 10.3.2.3/1	Moll, M.	2016e	GF-3308: Effects on the Parasitoid <i>Aphidius rhopalosiphii</i> , Extended Laboratory Study (Tier II) - Aged Residue Test DAS# 160187 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.3/2	Vaughan, R.	2017	GF-3308: Aged-residue extended laboratory tests to determine effects on the ladybird beetle, <i>Coccinella septempunctata</i> (Coleoptera, Coccinellidae) DAS# 170779 Mambo-Tox Ltd.Southampton SO16 7NP, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.4.1.1/1	Ganßmann, M.	2016a	GF-3308: Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 10% peat DAS# 160193 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.4.1.2/1	Ganßmann, M.	2016b	GF-3308: Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat DAS# 160191 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.4.1.2/2	Ganßmann, M.	2016c	GF-3308: Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat DAS# 160192 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.5/1	Hammesfahr, U.	2016	GF-3308: Effects on the Activity of the Soil Microflora in the Laboratory DAS# 160194 Institut für Biologische Analytik und Consulting IBACON GmbH, 64380 Rossdorf, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

KCP 10.6.2/1	Stromel, Friedemann	2016a	GF-3308 (DE-777 50 g a.s/L, EC): A Vegetative Vigour Test with ten Non Target Plant Species, GLP Terrestrial Non Target Plants (based on OECD Guideline 227) – Europe 2016 DAS# 160372 agro-check Dr. Teresiak & Erdmann GbR, Dorfstr.15D-16833 Lentzke, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 10.6.2/2	Stromel, Friedemann	2016b	GF-3308 (DE-777 50 g a.s/L, EC): A Seedling Emergence and Seedling Growth Test with ten Non Target Plant Species, GLP Terrestrial Non Target Plants (based on OECD Guideline 208) – Europe 2016 DAS# 160373 agro-check Dr. Teresiak & Erdmann GbR, Dorfstr.15D-16833 Lentzke, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

**List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review**

**zRMS comments:**

Please note that majority of toxicity data for fenpicoxamid were taken from EFSA Journal 2018;16(1):5146 and were thus evaluated at the EU level. Since the full list of respective studies performed with the active compound and evaluated at the EU level may be found in Vol. 2 of the monograph, the list below was not validated by the zRMS.

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CA 8.1.1.1 /1	xxx	2012	XR-777: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential testing Procedure xxx DAS Report No.: 110247 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience



CA 8.1.1.3 /1	xxx	2013	XDE-777 TGAI: A Reproduction Study with the Northern Bobwhite xxx DAS Report No.: 120384 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.1.1.3/2	xxx	2015	XDE-777: Reproductive Toxicity Test with the Northern Bobwhite ( <i>Colinus virginianus</i> ) xxx Smithers Viscient DAS Report No.: 140424 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.1.1.3/3	Valverde P	2016	XDE-777: Comparative analysis of historical control data in the reproductive toxicity tests with the northern bobwhite ( <i>Colinus virginianus</i> ). Lab: Wildlife International; Dow AgroSciences; DAS Study No. 120384A GLP/GEP (Y/N): N Published (Y/N): N	N	DAS/Corteva Agriscience
CA 8.1.1.3/4	Valverde P	2016	XDE-777: Comparative analysis of historical control data in the reproductive toxicity tests with the northern bobwhite ( <i>Colinus virginianus</i> ). Lab: Smithers Viscient; Dow AgroSciences; DAS Study No. 140424B GLP/GEP (Y/N): N Published (Y/N): N	N	DAS/Corteva Agriscience
CA 8.2.1 /1	xxx	2012	XR-777 - Acute Toxicity to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) Under Flow-Through Conditions, Following xxx Smithers Viscient DAS Report No.: 110213 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

CA 8.2.1 /2	xxx	2012	XDE-777 Technical: Acute Toxicity to the Common Carp, <i>Cyprinus carpio</i> , Determined Under Flow-Through Test Conditions xxx DAS Report No.: 120392 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1 /3	xxx	2012	X642188 Metabolite: Acute Toxicity Test with the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Flow-Through Test Conditions xxx DAS Report No.: 120382 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1/4	xxx	2014	X11963422 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions (Revision) xxx DAS Report No.: 130361 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.2.1/5	xxx	2014	X12264475 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions xxx DAS Report No.: 130360 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1/6	xxx	2014	X12313581 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions xxx DAS Report No.: 130362 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

CA 8.2.1/7	xxx	2014	X696872 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions xxx DAS Report No.: 130363 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1/8	xxx	2014	X696476 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions xxx DAS Report No.: 130364 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1/9	xxx	2014	X12314005 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions xxx DAS Report No.: 130365 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1/ 10	xxx	2015	X12255349 (a metabolite of XDE-777): Acute toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions DAS Report No.: 141000 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.2.1	xxx	2016	XDE-777: Acute Toxicity to the Zebra Fish, <i>Danio rerio</i> , Determined Under Flow-Through Test Conditions DAS Report No. 160129 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

CA 8.2.1	xxx	2016	XDE-777: Acute Toxicity to the Fathead minnow, Pimephales promelas, Determined Under Flow-Through Test Conditions DAS Report No. 160130 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.1	xxx	2016	XDE-777: Acute Toxicity to the Bluegill, Lepomis macrochirus, Determined Under Flow-Through Test Conditions DAS Report No. 161022 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.2.1 /1	xxx	2012	XR-777 TGAI – Early Life-Stage Toxicity Test with Fathead Minnow, Pimephales promelas, Following OECD Guideline #210 Smithers Viscient DAS Report No.: 110214 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.2.1/2	xxx	2016	XDE-777: Investigation of Larval Toxicity to the Fathead Minnow (Pimephales promelas) Under Static Conditions in a Water-Sediment System DAS Report No. 160128 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.2.3/1	xxx	2014	XDE-777: Investigation of bioconcentration in zebrafish ( <i>Danio rerio</i> ) under flow-through conditions xxx DAS Report No.: 130983 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
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CA 8.2.2.3/2	xxx	2015	14C-X696476: Bioconcentration and Metabolism Study with Zebrafish, <i>Danio rerio</i> xxx DAS Report No.: 140481 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.2.3/3	xxx	2014	14C-X12019520: Bioconcentration and Metabolism Study with Zebrafish, <i>Danio rerio</i> xxx DAS Report No.: 140480 GLP/GEP (Y/N): Yes Published (Y/N): No	Yes	DAS/Corteva Agriscience
CA 8.2.4.1 /1	Fournier A	2012	XR-777 TGAI - Acute Toxicity to Water Fleas ( <i>Daphnia magna</i> ) Under Static-Renewal Conditions, Following OECD Guideline #202 and JMAFF 12 NohSan, No. 8147 <i>Daphnia</i> Acute Immobilization Test (2-7-2-1) Data Requirement OECD Guideline 202 JMAFF 12 NohSan, No. 8147 (Revision) Smithers Viscient DAS Report No.: 110215 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1 /2	Holou M	2013	X642188 Metabolite: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 120381 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/03	Romine J	2014	X11963422 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: <del>130386</del> 130372 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/04	Huffman	2014	X12264475 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130371 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CA 8.2.4.1/05	Romine J	2014	X12313581 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130373 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/06	Stadler T	2014	X696872 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under StaticRenewal Test Conditions ABC Laboratories DAS Report No.: 130374 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/07	Stadler T	2014	X696476 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under StaticRenewal Test Conditions ABC Laboratories DAS Report No.: 130375 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/08	Dinehart S	2014	X12314005 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determine Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130376 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/09	Stadler T	2014	X12386481 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130379 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

CA 8.2.4.1/10	Romine J	2014	X763024 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under StaticRenewal Test Conditions ABC Laboratories DAS Report No.: 130378 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
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<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CA 8.2.4.1/11	Romine J	2014	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130380 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/12	Dinehart S	2014	X12335723 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130377 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/13	Romine J	2014	X12393285 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130383 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/14	Lamichhane K	2014	X12255349 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Exposed Under StaticRenewal Test Conditions ABC Laboratories DAS Report No.: 140484 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

CA 8.2.4.1/15	Lamichhane K	2014	X12446477 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Exposed Under StaticRenewal Test Conditions ABC Laboratories DAS Report No.: 140485 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.1/16	Romine J	2014	X12442397 (sodium salt of X12399889, a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 130382 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CA 8.2.4.1/17	Dinehart S	2015	X12442403 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 140486 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.2/1	Lamichhane K	2014	XDE-777 TGAI: Acute Toxicity to the Cladoceran, <i>Daphnia pulex</i> , Exposed Under Static-Renewal Test Conditions ABC Laboratories DAS Report No.: 140483 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.2/2	VanHooser, A.	2015a	XDE-777: Acute toxicity to the Freshwater Midge, <i>Chironomus riparius</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 141002 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience



CA 8.2.4.2/3	VanHooser, A.	2015b	X642188 (a metabolite of XDE-777): Acute toxicity to the Freshwater Midge, Chironomus riparius, Determined Under Static-Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 141003 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.4.2/4	Hadsell, R.	2015	X12255349 (a metabolite of XDE-777): Acute toxicity to the Freshwater Midge, Chironomus riparius, Determined Under Static-Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 141004 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.5/2	Lamichhane, K.	2015	X12255349 (a metabolite of XDE-777): Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS Report No. 140999 ABC Laboratories GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.2.5.1 /1	Fournier A	2012	XR-777 TGAI: Full Life-Cycle Toxicity Test with Water Fleas, Daphnia magna, Under Static Renewal Conditions Following OECD Guideline #211 Smithers Viscient DAS Report No.: 110216 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.6.1 /1	Rebstock M	2013	XDE-777: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata ABC Laboratories, Inc DAS Report No.: 120383 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

CA 8.2.6.1 /2	Rebstock M	2013	X642188 metabolite: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc DAS Report No.: 120380 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.6.1 /3	Bergfield A	2014	X11963422 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc DAS Report No.: 130385 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.6.1 /4	Aufderheide, J.	2014	X12264475 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc DAS Report No.: 130384 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.2.6.1 /5	Aufderheide, J.	2015	X12255349 (a metabolite of XDE-777): Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc DAS Report No.: 141001 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.3.1.1.1/1 CA 8.3.1.1.2/1	Schmitzer S	2012	Effects of XR-777 (Acute Contact and Oral) on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110168/110169 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

CA 8.3.1.1.1/2 CA 8.3.1.1.2/2	Schmitzer S	2014	XDE-777: Acute Contact and Oral Effects on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140217/140221 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.3.1.1.1/3	Schmitzer S	2012	Effects of X642188 (metabolite of XR-777) (Acute Oral Test) on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120379 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.3.1.1.1/4	Schmitzer S	2014	X696476 (a metabolite of XDE-777): Acute Oral Effects on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140215 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.3.1.1.1/5	Schmitzer S	2014	X12019520 (a metabolite of XDE-777): Acute Oral Effects on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140216 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.3.2.1 /1	Moll M	2013	Effects of XDE-777 on the Parasitoid <i>Aphidius rhopalosiphi</i> in the Laboratory (Tier I) - Dose Response Test - (Revision) Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110170 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.3.2.2 /1	Schwarz A	2013	Effects of XDE-777 on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory (Tier I) - Dose Response Test – (Revision) Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110171 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

CA 8.4.1 /1	Ganßmann M	2012	Effects of XDE-777 TGAI on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110172 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.1 /2	Ganßmann M	2012	Effects of X642188 (metabolite of XDE-777) on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120378 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.1 /3	Ganßmann M	2013	X11963422 (a metabolite of XDE-777): Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 10% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130204 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.1 /4	Ganßmann M	2013	X12264475 (a metabolite of XDE-777): Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 10% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130203 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.1 /5	Ganßmann M	2014	X696476 (a metabolite of XDE-777) Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 10% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140235 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
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CA 8.4.1 /6	Witte, B	2015	X12255349 (a metabolite of XDE-777) Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 141006 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /1	Ganßmann M	2012	Effects of XDE-777 TGAI on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120385 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /2	Ganßmann M	2012	Effects of XDE-777 TGAI on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120386 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /3	Ganßmann M	2012	Effects of X642188 (metabolite of XDE-777) on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120387 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /4	Ganßmann M	2012	Effects of X642188 (metabolite of XDE-777) on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120388 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /5	Ganßmann M	2013	X11963422 (a metabolite of XDE-777): Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130208 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.4.2.1 /6	Ganßmann M	2013	X11963422 (a metabolite of XDE-777): Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130210 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /7	Ganßmann M	2013	X12264475 (a metabolite of XDE-777): Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130207 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /8	Ganßmann M	2013	X12264475 (a metabolite of XDE-777) on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130209 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /9	Ganßmann M	2014	X696476 (a metabolite of XDE-777): Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140229 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.4.2.1 /10	Ganßmann M	2014	X696476 (a metabolite of XDE-777): Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140232 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience

CA 8.4.2.1 /11	Witte, B	2015 a	X12255349 (a metabolite of XDE-777): Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 141007 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.4.2.1 /12	Witte, B	2015 b	X12255349 (a metabolite of XDE-777): Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> <i>Folsomia candida</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 141008 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CA 8.5 /1	Hammesfahr U	2012	Effects of XDE-777 on the Activity of the Soil Microflora in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 110173 GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	DAS/Corteva Agriscience
CA 8.5 /2	Hammesfahr U	2012	Effects of X642188 (metabolite of XDE-777) on the Activity of the Soil Microflora in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 120377 GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	DAS/Corteva Agriscience
CA 8.5 /3	Hammesfahr U	2013	X11963422 (a metabolite of XDE-777): Effects on the Activity of the Soil Microflora in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130206 GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	DAS/Corteva Agriscience
CA 8.5 /4	Hammesfahr U	2013	X12264475 (a metabolite of XDE-777): Effects on the Activity of the Soil Microflora in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 130205 GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	DAS/Corteva Agriscience

CA 8.5 /5	Hammesfahr U	2014 a	X696476 (a metabolite of XDE-777): Effects on the Activity of the Soil Microflora in the Laboratory Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 140238 GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	DAS/Corteva Agriscience
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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CA 8.5 /6	Hammesfahr U	2014 b	X12255349 (a metabolite of XDE-777): Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation) Institut für Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 141009 GLP/GEP (Y/N): Yes Published (Y/N): Yes	No	DAS/Corteva Agriscience
CA 8.8 /1	Griffith A	2012	XR-777 TGAI - Activated Sludge Respiration Inhibition Test Following OECD Guideline 209 Smithers Viscient DAS Report No.: 110217 GLP/GEP (Y/N): Yes Published (Y/N): No	No	DAS/Corteva Agriscience
CP 10.1.1.1/1	xxx	2012	GF-2925: An acute oral toxicity study with the Northern Bobwhite using a sequential testing procedure xxx DAS Report No.: 120389 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
CP 10.2.1/1	xxx	2013	GF-2925: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions (Revision) xxx. DAS Report No.: 120374 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience



CP 10.2.1/2	Stadler T Lamichhane K	2014	GF-2925: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions (Revision) ABC Laboratories DAS Report No.: 120375 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.2.1/3	Holou M	2013	GF-2925: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. DAS Report No.: 120376 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CP 10.2.3/01	xxx	2014	GF-2925 (126 g/L): GF-2925 (126 g/L XDE-777): Investigation of larvae toxicity of fathead minnow ( <i>Pimephales promelas</i> ) under static conditions in a water sediment system xxx DAS Report No.: 130367 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
CP 10.2.3/02	xxx	2014	GF-2925 (132 g/L): GF-2925 (132 g/L XDE-777): Investigation of larvae toxicity of rainbow trout ( <i>Oncorhynchus mykiss</i> ) under static conditions in a water sediment system xxx DAS Report No.: 130368 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
CP 10.2.3/03	xxx	2014	GF-2925 (126 g/L XDE-777): Full Life Cycle test with the Zebrafish ( <i>Danio rerio</i> ) under static conditions in a water sediment system xxx DAS Report No.: 121049 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience

CP 10.2.3/04	xxx	2014	XDE-777: Community level study in outdoor aquatic mesocosms xxx DAS Report No.: 130984 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
CP 10.2.3/05	Kramer V	2014	Prediction of Octanol-Water Partition Coefficient, Acid Dissociation Constant, Fish Bioconcentration and Aquatic Toxicity of Metabolites of XDE-777 using Computerized Quantitative Structure-Activity Relationships Dow AgroSciences LLC DAS Report No.: 141106 GLP/GEP (Y/N): N Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.2.3/06	Mueller, J.	2015	XDE-777 metabolites: Analysis in aqueous and sediment samples of the outdoor mesocosm study Fraunhofer Institute DAS Report No.: 140860 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CP 10.2.3/07	xxx	2016	GF-2925 (126 g/L XDE-777): Investigation of larvae toxicity of fathead minnow ( <i>Pimephales promelas</i> ) under static conditions in a water sediment system xxx DAS Report No.: 130367, 1st study report ammendment GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
CP 10.2.3/08	xxx	2014	GF-2925 (132 g/L XDE-777): Investigation of larvae toxicity of rainbow trout ( <i>Oncorhynchus mykiss</i> ) under static conditions in a water sediment system xxx DAS Report No.: 130368, 1st study report ammendment GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience

CP 10.2.3/09	xxx	2014	GF-2925 (126 g/L XDE-777): Full Life Cycle test with the Zebrafish ( <i>Danio rerio</i> ) under static conditions in a water sediment system xxx DAS Report No.: 121049, 1st study report ammendment GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
CP 10.2.3/10	Kramer V, LopezMancisidor P	2016	Additional Summary Information on the Scientific Reliability of the XDE-777 Mesocosm Study Supporting the Assignment of an Assessment Factor of 2 for Derivation of the ETO-RAC for Aquatic Invertebrate and Plant Communities Dow AgroSciences No study number GLP/GEP (Y/N): N Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.3.1.1.1/1 CP 10.3.1.1.2/1	Schmitzer S	2012	Effects of GF-2925 (Acute Contact and Oral) on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory IBACON GmbH DAS Report No.: 120370, 120371 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.3.1.1.1/2 CP 10.3.1.1.2/2	Schmitzer S	2014	GF-2925: Acute Contact and Oral Effects on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory DAS Report No.: 140218, 140222 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
CP 10.3.2.1/1	Schwarz A	2012	Effects of GF-2925 on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory (Tier I) - Dose Response Test IBACON GmbH DAS Report No.: 110174 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.3.2.1/2	Moll M	2012	Effects of GF-2925 on the Parasitoid <i>Aphidius rhopalosiphi</i> in the Laboratory (Tier I) - Dose Response Test IBACON GmbH DAS Report No.: 110175 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience

CP 10.4.1.1/1	Ganßmann M	2012a	Effects of GF-2925 on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 5% Peat IBACON GmbH DAS Report No.: 120373 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.4.2.1/1	Ganßmann M	2012b	Effects of GF-2925 on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat IBACON GmbH DAS Report No.: 120390 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.4.2.1/2	Ganßmann M	2012c	Effects of GF-2925 on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat IBACON GmbH DAS Report No.: 120391 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.5/1	Hammesfahr U	2012	Effects of GF-2925 on the Activity of the Soil Microflora in the Laboratory IBACON GmbH DAS Report No.: 120372 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
CP 10.6.2/1	Friedemann Teresiak H	A 2012a	Evaluation of the Phytotoxicity of GF-2925 (XDE-777 130 g as/L, SC), GLP Vegetative Vigour Test agro- check DAS Report No.: 110093 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
CP 10.6.2/2	Friedemann Teresiak H	A 2012b	Evaluation of the Phytotoxicity of GF-2925 (XDE-777 130 g as/L SC), GLP Seedling Emergence and Seedling Growth Test agro- check DAS Report No.: 110094 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience

**List of data submitted by the applicant and not relied on**

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
KCP 10.2.1/7	Hadsell, R. L., Hoover, E.	2014, revised 2018	GF-3307: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions DAS Report No.140489 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.2/2	Dinehart, S.	2019	X642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with <i>Lumbriculus variegatus</i> Using Spiked Sediment DAS Study No. 180639 Eurofins EAG Agriscience, LLC, Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience

zRMS version

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**List of data relied on not submitted by the applicant but necessary for evaluation**

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
There were no data not submitted by the Applicant and relied on.					

zRMS version

## Appendix 2 Detailed evaluation of the new studies

### zRMS comments:

In case considered necessary, additional information has been added by the zRMS in the summaries of the studies for completeness

### A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

#### A 2.1.1 KCP 10.1.1 Effects on birds A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity A 2.1.1.1.1 GF-3308: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure

Comments of zRMS:	<p>The study was conducted in line with OECD 223 with no deviations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LD<sub>50</sub> &gt; 2000 mg product/kg b.w.</p>
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Reference:	KCP 10.1.1.1/1
Report:	xxx.; 2016; GF-3308: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure; xxx; Lab Study No. 379B-409; 160146; 20 April 2016; Unpublished
Guideline(s):	OECD Guideline 223
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

## MATERIALS AND METHODS

### Test System

Organism ( <i>Species</i> ):	Northern Bobwhite ( <i>Colinus virginianus</i> )
Study type:	Acute oral
Study duration:	14 days
Parameters measured:	Body weight, feed consumption
Observation intervals:	Multiple observations on Day 0 and twice daily observations on remaining days.
Age range of birds at test initiation:	38 weeks
Weight range of birds at study initiation:	185 to 248 grams
Test concentrations:	0 and 2000 mg/kg
No. of feed withholding days before dosing:	Birds were fasted for approximately 17.5 hours prior to dosing
Method of test item administration:	gavage

Diet:	Wildlife International basal ration
Number of birds per dose group:	5
Number of birds per control group:	5
Housing:	GQF Manufacturing Co. Model No. 0330
Environmental conditions:	Temperature: average 22.0 ± 0.5 °C (SD), maximum 23°C, minimum 21.4°C
	Photoperiod: 8 hrs of light per day/16 hours dark
	Humidity: average 39 ± 10% (SD), maximum 53%, minimum 23%

## Methodology

This test was conducted according to the sequential design OECD Test Guideline 223. The limit dosage of 2000 mg formulation/kg of body weight was tested with five birds. Birds were acclimated to the facility for 22 weeks and to the caging for five weeks prior to test initiation. The birds were fasted for approximately 17.5 hours prior to dosing. At experimental start, a single dose of the test substance was orally administered by gavage directly into the crop or proventriculus of each bird. Each bird was individually weighed and dosed on the basis of milligrams of GF-3308 per kilogram of body weight (mg/kg). The control birds received a corresponding volume of water that was filtered by reverse osmosis and deionized. All test and control birds received a volume of 4 mL/kg of body weight.

From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity, and abnormal behavior. Body weights were measured individually on the day of dosing (Day 0) and on Days 3, 7 and 14 of the test. Feed consumption was determined by pen for approximately 24-hour intervals from Day 0 to 1, Day 1 to 2, and Day 2 to 3. Average daily feed consumption was then determined from Days 3 to 7 and from Days 7 to 14.

## RESULTS AND DISCUSSION

The acute oral LD<sub>50</sub> value for northern bobwhite exposed to GF-3308 as a single oral dose was determined to be greater than 2000 mg/kg. The no-mortality level was 2000 mg/kg.

**Table 1: Effect of GF-3308 on mortality of Northern Bobwhite**

Treatment (mg/kg bw)	No. of birds	Cumulative mortality		
		At day 7	At day 14	Total (%)
Negative control	5	0	0	0
2000	5	0	0	0
LD <sub>50</sub>	> 2000 mg/kg			
95% C.I.	> 2000 mg/kg			
NOEL	Not Determined			

**Table 2: Effect of GF-3308 on body weight and feed consumption of Northern Bobwhite**

Treatment (mg/kg bw)	Observation								
	Mean body weight (g)				Feed consumption (g/bird/day)				
Day(s)	0	3	7	14	0-1	1-2	2-3	3-7	7-14
0	210	213	213	212	19	14	14	15	12
2000	206	199	203	208	8	10	11	14	14



## CONCLUSION

The acute oral LD<sub>50</sub> value for northern bobwhite exposed to GF-3308 as a single oral dose was determined to be greater than 2000 mg/kg. The no-mortality level was 2000 mg/kg.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Bobwhite quail	<i>Colinus virginianus</i>	GF-3308	14 day	LD <sub>50</sub>	>2000	mg/kg

**A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds A 2.1.2 KCP 10.1.2  
Effects on terrestrial vertebrates other than birds**

zRMS version \_\_\_\_\_

**A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals A 2.1.2.2 KCP 10.1.2.2  
Higher tier data on mammals A 2.1.3 KCP 10.1.3 Effects on other  
terrestrial vertebrate wildlife (reptiles and amphibians)**

## A 2.2 KCP 10.2 Effects on aquatic organisms

### A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes A 2.2.1.1 Study 1 - GF-3308: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Comments of zRMS:	<p>The study was conducted in line with OECD 201 with no deviations.</p> <p>The mean measured concentrations of the active substance were not maintained within 80-120% of nominal; therefore, the endpoints are expressed based on geometric mean concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p><math>E_rC_{50} &gt; 30</math> mg product/L (based on geometric mean concentration), corresponding to 1.48 mg a.s./L  <math>E_rC_{20} = 28</math> mg product/L (based on geometric mean concentration)  <math>E_rC_{10} = 18</math> mg product/L (based on geometric mean concentration)  <math>NOE_rC = 2.4</math> mg product/L (based on geometric mean concentration)</p> <p><math>E_yC_{50} = 21</math> mg product/L (based on geometric mean concentration), corresponding to 1.48 mg a.s./L  <math>E_yC_{20} = 12</math> mg product/L (based on geometric mean concentration)  <math>E_yC_{10} = 9.3</math> mg product/L (based on geometric mean concentration)  <math>NOE_yC = 2.4</math> mg product/L (based on geometric mean concentration)</p>
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Reference:	KCP 10.2.1/1
Report:	Bergfield, A.; 2016; GF-3308: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ; ABC Laboratories, Inc., Columbia, Missouri, USA; Lab Study No. 83496; DAS Study No. 160103 ; 09 June 2016; Unpublished
Guideline(s):	OECD Guideline 201
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: 4.9 wt% XDE-777  
Description (physical state): brown liquid with a fragrant odor  
Lot/batch no.: ENBK-148585-032A (TSN309730)

### Test system

Organism (*Species*): Green alga (*Pseudokirchneriella subcapitata*)  
Study type: Laboratory study assessing algal growth  
Study design: Static

Test concentrations:	Nominal: 0 (control), 2.5, 5.0, 10, 20, and 40 mg GF-3308/L 0 (control), 0.12, 0.25, 0.49, 0.98, and 2.0 mg a.i. (XDE-777)/L 0- and 72-hour geometric mean calculated: <MQL, 0.25, 0.85, 2.4, 12, and 30 mg GF-3308/L <MQL, 0.0123, 0.414, 0.115, 0.574, and 1.48 mg a.i./L
Duration:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield
Environmental conditions:	Test solution pH (range): 7.4 to 7.9 Test solution temperature (range): 22.9 to 24.8°C Temperature (range): 24 ± 2 °C Photoperiod: continuous light Light intensity (range): 7,406 to 7,675 lux
Observation intervals:	0, 24, 48, 72 hours
Age of inoculum:	two days old
Acclimation period/conditions:	All cultures were maintained under the same conditions as those used for testing.
Initial cell density:	$5.1 \times 10^3$ cells/mL
Growth medium:	Name: freshwater algal nutrient medium (FWAM) pH at test initiation: 7.4 to 7.5 pH at test termination: 7.4 to 7.9 Constant stirring <sup>2</sup> : Swirled on an orbital shaker table at 100 rpm
Method of test item added to the test medium:	A 0.040 mg GF-3308/mL primary standard was prepared on 18 February 2016 by transferring 0.0400 g of GF-3308 (total product) to a 1,000-mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was inverted to mix and sonicated for approximately 10 minutes following preparation. Appropriate aliquots of the primary standard solution were diluted with test medium to a volume of 1.0 or 0.50 L to prepare the test substance treatments at concentrations of 2.5, 5.0, 10, and 20 mg GF3308/L. The primary standard was used for the 40 mg GF-3308/L test substance treatment solution. The control consisted of test medium only.
No. of control replicates:	6
No. of test concentration replicates:	3
Analytical verification:	Method: measuring concentrations of XDE-777, active ingredient in GF-3308 using HPLC-MS/MS Samples taken : 0, 24, and 72 hrs Limit of Detection: Not Provided Limit of Quantitation: At 0 hour, MQL = 20.0 ng XDE-777/mL = 0.41 mg GF-3308/L At 24 hours, MQL = 1.00 ng XDE-777/mL = 0.020 mg GF-3308/L At 72 hours, MQL = 0.0200 ng XDE-777/mL = 0.00041 mg GF3308/L Recoveries from QC fortifications: 100-111%
Reference substance:	XDE-777

## Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labelled with study number, treatment, replicate, and grid position. The control was replicated six times (i.e., replicates A, B, C, D, E, and F) and each test substance treatment was replicated three times (replicates A, B, and

C). Each replicate contained 100 mL of the appropriate parent solution. An additional replicate (“83496, Level 1, Abiotic”) of the 2.5 mg GF-3308/L test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass. Additional replicates (“83496, Level X, Analytical”) of all control and test substance treatment levels, containing 100 mL of the appropriate parent solutions, were also prepared to be used for 24-hour solution analysis. At test initiation, all replicates of the control and the A, B, and C replicates and the analytical replicate of each test substance treatment were inoculated with 1.0 mL of an algal concentrate containing approximately  $5.1 \times 10^5$  cells/mL, resulting in a final density of approximately  $5.1 \times 10^3$  cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, and 72 hours ( $\pm 1$  hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer.

## RESULTS AND DISCUSSION

The 72 hour parameters were reported based upon the **geometric mean calculated** GF-3308 concentrations (from 0- and 72-hour analyses) and geometric mean measured XDE-777 concentrations (from 0- and 72-hour analyses).

**Table 3.** Calculated Concentrations of GF-3308, Based on Analysis of XDE-777, During a 72-Hour Growth Inhibition Toxicity Test with Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Nominal Concentration (mg GF-3308/L)	Calculated Concentration as mg GF-3308/L (Percent Nominal) <sup>a</sup>				
	0 Hour (Initial)	24 Hour	72 Hour	72-hour Mean <sup>b</sup>	72-hour Geometric Mean <sup>b</sup>
Control	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>
2.5	2.8 (112)	0.48 (19) <sup>d</sup>	0.023 (1) <sup>d</sup>	1.4 (56)	0.25 (10)
2.5 Abiotic	NA	NA	0.062 (2)	1.4 (56) <sup>e</sup>	0.42 (17) <sup>e</sup>
5.0	5.5 (110)	1.4 (28) <sup>d</sup>	0.13 (3)	2.8 (56)	0.85 (17)
10	9.4 (94)	1.6 (16) <sup>d</sup>	0.59 (6)	5.0 (50)	2.4 (24)
20	21 (105)	8.5 (43) <sup>d</sup>	6.6 (33) <sup>d</sup>	14 (70)	12 (60)
40	39 (98)	24 (60) <sup>d</sup>	23 (58) <sup>d</sup>	31 (78)	30 (75)
QC Fortification Spikes (% Recovery)					
Low Spike (2.25)	2.5 (111)	2.3 (102)	2.3 (102)	---	---
High Spike (45.9)	49 (107)	47 (102)	46 (100)	---	---

<sup>a</sup> Calculated Concentration = ((Measured Conc, ng a.i./mL × Analysis Vol) / Sample Vol) / purity of XDE-777, 4.9%/1000 ng/mL/mg/L

<sup>b</sup> Mean and geometric mean were calculated using the 0- and 72-hour concentrations

<sup>c</sup> At 0 hour, MQL = 20.0 ng XDE-777/mL = 0.41 mg GF-3308/L

At 24 hours, MQL = 1.00 ng XDE-777/mL = 0.020 mg GF-3308/L

At 72 hours, MQL = 0.0200 ng XDE-777/mL = 0.00041 mg GF-3308/L

<sup>d</sup> The sample concentrations were outside of the standard curve, so the samples were re-diluted at a higher dilution factor and reanalyzed. Original results not reported.

<sup>e</sup> The reported 72-hour mean and 72-hour geometric mean for 2.5 mg GF-3308/L abiotic uses the 2.5 mg GF-3308/L for the 0-Hour (Initial) values for calculations.

**Table 4: Mean cell density**

Concentration (mg GF-3308/L)	Concentration (mg XDE-777/L)	Mean cell density (cells/mL x 10 <sup>4</sup> )	% inhibition
		72 h	72 h
control	control	69.0	--
0.25	0.0123	68.8	0
0.85	0.0414	74.2	-8
2.4	0.115	70.2	-2
12	0.574	55.7	19
30	1.48	23.5	66

**Table 5: Mean growth rate and yield**

Concentration (mg GF-3308/L)	Concentration (mg XDE-777/L)	Mean growth rate (cell/ml/h)	% inhibition	Mean yield (cell/ml x10 <sup>4</sup> )	% inhibition
		0-72 h	72 h	72 h	72 h
control	control	0.0681	---	68.5	---
0.25	0.0123	0.0681	0	68.3	0
0.85	0.0414	0.0691	-1	73.7	-8
2.4	0.115	0.0684	0	69.7	-2
12	0.574	0.0652	4	55.2	19
30	1.48	0.0532	22	23.0	66

**Table 6: Effects of GF-3308 on algal growth based on geometric mean measured concentrations**

Hour	EC Type	EC Value [mg GF-3308/L]	95% Confidence Limits [mg GF-3308/L]	NOEC [mg GF-3308/L]
72	ErC <sub>10</sub>	18	17 – 20	
	ErC <sub>20</sub>	28	27 – 29	
	ErC <sub>50</sub>	>30	Not Statistically Sound	2.4
	EyC <sub>10</sub>	9.3	6.9 – 13	
	EyC <sub>20</sub>	12	9.3 – 15	
	EyC <sub>50</sub>	21	18 – 25	2.4

**Table 7: Effects of XDE-777 on algal growth based on geometric mean measured concentrations**

Hour	EC Type	EC Value [mg XDE-777/L]	95% Confidence Limits [mg XDE-777/L]	NOEC [mg XDE-777/L]
72	ErC <sub>10</sub>	0.898	0.807 – 0.989	
	ErC <sub>20</sub>	1.38	1.32 – 1.44	
	ErC <sub>50</sub>	>1.48	Not Statistically Sound	0.115
	EyC <sub>10</sub>	0.440	0.325 – 0.597	
	EyC <sub>20</sub>	0.562	0.445 – 0.711	
	EyC <sub>50</sub>	1.03	0.868 – 1.22	0.115

## CONCLUSION

The test acceptability criteria were met for this study. The number of algal cells in the control at test termination was greater than 16 times (actually 135.3 times) the number initially inoculated to verify logarithmic phase growth. The overall mean coefficient of variation for daily growth rates in the control replicates during the course of the test did not exceed 35% (actually 18%). The coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7% (actually 0%). The pH in the control did not increase more than 1.5 units during the study. This study satisfies the OECD guideline requirement for a growth inhibition test with *Pseudokirchneriella subcapitata*.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Freshwater green algae	<i>Pseudokirchneriella subcapitata</i>	GF-3308	72-hr	ErC <sub>50</sub>	>30	mg/L, gm

Freshwater green algae	<i>Pseudokirchneriella subcapitata</i>	GF-3308	72-hr	E <sub>y</sub> C <sub>50</sub>	21	mg/L
Freshwater green algae	<i>Pseudokirchneriella subcapitata</i>	XDE-777	72-hr	E <sub>t</sub> C <sub>50</sub>	>1.48	mg a.i./L
Freshwater green algae	<i>Pseudokirchneriella subcapitata</i>	XDE-777	72-hr	E <sub>y</sub> C <sub>50</sub>	1.03	mg a.i./L

#### A 2.2.1.2 Study 2 - GF-3308: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Flow-Through Test Conditions

Comments of zRMS:	<p>The study was conducted in line with OECD 203 (1992) with no deviations.</p> <p>Throughout the test the concentrations of the active substance were maintained above 80% of initial calculated concentrations; therefore the endpoint is expressed in terms of the initial measured concentration.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LC<sub>50</sub> = 0.078 mg product/L (based on initial measured concentration), corresponding to 0.0038 mg a.s./L</p>
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Reference:	KCP 10.2.1/2
Report:	xxx; 2016; GF-3308: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Flow-Through Test Conditions; xxx; Lab Study No. 83494; DAS Study No. 160101 ; 08 July 2016; Unpublished
Guideline(s):	OECD Guideline 203
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): GF-3308  
Purity: 4.9 wt% XDE-777 (synonym: XDE-777)  
Description (physical state): Fragrant brown liquid  
Lot/batch no.: ENBK-148585-032A [TSN309730]

### Test system

Organism (*Species*): Rainbow trout (*Oncorhynchus mykiss*)  
Study type: Acute  
Study design: Flow through  
Test concentrations: Nominal: 0 (control), 0.016, 0.031, 0.063, 0.13, and 0.25 mg GF-3308/L  
Mean calculated: <MQL (control), 0.0070, 0.013, 0.028, 0.055, and 0.14 mg GF-3308/L

Parameters measured:	Mortality
Observation intervals:	24 hours
Age, weight and length of fish at test initiation:	Age: >14 days Mean blotted wet weight: 0.8424 ± 0.1987 g (0.5776 to 1.2761 g) Mean total length: 46 ± 2.9 mm (41 to 51 mm)
Analytical confirmation of test concentrations:	On day -7, and 0, 48, and 96 hours
No. of holding days before dosing:	14
Number of fish per dose group:	7
Number of fish per control group:	7
Feeding regime:	None
Environmental conditions:	Loading rate: 0.0240 g/L/day Temperature: 14.3 to 15.3°C Photoperiod: 16-hr light:8-hr dark Dissolved oxygen concentration: 9.1 to 10.1 mg/L (92 to 104% saturation) pH: 8.3 to 8.4 Total hardness: 142 mg CaCO <sub>3</sub> /L Salinity: Not applicable
Reference substance:	XDE-777 [TSN302306]

## Methodology

A flow-through definitive test was performed from 14 to 18 March 2016 at nominal concentrations of 0 (control), 0.016, 0.031, 0.063, 0.13, and 0.25 mg GF-3308/L. Seven fish were impartially assigned to treatments by adding one fish per chamber, proceeding from control, then low to high test substance treatments, and repeating steps as necessary until seven fish were present in each test chamber. A single replicate was used for each of the control and test substance treatments. Observations for mortality and sublethal responses were made at approximately 24, 48, 72, and 96 hours.

Temperature, dissolved oxygen, and pH were measured in each test chamber daily. A continuous record of temperature from a centrally located test chamber was also maintained. Total hardness and alkalinity of the dilution water were measured using titrimetric methods adapted from Standard Methods. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods. The measured light intensity during the definitive test was 539.7 lux.

## RESULTS AND DISCUSSION

The mean calculated concentrations of GF-3308, based on XDE-777 analysis, in the test substance treatment solutions during the 96-hour exposure were 0.0070, 0.013, 0.028, 0.055, and 0.14 mg GF3308/L, which represented recoveries of 42 to 56% of the nominal concentrations. The biological response results were reported **based upon the mean calculated GF-3308 concentrations**.

The probit model was not suitable for estimates of LC<sub>50</sub> values based on the data, therefore, the UnTrimmed or Trimmed Spearman-Kärber method was used for the statistical analysis. Based on mean calculated concentrations, the estimated 24 hour LC<sub>50</sub> value was >0.14 mg GF-3308/L, the highest concentration tested. Based on mean calculated concentrations and the Trimmed Spearman Karber method, the estimated 48 hour LC<sub>50</sub> value was 0.095 mg GF-3308/L, with 95% confidence limits of 0.080 and 0.11 mg GF-3308/L. Based on mean calculated concentrations and the Un-Trimmed Spearman Karber method, the estimated 72 and 96 hour LC<sub>50</sub> values were identical at 0.078 mg GF-3308/L, with 95% confidence limits of 0.063 and 0.097 mg GF-3308/L. The slope of the 96-hour



concentration-response line was not determined. The 96-hour NOEC was 0.055 mg GF-3308/L based on mean calculated concentrations and a lack of statistically significant mortality and sublethal effects at this and all lower test substance concentrations, as compared to the control. The 96-hour LC<sub>50</sub> and NOEC were 0.0038 and 0.0027 mg XDE-777/L, respectively, as expressed as the active ingredient purity of 4.9%.

**Table 8: Calculated Concentrations of GF-3308 During the Flow-through Acute Toxicity Test with Rainbow Trout**

Target Nominal Concentration (mg GF-3308/L)	Calculated Concentration as mg GF-3308/L <sup>a</sup> (Percent Target Nominal)				Mean Calculated Concentration <sup>b</sup>
	Day -7	0 Hour	48 Hours	96 Hours	
0 (Control)	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>
0.016	0.0055 (34)	0.0064 (40)	0.0075 (47)	0.0070 (44)	0.0070 (44)
0.016 (Delivery Line)	0.011 (69)	N/A	N/A	N/A	N/A
0.031	0.010 (32)	0.011 (35)	0.013 (42)	0.014 (45)	0.013 (42)
0.063	0.021 (33)	0.025 (40)	0.027 (43)	0.031 (49)	0.028 (44)
0.13	0.045 (35)	0.046 (35)	0.059 (45)	0.059 (45)	0.055 (42)
0.25	0.096 (38)	0.12 (48)	0.17 (68)	0.12 (48)	0.14 (56)
0.25 (Delivery Line)	0.21 (84)	N/A	N/A	N/A	N/A
QC Fortification Spikes (% Recovery)					
Low Spike (0.0140)	0.015 (107)	0.015 (107) <sup>d</sup>	0.015 (107)	0.015 (107)	N/A
High Spike (0.299)	0.33 (110)	0.29 (97)	0.31 (104)	0.31 (104)	N/A

<sup>a</sup> Calculated Concentration (mg total product GF-3308/L) = Measured Conc. From Curve (ng/mL) x Analysis Volume (mL) / Sample Volume (mL) / 4.9%(XDE-777) / 1000

<sup>b</sup> Mean of 0, 48, and 96 hour measured concentrations. The Day -7 time point was not included in the mean calculation.

<sup>c</sup> MQL = 0.0020 mg/L

<sup>d</sup> Re-diluted in duplicate and analyzed. The mean of the three analyses was reported.

Note: N/A = Not applicable

**Table 9: Effect of GF-3308 on mortality of rainbow trout**

Treatment (mg GF-3308/L)		No. of fish	Cumulative mortality (%)			
Nominal	Mean calculated		24-hr	48-hr	72-hr	96-hr
Negative control	Negative control	7	0 (0)	0 (0)	0 (0)	0 (0)
0.016	0.0070	7	0 (0)	0 (0)	0 (0)	0 (0)
0.031	0.013	7	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.028	7	0 (0)	0 (0)	0 (0)	0 (0)
0.13	0.055	7	0 (0)	0 (0)	1 (14)	1 (14)
0.25	0.14	7	2 (29)	6 (86)	7 (100)	7 (100)
LC <sub>50</sub>		0.078 mg GF-3308/L				
95% C.I.		0.063 to 0.097 mg GF-3308/L				
NOEC		0.055 mg GF-3308/L				

**Table 10: Sub-lethal effects of GF-3308 in rainbow trout**

Treatment (mg GF-3308/L)	Observation period
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Nominal	Mean calculated	On Bottom of Test Chamber (% affected)				Loss of equilibrium (% affected)			
		24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr
Negative control	Negative control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.016	0.0070	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.031	0.013	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.063	0.028	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.13	0.055	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (14)
0.25	0.14	0 (0)	1 (14)	0 (0)	--	0 (0)	0 (0)	0 (0)	--

## CONCLUSION

There was no mortality among control animals during the course of the study. Therefore, control animals satisfied test acceptability criteria for survival (i.e.,  $\geq 90\%$  or one fish) as stated in the study protocol and all testing guidelines. Based on mean calculated concentrations and the Un-Trimmed Spearman Karber method, the estimated 96 hour  $LC_{50}$  value was at 0.078 mg GF-3308/L, with 95% confidence limits of 0.063 and 0.097 mg GF-3308/L. The slope of the 96-hour concentration-response line was not determined. The 96-hour NOEC was 0.055 mg GF-3308/L based on mean calculated concentrations and a lack of statistically significant mortality and sublethal effects at this and all lower test substance concentrations, as compared to the control. The 96-hour  $LC_{50}$  and NOEC were 0.0038 and 0.0027 mg XDE-777/L, respectively, as expressed as the active ingredient purity of 4.9%.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rainbow trout	<i>Oncorhynchus mykiss</i>	GF-3308	96-hr	$LC_{50}$	0.078	mg/L, mm
Rainbow trout	<i>Oncorhynchus mykiss</i>	GF-3308	96-hr	$LC_{50}$	0.0038	mg a.i./L

### A 2.2.1.3 Study 3 – GF-3308: Acute Toxicity to the Cladoceran *Daphnia magna*, Determined Under Static-Renewal Test Conditions

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no deviations.</p> <p>Due to known instability of the active substance (fenpicoxamid) the study was performed in a semi-static design with renewal intervals of 8 hours. The analytical verification of the active compound in the test solution was carried out in fresh solutions at 0 and 40 hours and in spent solutions at 8 and 48 hours. In order to determine the decline pattern additional measurements were performed at 2 and 42 hours.</p> <p>It is noted by the zRMS that in general, the analytical measurements should be performed in all fresh and spent test solutions, but in this study no chemical analyses were performed for renewal intervals 8-16, 16-24, 24-32 and 32-40 hours. Nevertheless, the decline pattern at both intervals of 0-8 and 40-48 hours was comparable and in opinion of the zRMS it is not expected that it would be different at remaining renewals. Taking this into account, the performed analyses are considered sufficient to derive endpoints based on average time weighted geometric mean measured concentration.</p> <p>As already mentioned above, at both renewal intervals the decline pattern of fenpicoxamid was similar. As expected, the measured concentrations dropped rapidly already 2 hours after preparation of fresh test solutions (4-28% of nominal, depending on the test group). In aged solution the measured concentrations were at 0.2-0.6% of nominal in three highest treatment groups and &lt;LOQ at two lowest test concentrations (it should be, however, noted that LOD was not established).</p> <p>Due to additional sampling at 2 and 42 hours it was possible to calculate the degradation rate constant indicating <math>DT_{50}</math> of 1.05 hours in the test system. In opinion of the zRMS</p>
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	<p>derived rate constant should be considered supportive information only, since the DT<sub>50</sub> derived with consideration of only 3 data points is not fully reliable since at least 5 data points are required by FOCUS guidance on degradation kinetics (2014). Nevertheless, the same guidance indicates that for rapidly degrading compounds 5 data points may be difficult to achieve and for this reason lower number may be considered sufficient, provided that acceptable fit is achieved. Although not full kinetic evaluation in line with FOCUS has been performed based on the analytical results from this study, the statics are acceptable and the linear fit indicates that the degradation of fenpicoxamid would be sufficiently describe by SFO kinetics. Although additional sampling at 4 h (44 h) or 6 h (46 h) would add more certainty in the obtained results, the zRMS is of the opinion that the sampling was sufficient to derive the degradation rate constant used for estimation of the residues of fenpicoxamid at 8 h (48 h) in the two lowest treatment groups.</p> <p>In addition to that it should be noted that at the two lowest test concentrations no immobilisation of <i>Daphnia</i> was observed and for this reason calculation of degradation rate constant and the residue level was rather informative, since it would have no impact on obtained results with the dose-response curve starting from the middle concentration of 0.018 mg test item/L (with 5% effect).</p> <p>The residues estimated using method described above were at the same (or almost the same) level as ½ LOQ used in the original test report. Taking this into account, there was no need for recalculation of the endpoints.</p> <p>It is noted that the endpoints were expressed in terms of the time weighted geometric mean measured concentrations. No justification was given why this method was selected and not commonly agreed geometric mean measured concentrations, however possibility to express endpoints in terms of TWA concentrations is given in OECD 23 (point 9, page 59, second edition of 2019) and is thus accepted by the zRMS.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>EC<sub>50</sub> = 0.048 mg product/L (based on time weighted geometric mean concentration), corresponding to 0.0023 mg a.s./L</p>
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Reference:	KCP 10.2.1/3
Report:	xxx.; 2016; GF-3308: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static Renewal Test Conditions; xxx; Lab Study No. 83495; DAS Study No. 160102 ; 01 December 2016; Unpublished
Guideline(s):	OECD Guideline 202
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name):	GF-3308
Purity:	4.8 wt% XDE-777
Description (physical state):	Brown liquid with a fragrant odor
Lot/batch no.:	E3240-85-1 (TSN311166)

## Test System

Organism ( <i>Species</i> ):	Water flea ( <i>Daphnia magna</i> )
Study type:	Acute
Study design:	Static-renewal, every 8 hours (i.e. 6 renewals)
Test concentrations:	Nominal: 0 (control), 0.031, 0.063, 0.13, 0.25, and 0.50 mg GF-3308/L Average Time Weighted Geometric Mean Calculated: <MQL (control), 0.0034, 0.0081, 0.018, 0.034, and 0.073 mg GF-3308/L
Parameters measured:	Immobility
Observation intervals:	0, 8, 16, 24, 32, 40, and 48 hours (Tables 3 and D-1 in the full report)
Age of test organisms at test initiation:	<24-hours old
Analytical confirmation of test concentrations:	0 and 40 hours (fresh); 8 and 48 hours (spent). Intermediate analyses to characterize the decline curve taken at 2 hours and 42 hours. As required by the guideline (OECD 202 §23), analysis conducted at the beginning and end of the test for the first and last renewals. Intermediate renewals at 8, 16, 24 and 32 hours were not analytically confirmed.
No. of holding days before dosing:	None
Number of daphnia per dose group:	20
Number of daphnia per control group:	20
Environmental conditions:	Loading rate: not applicable Temperature: 19.6 to 20.6 °C Photoperiod: 16-hr light: 8-hr dark Dissolved oxygen concentration: (fresh solutions): 8.3 to 8.7 mg/L (95 to 100% saturation) (spent solutions): 8.1 to 8.7 mg/L (93 to 100% saturation) pH: 8.5 to 8.6 Hardness: 152 mg CaCO <sub>3</sub> /L
Reference substance:	XDE-777 <u>technical</u> (TSN302306)

## Methodology

A definitive test was performed from 07 to 09 June 2016 at nominal concentrations of 0 (control), 0.031, 0.063, 0.13, 0.25, and 0.50 mg GF-3308/L. Due to rapid degradation of XDE-777, the test design was modified from a standard OECD 202 static-renewal study. A total of 6 renewals every 8 hours were conducted as noted in Protocol Amendment 1 to the Study Plan. As required by the guideline (OECD 202 §23), analysis was conducted at the beginning and end of the test for the first and last renewals. Intermediate renewals at 8, 16, 24 and 32 hours were not analytically confirmed. Additionally, to characterize the decline curve, as suggested by the EFSA Peer Review Opinion on Recurring Issues in Ecotoxicology (EFSA, 2015), samples were taken at 2 hours after the first and last renewals. Five neonates (<24-hours old) were added to each of four test chambers per treatment at the start of the test. The daphnids were observed for immobility and sublethal effects at each test solution renewal period. The observations performed at approximately 24 and 48 hours after test initiation were used for reporting exposure effects. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in fresh parent solutions at initiation and each solution renewal, and in individual replicate test chambers

for the corresponding spent solutions at each solution renewal and test termination. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods. The measured light intensity at initiation of the definitive test was 308 lux.

## RESULTS AND DISCUSSION

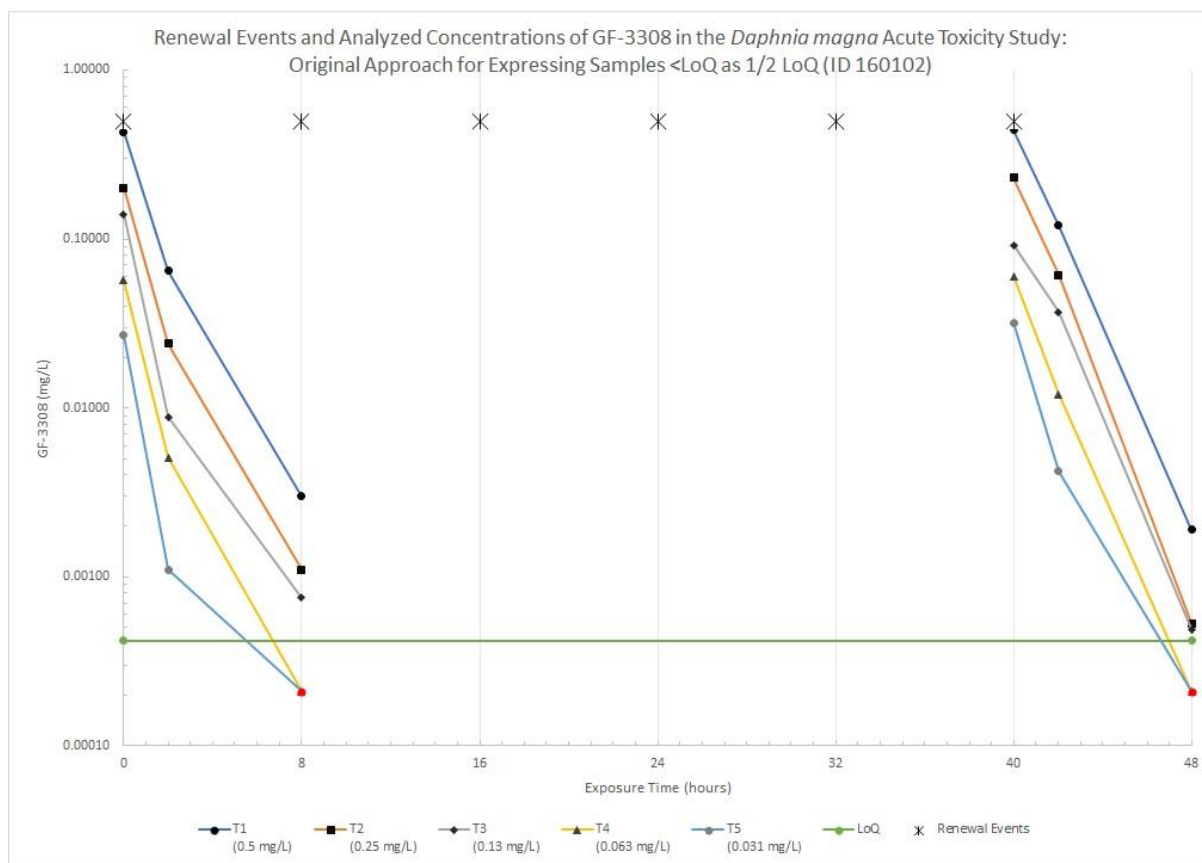
XDE-777 degrades very rapidly in water. To characterise the decline curve, additional analytical samples were taken 2 hours after the first and last renewal. Analytical recoveries were greater than the LOQ (referred to as MQL in the original study summary below) at all renewal and 2 hour time points in every treatment level. In the two lowest treatment levels, XDE-777 residues fell below the LOQ. Nevertheless, the exposure that occurred in the study is considered well-characterized because every treatment level had analytically confirmed > LOQ concentrations at 2 hours after renewal.

This ample analytical data set (Figure 1a) allows for the calculation of the degradation rate constant of XDE-777 in the test system (Figure 1b) which is determined by the slope of the regression of the natural logarithm of the percent recovery of XDE-777 concentration against the time after renewal. The rate constant for degradation of XDE-777 in this test system was  $-0.659 \text{ hr}^{-1}$  ( $R^2=0.94$ ;  $p<0.0001$ ; 95% CI -  $0.730$  to  $-0.587 \text{ hr}^{-1}$ ). This highly significant and consistent regression allows for a very confident estimation of the percent recovery and concentration that existed in the two lowest treatment levels with < LOQ analyses.

At 8 hours after renewal, the percent recovery was estimated to be 0.35% (Figure 1c). Therefore, the concentrations in the two sets of samples that were < LOQ can be calculated to be, in the 0.063 mg/L treatment at 8 hours after renewal, 0.00022 mg/L and in the 0.031 mg/L treatment, 0.00011 mg/L. As recommended by OECD 23 (2019) Guidance for Difficult to Test Substances, when exposure concentrations drop significantly after renewal, the geometric mean should be calculated to estimate the exposure value. These values were entered into the time-weighted geometric mean calculation for these two treatment levels, instead of using the  $\frac{1}{2}$  LOQ = 0.00021 mg/L value originally proposed in the study report.

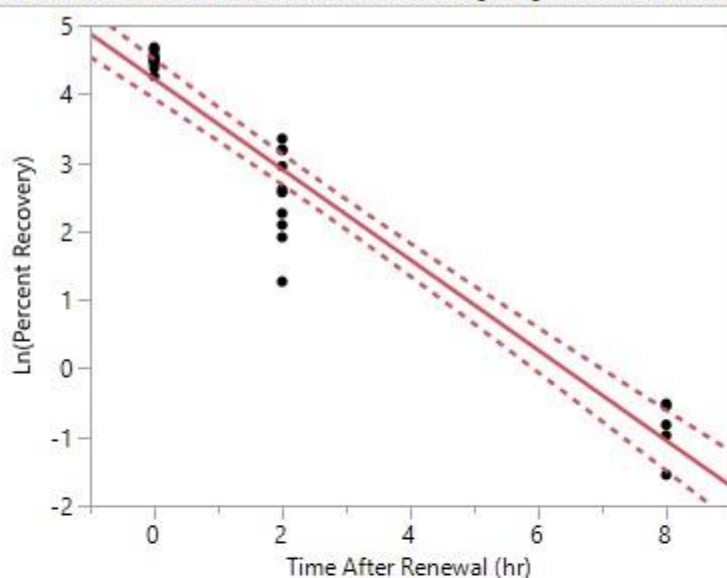
For the 0.063 mg/L treatment level, the use of 0.00022 mg/L instead of 0.00021 mg/L resulted in no difference in the calculated geometric mean value of 0.00081 mg/L. Similarly, in the 0.031 mg/L treatment level, the geometric mean calculated with the newly estimated 8 hour value of 0.00011 mg/L resulted in a slightly lower mean: 0.00033 mg/L instead of 0.00034 mg/L (Revised Table 11). As depicted in Figure 2, the difference in geometric mean exposure calculated using the accurately estimated 8 hour concentrations in these two lowest treatment levels has no effect whatsoever on the dose-response curve calculation of the EC<sub>50</sub>, since at both of these treatment levels, there was 0 response.

In any case, the EFSA Peer Review opinion noted that the geometric mean using  $\frac{1}{2}$  LOQ could be used in studies such as this one that had intermediate analyses conducted, as stated on page 7 of that document, *“The appropriateness of LOD or half of the LOQ, foreseen in OECD 23 for difficult substances, was also considered during the meeting. The experts considered that this approach could be used when intermediate measurements (e.g. more than one intermediate point or other information) are available. This information may allow using the LOD or half of the LOQ, to calculate a geometric mean concentration.”* (Emphasis added.) In conclusion, the study meets acceptance criteria outlined by the EFSA Peer Review Opinion, as well as the original OECD Test Guideline validity criteria and the originally reported endpoint is valid and unchanged using either method of calculating exposure concentrations.



**Figure 1a** Graphic depiction of analytically verified concentrations of GF-3308 (containing XDE-777 active ingredient) and the 6 dosing renewal events in the *Daphnia* acute toxicity study. Symbols in black/gray represent analytically verified samples >LOQ. Symbols in red represent the 4 samples that were reported as <LOQ but set at 1/2 LOQ for purposes of calculating the geometric mean.

### Bivariate Fit of Ln(Percent Recovery) By Time After Renewal (hr)



Linear Fit

#### Linear Fit

$\text{Ln(Percent Recovery)} = 4.2198247 - 0.6585915 \times \text{Time After Renewal (hr)}$

#### Summary of Fit

RSquare	0.938285
RSquare Adj	0.935713
Root Mean Square Error	0.540932
Mean of Response	2.497355
Observations (or Sum Wgts)	26

#### Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	106.76746	106.767	364.8835
Error	24	7.02257	0.293	Prob > F
C. Total	25	113.79003		<.0001*

#### Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t	Lower 95%	Upper 95%
Intercept	4.2198247	0.139231	30.31	<.0001*	3.9324665	4.5071829
Time After Renewal (hr)	-0.658592	0.034478	-19.10	<.0001*	-0.72975	-0.587433

**Figure 1b** Determination of the decline rate constant of GF-3308 in the *Daphnia* acute toxicity study. The slope of the regression line gives the decline rate constant =  $-0.659 \text{ hr}^{-1}$ . This is equivalent to a half-life of 1.05 hours in this test system.



Time After Renewal (hr)	Percent Recovery	Treatment	Ln(Percent Recovery)	Predicted Ln(Percent Recovery)	Predicted Percent Recovery
0	86	T1 (0.5 mg/L)	4.4543472963	4.2198246991	68.021559006
0	88	T1 (0.5 mg/L)	4.4773368145	4.2198246991	68.021559006
2	13	T1 (0.5 mg/L)	2.5649493575	2.9026416344	18.222218285
2	24	T1 (0.5 mg/L)	3.1780538303	2.9026416344	18.222218285
8	0.598	T1 (0.5 mg/L)	-0.514164525	-1.04890756	0.3503202441
8	0.38	T1 (0.5 mg/L)	-0.967584026	-1.04890756	0.3503202441
0	80	T2 (0.25 mg/L)	4.3820266347	4.2198246991	68.021559006
0	92	T2 (0.25 mg/L)	4.521788577	4.2198246991	68.021559006
2	9.6	T2 (0.25 mg/L)	2.2617630985	2.9026416344	18.222218285
2	24.4	T2 (0.25 mg/L)	3.1945831323	2.9026416344	18.222218285
8	0.44	T2 (0.25 mg/L)	-0.820980552	-1.04890756	0.3503202441
8	0.212	T2 (0.25 mg/L)	-1.551169004	-1.04890756	0.3503202441
0	107.69230769	T3 (0.13 mg/L)	4.6792781581	4.2198246991	68.021559006
0	70.769230769	T3 (0.13 mg/L)	4.2594243126	4.2198246991	68.021559006
2	6.7692307692	T3 (0.13 mg/L)	1.912387457	2.9026416344	18.222218285
2	28.461538462	T3 (0.13 mg/L)	3.3485536482	2.9026416344	18.222218285
8	0.5769230769	T3 (0.13 mg/L)	-0.550046337	-1.04890756	0.3503202441
8	0.3769230769	T3 (0.13 mg/L)	-0.975714152	-1.04890756	0.3503202441
0	90.476190476	T4 (0.063 mg/L)	4.5050867274	4.2198246991	68.021559006
0	95.238095238	T4 (0.063 mg/L)	4.5563800218	4.2198246991	68.021559006
2	8.0952380952	T4 (0.063 mg/L)	2.0912759993	2.9026416344	18.222218285
2	19.047619048	T4 (0.063 mg/L)	2.9469421094	2.9026416344	18.222218285
0	87.096774194	T5 (0.031 mg/L)	4.4670198475	4.2198246991	68.021559006
0	103.22580645	T5 (0.031 mg/L)	4.6369188843	4.2198246991	68.021559006
2	3.5483870968	T5 (0.031 mg/L)	1.2664931613	2.9026416344	18.222218285
2	13.548387097	T5 (0.031 mg/L)	2.6062675068	2.9026416344	18.222218285

**Figure 1c** Estimation of the percent recovery at 8 hours = 0.35% from the regression relationship determined in Figure 1b, marked with an arrow.

~~In any case, the EFSA Peer Review opinion noted that the geometric mean using 1/2LoQ could be used in studies such as this one that had intermediate analyses conducted, as stated on page 7 of that document, “The appropriateness of LOD or half of the LOQ, foreseen in OECD 23 for difficult substances, was also considered during the meeting. The experts considered that this approach could be used when intermediate measurements (e.g. more than one intermediate point or other information) are available. This information may allow using the LOD or half of the LOQ, to calculate a geometric mean concentration.” (Emphasis added.) In conclusion, the study meets acceptance criteria outlined by the EFSA Peer Review Opinion, as well as the original OECD Test Guideline validity criteria and the originally reported endpoint is valid and unchanged using either method of calculating exposure concentrations.~~

**Due to the rapid degradation of the XDE-777 active ingredient, a time weighted geometric mean concentration was determined for GF-3308 for 0-8 hours and 40-48 hours.** For the test treatments which had recoveries of <MQL in the 8 hour and 48 hour samples, half of the MQL was used for the purpose of calculating exposure concentrations. The overall average time weighted geometric mean concentrations in the test substance treatment solutions during the 48-hour exposure were 0.0034, 0.0081, 0.018, 0.034, and 0.073 mg GF-3308/L, which represented recoveries of 11 to 15% of the nominal concentrations.

The 24-hour EC<sub>50</sub> value was estimated to be >0.073 mg GF-3308/L, the highest concentration tested. The 48-hour EC<sub>50</sub> value was estimated to be 0.048 mg GF-3308/L (95% C.I.: 0.032 – 0.071 mg GF-3308/L). No sublethal effects were noted during the definitive test. The 48-hour NOEC was 0.018 mg GF-3308/L, based on the lack of statistical significance at this and all lower test treatment concentrations. The 48-hour EC<sub>50</sub> and NOEC were 0.0023 and 0.00086 mg XDE-777/L, respectively, as expressed as the active ingredient purity of 4.8%.



**Table 11: Revised Calculated Concentrations of GF-3308 (based on analysis of XDE-777) During the 48hour Acute Toxicity Test with *D. magna*. Revision of the geometric mean concentrations for the 0.031 and 0.063 mg/L treatment levels using the regression estimated percent recovery (0.0035%) at 8 hours derived from Figures 1b and 1c. Note: The only numerical difference in geometric mean occurs in the 0.031 mg/L treatment level by a single digit. Revisions marked by a box.**

		0.00011 (0.35)		0.00011 (0.35)	0.0023	0.0043	0.0033		
		0.00022 (0.35)		0.00022 (0.35)	0.0065	0.0097	0.0081		
Calculated Concentration as mg GF-3308/L (Percent Nominal) <sup>a</sup>									
Nominal Concentration (mg GF- 3308/L)	0-Hour	2-Hour (spent)	8-Hour (spent)	40-Hour (fresh)	42-Hour (spent)	48-Hour	Time Weighted Geometric Mean Concentration: 0-8 hours <sup>c</sup>	Time Weighted Geometric Mean Concentration: 40-48 hours <sup>c</sup>	Average Time Weighted Geometric Mean Concentration <sup>d</sup>
Control	<MQL <sup>b</sup>	<MQL <sup>b,i</sup>	<MQL <sup>b,i</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,g</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
0.031	0.027 (87) <sup>e</sup>	0.0011 (4) <sup>i</sup>	<MQL <sup>b,i</sup>	0.032 (103)	0.0042 (14)	<MQL <sup>b,g</sup>	0.0024 (8)	0.0044 (14)	0.0034 (11)
0.063	0.057 (90) <sup>e</sup>	0.0051 (8) <sup>i</sup>	<MQL <sup>b,i</sup>	0.060 (95)	0.012 (19)	<MQL <sup>b,g</sup>	0.0065 (10)	0.0096 (15)	0.0081 (13)
0.13	0.14 (108)	0.0088 (7) <sup>i</sup>	0.00075 (0.6) <sup>i</sup>	0.092 (71) <sup>f</sup>	0.037 (28)	0.00049 (0.4) <sup>g</sup>	0.014 (11)	0.021 (16)	0.018 (14)
0.25	0.20 (80) <sup>e</sup>	0.024 (10) <sup>i</sup>	0.0011 (0.4) <sup>i</sup>	0.23 (92)	0.061 (24)	0.00053 (0.2) <sup>g</sup>	0.026 (11)	0.041 (17)	0.034 (14)
0.50	0.43 (86) <sup>e</sup>	0.065 (13) <sup>i</sup>	0.00299 (0.6) <sup>i</sup>	0.44 (88)	0.12 (24)	0.0019 (0.4) <sup>g</sup>	0.063 (13)	0.083 (17)	0.073 (15)
Primary Stock (1.0)	1.2 (120)	NA	NA	1.1 (110)	NA	NA	NA	NA	NA
QC Fortification Spikes (% Recovery)									
Low Spike (0.0279)	0.031 (111)	0.035 (125) <sup>f</sup>	0.033 (118) <sup>f</sup>	0.031 (111)	0.032 (115) <sup>f</sup>	0.027 (97) <sup>e</sup>	NA	NA	NA
High Spike (0.572)	0.63 (110) <sup>e</sup>	0.68 (119) <sup>f</sup>	0.60 (105) <sup>f</sup>	0.62 (108)	0.60 <sup>e</sup> (105)	<MQL <sup>b,h</sup>	NA	NA	NA

<sup>a</sup> The Calculated Concentration, mg GF-3308/L = (Measured conc. from curve × Analysis Vol / Sample Vol) / 1000 / purity of XDE-777, 4.8%

<sup>b</sup> MQL = 0.0200 ng XDE-777/L = 0.00042 mg GF-3308/L

<sup>c</sup> For <MQL values, ½ MQL (0.00021 mg GF-3308/L used for calculations.

<sup>d</sup> Average of 0-8 and 40-48 hour time weighted geometric mean concentrations.

<sup>e</sup> Re-diluted in duplicate and re-analyzed, mean of duplicate re-analysis reported.

<sup>f</sup> Re-diluted in duplicate and re-analyzed, mean of original and duplicate re-analysis reported.

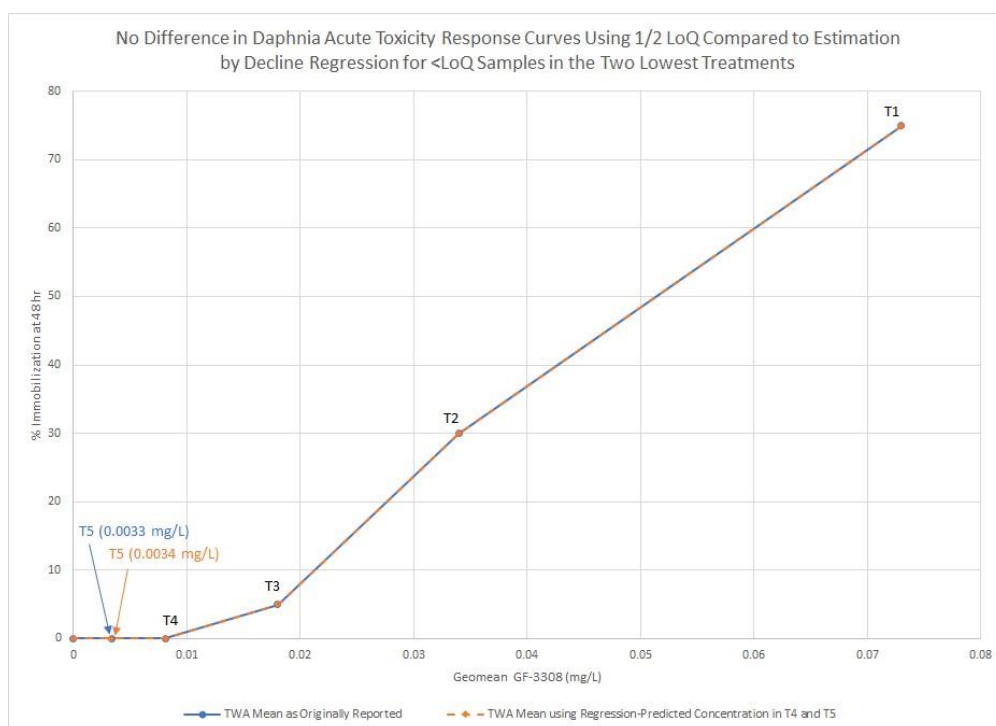
<sup>g</sup> Original concentration from curve had no peak. Samples re-analyzed twice. The third analysis is reported.

<sup>h</sup> Sample re-diluted in duplicate and re-analyzed, results matched the original.

<sup>i</sup> Sample re-diluted and re-analyzed, results of the re-analyses reported.

<sup>j</sup> Sample re-analyzed, results of the re-analyses reported.

NA = Not applicable.



**Figure 2** Comparison of dose response of *Daphnia magna* to GF-3308 using the originally calculated geometric mean concentrations (using  $\frac{1}{2}$  LOQ for 4 samples at the two lowest treatment levels) and geometric means concentrations calculated using the decline regression estimated values (0.35% recovery at 8 hours).

**Table 12: Calculated Concentrations of GF-3308 (based on analysis of XDE-777) During the 48hour Acute Toxicity Test with *D. magna***

Nominal Concentration (mg GF-3308/L)	Calculated Concentration as mg GF-3308/L (Percent Nominal) <sup>a</sup>						Time Weighted Geometric Mean Concentration: 0-8 hours <sup>c</sup>	Time Weighted Geometric Mean Concentration: 40-48 hours <sup>c</sup>	Average Time Weighted Geometric Mean Concentration <sup>d</sup>
	0-Hour	2-Hour (spent)	8-Hour (spent)	40-Hour (fresh)	42-Hour (spent)	48-Hour			
Control	<MQL <sup>b</sup>	<MQL <sup>b,i</sup>	<MQL <sup>b,i</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,g</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
0.031	0.027 (87) <sup>e</sup>	0.0011 (4) <sup>i</sup>	<MQL <sup>b,i</sup>	0.032 (103)	0.0042 (14)	<MQL <sup>b,g</sup>	0.0024 (8)	0.0044 (14)	0.0034 (11)
0.063	0.057 (90) <sup>e</sup>	0.0051 (8) <sup>i</sup>	<MQL <sup>b,i</sup>	0.060 (95)	0.012 (19)	<MQL <sup>b,g</sup>	0.0065 (10)	0.0096 (15)	0.0081 (13)
0.13	0.14 (108)	0.0088 (7) <sup>i</sup>	0.00075 (0.6) <sup>j</sup>	0.092 (71) <sup>f</sup>	0.037 (28)	0.00049 (0.4) <sup>g</sup>	0.014 (11)	0.021 (16)	0.018 (14)
0.25	0.20 (80) <sup>e</sup>	0.024 (10) <sup>i</sup>	0.0011 (0.4) <sup>j</sup>	0.23 (92)	0.061 (24)	0.00053 (0.2) <sup>g</sup>	0.026 (11)	0.041 (17)	0.034 (14)
0.50	0.43 (86) <sup>e</sup>	0.065 (13) <sup>i</sup>	0.00299 (0.6) <sup>j</sup>	0.44 (88)	0.12 (24)	0.0019 (0.4) <sup>g</sup>	0.063 (13)	0.083 (17)	0.073 (15)
Primary Stock (1.0)	1.2 (120)	NA	NA	1.1 (110)	NA	NA	NA	NA	NA
QC Fortification Spikes (% Recovery)									
Low Spike (0.0279)	0.031 (111)	0.035 (125) <sup>f</sup>	0.033 (118) <sup>f</sup>	0.031 (111)	0.032 (115) <sup>f</sup>	0.027 (97) <sup>e</sup>	NA	NA	NA
High Spike (0.572)	0.63 (110) <sup>e</sup>	0.68 (119) <sup>f</sup>	0.60 (105) <sup>f</sup>	0.62 (108)	0.60 <sup>e</sup> (105)	<MQL <sup>b,h</sup>	NA	NA	NA

<sup>a</sup> The Calculated Concentration, mg GF-3308/L = (Measured conc. from curve × Analysis Vol / Sample Vol) / 1000 / purity of XDE-777, 4.8%<sup>b</sup> MQL = 0.0200 ng XDE-777/L = 0.00042 mg GF-3308/L<sup>c</sup> For <MQL values, ½ MQL (0.00021 mg GF-3308/L used for calculations.<sup>d</sup> Average of 0-8 and 40-48 hour time weighted geometric mean concentrations.<sup>e</sup> Re-diluted in duplicate and re-analyzed, mean of duplicate re-analysis reported.<sup>f</sup> Re-diluted in duplicate and re-analyzed, mean of original and duplicate re-analysis reported.<sup>g</sup> Original concentration from curve had no peak. Samples re-analyzed twice. The third analysis is reported.<sup>h</sup> Sample re-diluted in duplicate and re-analyzed, results matched the original.<sup>i</sup> Sample re-diluted and re-analyzed, results of the re-analyses reported.<sup>j</sup> Sample re-analyzed, results of the re-analyses reported.

NA = Not applicable.

**Table 13: Effect of GF-3308 on Immobilization**

Average Time Weighted Geometric Mean Calculated Treatment (mg GF-3308/L)	24-hr		48-hr	
	No. immobile	% Immobility	No. immobile	% Immobility
Negative control	0	0	0	0
0.0034	0	0	0	0
0.0081	0	0	0	0
0.018	1	5	1	5
0.034	1	5	6	30*
0.073	1	5	15	75*
NOEC			0.018 mg GF-3308/L	
EC <sub>50</sub>	>0.073 mg GF-3308/L		0.048 mg GF-3308/L	

\* Statistically significant immobility as compared to the control (Fisher's one-tailed exact test,  $p \leq 0.05$ )**Table 14: Sub-lethal effects of GF-3308**

Average Time Weighted Geometric Mean Calculated Treatment (mg GF-3308/L)	Observation period	
	Observation 1 (% affected)	
	24-hr	48-hr
Negative control	0 (0)	0 (0)
0.0034	0 (0)	0 (0)
0.0081	0 (0)	0 (0)
0.018	0 (0)	0 (0)

0.034	0 (0)	0 (0)
0.073	0 (0)	0 (0)

## CONCLUSION

All test acceptability criteria were met for this study. Immobilization among control daphnids was 0%, which is below the acceptability limit of 10% as stated in the protocol and the OECD 202 test guideline. The dissolved oxygen concentration at the end of the test was  $\geq 8.4$  mg/L in control and test substance treatments, higher than the acceptability minimum of  $\geq 3$  mg/L. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*.

The 24-hour EC<sub>50</sub> value was estimated to be  $>0.073$  mg GF-3308/L, the highest concentration tested. The 48-hour EC<sub>50</sub> value was estimated to be 0.048 mg GF-3308/L (95% C.I.: 0.032 – 0.071 mg GF-3308/L). No sublethal effects were noted during the definitive test. The 48-hour NOEC was 0.018 mg GF-3308/L, based on the lack of statistical significance at this and all lower test treatment concentrations. The 48-hour EC<sub>50</sub> and NOEC were 0.0023 and 0.00086 mg XDE-777/L, respectively, as expressed as the active ingredient purity of 4.8%.

In conclusion, the study meets acceptance criteria outlined by the EFSA Peer Review Opinion, as well as the original OECD Test Guideline validity criteria and the originally reported endpoint is valid and unchanged using either method of calculating exposure concentrations.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	<i>Daphnia magna</i>	GF-3308	48-hr	EC <sub>50</sub>	0.048	mg/L twgm
Water flea	<i>Daphnia magna</i>	GF-3308	48-hr	EC <sub>50</sub>	0.0023	mg a.i./L

### A 2.2.1.4 Study 4 – X1642188 (a metabolite of XDE-777): Acute Toxicity Test to Cladoceran, *Daphnia magna*, Determined Under Flow-Through Test Conditions

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no deviations.</p> <p>The measured concentrations of the X642188 metabolite did not remain within 80-120% of nominal throughout the test. Therefore, the endpoint is expressed in terms of the overall mean measured metabolite concentrations.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>EC<sub>50</sub> = 0.79 µg metabolite/L (based on mean measured concentration)</p>
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Reference:	KCP 10.2.1/4
Report:	Goudie, O.; 2018; X642188 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Flow-Through Test Conditions; Analytical Bio-Chemistry Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, USA; Lab Study No. 87148; DAS Study No. 180562 ; 30 August 2018; Unpublished

Guideline(s):	OECD Guideline 202
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): X642188  
Purity: 99%  
Description (physical state): White solid with a mild odor  
Lot/batch no.: Lot No. SYN-FS08353-048 [TSN303567]

### Test System

Organism (*Species*): Water flea (*Daphnia magna*)  
Study type: Acute  
Study design: Flow through  
Test concentrations: Nominal: 0 (control), 0 (vehicle control), 0.049, 0.11, 0.23, 0.52, 1.1, and 2.5 µg X642188/L  
Mean measured: <MQL (control), <MQL (vehicle control), 0.034, 0.068, 0.14, 0.33, 0.62, and 1.3 µg X642188/L  
Parameters measured: Mobility  
Observation intervals: 24 and 48 hours  
Age of test organisms at test initiation: <24 hours  
Analytical confirmation of test concentrations: At 0, 24, and 48 hours  
No. of holding days before dosing: None  
Number of daphnia per dose group: 20  
Number of daphnia per control group: 20  
Environmental conditions: Loading rate: not applicable  
Temperature: 20.5 to 21.0 °C  
Photoperiod: 16-hr light: 8-hr dark  
Dissolved oxygen concentration: 6.9 to 8.4 mg/L (81 to 99% saturation) pH: 6.9 to 7.1  
Reference substance: none

### Methodology

A definitive test was performed from 15 to 17 May 2018 at nominal concentrations of 0 (control), 0 (vehicle control), 0.049, 0.11, 0.23, 0.52, 1.1, and 2.5 µg X642188/L. Five neonates (<24-hours old) were added to each replicate retention basket per treatment, four baskets per treatment, for a total of 20 neonates per treatment at the start of the test. The daphnids were observed for immobility and sublethal effects at approximately 24 and 48 hours after test initiation.

Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured daily in all replicates. The measured light intensity at the start of the definitive test was 219 lux.

Daphnids were maintained within retention baskets to facilitate daily observations and enumeration. One retention basket was used in each replicate test chamber at initiation. Measured concentrations of X642188 were determined in post (collected promptly following diluter cycle and delivery of fresh test solution to test chamber) and pre (collected as near as possible to the following diluter cycle [i.e., prior to delivery of fresh test solutions to test chamber]) samples in the test substance treatment solutions.

## RESULTS AND DISCUSSION

Mean measured concentrations of X642188 in the test solutions at 0-hour (initiation) were 0.040, 0.070, 0.15, 0.39, 0.73, and 1.4 µg X642188/L in the 0.049, 0.11, 0.23, 0.52, 1.1, and 2.5 µg X642188/L test treatments, which represented recoveries of 56 to 82% of the nominal concentrations. Mean measured concentrations of X642188 in test solutions at 24 hours were 0.029, 0.070, 0.13, 0.30, 0.56, and 1.3 µg X642188/L, which represented recoveries of 51 to 64% of the nominal concentrations. Mean measured concentrations of X642188 in test solutions at 48 hours (termination) were 0.032, 0.065, 0.13, 0.31, 0.57, and 1.2 µg X642188/L, which represented recoveries of 48 to 65% of the nominal concentrations. The overall mean measured concentrations in the test substance treatment solutions during the 48-hour exposure were 0.034, 0.068, 0.14, 0.33, 0.62, and 1.3 µg X642188/L, which represented recoveries of 52 to 69% of the nominal concentrations.

The biological response results were reported based upon the overall mean measured X642188 concentrations. After 48 hours of exposure, immobility was 5% in the control and vehicle control, and 5, 0, 10, 0, 25, and 95% in 0.034, 0.068, 0.14, 0.33, 0.62, and 1.3 µg X642188/L treatments, respectively. The estimated 24-hour EC<sub>50</sub> value was >1.3 µg X642188/L, the highest concentration tested. The slope of the 24-hour concentration-response line was not calculated. The estimated 48-hour EC<sub>50</sub> value was 0.79 µg X642188/L, with 95% confidence limits of 0.66 and 0.95 µg X642188/L. The slope of the 48-hour concentration-response line was 7.6.

The 48-hour NOEC was 0.33 µg X642188/L, based on the mean measured concentrations and the lack of statistically significant effects at this, and all lower test substance concentrations.

**Table 1.** Mean Measured Concentrations of X642188 During the 48-Hour Flow-Through Acute Toxicity Test with *Daphnia magna*

Nominal Concentration (µg X642188/L)	Mean Measured Concentration as µg X642188/L (Percent Nominal) <sup>a</sup>			Overall Mean
	0-Hour	24-Hour	48-Hour	
Control (0)	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Vehicle Control (0)	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
0.049	0.040 (82)	0.029 (59)	0.032 (65)	0.034 (69)
0.11	0.070 (64)	0.070 (64)	0.065 (59)	0.068 (62)
0.23	0.15 (65)	0.13 (57)	0.13 (57)	0.14 (61)
0.52	0.39 (75)	0.30 (58)	0.31 (60)	0.33 (63)
1.1	0.73 (66)	0.56 (51)	0.57 (52)	0.62 (56)
2.5	1.4 (56)	1.3 (52)	1.2 (48)	1.3 (52)

<sup>a</sup> See [Appendix D](#) for individual replicate analytical results.

<sup>b</sup> MQL = 0.010 µg X642188/L.

**Table 15: Effect of X642188 on immobilisation**

Mean Measured Treatment (µg X642188/L)	24-hr		48-hr	
	No. immobile	% Immobility	No. immobile	% Immobility
Negative control	0	0	1	5
Vehicle control	0	0	1	5
0.034	0	0	1	5
0.068	0	0	0	0
0.14	1	5	2	10
0.33	0	0	0	0
0.62	1	5	5	25*
1.3	8	40	19	95*
NOEC	NA		0.33 µg X642188/L	
EC <sub>50</sub>	>1.3 µg X642188/L		0.79 µg X642188/L (95% confidence limits 0.66 and 0.95 µg X642188/L)	

\* Statistically significant immobility as compared to the pooled control (Williams' test,  $p \leq 0.05$ )

**Table 16: Sub-lethal effects of X642188**

Mean Measured Treatment (µg X642188/L)	Observation period	
	Observation 1 (% affected)	
	24-hr	48-hr
Negative control	0 (0)	0 (0)
Vehicle control	0 (0)	0 (0)
0.034	0 (0)	0 (0)
0.068	0 (0)	0 (0)
0.14	0 (0)	0 (0)
0.33	0 (0)	0 (0)
0.62	0 (0)	0 (0)
1.3	0 (0)	0 (0)

## CONCLUSION

All test acceptability criteria were met for this study. Immobilization among control and vehicle control daphnids was 5%, respectively, which is below the acceptability limit of 10% as stated in the protocol and the OECD 202 test guideline. The dissolved oxygen concentration at the end of the test was  $\geq 6.9$  mg/L in control, vehicle control, and test substance treatments, which satisfies the acceptability criterion of  $\geq 3$  mg/L. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*. The 24-hour EC<sub>50</sub> value was estimated to be  $>1.3$  µg X642188/L, the highest concentration tested. The estimated 48-hour EC<sub>50</sub> value was 0.79 µg X642188/L, with 95% confidence limits of 0.66 and 0.95 µg X642188/L. No sublethal effects were noted during the definitive test. The 48-hour NOEC was 0.33 µg X642188/L.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	<i>Daphnia magna</i>	X642188	48-hr	EC <sub>50</sub>	0.79	µg X642188/L

### A 2.2.1.5 Study 5 – GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no deviations.</p> <p>Due to known instability of one of the active substances (fenpicoxamid) the study was performed in a semi-static design with renewal intervals of 8 hours. The analytical verification of both active compounds in the test solutions was performed at following time intervals:</p> <ul style="list-style-type: none"> <li>- at 0, 2 and 8 hours: in all test groups,</li> <li>- at 40, 42 and 48 h: in the two lowest concentration groups (31 and 63 µg test item/L, nominal),</li> <li>- in the highest concentration group additional measurements were performed at 16, 24 and 32 hours in fresh test solutions.</li> </ul> <p>It is noted by the zRMS that in general, the analytical measurements should be performed in all fresh and spent test solutions, but in this study no chemical analyses were performed for renewal intervals 8-16, 16-24, 24-32 and 32-40 hours, with exception of fresh test solutions of the highest treatment level at 16, 24 and 32 hours. Nevertheless, the decline pattern at both intervals of 0-8 and 40-48 hours was comparable and in opinion of the zRMS it is not expected that it would be different at remaining renewals. Taking this into account, the performed analyses are considered sufficient to derive endpoints based on average time weighted geometric mean measured concentration.</p> <p>As already mentioned above, at both renewal intervals the decline pattern of fenpicoxamid was similar. As expected, the measured concentrations dropped already 2 hours after preparation of fresh test solutions (45.8-61.1% of nominal, depending on the test group). In aged solutions the measured concentrations were at 13.5 to 16.2% of</p>
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	<p>nominal in three highest treatment groups, &lt;LOQ or between LOD and LOQ in the lowest test concentration and between LOD and LOQ to 15.9% of nominal at the second test concentration (63 µg test item/L, nominal).</p> <p>Prothioconazole was more stable in test solutions with measured concentrations ranging from 87.8 to 109% of nominal in fresh test solutions and from 65.6 to 86.9% of nominal in aged test solutions.</p> <p>The additional sampling at 2 and 42 hours enabled to consider ½ LOD or ½ LOQ in calculation of the time weighted geometric mean measured concentrations, in line with indications of EFSA Supporting publication 2015:EN-924 and OECD 23.</p> <p>Since fenpicoxamid was much less stable than prothioconazole, the endpoints for the formulated product were based on time weighted geometric mean measured concentration of fenpicoxamid.</p> <p>In the test report no justification was given why endpoints were based on TWA concentrations and not on commonly agreed geometric mean measured concentrations, however possibility to express endpoints in terms of TWA concentrations is given in OECD 23 (point 9, page 59, second edition of 2019) and is thus accepted by the zRMS..</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint:</p> <p>EC<sub>50</sub> = 0.015 mg product/L (based on time weighted geometric mean concentration), corresponding to 0.00071 mg a.s./L</p> <p>It has to be noted that results of this study were not considered in the risk assessment but were used to compare toxicity of three fenpicoxamid formulations (GF-2925, GF-3308 and GF-3307) to <i>Daphnia magna</i> in order to demonstrate that GF-2925 is most toxic and that the EU agreed endpoint from the mesocosm study performed with GF-2925 may be used in the higher tier refinement for GF-3308.</p>
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Reference:	KCP 10.2.1/5
Report:	Goudie, O.J., Schneider, S.Z., Zhang, L, Martin, K.H.; 2020; GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> ); Eurofins EAG Agrosience, LLC, Easton, Maryland, USA; Lab Study No. 379A-305; DAS Study No. 191366 ; 20 February 2020; Unpublished
Guideline(s):	OECD Guideline 202
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable. Please note that in assessment for GF-3308 endpoint from study with GF-3307 is used for comparative purposes only (see point 9.5 of this report for details).
Duplication (if vertebrate study)	No

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name):	GF-3307
Purity:	4.7 wt% (49 g/L) Fenpicoxamid, 9.7 wt% (101 g/L) Prothioconazole
Description (physical state):	Liquid
Lot/batch no.:	MAR19CE01Q (TSN400550)

## Test System

Organism ( <i>Species</i> ):	Cladoceran ( <i>Daphnia magna</i> )
Study type:	Acute
Study design:	Static-renewal (every 8 hours)
Test concentrations:	Nominal: 31, 63, 125, 250, 500 µg GF-3307/L Time weighted geometric mean calculated: 13, 24, 53, 100, 180 µg GF-3307/L
Parameters measured:	Immobility
Observation intervals:	24 and 48 hours
Age of test organisms at test initiation:	Neonate <24 hours old
Analytical confirmation of test concentrations:	0, 16, 24, 32, and 40 hours (fresh), 2, 8, 42, and 48 hours (spent). Only the high treatment was sampled at 16, 24, and 32 hours to confirm appropriate dosing.
No. of holding days before dosing:	None
Number of daphnia per dose group:	20
Number of daphnia per control group:	20
Environmental conditions:	Loading rate: 40 mL/ daphnid Temperature: 19.2 – 21.0°C Photoperiod: 16-hr light: 8-hr dark Dissolved oxygen concentration: (fresh solutions): 8.9 to 9.1 mg/L (98 to 100% saturation) (spent solutions): 8.8 to 9.1 mg/L (97 to 100% saturation) pH: 7.9 – 8.3
Reference substance:	Fenpicoxamid (TSN302306) and prothioconazole (TSN312881)

## Methodology

A definitive test was performed from 29 to 31 October 2019 at nominal concentrations of 0 (control), 31, 63, 125, 250, and 500 µg GF-3307/L. The daphnids used in the test were neonates (<24-hours old) obtained from cultures maintained by Eurofins-Easton. Daphnids were transferred to newly-prepared test solutions approximately every 8 hours during the exposure. Four replicate test chambers were maintained in each treatment and control group, with five neonates in each test chamber, for a total of 20 daphnids per concentration. The daphnids were observed for immobility and sublethal effects at each test solution renewal period. The observations performed at approximately 24 and 48 hours after test initiation were used for reporting exposure effects.

Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in fresh parent solutions at initiation and each solution renewal, and in individual replicate test chambers for the corresponding spent solutions at each solution renewal and test termination. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods. The measured light intensity at initiation of the definitive test was 568 lux.

## RESULTS AND DISCUSSION

Due to the rapid degradation of the fenpicoxamid active ingredient, a time weighted geometric mean calculated concentration based on fenpicoxamid analysis was determined for GF-3307 for 0-8 hours, and 40-48 hours in treatments with surviving organisms. A mean of the two exposure periods was then calculated where applicable to provide an overall time weighted geometric mean concentration. The

overall time weighted geometric mean fenpicoxamid concentrations were 0.633, 1.13, 2.50, 4.73, and 8.60 µg a.i./L, which represented 36 to 43% of the nominal fenpicoxamid concentrations. Based on the calculated fenpicoxamid concentrations, the time weighted geometric mean calculated GF-3307 concentrations in the test substance treatment solutions during the 48-hour exposure were 13, 24, 53, 100, and 180 µg GF-3307/L, which represented recoveries of 37 to 43% of the nominal concentrations.

One immobile daphnid was observed in the negative control group at 16 hours following test initiation. All other daphnids in the negative control group appeared normal throughout the test. Percent immobility in the 13, 24, 53, 100, and 180 µg GF-3307/L treatment groups at test termination was 35, 85, 100, 100, and 100%, respectively.

The 24-hour EC<sub>50</sub> value was estimated to be 32 µg GF-3307/L (95% CI: 24 and 53 µg GF-3307/L). The 48-hour EC<sub>50</sub> value was estimated to be 15 µg GF-3307/L (95% CI: 1.7 and 18 µg GF-3307/L). The sub lethal effect of lethargy was observed among the surviving daphnids in the 13, 24, and 180 µg GF-3307/L treatment groups during the exposure. A no-immobility concentration and NOEC were not determined. The lowest 100% immobility concentration was 53 µg GF-3307/L.

TABLE 1  
MEASURED CONCENTRATIONS OF FENPICOXAMID IN TEST SOLUTION SAMPLES

Nominal GF-3307 Concentration (µg GF-3307/L)	Nominal Fenpicoxamid Concentration (µg a.i./L)	Sample Number (379A-305-)	Sampling Time (Hour)	Measured Concentration <sup>1,2</sup> Fenpicoxamid (µg a.i./L)	Fenpicoxamid Percent of Nominal <sup>2</sup>	Fenpicoxamid Time-Weighted Geometric Mean Measured Concentration <sup>3</sup> (µg a.i./L)	Fenpicoxamid Time-Weighted Geometric Mean Measured Percent of Nominal <sup>1</sup>
Negative Control (0.0)	Negative Control (0.0)	1	0	< LOD	--	--	--
		7	2	< LOD	--		
		13	8	< LOD	--		
		22	40	< LOD	--		
		25	42	< LOD	--		
		28	48	< LOD	--		
31	1.5	2	0	1.48	98.8	0.633	43
		8	2	0.917	61.1		
		14	8	< LOD	--		
		23	40	1.33	88.7		
		26	42	0.846	56.4		
		29	48	> LOD but < LOQ	--		
63	3.0	3	0	2.97	98.9	1.13	38
		9	2	1.37	45.8		
		15	8	> LOD but < LOQ	--		
		24	40	2.64	87.9		
		27	42	1.73	57.6		
		30	48	0.478 <sup>4</sup>	15.9		
125	5.9	4	0	5.67	96.1	2.50	42
		10	2	3.27	55.4		
		16	8	0.957	16.2		
250	12	5	0	9.99	83.3	4.73	39
		11	2	6.53	54.4		
		17	8	1.70	14.2		
500	24	6	0	19.7	82.2	8.60 <sup>5</sup>	36
		12	2	11.3	46.9		
		18	8	3.25	13.5		
		19	16	20.6	86.0		
		20	24	20.7	86.2		
		21	32	22.0	91.5		

<sup>1</sup> The method limit of quantitation (LOQ) for the analysis of fenpicoxamid in freshwater was 0.705 µg a.i./L, defined as the lowest nominal concentration in a fortified sample in which a mean recovery of 70-110% was obtained. The method limit of detection (LOD) is defined as 30% of LOQ. The LOD was 0.212 µg a.i./L for fenpicoxamid.

<sup>2</sup> Results were generated using Analyst Version 1.6.3 and Excel 2010 in full precision mode. Manual calculations may differ slightly.

<sup>3</sup> Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

<sup>4</sup> Sample result is below LOQ but quantifiable because the peak area count is within the calibration curve range.

<sup>5</sup> The reported result was calculated using measured concentrations from the 0, 2, and 8-hour samples only.

Note: Where measured concentrations in test treatment samples were either <LOD, or >LOD but <LOQ, the LOD value (0.212 µg a.i./L) was used for the calculation of time weighted geometric mean exposures.

TABLE 2  
MEASURED CONCENTRATIONS OF PROTHIOCONAZOLE IN TEST SOLUTION  
SAMPLES

Nominal GF-3307 Concentration (µg GF-3307/L)	Nominal Prothioconazole Concentration (µg a.i./L)	Sample Number (379A-305-)	Sampling Time (Hour)	Measured Concentration <sup>1,2</sup> Prothioconazole (µg a.i./L)	Prothioconazole Percent of Nominal <sup>2</sup>	Prothioconazole Time-Weighted Geometric Mean Measured Concentration <sup>3</sup> (µg a.i./L)	Prothioconazole Time-Weighted Geometric Mean Measured Percent of Nominal <sup>1</sup>
Negative Control (0.0)	Negative Control (0.0)	1	0	< LOD	--	--	--
		7	2	< LOD	--	--	--
		13	8	< LOD	--	--	--
		22	40	< LOD	--	--	--
		25	42	< LOD	--	--	--
		28	48	< LOD	--	--	--
31	3.0	2	0	3.26	109	2.67	89
		8	2	2.93	97.8		
		14	8	1.97	65.6		
		23	40	3.01	100		
		26	42	2.81	93.7		
		29	48	2.61	86.9		
63	6.1	3	0	6.35	104	5.40	89
		9	2	5.64	92.5		
		15	8	4.49	73.7		
		24	40	6.14	101		
		27	42	5.60	91.7		
		30	48	5.23	85.7		
125	12	4	0	11.2	93.2	9.81	82
		10	2	10.5	87.3		
		16	8	8.50	70.9		
250	24	5	0	21.9	91.4	19.7	82
		11	2	20.1	83.7		
		17	8	18.5	76.9		
500	49	6	0	43.0	87.8	38.5 <sup>4</sup>	79
		12	2	40.0	81.6		
		18	8	35.2	71.9		
		19	16	44.6	91.1		
		20	24	45.9	93.6		
		21	32	44.6	91.0		

<sup>1</sup> The method limit of quantitation (LOQ) for the analysis of prothioconazole in freshwater was 1.46 µg a.i./L, defined as the lowest nominal concentration in a fortified sample in which a mean recovery of 70-110% was obtained. The method limit of detection (LOD) is defined as 30% of LOQ. The LOD was 0.437 µg a.i./L for prothioconazole.

<sup>2</sup> Results were generated using Analyst Version 1.6.3 and Excel 2010 in full precision mode. Manual calculations may differ slightly.

<sup>3</sup> Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

<sup>4</sup> The reported result was calculated using measured concentrations from the 0, 2, and 8-hour samples only.

TABLE 3  
CALCULATED OVERALL CONCENTRATIONS OF GF-3307 IN TEST SOLUTION  
SAMPLES

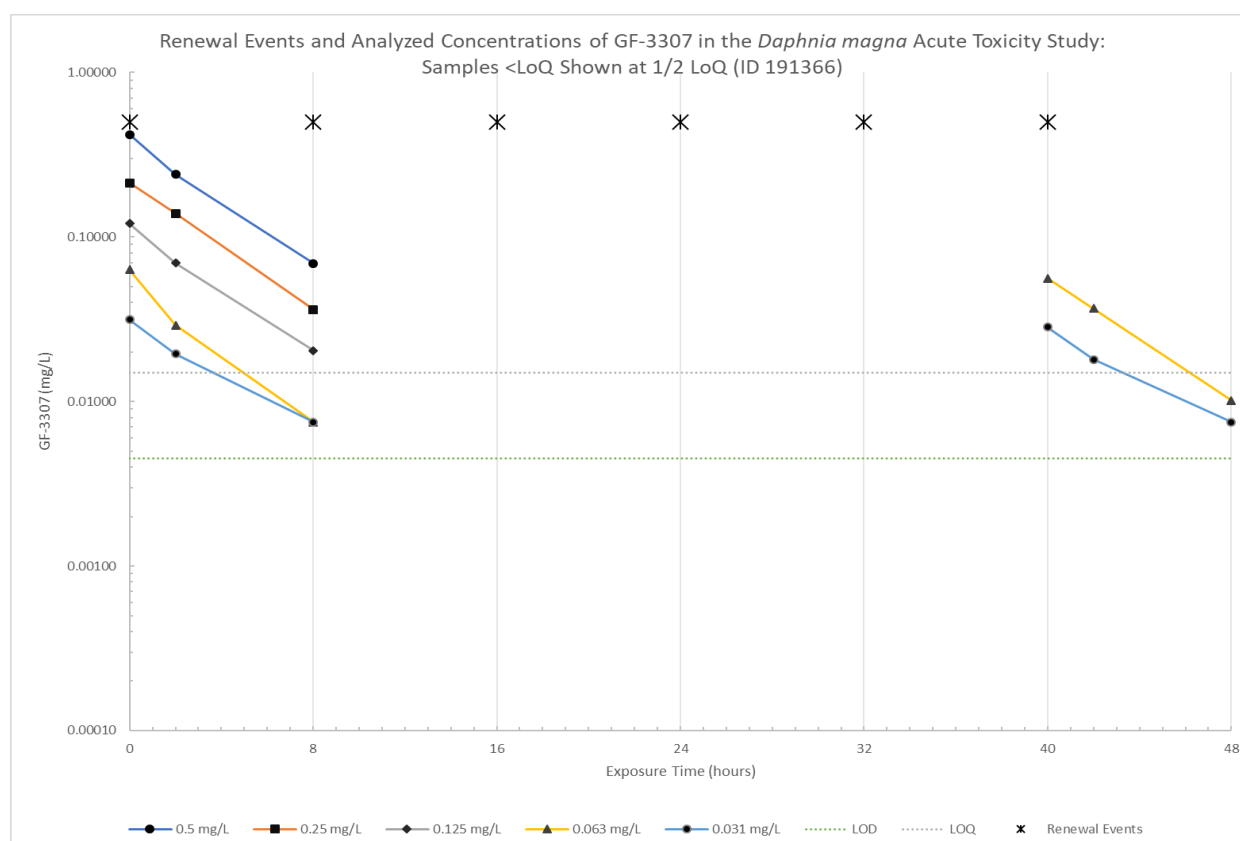
Nominal GF-3307 Concentration ( $\mu\text{g GF-3307/L}$ )	Nominal Fenpicoxamid Concentration ( $\mu\text{g a.i./L}$ )	Nominal Prothioconazole Concentration ( $\mu\text{g a.i./L}$ )	Time-Weighted Geometric Mean Measured Concentration <sup>1,2</sup>			GF-3307 Time- Weighted Mean Percent of Nominal <sup>2</sup>
			Fenpicoxamid ( $\mu\text{g a.i./L}$ )	Prothioconazole ( $\mu\text{g a.i./L}$ )	Measured GF-3307 Based on Fenpicoxamid ( $\mu\text{g GF-3307/L}$ ) <sup>3,4</sup>	
Negative Control (0.0)	Negative Control (0.0)	Negative Control (0.0)	--	--	--	--
31	1.5	3.0	0.633	2.67	13	43
63	3.0	6.1	1.13	5.40	24	38
125	5.9	12	2.50	9.81	53	43
250	12	24	4.73	19.7	100	40
500	24	49	8.60	38.5	180	37

<sup>1</sup> The limit of quantitation (LOQ) of GF-3307 for these analyses was set at 15.0  $\mu\text{g GF-3307/L}$  (0.705 and 1.46  $\mu\text{g a.i./L}$  for fenpicoxamid and prothioconazole, respectively), defined as the lowest fortified formulation concentration in a sample with an acceptable mean recovery between 70-110%.

<sup>2</sup> Results were generated using Analyst Version 1.6.3 and Excel 2010 in full precision mode. Manual calculations may differ slightly.

<sup>3</sup> The results for fenpicoxamid were divided by its weight percent in GF-3307 (4.7 wt%) to calculate the time-weighted geometric mean measured concentration of GF-3307. Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

<sup>4</sup> Reported to two significant figures.



**Figure 3. Decline of fenpicoxamid in the GF-3307 acute daphnid toxicity test with 8 hour renewals (Goudie, 2020, DAS 191366).** Test solutions were measured at 0, 2, 8 hours and 40, 42, and 48 hours. Low QC spike (LOQ = 0.015 mg GF-3307/L; limit of detection, LOD = 0.0045 mg GF-3307/L)

**Table 17: Effect of GF-3307 on immobilisation**

Average Time Weighted Geometric Mean Calculated Treatment ( $\mu\text{g GF-3307/L}$ )	24-hr		48-hr	
	No. immobile	% Immobility	No. immobile	% Immobility
Negative control	1	5	1	5
13	1	5	7	35
24	6	30	17	85
53	17	85	20	100
100	20	100	20	100
180	19	95	20	100
NOEC	--		--	
EC <sub>50</sub>	32 $\mu\text{g GF-3307/L}$		15 $\mu\text{g GF-3307/L}$	

**Table 18: Sub-lethal effects of GF-3307**

Average Time Weighted Geometric Mean Calculated Treatment ( $\mu\text{g GF-3307/L}$ )	Observation period	
	Lethargy (% affected)	
	24-hr	48-hr
Negative control	0	0
13	0	0
24	10	10
53	0	0
100	0	0
180	5	0

## CONCLUSION

All test acceptability criteria were met for this study. Immobilization among control daphnids was 5%, which is below the acceptability limit of 10% as stated in the protocol and the OECD 202 test guideline. The dissolved oxygen concentration at the end of the test was  $\geq 8.8$  mg/L in control and test substance treatments, higher than the acceptability minimum of  $\geq 3$  mg/L. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*. Based on the time-weighted geometric mean concentrations, the 48-hour EC<sub>50</sub> value was 15  $\mu\text{g GF3307/L}$ , with a 95% confidence interval of 1.7 to 18  $\mu\text{g GF-3307/L}$ . The slope of the concentrationresponse curve was calculated to be 5.49. The NOEC and no-immobility concentration could not be determined. The lowest 100% immobility concentration was 53  $\mu\text{g GF-3307/L}$ .

Common name	Species	Test Substance	Time-scale	Endpoint	Toxicity value	Units of GF3307
Cladoceran	<i>Daphnia magna</i>	GF-3307	48-hr	EC <sub>50</sub>	15	µg/L, twgm

**A 2.2.1.6 Study 6 – GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*)**

Comments of zRMS:	<p>The study was conducted in line with OECD 202 with no deviations regarding the test conditions.</p> <p>However, the in the test design only two test item concentrations were included, while OECD 202 clearly indicates that at least five test concentrations should be used. In the test report no justification for selection of only 2 test concentrations is given, but it may be related to high toxicity of fenpicoxamid at higher concentrations and difficulties with analytical verification of the active compound at lower concentrations, which are unquantifiable or undetectable in aged test solutions even when the renewal intervals of 8 hours are selected (with more frequent intervals the mortality in controls exceeds the validity criterion of 10%). Problems with analytical verification of fenpicoxamid</p>
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	<p>concentrations at lower treatment levels were observed already in two studies summarised above under KCP 10.2.1/03 (test with GF-3308) and KCP 10.2.1/5 (test with GF-3307).</p> <p>Nevertheless, study with GF-2925 was performed in order to compare toxicity of three fenpicoxamid formulations (GF-2925, GF-3308 and GF-3307) to <i>Daphnia magna</i> and demonstrate that GF-2925 is most toxic to justify that the EU agreed endpoint from the mesocosm study performed with GF-2925 may be used in the higher tier refinement for GF-3308. The results of the acute study with GF-2925 were thus not used directly in the risk assessment and the major deviation concerning too low number of tested concentrations is not a major issue in evaluation performed for GF-3308.</p> <p>Due to known instability of fenpicoxamid the study was performed in a semi-static design with renewal intervals of 8 hours. The analytical verification of the active compound was performed in all fresh and spent test solutions at each renewal interval in the lowest treatment group (0-8, 8-16, 16-24 and 24-32 hours). Additional chemical analyses were performed in aged solutions 2 hours after beginning each renewal interval (i.e. at 2, 10, 18 and 26 hours). In the highest treatment group chemical analyses were performed only for the first renewal interval at 0, 2 and 8 hours due to 100% immobilisation observed after 8 hours of exposure.</p> <p>Based on the analytical results, the time weighted geometric mean measured concentrations were calculated, in line with indications of EFSA Supporting publication 2015:EN-924 and OECD 23. In the test report no justification was given why endpoints were based on TWA concentrations and not on commonly agreed geometric mean measured concentrations, however possibility to express endpoints in terms of TWA concentrations is given in OECD 23 (point 9, page 59, second edition of 2019) and is thus accepted by the zRMS.</p> <p>The study was terminated after 32 hours due to 100% mortality observed in the lowest treatment level. At the highest test concentration 100% mortality was observed already after 8 hours.</p> <p>All validity criteria in controls were fulfilled, but due to only 2 tested concentrations and 100% mortality at both treatment levels, results of the study are not suitable for the risk assessment purposes. Nevertheless, they may be used as additional information to compare toxicity of three fenpicoxamid formulation (GF-2925, GF-3307 and GF-3308).</p> <p>Due to reasons mentioned above, the 48h EC<sub>50</sub> could not be determined, but it may be concluded that it is &lt;0.00165 mg test item/L (based on time weighted geometric mean concentration), corresponding to &lt;0.000203 mg a.s./L</p>
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Reference:	KCP 10.2.1/6
Report:	Goudie, O.J., Schneider, S.Z., Sneckenberger, G.W., Zhang, L.; 2021; GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran ( <i>Daphnia magna</i> ); Eurofins EAG Agrosience, LLC, Easton, Maryland, USA; Lab Study No. 379A-343; DAS Study No. 202284 ; 01 March 2021; Unpublished
Guideline(s):	OECD Guideline 202
Deviations:	Major (see the commenting box above)
GLP:	Yes
Acceptability:	Results of the study not suitable for risk assessment purposes since no reliable endpoint could be derived. Nevertheless, results may be used as supportive information in comparison of toxicity of various fenpicoxamid formulations (see point 9.5 of this report for details).



Duplication (if vertebrate study)	NA
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## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-2925  
Purity: 12.3 wt% (129 g/L) Fenpicoxamid  
Description (physical state): Liquid  
Lot/batch no.: F479I9O001 (TSN403981)

### Test System

Organism (*Species*): Cladoceran (*Daphnia magna*)  
Study type: Acute  
Study design: Static-renewal  
Test concentrations: Nominal: 2.44, 12.2 µg GF-2925/L (0.300, 1.50 µg fenpicoxamid/L)  
Time weighted geometric mean calculated: 1.65, 9.50 µg GF-2925/L (0.203, 1.17 µg fenpicoxamid/L)  
Parameters measured: Immobility  
Observation intervals: 8, 16, 24 and 32 hours  
Age of test organisms at test initiation: Neonate <24 hours old  
Analytical confirmation of test concentrations: 0, 8, 16 and 24 hours (fresh), 2, 8, 10, 16, 18, 24, 26, and 32 hours (spent). Only the low treatment was sampled after 8 hours due to 100% immobility occurring in the high treatment.  
No. of holding days before dosing: None  
Number of daphnia per dose group: 20  
Number of daphnia per control group: 20  
Environmental conditions: Loading rate: 40 mL/ daphnid  
Temperature: 19.2 – 20.7°C Photoperiod: 16-hr light: 8-hr dark Dissolved oxygen concentration: (fresh solutions): 8.8 to 9.1 mg/L (97 to 100% saturation)  
(spent solutions): 8.8 to 9.1 mg/L (97 to 100% saturation) pH: 7.9 – 8.3  
Reference substance: Fenpicoxamid (TSN302306)

### Methodology

A definitive test was performed from 21 to 22 January 2021 at nominal concentrations of 0 (control), 2.44, and 12.2 µg GF-2925/L. The daphnids used in the test were neonates (<24-hours old) obtained from cultures maintained by Eurofins-Easton. Surviving daphnids were transferred to newly-prepared test solutions approximately every 8 hours during the exposure. Four replicate test chambers were maintained in each treatment and control group, with five neonates in each test chamber, for a total of 20 daphnids per concentration. The daphnids were observed for immobility and sublethal effects at each test solution renewal period. The cumulative observations performed at approximately 32 hours after test initiation were used for reporting exposure effects.

Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in fresh parent solutions at initiation and each solution renewal, and in individual replicate test chambers for the corresponding spent solutions at each solution renewal and test termination. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with 30-minute simulated dawn and dusk periods. The mean measured light intensity at initiation of the definitive test was 880 lux.

## RESULTS AND DISCUSSION

Due to the rapid degradation of the fenpicoxamid active ingredient, a time weighted geometric mean calculated GF-2925 concentration based on fenpicoxamid analysis was determined for each 8-hour solution renewal period. Measured concentrations of fenpicoxamid were converted to calculated GF2925 concentrations based on the fenpicoxamid active ingredient of 12.3%, and time-weighted geometric mean calculated GF-2925 concentrations subsequently calculated. A mean of the applicable exposure periods was then calculated to provide an overall time weighted geometric mean concentration. Based on measured fenpicoxamid concentrations, the time weighted geometric mean calculated GF-2925 concentrations in the test substance treatment solutions during the 32-hour exposure were 1.65 and 9.50 µg GF-2925/L (0.203 and 1.17 µg fenpicoxamid/L), which represented recoveries of 67.5 and 77.8% of the nominal concentrations, respectively.

Due to 100% immobility, the time weighted geometric mean calculated 9.50 µg GF-2925/L treatment solution was not renewed at the 8-hour observation point. The biological response results are reported based upon the time weighted geometric mean calculated GF-2925 test solution concentrations. The control and all test treatment solutions were clear and colourless throughout the test. Water quality parameters of temperature, dissolved oxygen concentration and pH remained within acceptable limits throughout the definitive test. Based on time weighted geometric mean calculated GF-2925 concentrations, the 32-hour EC<sub>50</sub> value was estimated to be < 1.65 µg GF-2925/L (< 0.203 µg fenpicoxamid/L).

All daphnids in the negative control group appeared normal throughout the test. Percent immobility in the 1.65 and 9.50 µg GF-2925/L treatment groups at test termination was 100%. The sub lethal effect of lethargy was observed in the 1.65 µg GF-2925/L treatment group during the exposure. The 32-hour NOEC was not determined.

**Table 19: Calculated Concentrations of GF-2925 in Test Solution Samples**

Nominal Test Substance		Calculated GF-2925 Concentration as µg/L (Percent of Nominal) <sup>1,2,3</sup>										
Concentration	Sample ID	0-Hour	Sample ID	2-(Hour old)	Sample ID	8-(Hour old)	Sample ID	8(-newHour )	Sample ID	10(-oldHour )	Sample ID	16(-oldHour ) (µg GF-2925/L)
Negative Control	1	0 (N/A)	6	0 (N/A)	13	0 (N/A)	9	0 (N/A)	16	0 (N/A)	22	0 (N/A)
2.44	2	2.97 (122)	7	1.98 (81.3)	14	0.767 (31.4)	10	3.05 (125)	17	2.00 (82.0)	23	0.810 (33.2)
12.2 <sup>4</sup>	3	14.7 (121)	8	13.3 (109)	15 <sup>6</sup>	4.31 (35.3)	---	---	---	---	---	---
122 <sup>5</sup>	5	120 (98.6)	---	---	---	---	12	131 (107)	---	---	---	---
8000 <sup>5</sup>	4	8460 (106)	---	---	---	---	11	11380 (142)	---	---	---	---

<sup>1</sup> The limit of detection (LOD) was 0.0480 µg GF-2925/L (0.00590 µg a.i./L fenpicoxamid), defined as 30% of the LOQ.

<sup>2</sup> Results were generated using Microsoft Excel, based on the fenpicoxamid active ingredient at 12.3%. Manual calculations may differ slightly. <sup>3</sup> Sample ID: 379A-343-n, where n = sample number.

<sup>4</sup> Not sampled after 8-Hour old solutions due to 100% immobility in treatment.

<sup>5</sup> Stock solutions sampled at fresh preparation only.

**Table 20: Calculated Concentrations of GF-2925 in Test Solution Samples (cont.)**

Calculated GF-2925 Concentration as µg/L (Percent of Nominal) <sup>1,2,3</sup>											
Test	Concentration Substance ID <sup>3</sup>	Sample ID <sup>3</sup>	16(new-Hour ) Sample ID <sup>3</sup>	18(-oHour Id) Sample ID <sup>3</sup>	24(-oldHour ) Sample ID <sup>3</sup>	24(new-Hour ) Sample ID <sup>3</sup>	26(-oldHour ) Sample ID <sup>3</sup>	32(new-Hour ) Sample ID <sup>3</sup>	38(-oldHour ) Sample ID <sup>3</sup>	46(new-Hour ) Sample ID <sup>3</sup>	Sample ID <sup>3</sup>
(µg GF-2925/L)											
Negative Control	18	0	24	0	30	0	26	0	32	0	38

GF-3308

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		(N/A)		(N/A)		(N/A)		(N/A)		(N/A)		(N/A)
2.44	19 <sub>6</sub>	2.20 (90.3)	25 <sub>6</sub>	2.37 (97.3)	31	0.984 (40.3)	27	3.09 (127)	33	1.85 (76.0)	39	1.03 (42.3)
12.2 <sup>4</sup>	---	---	---	---	---	---	---	---	---	---	---	---
122 <sup>5</sup>	21	146 (119)	---	---	---	---	30	141 (116)	---	---	---	---
8000 <sup>5</sup>	20	12850 (161)	---	---	---	---	29	8700 (109)	---	---	---	---

<sup>1</sup> The limit of detection (LOD) was 0.0480 µg GF-2925/L (0.00590 µg a.i./L fenpicoxamid), defined as 30% of the LOQ.

<sup>2</sup> Results were generated using Microsoft Excel, based on the fenpicoxamid active ingredient at 12.3%. Manual calculations may differ slightly. <sup>3</sup>

ID: 379A-343-n, where n = sample number.

<sup>4</sup> Not sampled after 8-Hour old solutions due to 100% immobility in treatment.

<sup>5</sup> Stock solutions sampled at fresh preparation only.

<sup>6</sup> Backup samples were analyzed to confirm original results. Average of the original and backup analyses is reported.

Sample

**Table 21: Overall Test Treatment Exposure Concentrations of GF-2925**

Nominal GF-2925 Concentration (µg/L)	Time-Weighted Geometric Mean Concentration in µg GF-2925/L <sup>1</sup>				Overall Time Weighted Geometric Mean Concentration (% Nominal) in µg GF-2925/L
	0-8 Hours	8-16 Hours	16-24 Hours	24-32 Hours	
0.0	0	0	0	0	0 (N/A)
2.44	1.57	1.61	1.76	1.66	1.65 (67.5)
12.2 <sup>2</sup>	9.50	---	---	---	9.50 (77.8)

<sup>1</sup> Results were generated using Microsoft Excel, based on calculated individual GF-2925 concentrations. Manual calculations may differ slightly.

<sup>2</sup> Not sampled after the 8-hour time point due to 100% immobility in treatment.

**Table 22: Effect of GF-2925 on immobilisation**

Average Time Weighted Geometric Mean Calculated Treatment (µg GF-2925/L)	Average Time Weighted Geometric Mean Calculated Treatment (µg a.s./L)	8-hr		32-hr	
		No. immobile	% Immobility	No. immobile	% Immobility
Negative control	Negative control	0	0	0	0
1.65	0.203	11	55	20	100
9.50	1.17	20	100	20	100
NOEC		--		--	
EC <sub>50</sub>		--		< 1.65 µg GF-2925/L < 0.203 µg a.s./L	

**Table 23: Sub-lethal effects of GF-2925**

Average Time Weighted Geometric Mean Calculated Treatment (µg GF-2925/L)	Average Time Weighted Geometric Mean Calculated Treatment (µg a.s./L)	Observation period			
		Cumulative No. Lethargic (% affected)			
		8-hr	16-hr	24-hr	32-hr
Negative control	Negative control	0	0	0	0
1.65	0.203	1 (5)	0	3 (15)	--
9.50	1.17	--	--	--	--

## CONCLUSION

All test acceptability criteria were met for this study. Immobilization among control daphnids was 0%, which is below the acceptability limit of 10% as stated in the protocol and the OECD 202 (2004) test guideline. The dissolved oxygen concentration at the end of the test was  $\geq 8.7$  mg/L in control and test substance treatments, higher than the acceptability minimum of  $\geq 3$  mg/L. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*. Based on the time-weighted geometric mean concentrations, the 32-hour EC<sub>50</sub> value was  $<1.65$  µg GF2925/L ( $<0.203$  a.s./L). The NOEC was not determined. The lowest 100% immobility concentration was 1.65 µg GF-2925/L (0.203 a.s./L).

Common name	Species	Test Substance	Time-scale	Endpoint	Toxicity value	Units
Cladoceran	<i>Daphnia magna</i>	GF-2925	32-hr	EC <sub>50</sub>	$< 1.65$	µg GF-2925/L, twgm
Cladoceran	<i>Daphnia magna</i>	GF-2925	32-hr	EC <sub>50</sub>	$< 0.203$	µg fenpicoxamid/L, twgm

#### A 2.2.1.7 Study 7 - GF-3307: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static-Renewal Test Conditions

Comments of zRMS:	<p>Results of the study on toxicity of GF-3308 to <i>Daphnia magna</i> in semi-static test with 24 hour renewals were used by the Applicant to demonstrate that the representative formulation (GF-2925) is most toxic among three formulated products (GF-2925, GF3307 and GF-3308). However, no study with 24 hour renewal intervals was performed with the formulation evaluated in this report (GF-3308) and for this reason results of the study performed with GF-3307 were not useful in this comparison.</p> <p>Studies performed with 8 hour renewal intervals were performed with all three formulations and are deemed sufficient for comparative purposes. Taking this into account, the study below was not evaluated by the zRMS as not necessary. The study summary below was struck through as being not validated.</p>
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Reference:	KCP 10.2.1/7
Report:	xxx 2014; GF-3307: Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions; xxx; Lab Study No. 81070; DAS Study No. 140489; 09 January 2018; Revised ; Unpublished
Guideline(s):	OECD Guideline 202
Deviations:	Not validated
GLP:	Yes
Acceptability:	Not evaluated as being not necessary for purposes of the risk assessment performed for GF-3308.
Duplication (if vertebrate study)	No

#### ~~MATERIALS AND METHODS~~ Test Item(s)

~~ISO Common name:~~

~~Test item — (chemical/other name):~~ GF-3307

~~Purity:~~ 4.8% w/w XDE 777 and 9.4% w/w prothioconazole

~~Description (physical state):~~ Not stated

~~Lot/batch no.:~~ F1281-135-1 (TSN307579)

CAS no.: Not applicable

### Test System

Organism ( <i>Species</i> ):	Cladoceran ( <i>Daphnia magna</i> )
Study type:	Acute
Study design:	Static renewal, every 24 hours
Test concentrations:	Nominal: 0 (control), 0.0097, 0.021, 0.047, 0.10, 0.23, and 0.50 mg GF 3307/L (0 (control), 9.7, 21, 47, 100, 230, and 500 µg GF 3307/L) Geometric mean calculated: <MQL (control), 1.59, 2.53, 3.68, 5.11, 7.81, and 11.8 µg GF 3307/L
Parameters measured:	Immobility
Observation intervals:	24 hours
Age of test organisms at test initiation:	<24 hours
Analytical confirmation of test concentrations:	0, 24 and 48 hours
No. of holding days before dosing:	None
Number of daphnia per dose group:	20
Number of daphnia per control group:	20
Environmental conditions:	Loading rate: Not provided Temperature: 20.0 to 20.5°C Photoperiod: 16 hour light:8 hour dark Dissolved oxygen concentration: New 8.1 to 8.8 mg/L (93 to 101% sat.) Old 8.3 to 8.7 mg/L (95 to 100% sat.) pH: 8.2 to 8.5
Reference substance:	Not applicable

### Methodology

A definitive test was performed at nominal concentrations 0 (control), 0.0097, 0.021, 0.047, 0.10, 0.23, and 0.50 mg GF 3307/L (0 (control), 9.7, 21, 47, 100, 230, and 500 µg GF 3307/L). Five neonates (<24 hours old) were added to each of four test chambers per treatment at the start of the test. The daphnids were observed for immobility and sublethal effects at approximately 24 and 48 hours after test initiation. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in all treatment replicates daily. A thermistor probe was located in a surrogate test chamber to continuously record temperature.



## RESULTS AND DISCUSSION

Table 1. Calculated Concentrations of Total Product GF-3307 from Measured Concentrations of XDE-777 During the 48-Hour Acute Toxicity Test with *Daphnia magna*

Nominal Concentration mg GF-3307/L / µg GF-3307/L	Calculated Concentration as mg GF-3307/L (Percent Nominal)				Geometric Mean Calculated Concentration mg TP/L / µg TP/L
	0-Hour	24-Hour (old)	24-Hour (new)	48-Hour (old)	
Control	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
0.0097 / 9.7	0.00596 (61)	<MQL <sup>b, c</sup>	0.00621 (64)	<MQL <sup>b, c</sup>	0.00159 / 1.59 (16)
0.021 / 21	0.0157 (75)	<MQL <sup>b, c</sup>	0.0150 (71)	<MQL <sup>b, c</sup>	0.00253 / 2.53 (12)
0.047 / 47	0.0329 (70)	<MQL <sup>b, c</sup>	0.0321 (68)	<MQL <sup>b, c</sup>	0.00368 / 3.68 (8)
0.10 / 100	0.0638 (64)	<MQL <sup>b, c</sup>	0.0620 (62)	<MQL <sup>b, c</sup>	0.00511 / 5.11 (5)
0.23 / 230	0.150 (65)	<MQL <sup>b, c</sup>	0.143 (62)	<MQL <sup>b, c</sup>	0.00781 / 7.81 (3)
0.50 / 500	0.328 (66)	<MQL <sup>b, c</sup>	0.335 (67)	<MQL <sup>b, c</sup>	0.0118 / 11.8 (2)
<b>QC Fortification Spikes (% Recovery)</b>					
Low Spike (0.00900) / (9.00)	0.00842 (94)	0.00917 (102)	0.00896 (100)	0.00871 (97)	---
High Spike (0.560) / (560)	0.552 (99)	0.583 (104)	0.555 (99)	0.538 (96)	---

TP = Total Product (GF-3307)

<sup>a</sup> Initial mean measured concentrations are the average of the 0- and 24-hour (new) measured concentrations.<sup>b</sup> MQL = 0.000833 mg GF-3307/L (0.833 µg GF-3307/L)<sup>c</sup> When <MQL, ½ of MQL (0.000417 mg GF-3307/L (0.417 µg GF-3307/L)) used for mean calculations.

Note: 1,000 µg/L = 1 mg/L.

Table 24: Effect of GF-3307 on immobilisation

Treatment, geometric mean calculated concentration (µg GF-3307/L)	24-hr		48-hr	
	No. immobile	% Immobility	No. immobile	% Immobility
0 (control)	0	0	0	0
1.59	0	0	0	0
2.53	0	0	0	0
3.68	0	0	0	0
5.11	0	0	0	0
7.81	0	0	7	35 <sup>±</sup>
11.8	6	30	20	100 <sup>±</sup>
NOEC	Not calculated		5.11 µg GF-3307/L	
EC <sub>50</sub>	> 11.8 µg GF-3307/L		8.29 µg GF-3307/L (95% CI: 7.58 and 9.07 µg GF-3307/L)	

Table 25: Sub-lethal effects of GF-3307

Treatment, geometric mean calculated concentrations (µg GF-3307/L)	Observation period	
	Sub-lethal effects (% affected)	
	24-hr	48-hr
0 (control)	0	0
1.59	0	0
2.53	0	0

3.68	0	0
5.11	0	0
7.81	0	0
11.8	0	0

### CONCLUSION

All test acceptability criteria were met for this study. Immobilization among control daphnids was 0%, which is below the acceptability limit of 10% as stated in the protocol and the OECD 202 test guideline. The dissolved oxygen concentration at the end of the test was  $\geq 8.3$  mg/L in control and test substance treatments, higher than the acceptability minimum of 3 mg/L. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*. Based on the geometric mean calculated concentration, the 24-hour  $EC_{50}$  value was estimated to be  $>11.8$   $\mu$ g GF-3307/L, the highest concentration (95% confidence limits could not be calculated). The 48-hour  $EC_{50}$  value was estimated to be 8.29  $\mu$ g GF-3307/L (95% confidence limits of 7.58 and 9.07  $\mu$ g GF-3307/L) based on geometric mean calculated concentrations. The 48-hour NOEC was 5.11  $\mu$ g GF-3307/L, based on the absence of statistically significant immobility and sublethal effects at this, and all lower test substance concentrations.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	<i>Daphnia magna</i>	GF-3307	48 hr	$EC_{50}$	8.29	$\mu$ g/L gm

### A 2.2.1.8 Study 8 – X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions

Comments of zRMS:	<p>The study was conducted in line with OECD 203 (1992) with no deviations.</p> <p>Throughout the test the concentrations of metabolite X12019520 were maintained within 80-120% of nominal concentration; therefore, the endpoint is expressed in terms of the nominal concentration.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p><math>LC_{50} &gt; 10</math> mg pm/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/8
Report:	xxx.; 2018; X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions; xxx; Lab Study No. 87146; DAS Study No. 180560 ; 07 August 2018; Unpublished
Guideline(s):	OECD Guideline 203
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

Duplication (if vertebrate study)	No
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## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): X12019520 (a metabolite of XDE-777)  
Purity: 80%  
Description (physical state): Not provided  
Lot/batch no.: SYN-FS10703-098 [TSN307264]

### Test System

Organism (*Species*): Rainbow trout (*Oncorhynchus mykiss*)

Study type: Acute

Study design: Static-renewal

Test concentrations: Nominal: 0 (control) and 10 mg X12019520/L

Mean measured: <MQL (control) and 9.8 mg X12019520/L

Parameters measured: Mortality

Observation intervals: 24 hours

Age, weight and length of fish at test initiation: Age: >14 days

Mean blotted wet weight: 1.4523 ± 0.2795 g (1.0936 to 1.8092 g)

Mean total length: 53 ± 3.3 mm (48 to 57 mm)

Analytical confirmation of test concentrations: On days: 0 hour (fresh), 48 hours (spent and fresh), and 96 hours(spent)

No. of holding days before dosing: 14

Number of fish per dose group: 7

Number of fish per control group: 7

Feeding regime: Fish were fed salmon starter daily during holding, none during exposure

Environmental conditions: Loading rate: Instantaneous biomass loading: 0.5648 g/L

Temperature: 15.0 to 15.6 °C Photoperiod: 16-hr light:8-hr dark Dissolved oxygen concentration: Fresh 10.0 to 10.7 mg/L (103 to 113% sat.) Spent 6.9 to 9.9 mg/L (71 to 102% sat.) pH: 7.6 to 9.2

Total hardness: 150 mg CaCO<sub>3</sub>/L

Salinity: not applicable

Reference substance: none

### Methodology

A static-renewal definitive limit test was performed from 11 to 15 April 2018 at nominal concentrations of 0 (control) and 10 mg X12019520/L. Seven fish were impartially assigned to treatment replicates by adding one fish per chamber, proceeding from control, then test substance treatment, and repeating steps as necessary until seven fish were present in each replicate test chamber for a total of seven fish per test

treatment. Observations for mortality and sublethal responses were made at approximately 24, 48, 72, and 96 hours. In an effort to maintain maximal exposure to the test substance, the control and treated test solutions were freshly prepared and renewed at 48 hours. Temperature, dissolved oxygen, and pH were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was also maintained. Total hardness and alkalinity of the dilution water were measured using titrimetric methods adapted from Standard Methods. Conductivity of the dilution water was measured on test initiation day. The light intensity at definitive test initiation was 887 lux.

## RESULTS AND DISCUSSION

The concentration of X12019520 was measured in test solution samples collected from fresh solutions at initiation and 48-hours, and from spent solutions at 48- and 96-hours of the definitive test. The mean calculated concentrations in for the 10 mg X12019520/L test substance treatment solutions during the 96-hour exposure was 9.8 mg X12019520/L, which represented recoveries of 98% of the nominal concentrations. The biological response results were reported based upon the nominal X12019520 concentrations. After 96 hours of exposure, mortality was 0% in the control and test treatment. No sublethal effects were observed.

Table 2. Measured Concentrations of X12019520 During the Static-Renewal Acute Toxicity Test with Rainbow Trout, *Oncorhynchus mykiss*

Nominal Concentration (mg X12019520/L)	Measured Concentration as mg X12019520/L (Percent Nominal)				Arithmetic Mean Measured Concentration
	0 Hour	48 Hours (spent)	48 Hours (fresh)	96 Hours	
0 (control)	<MQL <sup>a</sup>	<MQL <sup>a</sup>	<MQL <sup>a</sup>	<MQL <sup>a</sup>	<MQL <sup>a</sup>
10	9.7 (97)	9.4 (94)	10 (103)	9.9 (99)	9.8 (98)
QC Fortification Spikes (% Recovery)					
Low Spike (4.9)	5.2 (106)	4.8 (98) <sup>b</sup>	5.1 (104) <sup>b</sup>	5.7 (116)	NA
High Spike (14)	15 (104)	15 (104) <sup>b</sup>	14 (100) <sup>b</sup>	15 (109)	NA

<sup>a</sup> MQL = 0.80 mg X12019520/L.

<sup>b</sup> Samples were re-diluted in duplicate, and the average of the two are reported.

NA= Not Applicable

Table 26: Effect of X12019520 on mortality of Rainbow trout

Treatment (mg X12019520/L)		No. of fish	Cumulative mortality (%)			
Nominal	Mean measured		24-hr	48-hr	72-hr	96-hr
Negative control	<MQL	7	0 (0)	0 (0)	0 (0)	0 (0)
10	9.8	7	0 (0)	0 (0)	0 (0)	0 (0)
LC <sub>50</sub>	>10 mg X12019520/L					
95% C.I.	Not calculated					
NOEC	10 mg X12019520/L					

Table 27: Sub-lethal effects of X12019520 in Rainbow trout

Treatment (mg X12019520/L)		Observation period			
Nominal	Mean measured	Observation 1 (% affected)			
		24-hr	48-hr	72-hr	96-hr
Negative control	<MQL	0 (0)	0 (0)	0 (0)	0 (0)
10	9.8	0 (0)	0 (0)	0 (0)	0 (0)

## CONCLUSION

There was no mortality among control animals during the course of the study. Therefore, control animals satisfied test acceptability criteria for survival (i.e.,  $\geq 90\%$  or one fish) as stated in the study protocol and the OECD 203 testing guidelines. Based on nominal X12019520 concentrations, the estimated 24-, 48-, 72-, and 96-hour  $LC_{50}$  value was  $>10$  mg X12019520/L, the only concentration tested. The slope of the 24-, 48-, 72-, and 96-hour concentration-response lines was not calculated. The 96-hour NOEC was 10 mg X12019520/L, based on nominal X12019520 concentrations and a lack of statistically significant mortality and sublethal effects at this, the only test substance concentration.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rainbow trout	<i>Oncorhynchus mykiss</i>	X12019520	96-hr	$LC_{50}$	$>10$	mg X12019520/L, nom

### A 2.2.1.9 Study 9 – X12446477 (metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions

Comments of zRMS:	<p>The study was conducted in line with OECD 203 (1992) with no deviations.</p> <p>Throughout the test the concentrations of metabolite X12446477 were maintained within 80-120% of nominal concentration; therefore, the endpoint is expressed in terms of the nominal concentration.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p><math>LC_{50} &gt; 10</math> mg pm/L (based on nominal concentration)</p>
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Reference:	KCP 10.2.1/9
Report:	xxx 2018; X12446477 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions; xxx; Lab Study No. 87147; DAS Study No. 180561 ; 18 July 2018; Unpublished
Guideline(s):	OECD Guideline 203
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): X12446477 (a metabolite of XDE-777)

Purity: 97%

Description (physical state): Not available

Lot/batch no.: XZ7-141472-71B [TSN307413]

### Test System

Organism (*Species*): Rainbow trout (*Oncorhynchus mykiss*)

Study type: Acute

Study design:	Static-renewal
Test concentrations:	Nominal: 0 (control), 0.63, 1.3, 2.5, 5.0, and 10 mg X12446477/L Mean measured: <MQL (control), 0.62, 1.2, 2.4, 4.9, and 9.6 mg X12446477/L
Parameters measured:	Mortality
Observation intervals:	24 hours
Age, weight and length of fish at test initiation:	Age: >14 days Mean blotted wet weight: $0.8685 \pm 0.1858$ g (0.6130 to 1.2036 g) Mean total length: $46 \pm 2.7$ mm (41 to 49 mm)
Analytical confirmation of test concentrations:	On days: 0 hour (fresh), 48 hours (spent and fresh), and 96 hours (spent)
No. of holding days before dosing:	14
Number of fish per dose group:	7
Number of fish per control group:	7
Feeding regime:	Fish were fed salmon starter daily during holding, none during exposure
Environmental conditions:	Loading rate: Instantaneous biomass loading: 0.34 g/L Temperature: 14.7 to 15.8 °C Photoperiod: 16-hr light:8-hr dark Dissolved oxygen concentration: 6.1 to 10.6 mg/L (64 to 109% sat.) pH: 7.6 to 8.3 Total hardness: 152 mg CaCO <sub>3</sub> /L Salinity: not applicable
Reference substance:	none

## Methodology

A static-renewal definitive test was performed from 26 to 30 March 2018 at nominal concentrations of 0 (control) 0.63, 1.3, 2.5, 5.0, and 10 mg X12446477/L. Seven fish were impartially assigned to treatment replicates by adding one fish per chamber, proceeding from control, then proceeding from low to high test substance treatments, and repeating steps as necessary until seven fish were present in each test chamber for a total of seven fish per test treatment. Observations for mortality and sublethal responses were made at approximately 24, 48, 72, and 96 hours. In an effort to maintain maximal exposure to the test substance, the control and treated test solutions were freshly prepared and renewed at 48 hours. Temperature, dissolved oxygen, and pH were measured in each test chamber. In addition, a continuous record of the temperature from the water bath was also maintained. Total hardness and alkalinity of the dilution water were measured using titrimetric methods adapted from Standard Methods on test initiation day. The light intensity at definitive test initiation was 983 lux.

## RESULTS AND DISCUSSION

The concentration of X12446477 was measured in test solution samples collected from fresh solutions at initiation and 48-hours, and from spent solutions at 48- and 96-hours of the definitive test. The arithmetic mean measured concentrations during the 96-hour exposure were 0.62, 1.2, 2.4, 4.9, and 9.6 mg X12446477/L, which represented recoveries of 96 to 98% of the nominal concentrations. The biological response results were reported based upon the nominal X12446477 concentrations. After 96 hours of exposure, mortality was 0% in the control and all test treatments. No sublethal effects were observed.

Table 2. Measured Concentrations of X12446477 During the Static-Renewal Acute Toxicity Test with Rainbow Trout, *Oncorhynchus mykiss*

Nominal Concentration (mg X12446477/L)	Measured Concentration as mg X12446477/L (Percent Nominal)				
	0 Hour	48 Hours (spent)	48 Hours (fresh)	96 Hours	Arithmetic Mean Measured Concentration
0 (control)	<MQL <sup>a</sup>	<MQL <sup>a</sup>	<MQL <sup>a</sup>	<MQL <sup>a</sup>	<MQL <sup>a</sup>
0.63	0.64 (102)	0.60 (95)	0.63 (100)	0.59 (94)	0.62 (98)
1.3	1.3 (104)	1.2 (92)	1.3 (96)	1.2 (92)	1.2 (96)
2.5	2.6 (106)	2.3 (93)	2.5 (100)	2.3 (92)	2.4 (98)
5.0	5.2 (104)	4.8 (96)	5.0 (99)	4.7 (94)	4.9 (98)
10	9.9 (99)	9.4 (94)	9.9 (99)	9.2 (92)	9.6 (96)
QC Fortification Spikes (% Recovery)					
Low Spike (0.096)	0.098 (102)	0.097 (101)	0.097 (101)	0.096 (100)	NA
High Spike (17)	18 (104)	18 (108)	18 (105)	18 (106)	NA

<sup>a</sup> MQL = 0.050 mg X12446477/L.

NA= Not Applicable

Table 28: Effect of X12446477 on mortality of rainbow trout

Treatment (mg X12446477/L)		No. of fish	Cumulative mortality (%)			
Nominal	Mean measured		24-hr	48-hr	72-hr	96-hr
Negative control	<MQL	7	0 (0)	0 (0)	0 (0)	0 (0)
0.63	0.62	7	0 (0)	0 (0)	0 (0)	0 (0)
1.3	1.2	7	0 (0)	0 (0)	0 (0)	0 (0)
2.5	2.4	7	0 (0)	0 (0)	0 (0)	0 (0)
5.0	4.9	7	0 (0)	0 (0)	0 (0)	0 (0)
10	9.6	7	0 (0)	0 (0)	0 (0)	0 (0)
LC <sub>50</sub>		>10 mg X12446477/L				
95% C.I.		Not calculated				
NOEC		10 mg X12446477/L				

Table 29: Sub-lethal effects of X12446477 in rainbow trout

Treatment (mg X12446477/L)		Observation period			
Nominal	Mean measured	Observation 1 (% affected)			
		24-hr	48-hr	72-hr	96-hr
Negative control	<MQL	0 (0)	0 (0)	0 (0)	0 (0)
0.63	0.62	0 (0)	0 (0)	0 (0)	0 (0)
1.3	1.2	0 (0)	0 (0)	0 (0)	0 (0)
2.5	2.4	0 (0)	0 (0)	0 (0)	0 (0)
5.0	4.9	0 (0)	0 (0)	0 (0)	0 (0)
10	9.6	0 (0)	0 (0)	0 (0)	0 (0)

## CONCLUSION

There was no mortality among control animals during the course of the study. Therefore, control animals satisfied test acceptability criteria for survival (i.e.,  $\geq 90\%$  or one fish) as stated in the study protocol and the OECD 203 testing guidelines. Based on nominal X12446477 concentrations, the

estimated 24-, 48-, 72-, and 96-hour LC<sub>50</sub> value was >10 mg X12446477/L, the highest concentration tested. The slope of the 24-, 48-, 72-, and 96-hour concentration-response lines was not calculated. The 96-hour NOEC was 10 mg X12446477/L, based on nominal X12446477 concentration and a lack of statistically significant mortality and sublethal effects at this, the highest test substance concentration.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rainbow trout	<i>Oncorhynchus mykiss</i>	X12446477	96-hr	LC <sub>50</sub>	>10	mg X12446477/L, nom

## A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

### A 2.2.2.1 Study 1 – X1642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, *Chironomus riparius*, Using Spiked Sediment

Comments of zRMS:	<p>The study was conducted in line with OECD 218 with no deviations.</p> <p>Throughout the test the concentrations of metabolite X642188 were not maintained within 80-120% of nominal concentration; therefore, the endpoints are expressed in terms of the initial measured concentrations (im) and time weighted mean measured concentrations (twm).</p> <p>Reliability of the EC<sub>10</sub> value was evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> <li>- NW (normalised width) of 0.19 (im) and 1.14 (twm) were calculated, which resulted in rating “excellent” in line with Table E9 in EFSA Supporting publication 2019:EN-1673,</li> <li>- although there is no EC<sub>20,low</sub> available for evaluation, the median EC<sub>10</sub> is lower than EC<sub>50,low</sub> which indicates a medium level of protection,</li> <li>- the dose-response curve is shallow with steepness of 0.22 (im) and 0.21 (twm) (i.e. &lt;0.33).</li> </ul> <p>Based on above indications the calculated EC<sub>10</sub> are considered to be sufficiently reliable.</p> <p>In the test report no justification was given why endpoints were based on TWA mean measured concentrations and not on commonly agreed geometric mean measured concentrations, however OECD 218 does not specify how the endpoints should be calculated in case the test item is not stable over the entire study period. Taking this into</p>
	<p>account, calculation of TWA mean measured concentrations is agreed by the zRMS.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>overall NOEC (all parameters) = 1.9 mg pm/kg (based on initial measured concentration) EC<sub>10</sub> (based on survival as most sensitive parameter) = 1.8 mg pm/kg (based on initial measured concentration)</p> <p>overall NOEC (all parameters)= 0.63 mg X642188/kg (based on time weighted mean measured concentration) EC<sub>10</sub> (based on survival as most sensitive parameter) = 0.58 mg X642188/kg (based on time weighted mean measured concentration)</p>



Reference:	KCP 10.2.2/1
Report:	xxx.; 2018; X642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment; xxx; Lab Study No. 87149; DAS Study No. 180563 ; 30 August 2018; Unpublished
Guideline(s):	OECD Guideline 218
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): X642188 (a metabolite of XDE-777)  
Purity: 99%  
Description (physical state): White solid with a mild odor  
Lot/batch no.: SYN-FS08353-048 (TSN303567)

### Test System

Organism (*Species*): Freshwater midge (*Chironomus riparius*)  
Study type: Chronic life cycle study Static

Duration of study: 28 days  
Method of test item application: Spiked sediment

Parameters measured: Survival, number emerged and time to emergence, and development rate  
Observation intervals: Daily  
Age of test organisms at test initiation: Approx. 3-day old first-instar larvae  
Test concentrations: Nominal: 0 (control), 0 (vehicle control), 0.63, 1.3, 2.5, 5.0, and 10 mg/kg  
Mean measured sediment at day 0: <MQL (control), <MQL (vehicle control), 0.42, 0.84, 1.9, 4.0, and 8.2 mg/kg  
Overlying water at day 0: ranged from 0.00098 to 0.018 mg/L  
Pore water at day 0: ranged from 0.00089 to 0.019 mg/L  
Time-weighted Mean Sediment: <MQL (control), <MQL (vehicle control), 0.15, 0.32, 0.63, 1.2, and 2.8 mg/kg

Feeding: 2.0 mL of 10 g/L algae was added to the test chambers on day 0, additionally the larvae were fed 5 mL of a 2.0 g/L flake food suspension daily on days 0 through 19. Feeding was suspended on day 20 due to excessive amounts of uneaten food.

Ratio of sediment layer to depth of overlying water: 1:4

Reference substance: none

No. of Chironomid per vessel: 20

No. of Chironomid per dose group: 120

group:

No. of Chironomid per control group: 120

group:

No. of replicates: 6 per treatment group and control

Environmental conditions: Temperature: 20.1 to 21.2 °C

Photoperiod: Dissolved Oxygen: 7.6 to 8.8 mg/L (90 to 101% saturation)

16 hr light: 8-hr dark pH: 8.0 to 8.5

Total Hardness: 152 to 270 mg CaCO<sub>3</sub>/L

Light intensity: 735 lux

Un-ionized Ammonia: 0.00 mg/L

Light

## Methodology

The test chambers were 1-L glass jars that were approximately 17 cm in height by 8.5 cm in diameter. Approximately 200 g (approximately 2 cm sediment depth) of prepared formulated sediment was added to each replicate test chamber. A 600-mL volume of dilution water (approximately 8 cm) was carefully added to the test chambers and a turbulence deflector was used during the water addition to minimize the disturbance of the sediment.

Six control, vehicle control, and test substance treatment replicate test chambers were prepared for the biological parameters. Eight additional control, vehicle control, and test substance treatment replicate chambers were prepared for the various analyses of the overlying water, pore water, and sediment samples.

The test chambers were placed in a temperature-controlled water bath arranged by treatment level. All replicates were covered with emergence traps to capture emerged adults. Gentle aeration was provided to each test chamber through a glass pipette. The pipette was inserted such that the tip was two to three centimeters from the sediment surface. Aeration was initiated approximately 24 hours after organism addition. During the course of the test, the aeration was adjusted as deemed necessary in an attempt to maintain the dissolved oxygen concentrations within each test chamber.

On study initiation day a target total of 20 midge larvae were added, five at a time, starting with the controls and proceeding to the high treatment, until the required number of individuals were added to each test chamber. Organisms were added 2-3 cm above the sediment/water interface using a widebore pipette. Observations of the biological replicates were recorded daily throughout the test. Any abnormal activity (i.e., sediment avoidance, inactivity, etc.) was noted, if observed.

The larvae were fed 2.0 mL of 10 g/L algae added to the test chambers on day 0, additionally the larvae were fed 5 mL of a 2.0 g/L flake food suspension daily on days 0 through 19. Feeding was suspended on day 20 due to excessive amounts of uneaten food.

## RESULTS AND DISCUSSION

The initial (day 0) mean measured concentrations of X642188 in the sediment samples were 0.42, 0.84, 1.9, 4.0, and 8.2 mg/kg dry sediment in the 0.63, 1.3, 2.5, 5.0, and 10 mg/kg treatments, respectively, which represented 65 to 82% of nominal. The mean measured concentrations of X642188 in sediment samples on day 2 were 0.33, 0.69, 1.3, 2.8, and 6.3 mg/kg dry sediment, which

represented 52 to 63% of the nominal concentrations. The mean measured concentrations of X642188 in sediment samples on day 10 were 0.16, 0.33, 0.65, 1.2, and 2.8 mg/kg dry sediment, which represented 24 to 28% of the nominal concentrations. The mean measured concentrations of X642188 in sediment samples on day 28 (termination) were 0.053, 0.10, 0.19, 0.39, and 0.86 mg/kg dry sediment, which represented 8 to 9% of the nominal concentrations. Results for sediment are given in Table 32, while in Tables 30 and 31 also analytical results for overlying water and pore water are reported.

Based on analytical results obtained in sediment, the time weighted mean concentrations were determined using the following calculation:

Area calculated by:  $((\text{Conc. 0} - \text{Conc. 1}) / (\ln \text{Conc. 0} - \ln \text{Conc. 1})) \times \text{time period (days)}$

where:

Conc. 0 is the concentration at the first sampling point (i.e., day 0, day 2, and day 10, respectively)

Conc. 1 the concentration at the corresponding second sampling point, (i.e., day 2, 10, and 28, respectively) for each sampled time period

Time weighted mean concentration = (day 0-2 area + day 2-10 area + day 10-28 area) / Total Days

The overall time-weighted mean measured concentrations of X642188 in the sediment samples were 0.15, 0.32, 0.63, 1.2, and 2.8 mg/kg dry sediment which represented 24, 25, 25, 24, and 28% of nominal. All biological response evaluations were calculated based on the initial (day 0) mean measured sediment concentrations and the time-weighted mean measured sediment concentrations.

**Table 30: Results from analysis of overlying water samples**

Nominal sediment concentration (mg/kg)	Measured concentrations (mg/L)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
0 (control)	<MQL	<MQL	<MQL	<MQL	Water type: moderately hard freshwater prepared by blending naturally hard well water with well water that was de-mineralized by reverse osmosis Dissolved oxygen: 7.6 to 8.8 mg/L (90 to 101% saturation) pH: 8.0 to 8.5  Hardness: 152 to 270 mg CaCO <sub>3</sub> /L  Alkalinity: not determined  Conductivity: not determined  Ammonia concentration: 0.00 mg/L (unionized) Water temperature: 20.1 to 21.2°C Renewal of water: none
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL	
0.63	0.00098, 0.0019	0.000015, 0.000017	<MQL	<MQL	
1.3	0.0018, 0.0023	0.000034, 0.000045	<MQL	<MQL	
2.5	0.0039, 0.0048	0.000064, 0.00011	0.000011, 0.000015	<MQL	
5.0	0.0093, 0.010	0.00013, 0.00014	0.000016, 0.000025	<MQL	
10	0.014, 0.018	0.00028, 0.00033	0.000041, 0.000070	<MQL	
MQL	0.000010 mg/L				
LOD	Not determined				

**Table 31: Results from analysis of pore water samples**

Nominal sediment concentration (mg/kg)	Measured concentrations (mg/L)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
0 (control)	<MQL	<MQL	<MQL	<MQL	Dissolved oxygen: not determined pH: not determined
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL	

0.63	0.00089, 0.0011	0.00034, 0.00038	0.00010, 0.00013	0.000013, 0.000014	Ammonia concentration: not determined  Hardness: not determined   Alkalinity: not determined  Conductivity: not determined  Water temperature: not determined
1.3	0.0027, 0.0028	0.00059, 0.00068	0.00017, 0.00018	0.000014, 0.000016	
2.5	0.0050, 0.0051	0.0012, 0.0013	0.00037, 0.00037	0.000039, 0.000046	
5.0	0.010, 0.011	0.0021, 0.0026	0.00078, 0.0012	0.000058, 0.000061	
10	0.018, 0.019	0.0064, 0.0066	0.0019, 0.0021	0.00015, 0.00022	
MQL	0.000010 mg/L				
LOD	Not determined				

**Table 32: Results from analysis of sediment samples**

Nominal sediment concentration (mg/kg)	Mean measured concentrations (mg/kg dry sediment)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
0 (control)	<MQL	<MQL	<MQL	<MQL	Type: artificial Total organic carbon: not determined Kaolin clay (%): 20 Fine industrial sand (%): 75 Sphagnum peat (%): 5 pH: 7.0 ± 0.5 units C/N ratio: not determined Deionised water: appropriate volume added to achieve a hydration level of approximately 35% Organic carbon: 2.5%
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL	
0.63	0.42	0.33	0.16	0.053	
1.3	0.84	0.69	0.33	0.10	
2.5	1.9	1.3	0.65	0.19	
5.0	4.0	2.8	1.2	0.39	
10	8.2	6.3	2.8	0.86	
MQL	0.0038 mg/kg				
LOD	Not determined				

The mean survival in replicates E and F at day 10 was 90 and 95% in the control and vehicle control, respectively, and ranged from 58 to 98% for the test substance treatment levels.

There was a statistically significant difference (Dunnett's test or Williams's test,  $p > 0.05$ ) in the mean day 10 survival in the 4.0 and 8.2 mg/kg day 0 mean measured sediment (1.2 and 2.8 mg/kg time-weighted mean measured sediment) test substance treatments as compared to the pooled control (i.e., 93%).

The mean adult biomass in replicates E and F at day 10 was 0.75 and 0.60 g in the control and vehicle control, respectively, and ranged from 0.54 to 0.90 g for the test substance treatment levels. There were no statistically significant differences (Dunnett's test and Williams's test,  $p > 0.05$ ) in mean adult biomass in the test substance treatments as compared to the pooled control (i.e., 0.67 g).

Percent emergence of adult freshwater midges in the control and vehicle control was 91 and 93% respectively. The mean percent emergence was 94, 90, 83, 69, and 49% in the 0.42, 0.84, 1.9, 4.0, and 8.2 mg/kg day 0 mean measured sediment treatments (0.15, 0.32, 0.63, 1.2, and 2.8 mg/kg timeweighted mean measured sediment treatments), respectively. There was a statistically significant difference (Dunnett's test and Williams' test,  $p > 0.05$ ) in mean percent emergence in the 4.0 and 8.2 mg/kg day 0 mean measured sediment (1.2 and 2.8 mg/kg time-weighted mean measured sediment) test substance treatments as compared to the pooled control (i.e., 92%). Emergence was observed starting on day 13 in the control, vehicle control, and all test substance treatments. The last emerged midge was observed on day 21.

The gender ratio for the control and vehicle control was 1.73 and 1.24 males to each female. The male to female emergence gender ratio for the treatments ranged from 1.03 in the 1.9 mg/kg day 0 mean measured sediment treatment (0.63 mg/kg time-weighted mean measured sediment treatment) to 1.62

in the 0.42 mg/kg day 0 mean measured sediment treatment (0.15 mg/kg time-weighted mean measured sediment treatment).

The mean development rates for the emergent males was 0.0759 and 0.0756 in the control and vehicle control, respectively, and ranged from 0.0723 to 0.0778 for the test substance treatment levels. The mean development rates for the emergent females was 0.0662 and 0.0665 in the control and vehicle control, respectively, and ranged from 0.0651 to 0.0720 for the test substance treatment levels. The mean total adult development rates were 0.0721 and 0.0712 in the control and vehicle control, respectively, and ranged from 0.0690 to 0.0743 for the test substance treatment levels. There was no statistically significant difference (Dunnett's test and Williams' test,  $p > 0.05$ ) in mean development rate for males, females, or the total midge population in any of the test substance treatments as compared to the pooled control.

**Table 33: Effect of X642188 on adult survival and biomass at day 10**

Day-0 Mean Measured Sediment Concentration (mg/kg)	Time-Weighted Mean Measured Sediment Concentration (mg/kg)	Parameter	Adult survival and biomass		
			Rep E	Rep F	Mean of all replicates
Negative control	Negative control	Survival (%)	100	80	90
		Biomass (mg)	0.55	0.94	0.75
Vehicle control	Vehicle control	Survival (%)	100	90	95
		Biomass (mg)	0.62	0.57	0.60
0.42	0.15	Survival (%)	100	95	98
		Biomass (mg)	0.62	0.74	0.68
0.84	0.32	Survival (%)	90	95	93
		Biomass (mg)	0.66	0.44	0.55
1.9	0.63	Survival (%)	95	75	85
		Biomass (mg)	0.49	0.59	0.54
4.0	1.2	Survival (%)	75	50	63 <sup>*</sup>
		Biomass (mg)	0.75	0.92	0.84
8.2	2.8	Survival (%)	40	75	58 <sup>*</sup>
		Biomass (mg)	1.04	0.76	0.90

\* Significant reduction in survival (Dunnett's or Williams' test,  $p \leq 0.05$ ) as compared to the pooled controls (mean survival 93%).

**Table 34: Effect of X642188 on adult emergence and development rate at day 28**

Day-0 Mean Measured Sediment (mg/kg)	Time-Weighted Mean Measured Sediment (mg/kg)	Sex of emerged midge	Adult emergence				Mean of all replicates
			Rep A	Rep B	Rep C	Rep D	
Negative control	Negative control	% Emerged	85	90	100	90	91
		M Dev. rate	0.0780	0.0760	0.0728	0.0766	0.0759
		F Dev. rate	0.0677	0.0627	0.0657	0.0687	0.0662
		Tot Dev. rate	0.0762	0.0690	0.0701	0.0731	0.0721
Vehicle control	Vehicle control	% Emerged	80	95	95	100	93
		M Dev. rate	0.0779	0.0744	0.0763	0.0736	0.0756
		F Dev. rate	0.0598	0.0672	0.0725	0.0664	0.0665
		Tot Dev. rate	0.0688	0.0710	0.0751	0.0700	0.0712
0.42	0.15	% Emerged	80	100	95	100	94
		M Dev. rate	0.0789	0.0762	0.0722	0.0732	0.0751
		F Dev. rate	0.0764	0.0667	0.0629	0.0573	0.0658
		Tot Dev. rate	0.0781	0.0729	0.0688	0.0657	0.0714
0.84	0.32	% Emerged	85	90	85	100	90
		M Dev. rate	0.0717	0.0779	0.0704	0.0693	0.0723
		F Dev. rate	0.0693	0.0724	0.0604	0.0582	0.0651
		Tot Dev. rate	0.0706	0.0758	0.0657	0.0640	0.0690

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1.9	0.63	% Emerged	70	100	95	65	83
		M Dev. rate	0.0793	0.0746	0.0757	0.0735	0.0758
		F Dev. rate	0.0772	0.0715	0.0643	0.0668	0.0700
		Tot Dev. rate	0.0784	0.0732	0.0697	0.0699	0.0728
4.0	1.2	% Emerged	60	60	70	85	69*
		M Dev. rate	0.0800	0.0758	0.0693	0.0772	0.0756
		F Dev. rate	0.0731	0.0734	0.0677	0.0736	0.0720
		Tot Dev. rate	0.0771	0.0746	0.0686	0.0755	0.0740
8.2	2.8	% Emerged	50	65	35	45	49*
		M Dev. rate	0.0790	0.0750	0.0770	0.0800	0.0778
		F Dev. rate	0.0770	0.0692	0.0735	0.0647	0.0711
		Tot Dev. rate	0.0782	0.0728	0.0745	0.0715	0.0743

\* Statistically significant (Dunnett's test and Williams' test,  $p \geq 0.05$ ) emergence effect as compared to the pooled controls. Total emergence includes emergent midge of unknown gender.

## CONCLUSION

The control organisms met the acceptability criterion for mean percent emergence (i.e., >70%) as specified by the study protocol and the OECD 218 testing guideline.

All effects concentrations were based on the day 0 mean measured sediment concentrations and the time-weighted mean measured sediment concentrations.

The day 10 survival NOEC and LOEC values, based the day 0 mean measured sediment concentrations, were 1.9 and 4.0 mg/kg, respectively, with EC<sub>10</sub>, EC<sub>15</sub>, and EC<sub>50</sub> values of 1.8 mg/kg (95% confidence limits 0.66 – 2.8 mg/kg), 2.5 mg/kg (95% confidence limits 1.2 – 3.7 mg/kg), and >8.2 mg/kg (6.4 – 24 mg/kg), respectively. The day 10 survival NOEC and LOEC values, based the time-weighted mean measured sediment concentrations, were 0.63 and 1.2 mg/kg, respectively, with EC<sub>10</sub>, EC<sub>15</sub>, and EC<sub>50</sub> values of 0.58 mg/kg (95% confidence limits 0.24 – 0.90 mg/kg), 0.80 mg/kg (95% confidence limits 0.41 – 1.2 mg/kg), and >2.8 mg/kg (2.1 – 7.9 mg/kg), respectively.

The emergence NOEC and LOEC values, based the day 0 mean measured sediment concentrations, were 1.9 and 4.0 mg/kg, respectively, with EC<sub>10</sub>, EC<sub>15</sub>, and EC<sub>50</sub> values of 1.9 mg/kg (95% confidence limits 0.86 - 2.7 mg/kg), 2.5 mg/kg (95% confidence limits 1.4 – 3.4 mg/kg), and >8.2 mg/kg (6.6 - 14 mg/kg), respectively. The emergence NOEC and LOEC values, based the time-weighted mean measured sediment concentrations, were 0.63 and 1.2 mg/kg, respectively, with EC<sub>10</sub>, EC<sub>15</sub>, and EC<sub>50</sub> values of 0.60 mg/kg (95% confidence limits 0.31 – 0.86 mg/kg), 0.81 mg/kg (95% confidence limits 0.48 – 1.1 mg/kg), and >2.8 mg/kg (2.2 – 4.6 mg/kg), respectively.

The day 10 adult biomass and the male, female and total development rate NOEC and LOEC values, based on the day 0 mean measured sediment concentrations (time-weighted mean measured sediment concentrations), were 8.2 and >8.2 mg/kg (2.8 and >2.8 mg/kg), respectively. The EC<sub>10</sub>, EC<sub>15</sub>, and EC<sub>50</sub> values for adult biomass and the male, female and total development rate data could not be calculated due to the lack of a concentration dependent response trend.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Midge	<i>Chironomus riparius</i>	X642188	10 day	Day 10 Survival NOEC	1.9	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	10 day	Day 10 Biomass NOEC	8.2	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Emergence NOEC	1.9	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Male Development Rate NOEC	8.2	mg/kg (day 0 mean sediment)

Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Female Development Rate NOEC	8.2	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Total Development Rate NOEC	8.2	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 10 Survival NOEC	0.63	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 10 Biomass NOEC	2.8	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Emergence NOEC	0.63	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Male Development Rate NOEC	2.8	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Female Development Rate NOEC	2.8	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X642188	28 day	Day 28 Total Development Rate NOEC	2.8	mg/kg (time-weighted mean sediment)

#### A 2.2.2.2 Study 3 – X1642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with *Lumbriculus variegatus* Using Spiked Sediment

Comments of zRMS:	The study below was not evaluated by the zRMS since study on toxicity of X642188 to <i>Chironomus riparius</i> was submitted and is deemed sufficient to address the data gap identified in EFSA Journal 2018;16(1):5146. Study on effects on second sediment dwelling species should be dealt with at the next renewal process of fenpicoxamid.
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Reference:	KCP 10.2.2/23
Report:	xxx.; 2019; X642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with <i>Lumbriculus variegatus</i> Using Spiked Sediment; xxx; Lab Study No. 87169; DAS Study No. 180639 ; 23 October 2019; Unpublished
Guideline(s):	OECD Guideline 225
Deviations:	Not validated
GLP:	Yes
Acceptability:	Not evaluated, not required to finalise the aquatic risk assessment from GF-3308 at the zonal level
Duplication (if vertebrate study)	NA

#### MATERIALS AND METHODS Test Item(s)

Test item (Common name):	X642188
Purity:	99%
Description (physical state):	white solid with a mild odour
Lot/batch no.:	SYN-FS08353-048 (TSN303567)

#### Test System

Organism ( <i>Species</i> ):	Blackworms (oligochaetes, <i>Lumbriculus variegatus</i> )
Study type:	Prolonged sediment toxicity test, static
Duration of study:	28 days
Method — of — test	Spiked sediment
— item application:	
Parameters measured:	Total number of surviving worms and mean total biomass (as dry weight) of the surviving worms
Observation intervals:	At 28 days
Age of test organisms at test initiation:	14 days post-synchronization
Test concentrations:	Nominal sediment: 0 (control), 0 (vehicle control, acetone), 2.5, 5.0, 10, 20, 40, and 80 mg/kg Initial mean measured in sediment: <LOD (control), <LOD (vehicle control), 1.9, 3.6, 8.4, 16, 34, and 62 mg/kg Time weighted mean measured in sediment: <LOD (control), <LOD (vehicle control), 0.64, 1.2, 3.2, 6.4, 14, and 33 mg/kg
Feeding:	A 1.0 mL volume of a wheat grass suspension (8.0 g of organic wheat grass blended into 1.0 L of deionized water to achieve an 8.0 g/L suspension) was added to each chamber on days 0, 1, 3, 7, 9, 12, 14, 16, 19, 22, 24, and 27.
Ratio of sediment layer to depth of overlying water:	~2.5 cm sediment to ~10 cm of dilution water (1:4)
Reference substance:	none
No. of <i>Lumbriculus</i> — per vessel:	10
No. of <i>Lumbriculus</i> per dose group:	100 (40 in biological replicates, 60 in analytical replicates)
No. of <i>Lumbriculus</i> — per control group:	120 (60 in biological replicates, 60 in analytical replicates)
Environmental conditions:	Temperature: 20.2 to 21.6 °C Photoperiod: 16 hr light:8 hr dark Light intensity: 330 lux

## Methodology

The in life phase of the definitive test was performed at nominal sediment concentrations of 0 (control), 0 (vehicle control, acetone), 2.5, 5.0, 10, 20, 40, and 80 mg/kg. Fourteen replicate test chambers were prepared for the control and vehicle control, and twelve replicate test chambers were prepared for each test substance treatment. Six control and vehicle control replicates and four test substance treatment replicates were prepared to assess the biological parameters. Eight additional replicates for each treatment were prepared for the various analyses of the sediment, pore water, and overlying water. Except for the day 0 analytical monitoring replicates (two per treatment), ten worms were added to each test chamber at initiation for a total of 120 worms per control and vehicle control, and 100 per test substance treatment. Behavioral observations of organisms in each chamber were made throughout the test and any behavioral abnormalities were noted. Behavioral observations were inadvertently not performed on day 8. At test termination (day 28), the entire contents of each chamber were poured through a 300 µm mesh sieve and the live and dead organisms were enumerated. Living organisms were assigned to one of three groups: a) large complete worms (adults) without regenerated body regions; b) complete worms with regenerated, lighter-colored body regions (i.e., with new posterior part, with new anterior part or with both new posterior and anterior parts); and c) incomplete worms (i.e., recently fragmented worms with non-regenerated body regions). All surviving organisms were euthanized in 70% ethanol and retained for weight measurements. Euthanized worms were pooled by replicate, placed into pre-weighed pans, dried overnight at approximately 102 °C, removed from the oven and placed in



a desiccator, and then weighed to the nearest 0.0001 g. Temperature, dissolved oxygen concentration, and pH of the overlying water were measured in one biological replicate of each treatment at least three times weekly during the study. Temperature was continuously measured in a single test chamber with an electronic data logger. Light intensity, measured at the test solution level, was 330 lux on day 0. Sediment, interstitial (pore) water, and overlying water samples were analysed for X642188 using a liquid chromatography system with tandem mass spectrometry (LC MS/MS).

## RESULTS AND DISCUSSION

The treatment mean measured X642188 concentration in sediment samples at day 0 was 1.9, 3.6, 8.4, 16, 34, and 62 mg/kg (73 to 84% of nominal) for the 2.5, 5.0, 10, 20, 40, and 80 mg/kg treatments, respectively. The treatment mean measured X642188 concentration in sediment samples at day 2 was 1.3, 2.8, 6.8, 13, 27, and 49 mg/kg (54 to 68% of nominal). The treatment mean measured X642188 concentration in sediment samples at day 10 was 0.63, 0.98, 3.1, 6.4, 15, and 33 mg/kg (20 to 41% of nominal). The treatment mean measured concentration of X642188 at day 28 was 0.24, 0.42, 1.2, 2.5, 6.8, and 22 mg/kg (8 to 27% of nominal). The time weighted mean measured X642188 concentration over the duration of the study was 0.64, 1.2, 3.2, 6.4, 14, and 33 mg/kg (23 to 41% of nominal) for the 2.5, 5.0, 10, 20, 40, and 80 mg/kg treatments, respectively. No residues of X642188 were detected in the control or vehicle control samples at or above the LOD of 0.014 mg/kg. The biological results were reported based on the initial (day 0) mean measured and overall time weighted mean measured X642188 concentrations in sediment. There was statistically significant reduction (Dunnett's and Williams' test,  $p = 0.05$  significance level) in the mean number of surviving worms in the 62 mg/kg initial mean measured treatment (33 mg/kg time weighted mean measured treatment) as compared to the vehicle control. The 28 day NOEC and LOEC for mean number of surviving worms was 34 and 62 mg/kg (14 and 33 mg/kg), based on initial mean measured (and time weighted mean measured) sediment values. There was a statistically significant reduction (Williams' test,  $p = 0.05$  significance level) for mean total biomass in the 62 mg/kg initial mean sediment treatment (33 mg/kg timeweighted mean measured sediment treatment) as compared to the pooled control. The 28 day NOEC and LOEC for mean total biomass was 34 and 62 mg/kg (14 and 33 mg/kg), based on initial mean measured (and time weighted mean measured) sediment values.

**Table 35: Results from analysis of sediment samples**

Nominal sediment concentration (mg/kg)	Mean measured concentrations (mg/kg)				Other parameters
	Day-0	Day-2	Day-10	Day-28	
Negative control	<LOD	<LOD	<LOD	<LOD	Type: artificial sandy loam Total organic carbon: not determined Kaolin clay (%): 20 Quartz sand (%): 77 Silt (%): 3 pH: 6.50 to 6.62 C/N ratio: not determined Deionised water: not determined Organic carbon: 2.5%
Vehicle control	<LOD	<LOD	<LOD	<LOD	
2.5	1.9	1.3	0.63	0.24	
5.0	3.6	2.8	0.98	0.42	
10	8.4	6.8	3.1	1.2	
20	16	13	6.4	2.5	
40	34	27	15	6.8	
80	62	49	33	22	
LOQ	0.046 mg/kg				
LOD	0.014 mg/kg				

**Table 36: Results from analysis of pore water samples**

Nominal sediment concentration (mg/kg)	Measured concentrations (mg/L)				Other parameters
	Day-0	Day-2	Day-10	Day-28	
Negative control	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	Dissolved oxygen: not determined  pH: not determined
Vehicle control	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	

2.5	0.0064, 0.0058	0.0024, 0.0025	0.00060, 0.00049	<LOD, <LOD	Ammonia concentration: not determined  Hardness: not determined  Alkalinity: not determined  Conductivity: not determined  Water temperature: not determined
5.0	0.011, 0.011	0.0038, 0.0045	0.00070, 0.0011	<LOD, <LOD	
10	0.024, 0.018	0.012, 0.013	0.0023, 0.0024	0.00057, 0.00038	
20	0.030, 0.025	0.024, 0.021	0.0042, 0.0044	0.00074, 0.00060	
40	0.053, 0.041	0.049, 0.044	0.011, 0.012	0.0017, 0.0017	
80	0.063, 0.073	0.086, 0.081	0.031, 0.024	0.0064, 0.0068	
LOQ	0.00033 mg/L				
LOD	0.000099 mg/L				

Table 37: Results from analysis of overlying water samples

Nominal sediment concentration (mg/kg)	Measured concentrations (mg/L)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
Negative control	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	Water type: moderately hard freshwater prepared by blending naturally hard well water with well water that was de-mineralized by reverse osmosis Dissolved oxygen: 5.9 to 8.4 mg/L (70 to 98% saturation) pH: 7.9 to 8.5  Hardness: 144 to 228 mg CaCO <sub>3</sub> /L Alkalinity: 136 to 224 mg CaCO <sub>3</sub> /L Conductivity: 355 to 512 µS/cm Ammonia concentration: <0.1 to 0.561 mg/L Water temperature: 20.2 to 21.6°C: Renewal of water: none
Vehicle control	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	<LOD, <LOD	
2.5	0.0030, 0.0025	<LOD, <LOD	<LOD, 0.00038	<LOD, <LOD	
5.0	0.0036, 0.0025	<LOQ>LOD, <LOQ>LOD	<LOD, <LOD	<LOD, <LOD	
10	0.011, 0.0087	0.00034, <LOQ>LOD	<LOD, <LOD	<LOD, <LOD	
20	0.011, 0.015	0.00049, 0.00040	<LOD, <LOD	<LOD, <LOD	
40	0.029, 0.035	0.00082, 0.00095	<LOD, <LOD	<LOD, <LOD	
80	0.088, 0.095	0.0036, 0.0014	<LOD, <LOQ>LOD	<LOD, <LOQ>LOD	
LOQ	0.00033 mg/L				
LOD	0.000099 mg/L				

Table 38: Effects of X642188 on total number of surviving worms and dry weight biomass after the 28-day exposure

Test concentrations (mg/kg)		Treatment mean of organisms surviving	Total biomass treatment mean wt. (g)
Initial mean measured	Time-weighted mean measured		
Negative control	Negative control	18	0.0144
Vehicle control	Vehicle control	23	0.0130
Pooled control	Pooled control	NA	0.0137
1.9	0.64	19	0.0130
3.6	1.2	22	0.0120

8.4	3.2	18	0.0116
16	6.4	21	0.0113
34	14	19	0.0115
62	33	15*	0.0106**

\* Statistically significant reduction compared to the vehicle control \*\*

Statistically significant reduction compared to the pooled control

## CONCLUSION

All test acceptability criteria were satisfied. The average number of living worms per replicate in the control and vehicle control treatments had increased by a factor of 1.8 and 2.3, respectively, at the end of exposure, which satisfied the minimum required factor of increase (at least 1.8). The pH of the overlying water was between 6 and 9 throughout the test (7.9 to 8.5 pH). Dissolved oxygen concentration in the overlying water was maintained above 30% of air saturation value (ASV) at test temperature during the test.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Survival NOEC (initial mean measured concentrations)	34	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Survival EC <sub>20</sub> (initial mean measured concentrations)	31	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Survival EC <sub>50</sub> (initial mean measured concentrations)	>62	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Biomass NOEC (initial mean measured concentrations)	34	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Biomass EC <sub>50</sub> (initial mean measured concentrations)	>62	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Survival NOEC (time-weighted mean measured concentrations)	14	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Survival EC <sub>20</sub> (time-weighted mean measured concentrations)	12	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Survival EC <sub>50</sub> (time-weighted mean measured concentrations)	>33	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Biomass NOEC (time-weighted mean measured concentrations)	14	mg/kg
Blackworms	<i>Lumbriculus variegatus</i>	X642188	28 day	Biomass EC <sub>50</sub> (time-weighted mean measured concentrations)	>33	mg/kg

### A 2.2.2.3 Study 3 – X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, *Chironomus riparius*, Using Spiked Sediment

Comments of zRMS:	<p>Although a data gap for submission of respective study on toxicity of metabolite X12335723 to sediment dwellers was identified in EFSA Journal 2018;16(1):5146, it seems that this was a mistake, since during the water/sediment studies X12335723 was not detected in sediment and exposure of sediment dwellers to this compound may be thus excluded.</p> <p>Nevertheless, some of metabolites present in sediment are formed from this compound and the study was submitted in order to demonstrate decreased toxicity to aquatic organisms from metabolites formed in a metabolic pathway including formation of</p>
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	<p>X12335723. Taking this into account, the study was evaluated by the zRMS. The study was performed in line with OECD 218 with no deviations.</p> <p>Throughout the test the concentrations of metabolite X12335723 were not maintained within 80-120% of nominal concentration; therefore, the endpoints are expressed in terms of the initial measured concentrations (im) and time weighted mean measured concentrations (twm).</p> <p>The test design was suitable to derive both, NOEC and ECx values, but due effects &lt;10% the ECx values could not be calculated.</p> <p>In the test report no justification was given why endpoints were based on TWA mean measured concentrations and not on commonly agreed geometric mean measured concentrations, however OECD 218 does not specify how the endpoints should be calculated in case the test item is not stable over the entire study period. Taking this into account, calculation of TWA mean measured concentrations is agreed by the zRMS.</p> <p>All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>overall NOEC (all parameters) = 6.8 mg pm/kg (based on initial measured concentration)</p> <p>overall NOEC (all parameters) = 2.2 mg X642188/kg (based on time weighted mean measured concentration)</p>
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Reference:	KCP 10.2.2/3
Report:	xxx.; 2018; X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> , Using Spiked Sediment; xxx; Lab Study No. 87150; DAS Study No. 180564 ; 31 August 2018; Unpublished
Guideline(s):	OECD Guideline 218
Deviations:	
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name):	X12335723
Purity:	77%
Description (physical state):	White powder
Lot/batch no.:	SYN-FS09270-061 (TSN304462)

### Test System

Organism ( <i>Species</i> ):	Freshwater midge ( <i>Chironomus riparius</i> )
Study type:	Chronic life cycle study Static
Duration of study:	28 days
Method of test item application:	Spiked sediment

Age of test organisms at test initiation:	Approx. 3-day old first-instar larvae
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.63, 1.3, 2.5, 5.0, and 10 mg/kg
Feeding:	Mean measured sediment at day 0: <MQL (control), <MQL (vehicle control), 0.69, 0.99, 1.8, 3.6, and 6.8 mg/kg Overlying water at day 0: ranged from <MQL to 0.025 mg/L Pore water at day 0: ranged from 0.52 to 7.3 mg/L Time-weighted Mean Sediment: <MQL (control), <MQL (vehicle control), 0.23, 0.33, 0.57, 1.2, and 2.2 mg/kg The larvae were fed 2.0 mL of 10 g/L algae on day 0, additionally the larvae were fed 5 mL of a 2.0 g/L flake food suspension daily, on days 0 through 20. Feeding was suspended on day 21 due to excessive amounts of uneaten food.
Ratio of sediment layer to depth of overlying water:	1:4
Reference substance:	none
No. of Chironomid per vessel:	20
No. of Chironomid per dose group:	120
No. of Chironomid per control group:	120
No. of replicates:	6 per treatment group and control
Environmental conditions:	Temperature: 20.2 to 20.7 °C Dissolved Oxygen: 7.1 to 8.8 mg/L (82 to 104% saturation) pH: 8.0 to 8.5 Total Hardness: 152 to 240 mg CaCO <sub>3</sub> /L
Parameters measured:	Survival, number emerged and time to emergence, and development rate
Observation intervals:	Daily Photoperiod: 16 hr light: 8-hr dark Light intensity: 702 lux Un-ionised Ammonia: 0.00 mg/L

## Methodology

The test chambers were 1-L glass jars that were approximately 17 cm in height by 8.5 cm in diameter. Approximately 200 g (approximately 2 cm sediment depth) of prepared formulated sediment (75% fine industrial sand, 20% kaolin clay, 4-5% sphagnum peat) thoroughly mixed with the test item was added to each replicate test chamber. A 600-mL volume of dilution water (approximately 8 cm) was carefully added to the test chambers and a turbulence deflector was used during the water addition to minimize the disturbance of the sediment.

Six control, vehicle control, and test substance treatment replicate test chambers were prepared for the biological parameters. Eight additional control, vehicle control, and test substance treatment replicate chambers were prepared for the various analyses of the overlying water, pore water, and sediment samples.

The test chambers were placed in a temperature-controlled water bath arranged by treatment level. All replicates were covered with emergence traps to capture emerged adults. Aeration was provided to each test chamber two to three centimetres from the sediment surface. Aeration was initiated approximately 24 hours after organism addition. During the test, the aeration was adjusted as deemed necessary in an attempt to maintain the dissolved oxygen concentrations within each test chamber.

On study initiation day a target total of 20 midge larvae were added, five at a time, starting with the controls and proceeding to the high treatment, until the required number of individuals were added to

each test chamber. Organisms were added 2-3 cm above the sediment/water interface using a widebore pipette.

Observations of the biological replicates were recorded daily throughout the test. Any abnormal activity (i.e., sediment avoidance, inactivity, etc.) was noted, if observed. At study day 10, two replicates (E and F) were sieved and surviving larvae or pupae, if any, were retained by the mesh and were recorded to determine the 10-day survival.

Temperature, dissolved oxygen concentration, and pH of the overlying water were measured in replicates A through F at test initiation, at day 10 in replicates E and F, and in replicates A through D at test termination. Temperature, pH, and dissolved oxygen were measured with a WTW Multi Meter 3430. The waterbath temperature was continuously measured starting on day 0 and recorded with an electronic data logger. On days 0 (replicates A through F) and 28 (replicates A through D), composite samples of overlying water were collected for measurement of total hardness and ammonia concentrations.

The concentrations of X12335723 was measured in all treatments within overlying water, pore water, and sediment samples collected at test initiation (day 0), day 2, day 10, and day 28 (termination). Overlying water, interstitial (pore) water, and sediment were analysed using a liquid chromatographic with tandem mass spectrometry (LC-MS/MS) system.

## RESULTS AND DISCUSSION

The initial (day 0) mean measured concentrations of X12335723 in the sediment samples were 0.69, 0.99, 1.8, 3.6, and 6.8 mg/kg dry sediment in the 0.63, 1.3, 2.5, 5.0, and 10 mg/kg treatments, respectively, which represented 68 to 109% of the nominal concentrations. The mean measured concentrations of X12335723 in sediment samples on day 2 were 0.52, 0.76, 1.3, 2.9, and 5.3 mg/kg dry sediment, which represented 53 to 82% of the nominal concentrations. The mean measured concentrations of X12335723 in sediment samples on day 10 were 0.24, 0.33, 0.59, 1.2, and 2.3 mg/kg dry sediment, which represented 23 to 38% of the nominal concentrations. The mean measured concentrations of X12335723 in sediment samples on day 28 (termination) were 0.056, 0.082, 0.14, 0.25, and 0.43 mg/kg dry sediment, which represented 4 to 9% of the nominal concentrations. Results for sediment are given in Table 41, while in Tables 39 and 40 also analytical results for overlying water and pore water are reported.

Based on analytical results obtained in sediment, the time weighted mean concentrations were determined using the following calculation:

Area calculated by:  $((\text{Conc. 0} - \text{Conc. 1}) / (\ln \text{Conc. 0}) - (\ln \text{Conc. 1})) \times \text{time period (days)}$

where:

Conc. 0 is the concentration at the first sampling point (i.e., day 0, day 2, and day 10, respectively)

Conc. 1 the concentration at the corresponding second sampling point, (i.e., day 2, 10, and 28, respectively) for each sampled time period

Time weighted mean concentration =  $(\text{day 0-2 area} + \text{day 2-10 area} + \text{day 10-28 area}) / \text{Total Days}$

The overall time-weighted mean measured concentrations of X12335723 in the sediment samples were 0.23, 0.33, 0.57, 1.2, and 2.2 mg/kg dry sediment which represented 37, 25, 23, 24 and 22% of nominal. All biological response evaluations were calculated based on the initial (day 0) mean measured sediment concentrations and the time-weighted mean measured sediment concentrations.

**Table 39: Results from analysis of overlying water samples**

Nominal sediment concentration (mg/kg)	Measured concentrations (mg/L)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
0 (control)	<MQL	<MQL	<MQL	<MQL	Water type: moderately hard freshwater prepared by blending naturally hard well water with well water that was de-mineralized by reverse osmosis Dissolved oxygen: 7.1 to 8.8 mg/L (82 to 103% saturation) pH: 8.0 to 8.5 Hardness: 156 to 240 mg CaCO <sub>3</sub> /L  Alkalinity: not determined  Conductivity: not determined  Ammonia concentration: 0.00 mg/L (unionized) Water temperature: 20.2 to 20.7 °C Renewal of water: none
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL	
0.63	<MQL	0.018, 0.021	<MQL	<MQL	
1.3	0.0038, 0.0050	0.030, 0.032	0.0081, 0.010	<MQL	
2.5	0.0086, 0.012	0.067, 0.082	0.013, 0.015	<MQL	
5.0	0.011, 0.021	0.16, 0.16	0.019, 0.024	<MQL	
10	0.019, 0.025	0.34, 0.36	0.048, 0.069	<MQL, 0.0054	
MQL	0.0040 mg/L				
LOD	Not determined				

**Table 40: Results from analysis of pore water samples**

Nominal sediment concentration (mg/kg)	Measured concentrations (mg/L)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
0 (control)	<MQL	<MQL	<MQL	<MQL	Dissolved oxygen: not determined pH: not determined Ammonia concentration: not determined Hardness: not determined  Alkalinity: not determined Conductivity: not determined Water temperature: not determined
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL	
0.63	0.52, 0.54	0.35, 0.36	0.11, 0.11	0.017, 0.018	
1.3	0.85, 0.86	0.58, 0.59	0.16, 0.17	0.028, 0.030	
2.5	1.6, 1.6	1.1, 1.1	0.33, 0.33	0.044, 0.045	
5.0	3.4, 3.4	2.3, 2.4	0.65, 0.66	0.086, 0.094	
10	7.2, 7.3	4.7, 5.1	1.4, 1.4	0.17, 0.18	
MQL	0.0040 mg/L				
LOD	Not determined				

**Table 41: Results from analysis of sediment samples**

Nominal sediment concentration (mg/kg)	Mean measured concentrations (mg/kg)				Other parameters
	Day 0	Day 2	Day 10	Day 28	
0 (control)	<MQL	<MQL	<MQL	<MQL	Type: artificial Total organic carbon: not determined Kaolin clay (%): 20 Fine industrial sand (%): 75 Sphagnum peat (%): 5 pH: 7.0 ± 0.5 units C/N ratio: not determined Deionised water: appropriate volume added to achieve a hydration level of approximately 35% Organic carbon: 2.5%
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL	
0.63	0.69	0.52	0.24	0.056	
1.3	0.99	0.76	0.33	0.082	
2.5	1.8	1.3	0.59	0.14	
5.0	3.6	2.9	1.2	0.25	
10	6.8	5.3	2.3	0.43	
MQL	0.0038 mg/kg				
LOD	Not determined				

The mean survival in replicates E and F at day 10 was 85 and 100% in the control and vehicle control, respectively, and ranged from 95 to 100 for the test substance treatment levels. There was a statistically significant difference between the control and vehicle control, therefore, statistical comparisons were made against the vehicle control. There was no statistically significant difference (Dunnett's test,  $p > 0.05$ ) in the mean day 10 survival in the test substance treatments as compared to the vehicle control (i.e., 100%).

The mean biomass in replicates E and F at day 10 was 0.59 and 0.59 g in the control and vehicle control, respectively, and ranged from 0.66 to 0.84 g for the test substance treatment levels. There were no statistically significant differences (Dunnett's test,  $p > 0.05$ ) in mean biomass in the test substance treatments as compared to the pooled control (i.e., 0.59 g).

Emergence was observed starting on day 13 in the control, vehicle control, and all test substance treatments. The last emerged midge was observed on day 23. Percent emergence of adult freshwater midges in the control and vehicle control was 90 and 90% respectively, which exceeded the minimum acceptability criterion of 70% control emergence specified by the protocol and the OECD 218 guideline. The mean percent emergence was 94, 93, 91, 93, and 96% in the 0.69, 0.99, 1.8, 3.6, and 6.8 mg/kg day 0 mean measured sediment treatments (0.23, 0.33, 0.57, 1.2, and 2.2 mg/kg timeweighted mean measured sediment treatments), respectively. There were no statistically significant differences (Dunnett's test,  $p > 0.05$ ) in mean percent emergence in the test substance treatments as compared to the pooled control.

The gender ratio for the control and vehicle control was 0.84 and 1.25 males to each female. The male to female emergence gender ratio for the treatments ranged from 0.83 in the 6.8 mg/kg day 0 mean measured sediment treatment (2.2 mg/kg time-weighted mean measured sediment treatment) to 1.03 in the 0.99 mg/kg day 0 mean measured sediment treatment (0.33 mg/kg time-weighted mean measured sediment treatment).

The mean development rates for the emergent males was 0.0730 and 0.0666 in the control and vehicle control, respectively, and ranged from 0.0670 to 0.0720 for the test substance treatment levels. The mean development rates for the emergent females was 0.0659 and 0.0580 in the control and vehicle control, respectively, and ranged from 0.0578 to 0.0637 for the test substance treatment levels. The mean total adult development rates was 0.0690 and 0.0625 in the control and vehicle control, respectively, and ranged from 0.0626 to 0.0674 for the test substance treatment levels. There were statistically significant differences between the control and vehicle control in male, female, and total adult development rates, therefore, statistical comparisons were made against the vehicle control. There was no statistically significant difference (Dunnett's test,  $p > 0.05$ ) in mean development rate, in any of the test substance treatments, for males, females, or total, as compared to the vehicle control.

**Table 42: Effect of X12335723 on adult survival and biomass at day 10**

Day-0 Mean Measured Sediment (mg/kg)	Time-Weighted Mean Measured Sediment (mg/kg)	Parameter	Adult survival and biomass		
			Rep E	Rep F	Mean of all replicates
Negative control	Negative control	Survival (%)	80	90	85
		Biomass (mg)	0.47	0.71	0.59
Vehicle control	Vehicle control	Survival (%)	100	100	100
		Biomass (mg)	0.64	0.54	0.59
0.69	0.23	Survival (%)	100	95	98
		Biomass (mg)	0.65	0.70	0.67
0.99	0.33	Survival (%)	95	100	98
		Biomass (mg)	0.65	0.67	0.66
1.8	0.57	Survival (%)	100	100	100
		Biomass (mg)	0.64	0.75	0.69
3.6	1.2	Survival (%)	100	90	95
		Biomass (mg)	0.72	0.79	0.76



6.8	2.2	Survival (%)	95	100	98
		Biomass (mg)	0.84	0.84	0.84

**Table 43: Effect of X12335723 on adult emergence and development rate at day 28**

Day-0 Mean Measured Sediment (mg/kg)	Time-Weighted Mean Measured Sediment (mg/kg)	Sex of emerged midge	Adult emergence				
			Rep A	Rep B	Rep C	Rep D	Mean of all replicates
Negative control	Negative control	% Emerged	85	85	100	90	90
		M Dev. rate	0.0742	0.0743	0.0713	0.0723	0.0730
		F Dev. rate	0.0710	0.0683	0.0614	0.0631	0.0659
		Tot Dev. rate	0.0727	0.0697	0.0658	0.0679	0.0690
Vehicle control	Vehicle control	% Emerged	90	100	85	85	90
		M Dev. rate	0.0625	0.0670	0.0648	0.0720	0.0666
		F Dev. rate	0.0585	0.0587	0.0550	0.0599	0.0580
		Tot Dev. rate	0.0610	0.0628	0.0607	0.0656	0.0625
0.69	0.23	% Emerged	95	90	100	90	94
		M Dev. rate	0.0728	0.0716	0.0689	0.0716	0.0712
		F Dev. rate	0.0604	0.0627	0.0616	0.0662	0.0627
		Tot Dev. rate	0.0630	0.0687	0.0657	0.0692	0.0666
0.99	0.33	% Emerged	85	90	95	100	93
		M Dev. rate	0.0696	0.0641	0.0655	0.0743	0.0684
		F Dev. rate	0.0568	0.0542	0.0535	0.0665	0.0578
		Tot Dev. rate	0.0651	0.0591	0.0575	0.0701	0.0629
1.8	0.57	% Emerged	90	95	90	90	91
		M Dev. rate	0.0678	0.0712	0.0663	0.0739	0.0698
		F Dev. rate	0.0548	0.0579	0.0575	0.0687	0.0597
		Tot Dev. rate	0.0606	0.0635	0.0615	0.0716	0.0643
3.6	1.2	% Emerged	95	95	90	90	93
		M Dev. rate	0.0631	0.0627	0.0692	0.0730	0.0670
		F Dev. rate	0.0573	0.0510	0.0589	0.0653	0.0581
		Tot Dev. rate	0.0591	0.0594	0.0629	0.0689	0.0626
6.8	2.2	% Emerged	95	95	100	95	96
		M Dev. rate	0.0747	0.0701	0.0710	0.0722	0.0720
		F Dev. rate	0.0695	0.0619	0.0626	0.0608	0.0637
		Tot Dev. rate	0.0725	0.0640	0.0663	0.0669	0.0674

## CONCLUSION

The control organisms met the acceptability criterion for mean percent emergence (i.e., >70%) as specified by the study protocol and the OECD 218 testing guideline.

All effects concentrations were based on the day 0 mean measured sediment concentrations and the time-weighted mean measured sediment concentrations.

The day 10 survival and biomass NOEC and LOEC values, based the day 0 mean measured sediment concentrations (time-weighted mean measured sediment concentrations), were 6.8 and >6.8 mg/kg (2.2 and >2.2 mg/kg), respectively, the highest concentration tested. The emergence and male, female, and total development rate NOEC and LOEC values, based the day 0 mean measured sediment concentrations (time-weighted mean measured sediment concentrations), were 6.8 and >6.8 mg/kg (2.2 and >2.2 mg/kg), respectively, the highest concentration tested. The EC<sub>10</sub>, EC<sub>15</sub>, and EC<sub>50</sub> values for day 10 survival, biomass, emergence, and the male, female and total development rate data could not be calculated due to the lack of a concentration dependent response trend.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 10 Survival NOEC	6.8	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 10 Biomass NOEC	6.8	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Emergence NOEC	6.8	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Male Development Rate NOEC	6.8	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Female Development Rate NOEC	6.8	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Total Development Rate NOEC	6.8	mg/kg (day 0 mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 10 Survival NOEC	2.2	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 10 Biomass NOEC	2.2	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Emergence NOEC	2.2	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Male Development Rate NOEC	2.2	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Female Development Rate NOEC	2.2	mg/kg (time-weighted mean sediment)
Midge	<i>Chironomus riparius</i>	X12335723	28 day	Day 28 Total Development Rate NOEC	2.2	mg/kg (time-weighted mean sediment)

### A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

#### A 2.2.3.1 Study 1 – X12433979 (a metabolite of XDE-777): Prediction of Octanol-Water Partition coefficient and Aquatic Toxicity using computerized Quantitative Structure-Activity Relationships

Comments of zRMS:	<p>This 'non-testing method' study was carried out using a Quantitative Structure Activity Relationship (QSAR) model for prediction of acute toxicity of metabolite X12433979 in fish (96h LC<sub>50</sub>), aquatic invertebrates (48h EC<sub>50</sub>), and algae (96h EC<sub>50</sub>). The zRMS agrees with the proposed endpoints and their justification.</p> <p>Fish LC<sub>50</sub> = 81.990 mg/L  Aquatic invertebrate EC<sub>50</sub> = 48.857 mg/L  Algae EC<sub>50</sub> = 44.437 mg/L</p> <p>Information regarding log Kow is not relevant for this part of the assessment and was struck through.</p>
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Reference:	KCP 10.2.3/1
Report:	Blickley, T.M., Kramer, V.J.; 2018; X12433979 (a metabolite of XDE-777): Prediction of Octanol-Water Partition Coefficient and Aquatic Toxicity using Computerized Quantitative Structure-Activity Relationships; Dow AgroSciences LLC, Zionsville, Indiana, USA; DAS Study No. 180910
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	No
Acceptability:	Acceptable

Duplication (if vertebrate study)	NA
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## Abstract

This document provides the results of “non-testing methods” for X12433979, a metabolite of XDE777. The “non-testing methods” included Quantitative Structure Property Relationship (QSPR) models for prediction of octanol-water partition coefficient (reported as  $\log_{10} K_{ow}$ ) and Quantitative Structure Activity Relationship (QSAR) models for prediction of acute toxicity in fish ( $LC_{50}$ ), aquatic invertebrates ( $EC_{50}$ ), and population-level toxicity ( $EC_{50}$ ) to algae. The parent XDE-777 and metabolite X12314005 were also evaluated for comparison and benchmarking. Predicted  $\log K_{ow}$  values for X12433979, X12314005, XDE-777 were 2.52, 2.61, and 4.15, respectively. Predicted fish 96 hr  $LC_{50}$ , daphnid 48 hr  $EC_{50}$ , and green algae 96 hr  $EC_{50}$  values for X12433979 were 81.990, 48.857, and 44.437 mg/L, respectively. Predicted fish 96 hr  $LC_{50}$ , mysid 96 hr  $EC_{50}$ , and green algae 96 hr  $EC_{50}$  values for X12314005 were 16.203, 17.264, and 12.182, respectively. Predicted fish 96 hr  $LC_{50}$ , mysid 96 hr  $EC_{50}$ , and green algae 96 hr  $EC_{50}$  values for XDE-777 were 4.569, 0.600, and 0.267 mg/L, respectively.

## Methodology

The  $\log_{10} K_{ow}$  and acute toxicity in fish ( $LC_{50}$ ), aquatic invertebrates ( $EC_{50}$ ), and population-level toxicity ( $EC_{50}$ ) to algae was estimated for metabolite X12433979, metabolite X12314005, and XDE777. The computer program KOWWIN v. 1.68 (Meylan and Howard, 1995) was used to calculate  $\log K_{ow}$ . Aquatic toxicity values were estimated using ECOSAR v1.11. These programs were executed within a suite of estimation programs known as EPI Suite v. 4.11 provided as an open access application (<https://www.epa.gov/tsca-screening-tools/epi-suite-tm-estimation-program-interface>) by the United States Environmental Protection Agency (US EPA).

## RESULTS AND DISCUSSION

The predicted  $\log K_{ow}$  value for X12433979 is presented in Table 1. Using the parent molecule XDE777 as a test case, the model performed well in predicting  $\log K_{ow}$  for the class of chemistry represented by XDE-777, providing an estimated value that was within 0.5 log units of the measured value at pH 7.

Table 44: Concordance of predicted with measured octanol-water partition coefficient ( $\log K_{ow}$ ) for XDE777.

DAS ID Number	Predicted $\log K_{ow}$	Measured $\log K_{ow}$	Study Reference
XDE-777	4.15	pH 5 — 4.2 pH 7 — 4.4 pH 9 4.3	Comb, 2012
X12314005	2.61	—	
X12433979	2.52	—	

Predicted acute toxicity values for fish, invertebrates, and algae are presented in Table 2 for metabolite X12433979. A small-scale verification exercise using XDE-777 and metabolite X12314005 is also presented and builds on previous work reported by Kramer, (2014)<sup>3</sup>. The lack of concordance between predicted and actual toxicity values for XDE-777 (2000X more toxic to fish and 100X more toxic to aquatic invertebrates than predicted) can be explained by the inability of the ECOSAR prediction tool

<sup>3</sup> Additional QSAR predicted aquatic toxicity values for XDE-777 metabolites X642188, X696872, X12255349, X12335723, X12446477, X12386481, X12264475, X763024, X12313581, X696476, X11963422 and X12019520 are available in Kramer, V.J. 2014. Prediction of Octanol-Water Partition Coefficient, Acid Dissociation Constant, Fish Bioconcentration and Aquatic Toxicity of Metabolites of XDE-777 using Computerized Quantitative Structure-Activity Relationships. Dow AgroSciences LLC. DAS ID# 141106. Unpublished., which was evaluated and accepted in the EFSA Peer Review Report (2017) and the endpoints included in the Fenpicoxamid LoEP (EFSA, 2018).

to take into account the specific mode of action of this pesticide molecule. Good concordance between predicted and measure values is observed for X12314005 for fish and aquatic invertebrates. For actual toxicity values observed to be greater than the maximum concentration tested, positive concordance was concluded in some cases even though the predicted value exceeded these thresholds because the risk assessment would still be protective using either value for these relatively low toxicity metabolites.

**Table 45: Concordance of predicted fish 96 hr LC<sub>50</sub>, aquatic invertebrate EC<sub>50</sub>, and algae EC<sub>50</sub> for XDE777, X12314005 and predicted values for X12433979.**

DAS ID Number	Pred. Fish 96 hr LC <sub>50</sub> (mg/L)	Actual Trout 96 hr LC <sub>50</sub> (mg/L)	Pred. Aq. Invert. EC <sub>50</sub> (mg/L)	Actual <i>D. magna</i> . EC <sub>50</sub> (mg/L)	Pred. Algae EC <sub>50</sub> (mg/L)	Actual Green Algae EC <sub>50</sub> (mg/L)	Study References
XDE-777	4.569	=0.0022	0.600	=0.00093	<b>0.267</b>	<b>&gt;0.522</b>	Fournier, 2012a Fournier, 2012b Rebstock, 2012
X12314005	<b>16.203</b>	<b>&gt;1.9</b>	<b>17.264</b>	<b>&gt;8.5</b>	12.182	--	Dinehart, 2014a Dinehard, 2014b
X12433979	81.990	--	48.857	--	44.437	--	
<sup>a</sup> >1.9 indicates a 50% effect level was not achieved in the study, hence the LC <sub>50</sub> was greater than (>) the highest concentration tested. Test concentrations were not expanded to encompass an observed 50% effect level due to various reasons including limited supply of test material and conduct of single-treatment level limit tests to reduce vertebrate animal testing. An “=” sign indicates that the 50% effect level was actually measured at the tested concentrations. Results in <b>bold</b> are considered to be concordant with predicted values. <sup>b</sup> -- indicates that an actual study result was not available.							

#### **A 2.2.3.2 Study 2 - GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna***

Comments of zRMS:	<p>The study was performed in order to investigate effects of GF-3308 on <i>Daphnia magna</i> population in an indoor aquatic microcosms. It should be, however, noted that the test system has not mimicked the natural conditions (as usually do microcosm/mesocosm studies) but the study was performed under standard laboratory conditions and the only difference from the 21-day reproductive toxicity study was inclusion of the various Daphnids stages (adults, juveniles and neonates).</p> <p>The study have not followed any recognised test guideline, but its design followed recommendations for the standard reproductive test (OECD 211) and mesocosm studies.</p> <p>Due to known instability of fenpicoxamid resulting from hydrolysis, the measured concentrations of the active compound in the test solutions of all treatments were intensively determined directly after dosing (at 0, 1, 2, 4, 8 and 24 hours) and then after 48 hours and 7 days.</p> <p>As expected, fenpicoxamid concentration declined rapidly and after 24 hours they represented 0-14% of nominal concentrations (depending on the test group). No residues of fenpicoxamid were found in any of the test solutions already 48 hours after dosing.</p> <p>In general, no treatment related effects were observed on the various developmental stages of <i>Daphnia magna</i> over the entire test period with exception of:</p> <ol style="list-style-type: none"> <li>1. Juveniles on day 2 with reduction of 30% in the highest treatment group comparing to controls. However, at remaining test concentrations the number of juveniles were variable with no dose-response. Taking this into account, the reduced abundance in the highest treatment group could be incidental. The abundance of adult and neonates was unaffected at this sampling day.</li> <li>2. Neonates on day 7 with clear reduction of abundance from second test concentration (0.017 mg test item/L) and observed dose response (reduction by 15, 15, 17 and 18% at 0.017, 0.052, 0,18 and 0.55 mg test item/L), reflected in calculated EC<sub>10</sub> (0.039 mg test item/L) being lower than the NOEC (0.55 mg test item/L). No effect was observed at 0.0054 mg test item/L. The abundance of adult and juveniles was unaffected at this sampling day.</li> </ol>
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	<p>At remaining samplings the abundance of all Daphnia stages was variable with no effects caused by the treatment.</p> <p>Due to lack of adverse effects and disappearance of the test item from the system, the study was terminated after 21 days.</p> <p>The performed MDD analysis resulted with MDD classes II-III for neonates and juveniles and I-II for adults (but MMD class I was observed on day 2 only), depending on the day of study. For total developmental stages MDD class III were calculated. Overall, the test design was sufficient to determine medium effects at most observations interval, as indicated in EFSA (2013). Lower MDD classes observed on some days do not invalidate the test results, especially the study was performed for bridging purposes only.</p> <p>It has to be noted that according to the EFSA aquatic guidance (2013), refined exposure laboratory studies with population of invertebrates are not recommended due to the rapid onset of recovery. The following is stated in the guidance:</p> <p><i>“Although refined exposure tests with standard test species that more or less resemble the design of tier 1 toxicity studies can be used for RAC derivation, the PPR Panel recommends not using refined exposure laboratory tests with populations of invertebrates (e.g. Daphnia) for this purpose when recovery is also considered. These population-level laboratory experiments with invertebrates are usually performed with individuals that differ in age and developmental state. As a result a rapid onset of recovery will occur after contamination under such test scenarios. Resources for surviving individuals will increase after contamination and will trigger an unrealistic strong recovery as no competitors are present (Knillmann et al., 2012b).”</i></p> <p>It should be, however, pointed out that this population study with GF-3308 together with similar study with fencicoxamid (Hicks, 2017, KCP 10.2.3/3, see below for summary) were performed in order to demonstrate that the active substance is more toxic than GF3308 and derived endpoints were not used directly in the risk assessment. Taking this into account, the zRMS is of the opinion that potential recovery is not an issue here and the study may be accepted for the comparative purposes with indication that the endpoints are not relevant for the risk assessment performed in line with EFSA (2013). For the same reasons the way of expression of the endpoints is considered not critical for this study and the zRMS agreed with endpoints expressed in terms of the initial measured concentrations. However, due to rapid recovery observed in such studies the endpoint from this study should not be read as an actual NOEC, but rather NOEAEC.</p> <p>Overall, the study is considered acceptable for comparative purposes with following endpoints (all based on initial measured concentrations):</p> <p>21-d NOEAEC = 550 µg prep/L (27 µg a.s./L)  EC<sub>10-neonates</sub> = 39 µg prep/L (1.9 µg a.s./L) (only on day 7, no effects at remaining samplings)  EC<sub>10-juveniles</sub> = 370 µg prep/L (18 µg a.s./L) (only on day 2, no effects at remaining samplings)</p> <p>Further discussion on the applicability of the study and reliability of the endpoints is presented in point 9.5 of this document.</p>
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Reference:	KCP 10.2.3/2
Report:	Hicks, S.; 2016; GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna; ABC Laboratories, Inc. a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, USA; Lab Study No. 83492; DAS Study No. 160126 ; 07 December 2016; Unpublished
Guideline(s):	None

Deviations:	Not relevant (no recognisable guideline followed)
GLP:	Yes
Acceptability:	Acceptable for the bridging purposes. Endpoints not relevant for the risk assessment performed in line with EFSA (2013) due to rapid onset of recovery in such tests.
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: 4.9 wt% DE-777 (synonym XDE-777)  
Description (physical state): Brown liquid  
Lot/batch no.: ENBK-14585-032A (TSN309730)

### Test System

Organism (*Species*): Water flea (*Daphnia magna*)  
Study type: Population effects  
Study design: Static  
Test concentrations: Nominal: 0 (control), 0.0072, 0.023, 0.065, 0.20, and 0.59 mg GF-3308/L  
Initial Mean Calculated: <MQL (control), 0.0054, 0.017, 0.052, 0.18, and 0.55 mg GF-3308/L  
Parameters measured: Daphnid abundance  
Observation intervals: Day 0, 2, 7, 14, and 21  
Age of test organisms at test initiation: ~4 and ~10 days old  
Analytical confirmation of test concentrations: XDE-777 and X642188: At initiation, 1 hour, 2 hour, 4 hour, 8 hour, 24 hour, 48 hour, and day 7  
X12255349: at initiation, 24, 48 hour and day 7, 14, and 21  
No. of holding days before dosing: 7 days  
Number of Daphnids per dose group: 800 juvenile and 800 adult (i.e., five per liter)  
Number of Daphnids per control group: 800 juvenile and 800 adult (i.e., five per liter)  
Feeding regime: Daily  
Environmental conditions: Loading rate: not applicable  
Temperature: 19.5 to 20.5 °C  
Photoperiod: 16.75-hr light: 7.25-hr dark (Day -7 to 10)  
16-hr light: 8-hr dark (Day 11 to 21)  
Dissolved oxygen concentration: 4.3 to 8.8 mg/L (49 to 101% sat.) pH: 7.9 to 8.6  
Total hardness: 178 to 204 mg CaCO<sub>3</sub>/L  
Salinity: not applicable  
Reference substance: XDE-777 technical (TSN302306)

### Methodology

A definitive test was performed from 22 February to 14 March 2016. Test chambers for the definitive test consisted of glass aquaria measuring approximately 30 cm wide by 60 cm long by 40 cm high with a test solution depth of 22 cm, for a test solution volume of approximately 40 L.

The control and all treatments were replicated four times. 200 adult and 200 juvenile daphnids were introduced into each aquarium on day -7 to allow a 7-day acclimation phase.

The test was initiated by administering the test substance to the dilution water within each test chamber on 22 February 2016 (study day 0). The test chambers were gently mixed with a glass rod while slowly adding the test solution over an approximate 2-minute period. Gentle mixing continued for approximately one minute after completing the solution return.

The daphnids were fed daily a diet consisting of increasing amounts of a concentrated algal suspension (*Pseudokirchneriella subcapitata*). Daphnids were also fed 20 or 40 mL per replicate of a suspension, ranging from approximately 2.14 to 2.39 g/L, of a prepared invertebrate food solution (wheat grass, salmon starter, and yeast suspension).

The definitive test was conducted for 21 days commencing seven days (i.e., Day -7; start of 7-day acclimatization phase) after the daphnids were added to the test chambers.

Observations were made daily for immobile daphnids and occurrence of abnormalities. Abundance (number of individuals per liter) of each life stage over time was determined by sampling the population prior to test initiation (days -5 and -3), at initiation (day 0), and on days 2, 7, 14, and 21 after test initiation.

Adult daphnids morphological sex was determined in each biological sampling. Each aquarium was divided into 18 equally sized areas by placing a plastic mesh grid on the top. Five areas were randomly selected to be sampled at each sampling event using a random number generator for each replicate. Approximately 2-L (~5% of the total volume) was collected at each biological sampling point. Sampling vessels, consisting of a 2" (~5.2 cm) diameter PVC pipe containing a silicone stopper attached to a length of string and wire were used to sample the water column at each selected area. The silicon stopper was lowered through the grid until it rested on the bottom of the aquarium. This was repeated until the 5 areas selected for sampling all had a silicone stopper placed accordingly. The PVC pipe was then lowered onto the silicone stopper and the seal tightened to retain the water column sample in the pipe. A 5-gal bucket was used to collect the samples from the water columns. The bucket contained three sieves, 1.00, 0.71, and 0.25 mm respectively, stacked in descending order of mesh size. The water column samples were emptied into the bucket and allowed to flow through the stack of sieves. Daphnids collected in the sample were trapped in a particular sieve, depending on the size of the daphnid. Each sieve was examined to determine the number of adult (>1.00 mm), juvenile (>0.71 to <1.00 mm), and neonate (>0.25 to <0.71 mm) daphnids collected. The collected daphnids were preserved with 1-2 mL 70% ethanol in appropriately labeled containers at room temperature for later counting. The collected water sample from each replicate was returned to the respective replicate test chamber following completion of daphnid segregation.

Test solutions were analysed for the concentration of GF-3308 using a liquid chromatography with tandem mass spectrometry (LC-MS/MS) system (LOQ = 0.00041 mg GF-3308/L). Concentrations of GF-3308, based on analysis of XDE-777, were measured in all control and test substance treatments at hour 0, 1, 2, 4, 8, 24, and 48, and day 7 time points. Concentrations of X642188 were measured in all control and test substance treatments at hour 0, 1, 2, 4, 8, 24, and 48, and day 7 time points (LOQ = 0.0000016 mg/L). Concentrations of X12255349 were measured in all control and test substance treatments at hour 0, 24, and 48, and day 7, 14, and 21 time points (LOQ = 0.0000042 mg/L).

The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) for the abundance data following test substance application were determined by using a one-tailed

Dunnett's and Williams' test with the alternate hypothesis being that the mean for the parameter was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance.



## RESULTS AND DISCUSSION

Mean calculated concentrations of GF-3308, based on analysis of XDE-777, in the test substance solution treatment replicate solutions at initiation (0 hour) were 0.0054, 0.017, 0.052, 0.18, and 0.55 mg GF-3308/L in the 0.0072, 0.023, 0.065, 0.20, and 0.59 mg GF-3308/L nominal treatments, respectively, representing 74 to 93% of nominal concentrations.

The mean calculated concentrations of GF-3308 in the test substance treatment replicate solutions at 1 hour were 0.0046, 0.013, 0.040, 0.13, and 0.39 mg GF-3308/L, respectively, representing 57 to 66% of nominal concentrations.

The mean calculated concentrations of GF-3308 in the test substance treatment replicate solutions at 2 hour were 0.0028, 0.011, 0.031, 0.096, and 0.30 mg GF-3308/L, respectively, representing 39 to 51% of nominal concentrations.

The mean calculated concentrations of GF-3308 in the test substance treatment replicate solutions at 4 hour were 0.0016, 0.0072, 0.018, 0.052, and 0.18 mg GF-3308/L, respectively, representing 22 to 31% of nominal concentrations.

The mean calculated concentrations of GF-3308 in the test substance treatment replicate solutions at 8 hour were 0.00062, 0.0027, 0.0068, 0.016, and 0.056 mg GF-3308/L, respectively, representing 8 to 12% of nominal concentrations.

The mean calculated concentrations of GF-3308 in the test substance treatment replicate solutions at 24 hour were <MQL, 0.0031, 0.0061, 0.0028, and 0.0028 mg GF-3308/L, respectively, representing 0 to 13% of nominal concentrations.

No residues of GF-3308 were detected in the control solutions above the MQL (0.00041 mg GF-3308/L) during the exposure period.

Mean measured concentrations of XDE-777, the active ingredient in GF-3308, in the test substance treatment replicate solutions at initiation (0 hour) were 0.00026, 0.00086, 0.0025, 0.0086, and 0.027 mg a.i./L in the 0.00035, 0.0011, 0.0032, 0.0098, and 0.029 mg a.i./L nominal treatments, respectively, representing 74 to 93% of nominal concentrations.

The mean measured concentrations of XDE-777 in the test substance treatment solutions at 1 hour were 0.00023, 0.00065, 0.0019, 0.0063, and 0.019 mg a.i./L, respectively, representing 59 to 66% of nominal concentrations.

The mean measured concentrations of XDE-777 in the test substance treatment solutions at 2 hour were 0.00014, 0.00056, 0.0015, 0.0048, and 0.015 mg a.i./L, respectively, representing 40 to 52% of nominal concentrations.

The mean measured concentrations of XDE-777 in the test substance treatment solutions at 4 hour were 0.000079, 0.00035, 0.00085, 0.0025, and 0.0086 mg a.i./L, respectively, representing 23 to 32% of nominal concentrations.

The mean measured concentrations of XDE-777 in the test substance treatment solutions at 8 hour were 0.000030, 0.00014, 0.00033, 0.00075, and 0.0028 mg a.i./L, respectively, representing 8 to 13% of nominal concentrations.

The mean measured concentrations of XDE-777 in the test substance treatment solutions at 24 hour were <MQL, 0.00015, 0.00030, 0.00014, and 0.00014 mg a.i./L, respectively, representing 0 to 14% of nominal concentrations.

The measured concentrations of XDE-777 in the test substance treatment solutions at 48 hours and day 7 were <MQL in all replicates from all test substance treatments.

No residues of XDE-777 were detected in the control solutions above the MQL (0.0000200 mg a.i./L) during the exposure period.

### The biological response results were reported based upon the initial mean measured GF-3308 and XDE-777 concentrations.

Table 2. Measured Concentrations of XDE-777 (Active Ingredient of GF-3308) During the Population Toxicity Test with *Daphnia magna*

Nominal Concentration (mg a.i./L)	R E P	Measured Concentrations Expressed as mg a.i./L <sup>a</sup>								
		Measured	0 hours % of Nominal	Mean (% nominal)	Measured	1 hour % of Nominal	Mean (% nominal)	Measured	2 hours % of Nominal	Mean (% nominal)
0 (Control)	A	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>
	B	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--	
	C	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--	
	D	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--	
0.00035	A	0.00027	77	0.00026 (74)	0.00021	60	0.00023 (66)	0.00012	34	0.00014 (40)
	B	0.00027	77		0.00023	66		0.00014	40	
	C	0.00026	74		0.00024	69		0.00014	40	
	D	0.00025	71		0.00022	63		0.00014	40	
0.0011	A	0.00090	82	0.00086 (78)	0.00075	68	0.00065 (59)	0.00061	55	0.00056 (51)
	B	0.00086	78		0.00069	63		0.00052	47	
	C	0.00085	77		0.00066	60		0.00055	50	
	D	0.00083	75		0.00051	46		0.00055	50	
0.0032	A	0.0025	78	0.0025 (78)	0.0020	63	0.0019 (59)	0.0015	47	0.0015 (47)
	B	0.0024	75		0.0018	56		0.0014	44	
	C	0.0026	81		0.0020	63		0.0017	53	
	D	0.0026	81		0.0019	59		0.0015	47	
0.0098	A	0.0085	87	0.0086 (88)	0.0064	65	0.0063 (64)	0.0051	52	0.0048 (49)
	B	0.0084	86		0.0064	65		0.0050	51	
	C	0.0085	87		0.0060	61		0.0043	44	
	D	0.0091	93		0.0062	63		0.0047	48	
0.029	A	0.027	93	0.027 (93)	0.019	66	0.019 (66)	0.015	52	0.015 (52)
	B	0.025	86		0.019	66		0.015	52	
	C	0.028	97		0.020	69		0.015	52	
	D	0.027	93		0.019	66		0.014	48	

<sup>a</sup> Sample Measured Concentration (mg a.i./L) = Measured Conc. from Curve (ng a.i./mL) × Analysis Volume (mL) / Sample Volume (mL) / 1000 ng/mg

<sup>b</sup> Minimum Quantifiable Limit (MQL) = 0.0000200 mg a.i./L.

Table 2. Measured Concentrations of XDE-777 (Active Ingredient of GF-3308) During the Population Toxicity Test with *Daphnia magna* (continued)

Nominal Concentration (mg a.i./L)	R E P	Measured Concentrations Expressed as mg a.i./L <sup>a</sup>								
		Measured	4 hours % of Nominal	Mean (% nominal)	Measured	8 hours % of Nominal	Mean (% nominal)	Measured	24 hours % of Nominal	Mean (% nominal)
0 (Control)	A	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>
	B	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--	
	C	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--	
	D	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	--	
0.00035	A	0.000068	19	0.000079 (23)	<MQL <sup>b</sup>	NA	0.000030 <sup>c</sup> (9)	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>
	B	0.000081	23		0.000036	10		<MQL <sup>b</sup>	--	
	C	0.000084	24		0.000035	10		<MQL <sup>b</sup>	--	
	D	0.000081	23		0.000040	11		<MQL <sup>b</sup>	--	
0.0011	A	0.00041	37	0.00035 (32)	0.00013	12	0.00014 (13)	0.00019	17	0.00015 (14)
	B	0.00035	32		0.00013	12		0.00011	10	
	C	0.00031	28		0.00014	13		0.00012	11	
	D	0.00033	30		0.00014	13		0.00018	16	
0.0032	A	0.00097	30	0.00085 (27)	0.00034	11	0.00033 (10)	0.00033	10	0.00030 (9)
	B	0.00087	27		0.00035	11		0.00026	8	
	C	0.00083	26		0.00031	10		0.00028	9	
	D	0.00074	23		0.00032	10		0.00032	10	
0.0098	A	0.0029	30	0.0025 (26)	0.00096 <sup>d</sup>	10	0.00075 (8)	0.00012 <sup>d</sup>	1	0.00014 (1)
	B	0.0025	26		0.00073 <sup>d</sup>	7		0.00014 <sup>d</sup>	1	
	C	0.0022	22		0.00059 <sup>d</sup>	6		0.00014 <sup>d</sup>	1	
	D	0.0025	26		0.00073 <sup>d</sup>	7		0.00015 <sup>d</sup>	2	
0.029	A	0.0085	29	0.0086 (30)	0.0024	8	0.0028 (10)	0.00036 <sup>d</sup>	1	0.00014 (0)
	B	0.0088	30		0.0030	10		0.00070 <sup>d</sup>	0	
	C	0.0081	28		0.0028	10		0.00070 <sup>d</sup>	0	
	D	0.0090	31		0.0028	10		0.00051 <sup>d</sup>	0	

<sup>a</sup> Sample Measured Concentration (mg a.i./L) = Measured Conc. from Curve (ng a.i./mL) × Analysis Volume (mL) / Sample Volume (mL) / 1000 ng/mg

<sup>b</sup> Minimum Quantifiable Limit (MQL) = 0.0000200 mg a.i./L.

<sup>c</sup> The Mean was calculated using 1/2 the MQL for Level 1A sample measured concentration which was <MQL.

<sup>d</sup> The original sample concentrations were below the lowest standard, so the samples were re-analyzed at a lower analysis volume, and the result was reported.

Table 2. Measured Concentrations of XDE-777 (Active Ingredient of GF-3308) During the Population Toxicity Test with *Daphnia magna* (continued)

Nominal Concentration (mg a.i./L)	R E P	Measured Concentrations Expressed as mg a.i./L <sup>a</sup>				
		Measured	48 hours % of Nominal	Mean (% nominal)	Day 7 % of Nominal	Mean (% nominal)
0 (Control)	A	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
	B	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	
	C	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	
	D	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	
0.00035	A	<MQL <sup>b</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
	B	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	
	C	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	
	D	<MQL <sup>b</sup>	--		<MQL <sup>b</sup>	
0.0011	A	<MQL <sup>b,d</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b,d</sup>	<MQL <sup>b</sup>
	B	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	C	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	D	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
0.0032	A	<MQL <sup>b,d</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b,d</sup>	<MQL <sup>b</sup>
	B	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	C	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	D	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
0.0098	A	<MQL <sup>b,d</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b,d</sup>	<MQL <sup>b</sup>
	B	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	C	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	D	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
0.029	A	<MQL <sup>b,d</sup>	--	<MQL <sup>b</sup>	<MQL <sup>b,d</sup>	<MQL <sup>b</sup>
	B	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	C	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	
	D	<MQL <sup>b,d</sup>	--		<MQL <sup>b,d</sup>	

<sup>a</sup> Sample Measured Concentration (mg a.i./L) = Measured Conc. from Curve (ng a.i./mL) × Analysis Volume (mL) / Sample Volume (mL) / 1000 ng/mg

<sup>b</sup> Minimum Quantifiable Limit (MQL) = 0.0000200 mg a.i./L.

<sup>d</sup> The original sample concentrations were below the lowest standard, so the samples were re-analyzed at a lower analysis volume, and the result was reported.

The measured concentrations of metabolites X642188 and X12255349 over the test period are presented in the below tables.

Analysis Timepoint	Mean Measured Concentrations Expressed as mg/L <sup>a</sup>					
	0 (Control) <sup>b</sup>	0.00035 <sup>b</sup>	0.0011 <sup>b</sup>	0.0032 <sup>b</sup>	0.0098 <sup>b</sup>	0.029 <sup>b</sup>
0 Hour	<MQL <sup>c</sup>	0.0000051	0.000016	0.000049	0.00013	0.00047
1 Hour	<MQL <sup>c</sup>	0.0000036	0.000010	0.000043	0.00014	0.00038
2 Hour	<MQL <sup>c</sup>	0.0000021	0.0000098	0.000023	0.000080	0.00022
4 Hour	<MQL <sup>c</sup>	0.0000018 <sup>d</sup>	0.0000062	0.000020	0.000044	0.00018
8 Hour	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.0000031	0.0000084	0.000021	0.000069
24 Hour	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.0000010 <sup>d</sup>	0.0000046
48 Hour	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.0000019 <sup>d</sup>
Day 7	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>
Day 14	<MQL <sup>c</sup>	---	---	---	---	---
Day 21	<MQL <sup>c</sup>	---	---	---	---	---

<sup>a</sup> Measured Concentration (mg X642188/L) = Measured Conc. from Curve (ng/mL) × Analysis Volume (mL) / Sample Volume (mL) / 1000 ng/mg

<sup>b</sup> Nominal concentration as mg XDE-777/L.

<sup>c</sup> MQL = 0.0016 ng/mL = 0.0000016 mg/L.

<sup>d</sup> The Mean was calculated using 1/2 the MQL for samples which were <MQL

--- Samples were not analyzed for X642188 because Day 7 showed all concentrations were <MQL

Analysis Timepoint	Mean Measured Concentrations Expressed as mg/L <sup>a</sup>					
	0 (Control) <sup>b</sup>	0.00035 <sup>b</sup>	0.0011 <sup>b</sup>	0.0032 <sup>b</sup>	0.0098 <sup>b</sup>	0.029 <sup>b</sup>
0 Hour	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>
24 Hour	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.000015	0.000040
48 Hour	<MQL <sup>c</sup>	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.000011	0.000027	0.000085
Day 7	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.0000061	0.000023	0.000068	0.00020
Day 14	<MQL <sup>c</sup>	0.0000046 <sup>d</sup>	0.000011	0.000034	0.000095	0.00029
Day 21	<MQL <sup>c</sup>	<MQL <sup>c</sup>	0.0000083 <sup>d</sup>	0.000027	0.000082	0.00028

<sup>a</sup> Measured Concentration (mg X12255349/L) = Measured Conc. from Curve (ng/mL) x Analysis Volume (mL) / Sample Volume (mL) / 1000 ng/mg

<sup>b</sup> Nominal concentration as mg XDE-777/L.

<sup>c</sup> MQL = 0.0042 ng/mL = 0.0000042 mg/L

<sup>d</sup> The Mean was calculated using 1/2 the MQL for samples which were <MQL

No statistically significant differences were noted in the abundance of any age groups (i.e., adults, juveniles, neonates, or all daphnid age groups combined) during the 21-day post-exposure (Williams' test or Dunnett's test;  $p = 0.05$ ). No males were observed in the adult daphnids population at any sampling time point. All juveniles and neonates were preserved in an appropriately labeled scintillation vial with 70% ethanol for future sexing, if necessary. No abnormal daphnids were observed over the course of the exposure.

**Table 46: Effects of GF-3308 on mean daphnid abundance (Daphnids/L)**

Initial Mean Calculated Treatment (mg GF-3308/L)	Initial Mean Measured Treatment (mg XDE-777/L)	Day -5				Day -3			
		No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	Negative control	7	2	0	8	10	1	1	11
0.0054	0.00026	6	1	0	7	8	0	0	8
0.017	0.00086	5	1	0	6	6	0	1	6
0.052	0.0025	4	1	0	5	9	0	0	9
0.18	0.0086	7	1	0	8	13	0	1	14
0.55	0.027	9	1	1	10	7	0	0	7
NOEC		Not Calculated				Not Calculated			
EC <sub>10</sub>		Not Calculated				Not Calculated			
EC <sub>20</sub>		Not Calculated				Not Calculated			

**Table 47: Effects of GF-3308 on mean daphnid abundance (Daphnids/L) (continued)**

Initial Mean Calculated Treatment (mg GF-3308/L)	Initial Mean Measured Treatment (mg XDE-777/L)	Day 0				Day 2			
		No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	Negative control	7	12	71	90	8	44	45	96
0.0054	0.00026	7	8	44	58	11	32	44	86
0.017	0.00086	3	6	41	50	16	52	76	144
0.052	0.0025	9	6	54	68	9	38	74	121

0.18	0.0086	6	11	52	69	13	44	83	139
0.55	0.027	5	5	45	54	11	31	80	122
NOEC		Not Calculated				0.55 mg GF-3308/L			
EC <sub>10</sub>		Not Calculated				0.37 mg GF-3308/L			
EC <sub>20</sub>		Not Calculated				0.47 mg GF-3308/L			

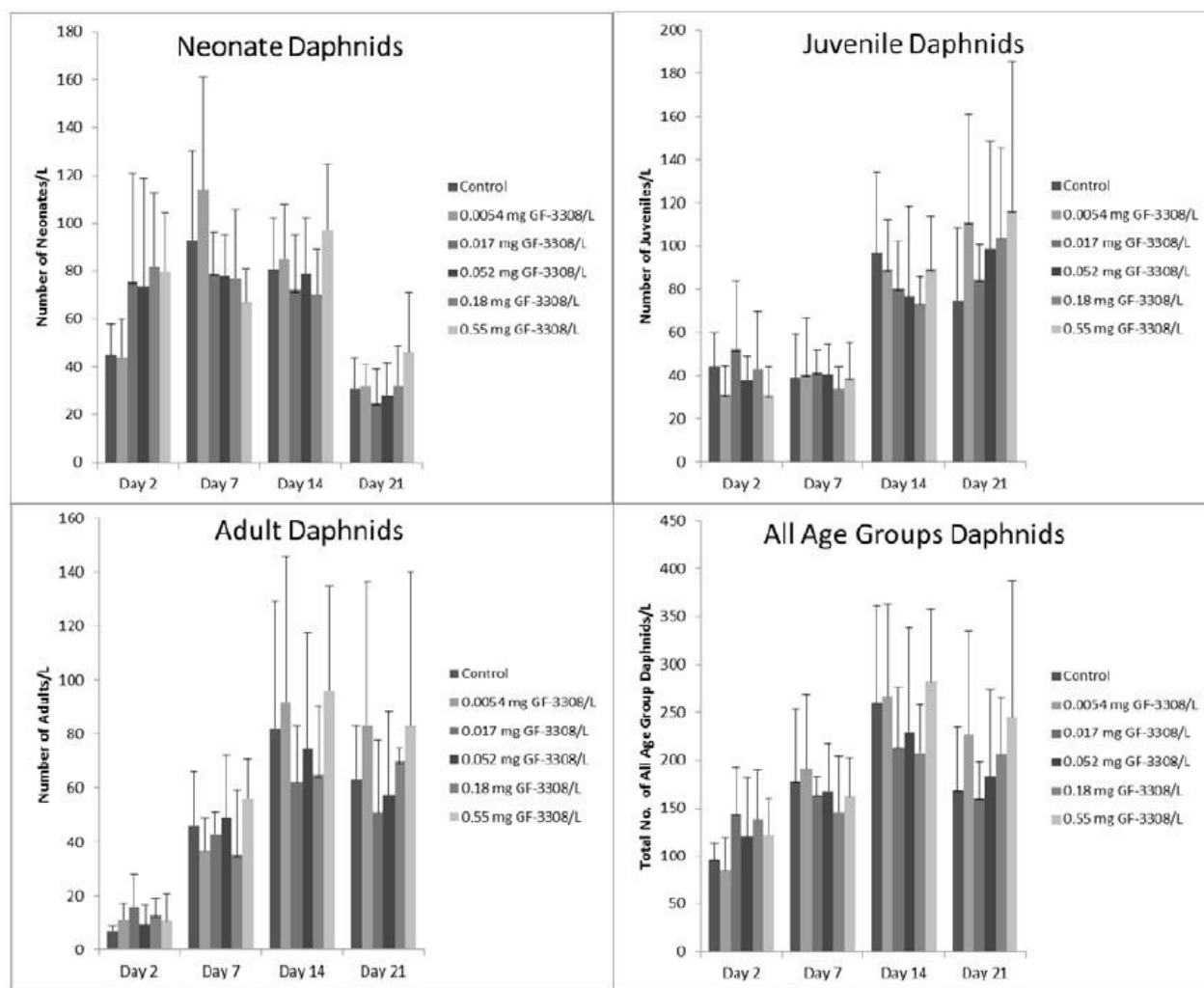
**Table 48: Effects of GF-3308 on mean daphnid abundance (Daphnids/L) (continued)**

Initial Mean Calculated Treatment (mg GF-3308/L)	Initial Mean Measured Treatment (mg XDE-777/L)	Day 7				Day 14			
		No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	Negative control	46	39	93	178	82	98	81	260
0.0054	0.00026	37	41	114	192	92	90	86	267
0.017	0.00086	43	42	79	164	62	81	73	215
0.052	0.0025	49	41	79	168	75	77	79	231
0.18	0.0086	36	34	77	146	65	73	70	208
0.55	0.027	56	40	67	162	96	90	97	283
NOEC		0.55 mg GF-3308/L				0.55 mg GF-3308/L			
EC <sub>10</sub>		0.039 mg GF-3308/L				Not Statistically Sound			
EC <sub>20</sub>		0.21 mg GF-3308/L				Not Statistically Sound			

**Table 49: Effects of GF-3308 on mean daphnid abundance (Daphnids/L) (continued)**

Initial Mean Calculated Treatment (mg GF-3308/L)	Initial Mean Measured Treatment (mg XDE-777/L)	Day 21			
		No. adults	No. juveniles	No. neonates	Total
Negative control	Negative control	64	75	31	169
0.0054	0.00026	83	112	33	227
0.017	0.00086	51	85	25	161
0.052	0.0025	57	99	29	184
0.18	0.0086	70	104	32	206
0.55	0.027	83	117	46	246
NOEC		0.55 mg GF-3308/L			
EC <sub>10</sub>		Not Statistically Sound			
EC <sub>20</sub>		Not Statistically Sound			

The abundance of particular Daphnids age groups over the test period is illustrated on the following figure.



## CONCLUSION

Daphnids abundance (all daphnid age groups, including neonates, juvenile, and adult) was unaffected in all test substance concentrations during the 21-day following test substance administration.

Based on initial mean measured concentrations the NOEC and LOEC value was 0.55 mg GF-3308/L (0.027 mg XDE-777/L) and >0.55 mg GF-3308/L (>0.027 mg XDE-777/L) for all age groups. For juvenile daphnid abundance at Day 2, the EC<sub>10</sub> and EC<sub>20</sub> were 0.37 mg GF-3308/L (95% CL: 0 – 2.95 mg GF-3308/L) and 0.47 mg GF-3308/L (95% CL: 0 - 1.73 mg GF-3308/L), respectively.

For neonate daphnid abundance at Day 7, the EC<sub>10</sub> and EC<sub>20</sub> were 0.039 mg GF-3308/L (95% CL: 0 - 0.19 mg GF-3308/L) and 0.21 mg GF-3308/L (95% CL: 0 – 0.56 mg GF-3308/L), respectively.

The EC<sub>10</sub> and EC<sub>20</sub> were not statistically sound for any other age groups at any time point.

Water quality parameters were within protocol specified limits throughout the exposure (dissolved oxygen was >40% of air saturation, temperatures remained within 20 ± 1°C and did not vary by more than ±1.0°C between chambers at any time point during the exposure).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	<i>Daphnia magna</i>	GF-3308	Days 2, 7, 14, and 21	NOEAEC <del>NOEC</del>	0.55	mg prep/L im
Water flea	<i>Daphnia magna</i>	GF-3308	Days 2, 7, 14, and 21	NOEAEC <del>NOEC</del>	0.027	mg a.s./L



### Additional information generated post-study completion:

A non-GLP Minimum Detectable Difference (MDD) analysis was conducted for the GF-3308 daphnid population study. The raw data was transformed using  $\ln(2y+1)$  and statistically evaluated using Dunnett's test. The program Community Analysis V4 (CA) was used for NOEC and MDD calculations. A former version of the CA program is described in Hommen et al. (1994).

Hommen, U., Veith, D. and Dülmer, U. (1994): A computer program to evaluate plankton data of freshwater field tests. In I.R. Hill, F. Heimbach, P. Leeuwangh, P. Matthiessen (eds.): Freshwater Field Tests for Hazard Assessment of Chemicals. Lewis Publ., Boca Raton, FL, USA

**Table 50: MDD analysis: Effects of GF-3308 on abundance of neonate, juvenile, adult , and total daphnids\_NOEC (MDD class)**

day	Neonate	Juvenile	Adult	Total
2	0.55 - (II)	0.55 - (II)	0.55 - (I)	0.55 - (III)
7	0.55 - (III)	0.55 - (II)	0.55 - (II)	0.55 - (III)
14	0.55 - (III)	0.55 - (III)	0.55 - (II)	0.55 - (III)
21	0.55 - (II)	0.55 - (II)	0.55 - (II)	0.55 - (III)

Class 0 is > 100%, no effects can be determined statistically; I is 90-100%, only large effects can be determined statistically; II is 70-90%, large to medium effects can be determined statistically; III is 50-70%, medium effects can be determined statistically; IV is <50%, small effects can be determined statistically.

**Table 51: MDD analysis: Effects of GF-3308 on abundance of neonate, juvenile, adult , and total daphnids\_NOEC (%MDD)**

day	Neonate	Juvenile	Adult	Total
2	0.55- (71)	0.55- (76)	0.55- (92)	0.55- (65)
7	0.55- (63)	0.55- (75)	0.55- (72)	0.55- (64)
14	0.55- (63)	0.55- (66)	0.55- (79)	0.55- (66)
21	0.55- (74)	0.55- (71)	0.55- (77)	0.55- (70)

**On all four sampling days, the MDD for the total abundance was 64-70% (class III) which allows the determination of medium effects according to the current aquatic guidance document (EFSA PPR 2013).**

### A 2.2.3.3 Study 3 – XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*

Comments of zRMS:	<p>The study was performed in order to investigate effects of fenpicoxamid on <i>Daphnia magna</i> population in an indoor aquatic microcosms. It should be, however, noted that the test system has not mimicked the natural conditions (as usually do microcosm/mesocosm studies) but the study was performed under standard laboratory conditions and the only difference from the 21-day reproductive toxicity study was inclusion of the various Daphnids stages (adults, juveniles and neonates).</p> <p>The study have not followed any recognised test guideline, but its design followed recommendations for the standard reproductive test (OECD 211) and mesocosm studies.</p> <p>Due to known instability of fenpicoxamid resulting from hydrolysis, the measured concentrations of the active compound in the test solutions of all treatments were intensively determined directly after dosing (at 0, 1, 2, 4, 8, 24 and 48 hours) and then after 4, 7, 10, 14, 18, 21, 28 and 35 days, when the analyses were focused on fenpicoxamid metabolites.</p> <p>As expected, fenpicoxamid concentration declined rapidly and after 24 hours they represented &lt;LOQ to 1% of nominal concentrations (depending on the test group). No residues of fenpicoxamid were found in any of the test solutions already 48 hours after dosing.</p> <p>In case there was statistically significant difference between performance in negative and vehicle control, the effects observed in the test item groups were compared with the</p>
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	<p>vehicle controls. In case there was no significant difference between controls, the effects were compared to performance in pooled controls.</p> <p>The zRMS in general agrees with the derived NOEC and EC<sub>10</sub> values with exception of the NOEC determined for the abundance of neonates (day 2 and 7) and juveniles (day 7) when clear effects &gt;20% were observed at concentration set as NOEC (1.88 µg a.s./L). Nevertheless, EC<sub>10</sub> values lower than the NOEC were calculated for these days and developmental stages and these may be used for comparative purposes as being more reliable than the statistical NOEC values.</p> <p>Similar situation was observed in case of the endpoints for population growth.</p> <p>It has to be noted that according to the EFSA aquatic guidance (2013), refined exposure laboratory studies with population of invertebrates are not recommended due to the rapid onset of recovery. The following is stated in the guidance:</p> <p><i>“Although refined exposure tests with standard test species that more or less resemble the design of tier 1 toxicity studies can be used for RAC derivation, the PPR Panel recommends not using refined exposure laboratory tests with populations of invertebrates (e.g. Daphnia) for this purpose when recovery is also considered. These population-level laboratory experiments with invertebrates are usually performed with individuals that differ in age and developmental state. As a result a rapid onset of recovery will occur after contamination under such test scenarios. Resources for surviving individuals will increase after contamination and will trigger an unrealistic strong recovery as no competitors are present (Knillmann et al., 2012b).”</i></p> <p>It should be, however, pointed out that in order to exclude impact of recovery on the derived NOEC, the results obtained after 21 days were statistically not analysed and served as supportive information only. Furthermore, this population study with fenpicoxamid together with similar study with GF-3308 (Hicks, 2016, KCP 10.2.3/2, see above for summary) were performed in order to demonstrate that the active substance is more toxic than GF-3308 and derived endpoints were not used directly in the risk assessment. Taking this into account, the zRMS is of the opinion that potential recovery is not an issue here and the study may be accepted for the comparative purposes with indication that the endpoints are not relevant for the risk assessment performed in line with EFSA (2013). For the same reasons the way of expression of the endpoints is considered not critical for this study and the zRMS agreed with endpoints expressed in terms of the initial measured concentrations.</p> <p>No MDD analysis was performed, but clear effects of fenpicoxamid on various stages of <i>Daphnia magna</i> were observed and this is considered sufficient for the comparative purposes between the active compound and formulation.</p> <p>Overall, the study is considered acceptable for comparative purposes with following endpoints (all based on initial measured concentrations):</p> <p>35-d NOEC = 1.88 µg a.s./L  EC<sub>10</sub>-juveniles = 0.770 µg a.s./L (based on clear reduction on day 7) EC<sub>10</sub>-juveniles = 1.11 µg a.s./L (based on clear reduction on day 7)</p>
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Reference:	KCP 10.2.3/3
Report:	Hicks, S.; 2017; XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with <i>Daphnia magna</i> ; ABC Laboratories, Inc., a wholly owned subsidiary of EAG, Inc., Columbia, Missouri, USA; Lab Study No. 83491; DAS Study No. 160125 ; 14 August 2017; Unpublished
Guideline(s):	None

Deviations:	Not relevant (no recognisable guideline followed)
GLP:	Yes
Acceptability:	Acceptable for the bridging purposes. Endpoints not relevant for the risk assessment performed in line with EFSA (2013) due to rapid onset of recovery in such tests.
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): XDE-777  
Purity: 73.2%  
Description (physical state): White solid with a slight odour  
Lot/batch no.: E3485-83 (TSN301100)

### Test System

Organism (*Species*): Water flea (*Daphnia magna*)  
Study type: Population effects  
Study design: Static  
Test concentrations: Nominal: 0 (control), 0 (vehicle control; 10 µL DMF/L), 1.0, 2.5, 6.3, 16, and 40 µg a.i./L  
Initial Mean measured: <MQL (control), <MQL (vehicle control; 10 µL DMF/L), 0.690, 1.88, 4.75, 13.3, and 34.6 µg a.i./L  
Parameters measured: Daphnid abundance  
Observation intervals: Day 0, 2, 7, 14, 21, 28, and 35  
Age of test organisms at test initiation: ~4 and ~10 days old  
Analytical confirmation of test concentrations: On days: 0, 1, 2, 4, 8, 24, and 48 hours, day 4, 7, 10, 14, 18, 21, 28, and 35  
No. of holding days before dosing: 7 days  
Number of Daphnids per dose group: 800 juvenile and 800 adult  
Number of Daphnids per control group: 800 juvenile and 800 adult  
Feeding regime: The daphnids were fed daily a diet consisting of increasing amounts (134-184 mL) of a concentrated ( $6.0 \times 10^7$  cells/mL) algal suspension (*Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*). Daphnids were also fed 20 or 40 mL per replicate of an approximately 2.34 to 2.83 g/L suspension of a prepared invertebrate food solution (wheat grass, salmon starter, and yeast suspension).  
Environmental conditions: Loading rate: not applicable  
Temperature: 19.9 to 21.0 °C  
Photoperiod: 16-hr light: 8-hr dark  
Dissolved oxygen concentration: 4.2 to 8.2 mg/L (49 to 96% sat.) pH: 7.7 to 8.7  
Total hardness: 176 to 200 mg CaCO<sub>3</sub>/L  
Salinity: not applicable  
Reference substance: XDE-777 technical (TSN302306)  
X642188 technical (TSN303567)

X12264475 (TSN307414)  
X12313581 (TSN306050)  
X696476 (TSN307152)  
X12019520 (TSN307264)  
X12255349 (TSN306954)  
X12386481 (TSN304620)  
X12335723 (TSN304462)

## Methodology

A definitive test was performed from 23 March to 27 April 2016 with target nominal concentrations of 0 (control), 0 (vehicle control), 1.0, 2.5, 6.3, 16, and 40 µg a.i./L. The solvent (i.e., DMF) concentration in the vehicle control and each test substance treatment solution was 10 µL/L. The definitive test was conducted for 35 days commencing seven days (i.e., Day -7; start of 7-day acclimation phase) after the daphnids were added to the test chambers.

A total of two hundred juvenile and two hundred adult daphnids were added to each test chamber. Test chambers for the definitive test consisted of glass aquaria measuring approximately 30.3 cm wide by 75.1 cm long by 30.7 cm high with a test solution depth of 17.6 cm, for a test solution volume of approximately 40 L. The control and all treatments were replicated four times. The entire volume of the appropriate dosing solution was slowly poured into each test chamber while gently mixing the water in the test chamber with a glass rod. Gentle mixing of the solution within the test chambers using a glass rod continued for one minute after dosing.

Abundance (number of individuals per liter) of each life stage over time was determined by sampling the population prior to test initiation (days -5 and -2), at initiation (day 0), and on days 2, 7, 14, 21, 28, and 35 after test initiation. Adult daphnids morphological sex was determined on days -5, -2, 0, 2, 7, 14, 21, 28, and 35.

Each aquarium was divided into 21 equally sized areas by placing a plastic mesh grid on the top. Six areas were randomly selected to be sampled at each sampling event using a random number generator for each replicate. Approximately 2-L (~5% of the total volume) was collected at each biological sampling point. Sampling vessels, consisting of a 2" (~5.2 cm) diameter PVC pipe containing a silicone stopper were used to sample the water column at each selected area. The silicon stopper was lowered through the grid until it rested on the bottom of the aquarium. This was repeated until the 6 areas selected for sampling all had a silicone stopper placed accordingly. The PVC pipe was then lowered onto the silicone stopper and the seal tightened to retain the water column sample in the pipe. A 5-gal bucket was used to collect the samples from the water columns. The bucket contained three sieves, 1.00, 0.71, and 0.25 mm respectively, stacked in descending order of mesh size. The water column samples were emptied into the bucket and allowed to flow through the stack of sieves. Daphnids collected in the sample were trapped in a particular sieve, depending on the size of the daphnid. Each sieve was examined to determine the number of adult (>1.00 mm), juvenile (>0.71 to <1.00 mm), and neonate (>0.25 to <0.71 mm) daphnids collected. Following counting, the adult daphnids were transferred by pipet to a petri dish with test medium and observations were performed on the morphological sex of the adults.

Data collected prior to the start of the chemical exposure and on the dosing day (i.e., days -5 and -2 and day 0) were not statistically analyzed. After the data met the terms for population recovery, there were no further statistical analyses performed on the subsequent time points. Prior to comparisons of the treatment groups to the control group for each endpoint, the control and vehicle control were compared to determine if differences between control groups were statistically significant using a ttest (two sided). Because there was a statistically significant difference between the control and vehicle control treatment for abundance of juveniles, adults, and all age groups on day 2 and adults on day 7, comparisons were made against the vehicle control treatment. Because there was no statistically significant difference

between the control and vehicle control for abundance of neonates (day 2, 7, 14, and 21), juveniles (day 7, 14, 21, and 28), adults (day 14 and 21), and all age groups (day 7, 14, and 21) the control and vehicle control groups were pooled for subsequent comparisons. Because there was no statistically significant difference between the control and vehicle control treatment for population growth rate data of neonates (day 0- 7, 0-14, 0-21), juveniles (day 0-7, 0-14, 0-21, 0-28, and 0-35), adults (day 0-7, 0-14, and 0-21), all age groups (day 0-7, 0-14, and 0-21) the control and vehicle control groups were pooled for subsequent comparisons.

Test solutions were analysed for the concentrations of XDE-777 and its metabolites X642188, X12264475, X12313581, X696476, X12019520, X12255349, X12386481, and X12335723, using a liquid chromatography with tandem mass spectrometry (LC-MS/MS) system.

The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) for the abundance and population growth rate data was determined by using a one-tailed Dunnett's and Williams' test with the alternate hypothesis being that the mean for the parameter was reduced in comparison to the control mean. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance.

## RESULTS AND DISCUSSION

The initial mean measured XDE-777 concentrations at test initiation (0 hour) were 0.690, 1.88, 4.75, 13.3, and 34.6 µg a.i./L in the 1.0, 2.5, 6.3, 16, and 40 µg a.i./L nominal test substance treatments, respectively, which represented recoveries of 69 to 87% of the nominal concentrations. Measured XDE-777 concentrations from individual replicate solutions at hour 0 were consistent within treatment (coefficient of variation was <10% for all treatments). XDE-777 concentrations declined rapidly in all test substance treatments (mean measured concentration were ≤1% of nominal in all treatments by 24 hours and <MQL in all treatments by 48 hours).

The biological response results and all subsequent analytical results were reported based upon the initial (i.e., 0 hour) mean measured XDE-777 concentrations.

Table 1. Measured Concentrations of XDE-777 During the Population Effects Study with *Daphnia magna*

Nominal Concentration (µg a.i./L)	R E P	Measured Concentrations Expressed as µg a.i./L						
		0 hour				1 hour		
		Measured	% of Nominal	Mean (% nominal)	%CV	Measured	% of Nominal	Mean (% nominal)
0 (Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--			<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--			<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--			<MQL <sup>a</sup>	--	
0 (Vehicle Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--			<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--			<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--			<MQL <sup>a</sup>	--	
1.0	A	0.692	69	0.690 (69)	4	0.532	53	0.526 (53)
	B	0.720	72			0.533	53	
	C	0.649	65			0.479	48	
	D	0.700	70			0.561	56	
2.5	A	1.77	71	1.88 (75)	4	1.59	64	1.52 (61)
	B	1.96	78			1.62	65	
	C	1.93	77			1.48	59	
	D	1.86	74			1.40	56	
6.3	A	4.74	75	4.75 (75)	5	3.66	58	3.68 (58)
	B	4.73	75			3.70	59	
	C	4.50	71			3.49	55	
	D	5.04	80			3.88	62	
16	A	13.3	83	13.3 (83)	1	10.5	66	10.2 (64)
	B	13.3	83			9.49	59	
	C	13.1	82			10.8	68	
	D	13.3	83			10.0	63	
40	A	34.2	86	34.6 (87)	3	26.1	65	26.1 (65)
	B	34.0	85			25.9	65	
	C	33.8	85			26.3	66	
	D	36.3	91			26.0	65	

<sup>a</sup> Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.

<sup>b</sup> Results from all samples collected from Day 4, Day 7, Day 10, Day 14, Day 18, Day 21, Day 28, and Day 35 were <MQL. These results are not included in table.

Table 1. Measured Concentrations of XDE-777 During the Population Effects Study with *Daphnia magna* (continued)

Nominal Concentration (µg a.i./L)	R E P	Measured Concentrations Expressed as µg a.i./L					
		2 hour			4 hour		
		Measured	% of Nominal	Mean (% nominal)	Measured	% of Nominal	Mean (% nominal)
0 (Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
0 (Vehicle Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
1.0	A	0.573	57	0.457 (46)	0.290	29	0.290 (29)
	B	0.421	42		0.291	29	
	C	0.384	38		0.242	24	
	D	0.449	45		0.338	34	
2.5	A	0.992	40	1.04 (42)	0.729	29	0.716 (29)
	B	1.14	46		0.723	29	
	C	1.03	41		0.718	29	
	D	0.997	40		0.692	28	
6.3	A	2.67	42	2.79 (44)	1.68	27	1.74 (28)
	B	2.79	44		1.49	24	
	C	2.83	45		1.86	30	
	D	2.86	45		1.91	30	
16	A	7.95	50	7.33 (46)	4.86	30	4.88 (31)
	B	6.42	40		4.17	26	
	C	8.19	51		6.03	38	
	D	6.77	42		4.47	28	
40	A	20.0	50	18.3 (46)	13.2	33	12.3 (31)
	B	14.3	36		9.85	25	
	C	19.4	49		13.5	34	
	D	19.3	48		12.8	32	

<sup>a</sup> Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.<sup>b</sup> Results from all samples collected from Day 4, Day 7, Day 10, Day 14, Day 18, Day 21, Day 28, and Day 35 were <MQL. These results are not included in table.Table 1. Measured Concentrations of XDE-777 During the Population Effects Study with *Daphnia magna* (continued)

Nominal Concentration (µg a.i./L)	R E P	Measured Concentrations Expressed as µg a.i./L					
		8 hour			24 hour		
		Measured	% of Nominal	Mean (% nominal)	Measured	% of Nominal	Mean (% nominal)
0 (Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
0 (Vehicle Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--		<MQL <sup>a</sup>	--	
1.0	A	0.107	11	0.117 (12)	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	0.125	13		<MQL <sup>a</sup>	--	
	C	0.0783	8		<MQL <sup>a</sup>	--	
	D	0.156	16		<MQL <sup>a</sup>	--	
2.5	A	0.318	13	0.340 (14)	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	0.392	16		<MQL <sup>a</sup>	--	
	C	0.305	12		<MQL <sup>a</sup>	--	
	D	0.346	14		<MQL <sup>a</sup>	--	
6.3	A	0.637	10	0.750 (12)	0.0376	1	0.0227 (0)
	B	0.682	11		<MQL <sup>a</sup>	0	
	C	0.773	12		<MQL <sup>a</sup>	0	
	D	0.907	14		0.0331	1	
16	A	2.31	14	2.31 (14)	0.220	1	0.125 (1)
	B	1.56	10		0.0224	0	
	C	3.30	21		0.218	1	
	D	2.05	13		0.0415	0	
40	A	5.87	15	5.24 (13)	0.302	1	0.164 (0)
	B	3.30	8		0.0404	0	
	C	6.27	16		0.185	0	
	D	5.53	14		0.127	0	

<sup>a</sup> Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.<sup>b</sup> Results from all samples collected from Day 4, Day 7, Day 10, Day 14, Day 18, Day 21, Day 28, and Day 35 were <MQL. These results are not included in table.

Table 1. Measured Concentrations of XDE-777 During the Population Effects Study with *Daphnia magna* (continued)

Nominal Concentration (µg a.i./L)	R E P	Measured Concentrations Expressed as µg a.i./L		
		48 hour <sup>b</sup>		Mean (% nominal)
		Measured	% of Nominal	
0 (Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	
0 (Vehicle Control)	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	
1.0	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	
2.5	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	
6.3	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	
16	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	
40	A	<MQL <sup>a</sup>	--	<MQL <sup>a</sup>
	B	<MQL <sup>a</sup>	--	
	C	<MQL <sup>a</sup>	--	
	D	<MQL <sup>a</sup>	--	

<sup>a</sup> Minimum Quantifiable Limit (MQL) = 0.0200 ng a.i./mL.<sup>b</sup> Results from all samples collected from Day 4, Day 7, Day 10, Day 14, Day 18, Day 21, Day 28, and Day 35 were <MQL. These results are not included in table.

The measured concentrations of metabolites X642188, X12255349, X12335723, X12313581, X12019520, X12386481, X12264475, X696476 over the test period are presented in the below tables.

Sample Day	Mean Measured Concentrations Expressed as µg X642188/L						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0066	0.024	0.051	0.16	0.41
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0066	0.022	0.043	0.16	0.40
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0075	0.015	0.036	0.12	0.32
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0084	0.027	0.053	0.17	0.38
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0074	0.021	0.052	0.15	0.25
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0072	0.025	0.033
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.11	0.30	0.75
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.14	0.40	0.78 <sup>c</sup>
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.012	0.034	0.042 <sup>c</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>

<sup>a</sup> Nominal XDE-777 Concentration (µg a.i./L)<sup>b</sup> MQL = 0.0016 ng/mL.<sup>c</sup> Mean calculated using ½ MQL (0.00080 ng/mL) for <MQL values.



Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X12255349/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.014	0.035	0.11	0.26	0.64
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.016	0.049	0.097	0.25	0.49
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.0099	0.039	0.091	0.27	0.79
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.019	0.045	0.11	0.31	0.67
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.017	0.051	0.13	0.37	0.46
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.029	0.068	0.20	0.62	1.3
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.029	0.076	0.23	0.65	1.7
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.044	0.11	0.34	0.95	2.4
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.046	0.13	0.38	1.0	2.8
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.057	0.14	0.33	0.97	2.6
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.047	0.15	0.37	1.1	2.7
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.035	0.10	0.26	0.65	1.3
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.027	0.070	0.14	0.041	0.11
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.0091 <sup>c</sup>	0.021	0.044
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>

<sup>a</sup> Initial mean measured XDE-777 concentration ( $\mu\text{g a.i./L}$ )<sup>b</sup> MQL = 0.0042 ng/mL.<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.0021 ng/mL) for <MQL values.

Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X12335723/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.39	0.99	2.2
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.37	1.2	2.8	7.4	19
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.90	1.8	4.2	10	25
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.1	2.5	6.5	15	41
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.2	3.2	8.6	20	51
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.2	3.3	7.1	23	60
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.60	2.0	4.1	10	30
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.27	0.59	1.6	4.2	17
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.25 <sup>c</sup>	0.59	1.9
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	<MQL <sup>b,c</sup>	0.61
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>

<sup>a</sup> Initial mean measured XDE-777 concentration ( $\mu\text{g a.i./L}$ ).<sup>b</sup> MQL = 0.20 ng/mL.<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.10 ng/mL) for <MQL values.

Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X12313581/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.3 <sup>c</sup>	2.2 <sup>c</sup>	4.6 <sup>c</sup>
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	<MQL <sup>b</sup>	0.85 <sup>c</sup>	<MQL <sup>b</sup>	4.6 <sup>c</sup>
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.23 <sup>c</sup>
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.20 <sup>c</sup>	0.40 <sup>c</sup>
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.39	0.59	1.1
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.36	0.85
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.23	0.41	0.99
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.22	0.44	1.1
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.24	0.49	1.4
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.39	0.46	0.56	0.88	1.9
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.24	0.51	1.1

<sup>a</sup> Nominal XDE-777 Concentration ( $\mu\text{g a.i./L}$ )<sup>b</sup> MQL = 0.20 ng/mL.<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.10 ng/mL) for <MQL values.

Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X12019520/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.14	0.37
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.13	0.37
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.10 <sup>c</sup>	0.25	0.51
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.20	0.74
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.24	0.57	0.85
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.19	0.55	1.9	4.6
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>c</sup>	0.29	0.80	2.0	5.1
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.24	0.72	1.8	5.1
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.48	0.98	4.6
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.35	1.1	3.9
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.39	3.8
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.5
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.14 <sup>c</sup>
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>

<sup>a</sup> Nominal XDE-777 Concentration ( $\mu\text{g a.i./L}$ )<sup>b</sup> MQL = 0.10 ng/mL.<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.050 ng/mL) for <MQL values.



Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X12386481/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.23	0.82	1.8
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.24	0.88	2.6
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b, c</sup>	0.37	0.82	2.5
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.38	1.3	3.1
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.48	1.3	3.7
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.26	0.60	1.8	5.0
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.35	1.5	3.5	5.3
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.36	1.3	2.3	5.2
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.73	1.9	2.4
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.45	1.1	2.7
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.52
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b, c</sup>
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>

<sup>a</sup> Nominal XDE-777 Concentration ( $\mu\text{g a.i./L}$ )<sup>b</sup> MQL = 0.20 ng/mL.<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.10 ng/mL) for <MQL values.

Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X12264475/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.440	1.11
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.366	0.454	0.542	1.05	2.08
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b, c</sup>	0.259	0.419	0.991
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.323	0.834	2.04
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.261	0.351	0.522	1.33	6.22
24 Hour	1.46 <sup>c</sup>	0.259 <sup>c</sup>	0.430	0.656	1.69	15.8	17.0
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.844	1.14	1.88	6.38	22.6
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.524	1.21	8.53	22.2
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.913	2.47	5.67	17.3	39.0
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.16	2.10	12.8	20.6
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.77	2.22	5.60	15.1	17.9
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.42	1.94	5.46	14.8	37.3
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	1.11	1.86	4.90	11.7	34.0
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.362	0.919	2.18	10.5	27.8
Day 35	0.303 <sup>c</sup>	<MQL <sup>b</sup>	1.46	1.86	6.26	15.2	17.3

<sup>a</sup> Nominal XDE-777 Concentration ( $\mu\text{g a.i./L}$ )<sup>b</sup> MQL = 0.208 ng/mL.<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.104 ng/mL) for <MQL values.

Sample Day	Mean Measured Concentrations Expressed as $\mu\text{g X696476/L}$						
	0 (Control) <sup>a</sup>	0 (Vehicle Control) <sup>a</sup>	1.0 <sup>a</sup>	2.5 <sup>a</sup>	6.3 <sup>a</sup>	16 <sup>a</sup>	40 <sup>a</sup>
0 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
1 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
2 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
4 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
8 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>
24 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.266	0.567
48 Hour	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.414	1.15
Day 4	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.216 <sup>c</sup>	0.703	1.98
Day 7	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.386	1.02	2.66
Day 10	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.352	1.03	2.77
Day 14	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.316	0.628	1.36	2.67
Day 18	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.344	0.674	1.53	2.55
Day 21	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.236	0.383	0.743	1.65	3.64
Day 28	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b</sup>	<MQL <sup>b,c</sup>	0.681	1.84	4.35
Day 35	<MQL <sup>b</sup>	<MQL <sup>b</sup>	0.296	0.453	0.883	2.13	5.26

<sup>a</sup> Nominal XDE-777 Concentration ( $\mu\text{g a.i./L}$ )

<sup>b</sup> MQL = 0.208 ng/mL.

<sup>c</sup> Mean calculated using  $\frac{1}{2}$  MQL (0.104 ng/mL) for <MQL values.

Daphnid abundance (i.e., neonate, juvenile, adults, and all age groups combined) was unaffected in the 0.690 and 1.88  $\mu\text{g a.i./L}$  test substance concentrations during the 35-day post-exposure.

A statistically significant difference was noted in the abundance of neonates on Day 2 and 7 in the 4.75, 13.3, and 34.6  $\mu\text{g a.i./L}$  groups.

A statistically significant difference was noted in the abundance of juveniles on Day 2, 7, and 14 in the 13.3 and 34.6  $\mu\text{g a.i./L}$  groups.

A statistically significant difference was noted in the abundance of adults on Day 2 in the 13.3 and 34.6  $\mu\text{g a.i./L}$  groups.

A statistically significant difference was noted in the abundance of total (all age groups combined) on Day 2 in the 13.3 and 34.6  $\mu\text{g a.i./L}$  groups and on Day 7 in the 4.75, 13.3, and 34.6  $\mu\text{g a.i./L}$  groups.

Daphnid growth rate (i.e., neonate, juvenile, adults, and all age groups combined) was unaffected in the 0.690 and 1.88  $\mu\text{g a.i./L}$  test substance concentrations during the 35-day post-exposure.

A statistically significant difference was noted in the growth rate of neonates, adults, and total (all age groups combined) on Day 0-7 in the 4.75  $\mu\text{g a.i./L}$  treatment group.

A statistically significant difference was noted in the growth rate of juveniles on days 0-7, 0-14, and 0-21 in the 13.3  $\mu\text{g a.i./L}$  treatment group.

Male daphnia were observed in the adult daphnids population on day -5 (1.88 and 13.3  $\mu\text{g a.i./L}$ ), day

-2 (vehicle control, 0.690, and 34.6 µg a.i./L), day 0 (control and 13.3 µg a.i./L), day 2 (control, vehicle control, and 13.3 µg a.i./L), day 7 (vehicle control), day 14 (vehicle control), day 21 (control), and day 35 (0.690 and 34.6 µg a.i./L).

All juveniles and neonates from these time points were preserved in an appropriately labeled scintillation vial with 70% ethanol for future sexing, if necessary. The number of male daphnids found in the samples was ≤ 1% of the replicate population during the study.

Following dosing on day 0, a majority of the daphnia in the 34.6 µg a.i./L test substance treatment were observed immobile. Approximately 24 hours after dosing, all surviving daphnia in the 34.6 µg a.i./L test substance treatment appeared normal. No other abnormal or immobile daphnids were observed over the course of the exposure.

**Table 52: Effects of XDE-777 on mean daphnid abundance (Daphnids/L)**

Initial Mean Measured Treatment (µg a.i./L)	Day 2				Day 7			
	No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	43.3	76.0	97.0	216	131	117	220	467
Vehicle control	19.5	29.3	70.8	119	61.3	92.3	256	409
Pooled control	31.4	52.6	83.9	167	96.1	105	238	438
0.690	30.0	53.5	109	192	114	118	225	456
1.88	14.3	28.3	61.8	103	59.0	76.8	174	309
4.75	20.0	28.5	46.5 *	94.5	27.0	57.8	140 *	225 *
13.3	6.50 **	14.5 **	50.5 *	70.8 **	88.5	40.5 *	104 *	232 *
34.6	0.750 **	3.75 **	10.0 *	14.5 **	31.0	14.5 *	15.0 *	60.5 *
NOEC	4.75	4.75	1.88	4.75	34.6	4.75	1.88	1.88
EC <sub>10</sub>	5.54	4.75	1.52	6.06	NE	0.770	1.11	NSS
EC <sub>20</sub>	7.10	6.98	3.13	8.53	NE	1.73	2.25	1.59

\*statistically different from pooled controls (Williams' test; p = 0.05)

\*\*statistically different from vehicle control (Williams' test; p = 0.05)

NE = could not be estimated; NSS = not statistically sound; NA = not applicable <sup>a</sup> Data on this day was not statistically analysed because the terms of recovery had been met prior to this day.

**Table 53: Effects of XDE-777 on mean daphnid abundance (Daphnids/L) (continued)**

Initial Mean Measured Treatment (µg a.i./L)	Day 14				Day 21			
	No. adults	No. juveniles	No. neonates	Total	No. adults	No. juveniles	No. neonates	Total
Negative control	112	173	111	396	96.3	166	52.0	314
Vehicle control	119	233	125	476	149	246	51.0	446
Pooled control	116	203	118	436	123	206	51.5	380
0.690	164	208	111	482	206	258	35.3	499
1.88	167	209	117	493	144	215	40.0	398
4.75	131	177	126	433	151	208	52.8	412
13.3	141	118 *	140	398	123	155	42.5	320
34.6	108	60.0 *	323	490	77.3	164	298	539
NOEC	34.6	4.75	34.6	34.6	34.6	34.6	34.6	34.6

EC <sub>10</sub>	NE	3.38	NE	NE	NE	NE	NE	NE
EC <sub>20</sub>	NE	6.22	NE	NE	NE	NE	NE	NE

\*statistically different from pooled controls (Williams' test;  $p = 0.05$ )

\*\*statistically different from vehicle control (Williams' test;  $p = 0.05$ )

NE = could not be estimated; NSS = not statistically sound; NA = not applicable <sup>a</sup> Data on this day was not statistically analysed because the terms of recovery had been met prior to this day.

**Table 54: Effects of XDE-777 on mean daphnid abundance (Daphnids/L) (continued)**

Initial Mean Measured Treatment (µg a.i./L)	Day 28				Day 35			
	No. adults <sup>a</sup>	No. juveniles	No. neonates	Total <sup>a</sup>	No. adults <sup>a</sup>	No. juveniles <sup>a</sup>	No. neonates <sup>a</sup>	Total <sup>a</sup>
Negative control	94.0	149	23.8	266	84.5	142	11.5	238
Vehicle control	48.5	140	17.5	205	86.8	146	7.00	239
Pooled control	71.3	144	20.6	235	85.6	144	9.25	238
0.690	122	213	16.3	351	58.3	136	3.25	197
1.88	96.5	174	15.3	285	77.3	187	5.25	269
4.75	131	202	26.0	357	92.8	186	7.25	286
13.3	121	194	23.5	338	94.0	145	6.25	245
34.6	50.8	185	119	355	48.0	187	53.0	288
NOEC	NA	34.6	NA	NA	NA	NA	NA	NA
EC <sub>10</sub>	NA	NE	NA	NA	NA	NA	NA	NA
EC <sub>20</sub>	NA	NE	NA	NA	NA	NA	NA	NA

\*statistically different from pooled controls (Williams' test;  $p = 0.05$ )

\*\*statistically different from vehicle control (Williams' test;  $p = 0.05$ )

NE = could not be estimated; NSS = not statistically sound; NA = not applicable <sup>a</sup> Data on this day was not statistically analysed because the terms of recovery had been met prior to this day.

**Table 55: Effects of XDE-777 on Population Growth Rate**

Initial Mean Measured Treatment (µg a.i./L)	Day 0-7				Day 0-14			
	Adults	Juveniles	Neonates	Total	Adults	Juveniles	Neonates	Total
Negative control	0.422	0.367	0.307	0.345	0.198	0.209	0.101	0.158
Vehicle control	0.319	0.306	0.303	0.302	0.210	0.224	0.101	0.165
Pooled control	0.371	0.337	0.305	0.324	0.204	0.217	0.101	0.162
0.690	0.393	0.376	0.311	0.335	0.222	0.232	0.106	0.173
1.88	0.266	0.228	0.258	0.252	0.216	0.194	0.0993	0.162
4.75	0.217 *	0.206	0.164 *	0.180*	0.221	0.190	0.0745	0.137
13.3	0.245 *	0.0925*	0.121 *	0.150*	0.164	0.122*	0.0819	0.113
34.6	0.218 *	-0.0272*	-0.161 *	0.0249*	0.195	0.0979*	0.137	0.139
NOEC	1.88	4.75	1.88	1.88	34.6	4.75	34.6	34.6
EC <sub>10</sub>	NSS	1.84	NE	NE	NE	1.86	NSS	NE
EC <sub>20</sub>	1.35	2.90	NE	NE	NE	4.83	NSS	NE

\*statistically different from pooled controls (Williams' test;  $p = 0.05$ )

NE = could not be estimated; NSS = not statistically sound; NA = not applicable <sup>a</sup>

The terms of recovery had been met prior to this day.

**Table 56: Effects of XDE-777 on Population Growth Rate ~~mean daphnid abundance~~ (Daphnids/L) (continued)**

Initial Mean	Day 0-21	Day 0-28
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Measured Treatment (µg a.i./L)	Adults	Juveniles	Neonates	Total	Adults <sup>a</sup>	Juveniles	Neonates <sup>a</sup>	Total <sup>a</sup>
Negative control	0.123	0.137	0.0324	0.0940	NA	0.0989	NA	NA
Vehicle control	0.151	0.158	0.0302	0.111	NA	0.0972	NA	NA
Pooled control	0.137	0.147	0.0313	0.103	NA	0.0981	NA	NA
0.690	0.161	0.161	0.00942	0.115	NA	0.116	NA	NA
1.88	0.137	0.130	0.0161	0.0981	NA	0.0901	NA	NA
4.75	0.155	0.135	0.00523	0.0895	NA	0.0993	NA	NA
13.3	0.107	0.0940*	-0.00224	0.0659	NA	0.0780	NA	NA
34.6	0.114	0.113*	0.0853	0.0962	NA	0.0891	NA	NA
NOEC	34.6	4.75	34.6	34.6	NA	34.6	NA	NA
EC <sub>10</sub>	NE	NE	NE	NE	NA	NE	NA	NA
EC <sub>20</sub>	NE	NE	NE	NE	NA	NE	NA	NA

\*statistically different from pooled controls (Williams' test; p = 0.05)

NE = could not be estimated; NSS = not statistically sound; NA = not applicable <sup>a</sup>

The terms of recovery had been met prior to this day.

**Table 57: Effects of XDE-777 on Population Growth Rate ~~mean-daphnid-abundance~~ (Daphnids/L) (continued)**

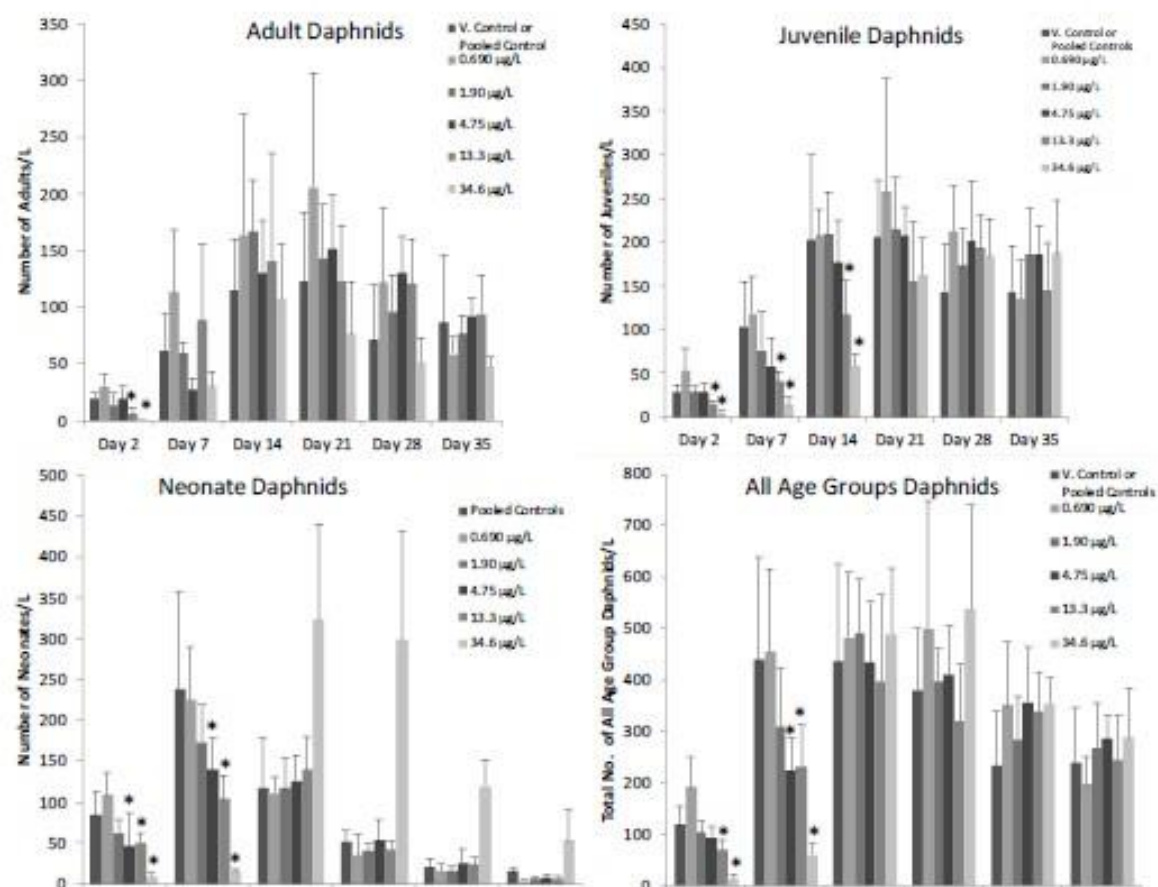
Initial Mean Measured Treatment (µg a.i./L)	Day 0-35			
	Adults <sup>a</sup>	Juveniles	Neonates <sup>a</sup>	Total <sup>a</sup>
Negative control	NA	0.0782	NA	NA
Vehicle control	NA	0.0777	NA	NA
Pooled control	NA	0.0779	NA	NA
0.690	NA	0.0798	NA	NA
1.88	NA	0.0741	NA	NA
4.75	NA	0.0781	NA	NA
13.3	NA	0.0547	NA	NA
34.6	NA	0.0712	NA	NA
NOEC	NA	34.6	NA	NA
EC <sub>10</sub>	NA	NE	NA	NA
EC <sub>20</sub>	NA	NE	NA	NA

\*statistically different from pooled controls (Williams' test; p = 0.05)

NE = could not be estimated; NSS = not statistically sound; NA = not applicable <sup>a</sup>

The terms of recovery had been met prior to this day.

The abundance of particular Daphnids age groups over the test period is illustrated on the following figure.



\* statistically significance differences between the means of pooled controls or vehicle control as compared to the means of treatment groups ( $p = 0.05$ ; Williams' Test)

## CONCLUSION

Daphnid abundance and population growth rate were unaffected in the 0.690 and 1.88 µg a.i./L test substance concentrations during the 35-day post-exposure. Based on abundance data, each age group (i.e., neonate, juvenile, and adults) had recovered in the 4.75, 13.3, and 34.6 µg a.i./L test substance concentrations by day 21 as there were no statistical differences between the abundance on days 21 and 28. Based on growth rate, all age groups had recovered by day 28.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Water flea	<i>Daphnia magna</i>	XDE-777	35 days	NOEAEC <del>NOEC</del>	1.88	µg a.i./L

### A 2.2.3.4 Study 4 – Efficacy of XDE-777 metabolites to *Septoria tritici* on wheat

Comments of zRMS:	<p>The study below was validated and agreed by the zRMS efficacy expert. Performed evaluation confirmed that none of the tested fenpicoxamid metabolites (X12019520, X12255349, X12313581, X12314005, X12335723, X12393285 and X696476) is biologically active.</p> <p>For full study summary and zRMS evaluation, please refer to the Core Assessment, Part B, Section 3.</p> <p>The summary below was struck through as no evaluated in area of Section 9.</p>
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Reference:	KCP 10.2.3/4
Report:	Mathieson, T. 2018; Efficacy of XDE-777 metabolites to Septoria tritici on wheat; Dow AgroSciences LLC, Zionsville, Indiana, USA; 30 July 2018; Unpublished
Acceptability:	Accepted by the zRMS efficacy expert during evaluation performed in area of Section 3
Duplication (if vertebrate study)	NA

## SUMMARY

XDE 777, a pro fungicide of the natural picolinamide UK 2A, is a fungicide of Dow AgroSciences. Seven metabolites of XDE 777 identified in soil metabolism, soil photolysis, or aqueous hydrolysis studies were evaluated for their biological activity vs. wheat leaf blotch caused by *Septoria tritici* (SEPTTR), the key driver disease in the European cereal fungicide market. In the current study, XDE777 was highly potent vs. SEPTTR when used as both protectant and curative treatments. UK 2A, from which XDE 777 was derived, showed high level of protectant activity but significantly weaker curative activity. None of the seven metabolites of XDE 777 showed any meaningful fungicidal activity vs. SEPTTR.

## MATERIALS AND METHODS Chemicals

XDE 777, UK 2A, and the seven metabolites of XDE 777, plus one formulated material were used in the studies Table 1. Their structures and lot information are listed in Figure 1. X696476, X2313581, X12019520, X12335723, X12314005, are soil metabolites (Hastings and Jackson, 2013), X12255349 is a soil photolysis metabolite (Cooke, 2013), and X12393285 is a hydrolysis metabolite (Yoder and Jackson, 2013).



**Table 1. Compounds tested for the control of SEPTTR.**

Compound	X number	Lot number	Use rate (ppm)
XDE-777	X772777	TSN303160	100, 25, 6.25, 1.56, 0.39, 0.10
UK-2A	X642188	TSN303567	100, 25, 6.25, 1.56, 0.39, 0.10
	X696476	TSN307152	100, 25, 6.25, 1.56, 0.39, 0.10
	X2313581	TSN306004	100, 25, 6.25, 1.56, 0.39, 0.10
	X12019520	TSN307264	100, 25, 6.25, 1.56, 0.39, 0.10
	X12255349	TSN306954	100, 25, 6.25, 1.56, 0.39, 0.10
	X12314005	TSN306252	100, 25, 6.25, 1.56, 0.39, 0.10
	X12335723	TSN304462	100, 25, 6.25, 1.56, 0.39, 0.10
	X12393285	TSN304332	100, 25, 6.25, 1.56, 0.39, 0.10
XDE-777	GF-3308		100, 25, 6.25, 1.56, 0.39, 0.10

**Table 2. Source of metabolites.**

Metabolite	Source
X12019520	hydrolysis, surface water mineralization, water/sediment
X12255349	soil photolysis
X12313581	aerobic soil, surface water mineralization, water/sediment
X12314005	hydrolysis, surface water mineralization, water/sediment
X12335723	hydrolysis, water/sediment
X12393285	hydrolysis
X696476	aerobic and anaerobic soil, surface water mineralization, water/sediment

### Plant Material

Wheat plants (variety ‘Yuma’) were grown from seeds in a greenhouse in plastic pots of surface area 27.5 cm<sup>2</sup> containing a mixture of 90% artificial soil and 10% field soil. The resulting seedlings (8–12 per pot) were used for testing when the primary or first leaves were fully emerged typically 8 to 9 days after planting.

### Compound application and evaluation of disease development.

Two mg of each compound were dissolved in 2 mL acetone, and 0.5 mL of the solution was sequentially mixed with 1.5 mL of acetone to make 4 fold dilutions. The acetone dilutions were mixed with 9 volumes of water containing 110 ppm Triton X 100 to obtain formulated high volume (HV) spray solutions. The solutions were applied to the plants at 15 mL per tray using an automated booth sprayer, which utilized two 6218 1/4 JAUPM spray nozzles operating at 20 psi and set at opposing angles to cover both sides of leaf surfaces. After application, the plants were allowed to air dry prior to further handling.

Wheat plants were inoculated with an aqueous spore suspension of *Septoria tritici* either three days prior to (3 DC) or one day after (1 DP) fungicide treatments. After inoculation the plants were maintained at 100% relative humidity (one day in a dark dew chamber followed by two days in a lighted dew chamber at 20 °C) to permit spores to germinate and infect the leaf. The plants were then transferred to a greenhouse set at 20 °C for disease to develop. When disease symptoms were fully expressed on the 1st leaves of the untreated plants, infection levels were assessed on a scale of 0 to 100 percent disease severity. Percent disease control was calculated using the ratio of disease severity on treated plants relative to untreated controls.

### RESULTS AND DISCUSSION Activity of UK-2A, XDE-777 and its seven metabolites vs. SEPTTR

High volume one day protectant (1 DP) and three day curative (3 DC) SEPTTR activity of XDE-777, UK-2A, and the seven metabolites of XDE-777 were evaluated using compound rates ranging from 100 to 0.1 ppm. The test results indicated: 1) XDE-777 was highly active vs. SEPTTR in both curative and protectant treatments; 2) UK-2A showed very strong protectant SEPTTR activity but curative efficacy



was much weaker; 3) none of the seven metabolites showed any meaningful biological activity vs. SEPTTR. One compound X772777 did show an anomaly in the curative test at the second rate, cause unknown. This result did not appear in the repeat test.

## CONCLUSION

In these studies, we evaluated the biological activity of XDE 777, UK 2A, the formulated product GF3308 as well as seven metabolites of XDE 777, vs. SEPTTR, the causal agent of wheat leaf blotch. The compound XDE 777 was highly potent as both protectant and curative treatments vs. SEPTTR the key driver disease. The natural product UK 2A, from which XDE 777 was derived, showed a high level of protectant activity but curative activity was weaker. With the metabolites tested none showed any meaningful fungicidal activity vs. SEPTTR. The formulated product was also highly active in both curative and protectant test.

### A 2.2.3.5 Study 5 – *Septoria tritici* Biological screening report for five mteabolites of XDE-777

Comments of zRMS:	<p>The study below was validated and agreed by the zRMS efficacy expert. Performed evaluation confirmed that none of the tested fenpicoxamid metabolites (X763024, X11963422, X12264475, X12255349 and X12393285) is biologically active.</p> <p>For full study summary and zRMS evaluation, please refer to the Core Assessment, Part B, Section 3.</p> <p>The summary below was struck through as no evaluated in area of Section 9.</p>
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Reference:	KCP 10.2.3/5
Report:	Yao, C.; 2014; <i>Septoria tritici</i> Biological Screening Report for Five Metabolites of XDE777; Dow AgroSciences LLC, Zionsville, Indiana, USA; Lab Study No. DAI 1370; 04 November 2014; Unpublished
Acceptability:	Accepted by the zRMS efficacy expert during evaluation performed in area of Section 3
Duplication (if vertebrate study)	NA

## SUMMARY

XDE 777, a pro fungicide of the natural picolinamide UK 2A, is a pre-development fungicide of Dow AgroSciences. Five metabolites of XDE 777 identified in soil metabolism, soil photolysis, or aqueous hydrolysis studies were evaluated for their biological activity vs. wheat leaf blotch caused by *Septoria tritici* (SEPTTR), the key driver disease in the European cereal fungicide market. In the current studies, XDE 777 was highly potent vs. SEPTTR when used as both protectant and curative treatments. UK 2A, from which XDE 777 was derived, showed high level of protectant activity but significantly weaker curative activity. However, none of the five metabolites of XDE 777 showed any meaningful fungicidal activity vs. SEPTTR.

## MATERIALS AND METHODS

### Chemicals

XDE 777, UK 2A, and the five metabolites of XDE 777 were used in the studies. Their structures and lot information are listed in Figure 1. X763024, X11963422 and X12264475 are soil metabolites (Hastings and Jackson, 2013), X12255349 is a soil photolysis metabolite (Cooke, 2013), and X12393285 is a hydrolysis metabolite (Yoder and Jackson, 2013).

**Plant Material:** Wheat plants (variety ‘Yuma’) were grown from seeds in a greenhouse in plastic pots of surface area

27.5 cm<sup>2</sup> containing 50% mineral soil and 50% soil less Metro mix. The resulting seedlings (8-12 per pot) were used for testing when the primary or first leaves were fully emerged, typically 7 to 8 days after planting.

#### Compound applications and evaluation of disease development

Two mg of each compound were dissolved in 2 mL acetone, and 0.5 mL of the solution was sequentially mixed with 1.5 mL of acetone to make 4 fold dilutions. The acetone dilutions were mixed with 9 volumes of water containing 110 ppm Triton X-100 to obtain formulated high volume (HV) spray solutions. The solutions were applied to the plants at 15 mL per tray using an automated booth sprayer, which utilized two 6218 1/4 JAUPM spray nozzles operating at 20 psi and set at opposing angles to cover both sides of leaf surfaces. After application, the plants were allowed to air dry prior to further handling.

Wheat plants were inoculated with an aqueous spore suspension of *Septoria tritici* either three days prior to (3-DC) or one day after (1-DP) fungicide treatments. After inoculation the plants were maintained at 100% relative humidity (one day in a dark dew chamber followed by two days in a lighted dew chamber at 20 °C) to permit spores to germinate and infect the leaf. The plants were then transferred to a greenhouse set at 20 °C for disease to develop. When disease symptoms were fully expressed on the 1st leaves of untreated plants, infection levels were assessed on a scale of 0 to 100 percent disease severity. Percent disease control was calculated using the ratio of disease severity on treated plants relative to untreated controls.

## **RESULTS AND DISCUSSION**

### Activity of UK-2A, XDE-777 and its three soil metabolites vs. SEPTTR

High volume one day protectant (1-DP) and three day curative (3-DC) SEPTTR activity of XDE-777, UK-2A, and the three soil metabolites of XDE-777 were evaluated using compound rates ranging from 100 to 0.1 ppm (Table 1). The test results indicated that: 1) XDE-777 was highly active vs. SEPTTR in both curative and protectant treatments; 2) UK-2A showed very strong protectant SEPTTR activity but curative efficacy was much weaker; 3) none of the three metabolites showed any meaningful biological activity vs. SEPTTR (activity levels of metabolites were < 1/1000 the activity levels observed for XDE-777).

### Activity of UK-2A, and XDE-777 soil photolysis and aqueous hydrolysis metabolites vs. SEPTTR

UK-2A, the soil photolytic product X12255349 and the hydrolysis metabolite X12393285 of XDE-777 were tested vs. SEPTTR in both protectant and curative treatments (Table 2). The test rates ranged from 100 to 6.25 ppm. The test results indicated that the two metabolites of XDE-777 were inactive against SEPTTR as curative and protectant treatments, while UK-2A was highly active as a protectant treatment.

## **CONCLUSIONS**

In this study, we evaluated the biological activity of XDE-777 and UK-2A, as well as that of five metabolites of XDE-777, vs. SEPTTR, the causal agent of wheat leaf blotch. XDE-777 was highly potent as both protectant and curative treatments vs. this key driver disease. UK-2A, the natural product from which XDE-777 was derived, showed a high level of protectant activity but curative activity was weaker. However, none of the five metabolites showed any meaningful fungicidal activity vs. SEPTTR.

## **A 2.3 KCP 10.3 Effects on arthropods**

### **A 2.3.1 KCP 10.3.1 Effects on bees A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees A 2.3.1.1.1.1**

## Study 1 - GF-3308: Acute contact and oral effects on Honeybees (*Apis mellifera* L.) in the laboratory

Comments of zRMS:	<p>The study was performed in line with OECD 213 and OECD 214 with a minor deviation.</p> <p>It was noted that in the contact test a 5 µL droplet was chosen instead of the guideline recommendation of a 1 µL droplet, since according to the testing facility's experience a higher volume ensured a more reliable dispersion of the test item and no adverse effects on the outcome of the study were expected. In zRMS opinion this deviation is considered to have no effect on the outcome of the study since all the validity criteria were met.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>48h oral LD<sub>50</sub> &gt; 205.6 µg product/bee  48h contact LD<sub>50</sub> = 53.4 µg product/bee</p>
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Reference:	KCP 10.3.1.1.1/1
Report:	Schmitzer, S.; 2016; GF-3308: Acute contact and oral effects on honeybees ( <i>Apis mellifera</i> L.) in the laboratory; ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 111271035; DAS Study No. 160184 ; 01 November 2016; Unpublished
Guideline(s):	OECD 213 and 214
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

### MATERIALS AND METHODS

Test item (Common name): GF-3308  
Purity: XDE-777: 4.8% (49 g/L)  
Description (physical state): Brown liquid  
Lot/batch no.: Lot No.: E3240-85-1 (Test Substance Number: TSN311166)

### Test System

Organism (*Species*): Honey bee (*Apis mellifera*)  
Study type: Acute oral and contact dose response study

Study design: Acute contact LD<sub>50</sub> test and oral limit test; duration 48 h; 5 replicates (oral limit test) or 3 replicates (contact dose response test), each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hours

Test concentrations:	Oral: [nominal]: 0 (untreated control), 200 µg GF3308/bee Oral [measured]: 0 (control), 205.6 µg GF-3308/bee Contact: 0 (control), 200, 100, 50, 25 and 12.5 µg GF3308/bee
Information on bee colony (health etc):	The bees used in the test were female worker bees from a single, disease-free colony. The hive had never been treated for varroa mites or for disease. The bees were maintained in a clean holding cage.
Amount of treated diet consumed:	Consumption of the treated diets resulted in calculated dosages ranging from 178.0 to 216.0 µg GF-3308/bee.
Feeding method:	50% w/v sucrose solution <i>ad libitum</i> ; was given directly after treatment using syringes; no replacements of the food was necessary during the experimental time of the experiments (48 h).
Environmental conditions:	Temperature: 25 - 26°C oral 26 - 27°C contact Relative Humidity: 58 - 61% oral 60 - 62% contact Photoperiod: The environmental chamber was kept dark except when room lighting was used during observation periods.
Reference substance:	0.30, 0.20, 0.15 and 0.10 µg Dimethoate per bee (contact test) 0.30, 0.15, 0.08 and 0.05 µg Dimethoate per bee (oral test)
Solvent substance (if applicable):	None.

### Methodology

Contact dose response study: a single 5 µL droplet of GF-3308 in an appropriate carrier (tap water + 0.5 % Adhäsit) was placed on the dorsal bee thorax using a Multipette®, Eppendorf. For the control one 5 µL droplet of tap water containing 0.5 % Adhäsit was used.

Oral limit study: after mixing the test solutions with 50 % w/v sucrose solution the final concentration of sucrose in the test item solution offered to the bees was 50 %. For the controls 50 % w/v sucrose solution was used.

## RESULTS AND DISCUSSION

Mortality and sublethal effects for the oral and contact studies are summarised below.

**Table 58: Toxicity of GF-3308 to honeybees in oral and contact toxicity test**

Treatment µg GF-3308 /bee		Contact	Oral
		Oral	Contact
Nominal	Mean consumed dose	Mortality (%)	
		48-hr	48-hr
Control (0)	205.6	0.0	0.0
200.0	-	90.0	14.0
100.0	-	83.3	-
50.0	-	33.3	-

25.0	-	23.3	-
12.5	-	10.0	-
Contact 48-hr LD <sub>50</sub>	53.4 µg/bee (95% CI 41.9 µg/bee – 68.3 µg/bee not available)		
Oral 48-hr LD <sub>50</sub>	≥ 205.6 µg/bee (95% CI not available)		
Contact LD <sub>50</sub> (24-hr) value of the reference item: 0.17 µg dimethoate/bee			
Oral LD <sub>50</sub> (24-hr) value of the reference item: 0.18 µg dimethoate/bee			

**Table 59: Sublethal effects of GF-3308 to honey bees oral and contact toxicity test**

		Treatment µg/bee		
Nominal	Consumed	Sublethal effects after 48 hrs (% number of bees)		
		On-back	Behavioural abnormalities* (mean) Lethargic	Other
Contact:				
Control (0)	-	0%	0%	0%
200.0	-	0%	0%	0%
100.0	-	0%	0%	0%
50.0	-	0%	6.7 3.3%	3.3%
25.0	-	0%	0%	0%
12.5	-	0%	0%	0%
Oral:				
Control (0)	0	0%	0%	0%
200	205.6	0%	0%	0%

\* behavioural abnormalities - observed symptoms such as moribund, affected, cramps, apathy, vomiting

## CONCLUSION

The toxicity of GF-3308 was tested in both an acute contact and an oral toxicity test on honey bees. In the contact toxicity test the LD<sub>50</sub> (48 h) value was 53.4 µg product/bee. In the oral toxicity test the LD<sub>50</sub> (48 h) value was > 205.6 µg product/bee.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3308	48 hr - contact	LD <sub>50</sub>	53.4	µg product/bee
Honey bee	<i>Apis mellifera</i>	GF-3308	48 hr - oral	LD <sub>50</sub>	> 205.6	µg product/bee

### A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees A 2.3.1.2.1 Study 1 - GF-3308 - Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure)

Comments of zRMS:	<p>The study was performed in line with OECD 239 with minor deviations.</p> <p>It was noted that for the toxic reference item groups no other observations except mortality were assessed. No emergence boxes were used as from day 15 to enable the assignment of each emerged bee to the respective replicate. However, these deviations had no impact on the study outcome.</p>
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	<p>All validity criteria were met:</p> <ul style="list-style-type: none"> <li>- mortality in controls from day 3 to day 8 was <math>\leq 15\%</math> (actually 2.1 % and 10.4 %),</li> <li>- emergence in controls on day 22 was <math>\geq 70\%</math> (actually 85.4 % and 72.9 %),</li> <li>- dimethoate reference item cumulative mortality on day 8 was <math>\geq 50\%</math> (actually 95.8 %),</li> <li>- fenoxycarb reference item adult emergence on day 22 was <math>\leq 20\%</math> (actually 0.0 %).</li> </ul> <p>The measured concentration of the active substance in the stock solution was maintained within 80-120% of nominal.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>NOEC = 55.6 mg product/kg food EC<sub>50</sub> = 172 mg product/kg food</p> <p>NOED = 8.56 µg product/larva ED<sub>50</sub> = 26.5 µg product/larva</p>
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Reference:	KCP 10.3.1.2/1
Report:	Emmanuelle Vergé; 2020; GF-3308 - Honey Bee ( <i>Apis mellifera</i> L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agrosience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S19-00184; DAS Study No. 190305 ; 07 May 2020; Unpublished
Guideline(s):	OECD 239
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): GF-3308  
Purity: 5.1% w/w (51 g/L) fenpicoxamid  
Description (physical state): liquid / brown  
Lot/batch no.: ENBK-169309-002 (TSN313638)

### Test System

Organism (*Species*): Honey bee (*Apis mellifera*)

Study type: Chronic Larval – repeated exposure Study design: Dose-response test; duration 22 days; 3 or more replicates, each starting with at least 12 synchronized 1<sup>st</sup> instar larvae per test concentration; for each treatment group (1 control group (C), 1 solvent control group (CS), 5 test item groups (T1-T5)), 48 test organisms from three different hives were tested; each hive equates to one replicate, 16 larvae for each replicate were used; assessment of mortality and behavioural effects daily after administration of the test item on days 3, 4, 5, and 6 and on days 7, 8, 15 and adult emergence on day 22. Visual assessment of uneaten food on day 8 prior to transfer of the test plates into pupal desiccator. Monitoring of pupal development and adult emergence (eclosion) until day 22. Weighing of emerged bees on day 22.

## Test concentrations:

0 (control, solvent control),  
 6.20 (corrected for analytical recovery 4.70), 18.5, 55.6, 167 and 500 mg GF-3308/kg diet equivalent to 0.95 (0.724), 2.85, 8.56, 25.7 and 77.0 µg GF-3308/larva per developmental period  
 48.0 mg dimethoate/kg diet, equivalent to 7.39 µg dimethoate/larva per developmental period and 0.320 mg fenoxycarb/kg diet, equivalent 0.0493 µg fenoxycarb/larva per developmental period

Information on bee colony (health, etc.): The larvae used in the test were from three disease-free colonies (one per replicate). The hive had not been treated for *Varroa* mites or for disease for at least 4 weeks prior to study initiation.

## Analytical verification:

Fenpicoxamid was analysed in the stock solution, the test item solutions and control solution as well as in the test item treated larval diet and the diet of the control group by liquid chromatography and mass spectrometric detection (HPLC-MS/MS). Additional verification of the homogeneity (top and bottom sampling of treated diet) and stability (sampling at  $24 \pm 1$  hours after preparation) of the test item in the larval diet. The analytical verification of fenpicoxamid resulted in recoveries of 71 to 108 % (solutions) and 73 to 97 % (diet) of the nominal values. The concentrations of fenpicoxamid in the homogeneity samples taken from the top and bottom of the treated diet of the lowest and highest test item group were equivalent to recoveries of 72 to 100 %. The measured recovery rate of fenpicoxamid in the aged larval diet of the lowest and highest test item group was 40 and 44 %.

The concentration of the test item in the larval diet of the four highest concentrations was confirmed. The recovery values for the larval diet samples of the lowest treatment group were not within the range of 80 to 120 % of the nominal concentrations, further evaluations were done with concentrations corrected for actual recovery.

## Feeding method:

Three different diets (A, B and C) were administered depending on the developmental stage of the larvae. The diets were based on 50 % fresh royal jelly and 50 % aqueous solution containing variable amounts of yeast extract, glucose and fructose in the three diets. The feeding solutions were prepared as needed.

Diets A and B (20 µL/larvae, each) were administered on days 1 and 3, respectively. Diet C was administered once on days 4 to 6 in increasing volumes of 30 to 50 µL/larvae. The test item was administered on days 3, 4, 5 and 6 homogeneously dispersed in 20 to 50 µL/larvae of diet B or C depending upon the day of incubation.

## Environmental conditions:

Temperature: 30.7 – 34.6°C

D1 to D8: control groups 33.4 – 34.5°C, mean 34.3°C; test item groups: 33.7 – 34.4°C, mean 34.3°C; reference item groups: 33.9 – 34.6°C, mean 34.4°C

D8 to D15: control groups 33.0 – 34.3°C, mean 34.3°C; test and reference item groups: 34.0 – 34.4°C, mean 34.3°C

D15 to D22: control, test and reference item groups: 30.7 – 34.2°C, mean 33.9°C

Relative Humidity: 46.5 - 100 %; mean 94.3-98.2% depending on a treatment group (day 1 to day 8), 52.3 – 89.8%; mean 80.0-85.0% depending on a treatment group (day 8 to day 15), 44.4 - 81.9 %; mean 73.1% (day 15 - day 22)

Photoperiod: constant darkness except during grafting, feeding and assessments.

Reference substance:

Dimethoate: 48.0 mg dimethoate/kg diet, 7.39 µg dimethoate/larva per developmental period

Fenoxycarb: 0.320 mg fenoxycarb/kg diet, 0.0493 µg fenoxycarb/larva per developmental period

## Methodology

On day 1 synchronised honey bee larvae (first instar, L1) were taken from the combs of 3 hives and were individually transferred into well-plates, where they were fed a standardised amount of artificial diet. From day 3 until day 6 GF-3308 was administered daily to the larvae in the diet in a range of increasing concentrations, which remained constant during the application period. The presence of uneaten food was qualitatively recorded on day 8. Cumulative mortalities during the larval phase were assessed daily from day 4 until day 8. Cumulative mortalities during the pupation phase were assessed on day 15 and on day 22. The adult emergence rate was assessed on day 22. Additionally, the weight of emerged bees was assessed on day 22. Other observations and any other adverse effects were recorded in comparison to the control group.

## RESULTS AND DISCUSSION

On day 8, larval mortality was 2.1 % in the control group and 10.4 % in the solvent control group. Larval mortality in the dimethoate reference item group was 95.8 %. On day 22, the adult emergence rate in the control and solvent control group was 85.4 and 72.9 %, respectively. The adult emergence rate in the fenoxycarb reference item group was 0.0 %.

Compared to the control group, the adult emergence rate on day 22 was statistically significantly different in the test item group T4 (167 mg GF-3308/kg diet) and T5 (500 mg GF-3308/kg diet) (Cochran-Armitage test with Rao-Scott adjustment, one sided greater,  $\alpha = 0.05$ ). Therefore, the NOEC for adult emergence on day 22 was determined to be 55.6 mg GF-3308/kg diet, equivalent to a NOED of 8.56 µg GF-3308/larva per developmental period.

The EC<sub>50</sub> for adult emergence on day 22 was determined to be 172 mg product/kg diet (95 % CL: 141 / 209), equivalent to an ED<sub>50</sub> of 26.5 µg product/larva per developmental period (95 % CL: 21.7 / 32.2) (Spearman-Kärber procedure).

**Table 60: Toxicity of GF-3308 to honey bee larvae in a chronic exposure toxicity test**

Nominal Treatment		Chronic larval exposure toxicity		
mg GF-3308/kg diet	µg GF-3308/larva per developmental period	Mortality (%) (Corrected Mortality (%))		Emergence (%) (Inhibition compared to control %)
		Day 8	Day 15	Day 22
Control (0)		2.1 (n.a.)	14.6 (n.a.)	85.4 (n.a.)
Solvent control (0)		10.4 (n.a.)	25.0 (n.a.)	72.9 (n.a.)



6.20 (4.70) <sup>a</sup>	0.95 (0.724)	4.2 (2.1)	20.8 (7.3)	79.2 (7.3)
18.5	2.85	4.2 (2.1)	12.5 (-2.5)	87.5 (-2.5)
55.6	8.56	4.2 (2.1)	20.8 (7.3)	79.2 (7.3)
167	25.7	22.9* (21.2)	39.6* (29.3)	58.3* (31.7)
500	77.0	93.8* (93.7)	100* (100)	0.0* (100)
Reference item (7.39 µg dimethoate/larva per developmental period, nominal)		95.8 (95.7)	---	---
Reference item (0.0493 µg fenoxycarb/larva per developmental period, nominal)		4.2 (-6.9)	31.3 (8.4)	0.0 (100)
22-day NOED/NOEC		8.56 µg GF-3308/larva per developmental period, equivalent to 55.6 mg GF-3308/kg diet		

<sup>a</sup> corrected for analytical recovery

\* Significantly different compared to control (Cochran-Armitage test, one sided greater,  $\alpha = 0.05$ ) n.a.: not applicable

**Table 61: Uneaten food, developmental and behavioural effects in the chronic exposure larval toxicity test for GF-3308**

Nominal Treatment		Chronic larval exposure toxicity		
mg GF-3308/kg diet	µg GF-3308/larva per developmental period	Uneaten food observed on day 8	Behavioural effects (day)	Developmental effects (day)
Control (0)		no	none	none
Solvent Control (0)		yes	none	none
6.20 (4.70) <sup>a</sup>	0.95 (0.724)	no	none	none
18.5	2.85	no	none	none
55.6	8.56	no	none	none
167	25.7	no	none	none
500	77.0	yes	none	none
Reference item (7.39 µg dimethoate/larva per developmental period)		no	none	none
Reference item (0.0493 µg fenoxycarb/larva per developmental period)		no	none	none

<sup>a</sup> corrected for analytical recovery

## CONCLUSION

In a repeated exposure larval toxicity test with GF-3308 and a duration of 22 days, the NOEC for adult emergence was determined as 55.6 mg GF-3308/kg diet, equivalent to a NOED of 8.56 µg GF-3308/larva per developmental period. The EC<sub>50</sub> for adult emergence on day 22 was 172 mg GF3308/kg diet (95% CL: 141 / 209 mg GF-3308/kg diet) equivalent to an ED<sub>50</sub> of 26.5 µg product/larva per developmental period (95 % CL: 21.7 / 32.2).

The study was deemed valid since all validity criteria were met.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3308	22 day	NOED	8.56	µg GF-3308/larva per developmental period
Honey bee	<i>Apis mellifera</i>	GF-3308	22 day	NOEC	55.6	mg GF-3308/kg diet

### A 2.3.1.2.2 Study 2 - GF-3308: Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L. in a 10 Day Chronic Feeding Test Under Laboratory Conditions

Comments of zRMS:	The study was performed in line with OECD (2016) Proposal for a New Guideline for the Testing of Chemicals, Honey Bee ( <i>Apis mellifera</i> L.), Chronic Oral Toxicity 10 Day Feeding Test in the Laboratory and was checked against compliance with OECD 245. No deviations were noted and all the validity criteria were met:					
	<div><div>-</div><div>the average mortality across replicates for the untreated control group was <math>\leq 15\%</math> at the end of the test (observed 7.5 % mortality),</div><div>-</div><div>the average mortality in the reference substance treated group was <math>\geq 50\%</math> at the end of the test (observed 97.5 %).</div></div>					
	Since the mean measured concentrations of the test item in spent diet dropped below 80%, the zRMS calculated the overall geometric mean measured concentrations in fresh and spent diet in particular test groups and the overall geometric mean measured concentration. Results are presented in table below.					
	Mean measured concentrations [%] of test item at					
	Test day	12.5 ppm	25 ppm	50 ppm	100 ppm	200 ppm
	1 (fresh)	99	91	92	93	100
	2 (aged)	70	78	90	79	83
	2 (fresh)	86	80	86	91	88
	3 (fresh)	86	84	93	90	83
	4 (aged)	71	63	63	72	79
4 (fresh)	89	86	89	85	80	
5 (fresh)	73	86	82	80	90	
6 (aged)	69	76	63	72	73	
6 (fresh)	86	73	84	89	97	
7 (fresh)	89	90	88	82	83	
8 (aged)	55	56	57	74	69	
8 (fresh)	86	87	80	86	96	
9 (fresh)	86	88	85	90	93	
10 (fresh)	80	80	84	83	91	
10 (aged)	64	45	74	59	78	
Mean %	78.4	76.3	79.8	81.1	85.1	
Overall mean %	80.1					
The overall mean measured concentration was 80.1% and for this reason endpoints were based on actual uptake of the test item by bees not corrected for measured concentration.						
Overall, the study is considered acceptable with following endpoints relevant for the risk assessment:						
LDD <sub>50</sub> = 0.71 µg a.s./bee/day (based on actual uptake)						
NOEDD = 0.49 µg a.s./bee/day (based on actual uptake)						

Reference:	KCP 10.3.1.2/2
Report:	Vergé, E.; 2017; GF-3308 - Assessment of Effects on the Adult Honey Bee, <i>Apis mellifera</i> L., in a 10 Day Chronic Feeding Test under Laboratory Conditions; Eurofins
	Agroscience Services EcoChem / Eurofins Agroscience Services Ecotox GmbH, 75223, Niefern-Öschelbronn, Germany; Lab Study No. S16-02528; DAS Study No. 160522 ; 20 February 2017; Unpublished
Guideline(s):	OECD (2016) Proposal for a New Guideline for the Testing of Chemicals, Honey Bee ( <i>Apis mellifera</i> L.), Chronic Oral Toxicity 10 Day Feeding Test in the Laboratory
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable

Duplication (if vertebrate study)	NA
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## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): GF-3308  
Purity: 49 g/L XDE-777  
Description (physical state): Liquid /brown  
Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism (Species): Honey bee (*Apis mellifera*); Young adult worker bees (newly hatched; 1 to 2 days old)

Study type: Chronic adult oral

Study design: Dose-response test; duration 10 days; one control group, five concentrations of the test item, one concentration of the reference item; 4 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality, food consumption and behavioural effects daily.

Four additional test units without bees but with full food syringes containing pure 50 % (w/v) aqueous sucrose solution for evaluation of the evaporation. One set each day of analytical samples of the control and test item feeding solutions for dose verification. Additionally, analytical samples (10 mL) of the spent diet (feeding solution left in the feeders of the test units at the end of the 24 h feeding interval) were taken at the end of the feeding intervals A1, A3, A5, A7 and A9.

Test concentrations: Oral: 0 (control), µg /bee

Control: C (pure 50 % (w/v) aqueous sucrose solution)

Test item: 12.5, 25, 50, 100 and 200 mg a.i./kg food

Information on bee colony (health etc): The bees used in the test were from a single, diseasefree colony. The hive had not been treated for *Varroa* mites or for disease in the last 4 weeks. The bees were maintained in a clean holding cage at a temperature of approximately 35°C and 50 to 70% humidity.

Amount of treated diet consumed: Consumption of the treated diets ranged from 12.9 to 39.1 mg of diet. Calculated daily dosages ranged from 0.49 to 2.57 µg XDE-777/bee.

Feeding method: During acclimation bees were provided *ad libitum* a 500 g/L (w/v) sucrose solution in water. The bees for the definitive test were housed in cages containing preweighed feeders (syringes) containing approximately 400 mg of the appropriate control or treated solutions. All control and treatment feeders were exchanged daily with freshly prepared diet. Consumption of the feeding solutions was monitored by weighing the syringe before and after feeding, correcting for evaporation.

Environmental conditions:

Temperature: 31.6 to 33.3°C

Relative humidity: 41.5\* to 67.3 %

**Photoperiod:** The environmental chamber was kept dark except when room lighting was used during observation periods.

\*short-term deviation (less than 2 hours)

Reference substance:

Dimethoate: 0.90 mg a.i./kg diet

Solvent substance (if applicable):

## Methodology

Honey bees were exposed to a 50 % (w/v) aqueous sucrose solution containing five concentrations of GF-3308 by continuous and *ad libitum* feeding over a period of 10 days. The control group was fed with pure 50 % (w/v) aqueous sucrose solution. Mortality and behavioural abnormalities were assessed daily during the 10 day exposure period. The chronic effects of GF-3308 were evaluated by comparing the results of the test item group to those of the control group. Additionally 4 test units without bees but with full food syringes containing pure 50 % (w/v) aqueous sucrose solution were placed in the climatic chamber for the evaluation of the evaporation.

## RESULTS AND DISCUSSION

In the control group C 7.5 % cumulative mortality was observed after 10 days of continuous feeding. In the test item groups, a cumulative mortality of 17.5, 45.0, 80.0, 92.5 and 100 % (corrected mortality 10.8, 40.5, 78.4, 91.9 and 100 %) was observed at the respective concentrations of 12.5, 25, 50, 100 and 200 mg XDE-777/kg diet at the end of the 10 day test period. Behavioural abnormalities were observed in all the test item treatment groups.

The overall mean daily consumption of feeding solution over the entire test period of the control group C was 40.5 mg/bee/day. At the concentrations of 12.5, 25, 50, 100 and 200 mg XDE-777/kg diet the overall mean daily consumption of feeding solution was 39.1, 27.0, 18.8, 14.7 and 12.9 mg/bee/day, respectively. The difference between the overall mean daily consumption of feeding solution of the control group C and the tested concentrations was statistically significant at the concentrations of 25, 50, 100 and 200 mg XDE-777/kg diet. In the toxic reference item group, the overall mean daily consumption of feeding solution was 20.2 mg/bee/day.

The actual mean concentrations of XDE-777 in the feeding solutions, determined from 0DBA1 (DBA - Day Before Application) to 0DBA10, were in a range from 85 to 90 % of the nominal concentrations. The actual concentrations of XDE-777 in the spent diet, determined 1DAA1 (DDA – Day After Application), 1DAA3, 1DAA5, 1DAA7 and 1DAA9 were in the range from 64 to 76 % of the nominal concentrations. No residues of XDE-777 above the LOD (0.360 mg a.i./L) were found in the control samples.

**Table 62: Toxicity of GF-3308 to honey bees in the chronic oral toxicity test**

[illegible]

100	1.47		2.5	5.0	17.5	37.5	52.5	62.5	72.5	77.5	87.5	(91.9)	
200	2.57	7.5	35.0	52.5	72.5	85.0	97.5	97.5	97.5	100	100*	(100)	Reference
Item	0.0	0.0	5.0	20.0	75.0	90.0	97.5	97.5	97.5	97.5	97.5	(97.3)	
10 day LDD <sub>10</sub> (95 %			0.41 µg a.i./bee/day (0.32 to 0.48 µg										
a.i./bee/day) confidence limits)			10 day LDD <sub>50</sub> (95 %										
a.i./bee/day (0.64 to 0.79 µg a.i./bee/day) confidence limits)			0.71 µg										
10 day LOEDD mortality			0.68 µg a.i./bee/day										
10 day NOEDD mortality			0.49 µg a.i./bee/day										
10 day LC <sub>10</sub> (95 %			9.53 mg a.i./kg diet (6.06 to 12.8 mg a.i./kg diet) confidence limits)										
10 day LC <sub>50</sub> (95 %			27.0 mg a.i./kg diet (22.0 to 32.4 mg a.i./kg diet) confidence limits)										
10 day LOEC mortality			25.0 µg a.i./kg diet										
10 day NOEC mortality			12.5 mg a.i./kg diet										

\*Significantly different compared to the control (Cochran-Armitage test with Rao-Scott adjustment, one-sided greater,  $\alpha = 0.05$ )

**Table 63: Effect of GF-3308 on diet consumption in honey bees in the chronic oral toxicity test**

Treatment		Oral 10 day test											
Nominal Concentration mg XDE-777/kg diet	Measured daily dose mg XDE-777/bee	Diet Consumption (mg/day)											
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean	
Control (0)	0	30.6	30.2	47.0	43.0	44.1	45.0	48.2	36.6	39.9	40.3	40.5	
12.5	0.49	26.1	40.7	38.5	41.3	49.0	48.1	43.8	39.6	29.6	34.4	39.1	
25	0.68	19.3	31.8	35.7	32.5	33.8	20.4	26.3	33.7	12.6	22.4	27.0	
50	0.94	13.6	28.5	32.3	26.7	23.6	20.4	6.60	16.5	12.7	5.10	18.8	
100	1.47	13.1	20.4	15.2	19.1	15.9	12.0	12.5	19.0	0.30	20.0	14.7	
200	2.57	16.3	18.4	12.9	9.30	12.3	4.70	16.5	17.8	0.00	-	12.9	
Reference Item		32.0	26.7	18.1	19.9	20.0	19.5	11.0	1.50	7.00	11.7	20.2	
10 day LOEC, diet consumption		25.0 µg a.i./kg diet											
10 day NOEC, diet consumption		12.5 µg a.i./kg diet											

- all bees were dead

**Table 64: Sublethal effects of GF-3308 to honey bees in the chronic oral toxicity test**

Treatment		Oral 10 day test		
Nominal Concentration mg XDE-777/kg diet	Measured daily dose µg XDE-777/bee	Sublethal effects (Number of bees, Day observed)		
		On back	Lethargic	Other*
Control (0)	0	0	0	1 (day 9)
12.5	0.49	0	0	1 (day 2), 2 (day 7), 2 (day 8), 1 (day 9), 1 (day 10)
25	0.68	0	0	1 (day 1), 2 (day 5), 3 (day 7), 1 (day 8), 1 (day 9), 1 (day 10)
50	0.94	0	0	2 (day 1), 1 (day 5), 5 (day 6), 5 (day 7), 1 (day 8), 6 (day 9), 5 (day 10)
100	1.47	0	0	2 (day 3), 1 (day 4), 5 (day 5), 3 (day 6), 5 (day 7), 6 (day 8), 4 (day 9), 3 (day 10)
200	2.57	0	0	4 (day 1), 1 (day 2), 4 (day 3), 1 (day 5), 1 (day 6), 1 (day 7), 1 (day 8)

\*affected, apathetic, moribund

## CONCLUSION

The continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item GF-3308 at the treatment levels of 12.5, 25, 50, 100 and 200 mg XDE-777/kg diet caused adverse effects regarding mortality and behavioural abnormalities. In the test item treatment groups cumulative mortality of 17.5, 45.0, 80.0, 92.5 and 100 % (corrected mortality 10.8, 40.5, 78.4, 91.9 and 100 %) was observed at the respective concentrations of 12.5, 25, 50, 100 and 200 mg XDE-777/kg diet. In the control group C after 10 days of continuous feeding, 7.5 % mortality was observed. No remarkable behavioural abnormalities were observed in the control and solvent control groups.

The LOEC and NOEC, based on overall mean consumption of feeding solution after 10 days of continuous exposure were determined to be 25.0 mg XDE-777/kg diet and 12.5 mg XDE-777/kg diet, respectively.

The LOEC and NOEC for mortality after 10 days of continuous exposure was determined to be 25.0 mg XDE-777/kg diet and 12.5 mg XDE-777/kg diet. The corresponding LOED and NOEDD, based on the actual consumption of the respective feeding solutions, were determined to be 0.68 µg XDE777/bee/day and 0.49 µg XDE-777/bee/day.

The LC<sub>10</sub>, LC<sub>50</sub> after 10 days of continuous exposure were determined to be 9.53 mg a.i./kg diet and 27.0 mg XDE-777/kg diet, respectively. The corresponding LDD<sub>10</sub>, LDD<sub>50</sub> based on the actual consumption of the respective feeding solutions were determined to be 0.41 µg XDE-777/bee/day and 0.71 µg XDE-777/bee/day, respectively.

Common name	Species	Test item	Timescale	Endpoint	Toxicity value	Units of test item
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	Food consumption LOEC	25.0	mg XDE-777/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	Food consumption NOEC	12.5	mg XDE-777/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	Mortality LOEC	25.0	mg XDE-777/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	Mortality NOEC	12.5	mg XDE-777/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	Mortality LOEDD	0.68	µg XDE-777/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	Mortality NOEDD	0.49	µg XDE-777/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	LC <sub>10</sub>	9.53	mg XDE-777/kg diet

Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	LC <sub>50</sub>	27.0	mg XDE-777/kg diet
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	LDD <sub>10</sub>	0.41	µg XDE-777/bee/day
Honey bee	<i>Apis mellifera</i>	GF-3308	10 days	LDD <sub>50</sub>	0.71	µg XDE-777/bee/day

**A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey  
bee life stages A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects A 2.3.1.5 KCP  
10.3.1.5 Cage and tunnel tests**

**A 2.3.1.5.1 Study 1 - GF-3308 (XDE-777): Brood Development of the Honey Bee (*Apis mellifera* L.) in a Semi-Field Tunnel Study in *Phacelia tanacetifolia* in Germany 2016**

Comments of zRMS:	<p>The study was performed according to OECD 75 with no deviations.</p> <p>The test item (GF-3308) was applied at two application rates corresponding to 65 and 130 g a.s./ha to flowering <i>Phacelia tanacetifolia</i> during the bee flight. The water control and toxic standard (Insegar containing fenoxycarb) groups were also included in the test design. Each treatment group consisted of 5 replicates.</p> <p>Bees were exposed to the treated <i>Phacelia</i> for 7 days in the tunnels and after that further monitoring of the colonies was performed for full 2 brood cycles.</p> <p>During the exposure phase rainfall at 9 and 8 mm was observed 1 and 3 days after application, respectively. Nevertheless, fenpicoxamid residues were still present in pollen and nectar collected from the worker bees after the rainfall.</p> <p>Plants of <i>Phacelia</i> were collected for analysis of residues of fenpicoxamid in nectar and pollen from flowers, but due to confusion of sample labels no residue analyses were performed on these specimens.</p> <p>It is noted that the study was performed rather late in the season (application of the test item was carried out on 18<sup>th</sup> of July) resulting with the brood assessments of the 2<sup>nd</sup> brood cycle performed in the middle of September and last brood assessments carried out in October, i.e. at the time of the natural decline of the bee colony before wintering. This may add some uncertainty in the brood parameters investigated at the end of the test. Both rates of the test item had statistically significant but transient effect on bee foraging</p>
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	<p>activity directly after the application. On the next days the foraging activity was at level comparable to controls (or to activity in the given group observed before the treatment) and for this reason observed effects are considered to be biologically not relevant.</p> <p>On the basis of analysis of the mortality data the zRMS agrees that increased mortality observed on some days was due handling of the bees/colonies necessary to derive respective endpoints from the study. It is also noted that increased mortality on those days was observed in all tested groups, including controls. Taking this into account, increased mortality is considered to be not treatment related.</p> <p>In both treatment groups behavioural effects were observed, especially during the exposure period. However, behavioural abnormalities had no impact on the colony performance and are thus considered to be biologically not relevant.</p> <p>Application of GF-3308 at 65 g a.s./ha (T1) had no effect on colony size. Exposure to higher rate of 130 g a.s./ha (T2) had no effect on colony size during the exposure period, but the number of bees in this treatment group clearly declined at the monitoring site. Observed effect was statistically significant and on this basis it is concluded that GF3308 applied at 130 g a.s./ha had adverse effect on colony strength.</p> <p>Lower rate of GF-3308 had no effect on the amount of brood (eggs, larvae and pupae). At higher rate some effect on brood could be observed.</p> <p>No statistically significant differences between the brood termination rates in control and test item groups were observed over the whole study period. However, during the 1<sup>st</sup> brood cycle the BTR in the T2 group were higher comparing to controls (especially for cells initially containing eggs and old larvae). This confirms the observations made on the brood area in the higher rate treatment group. During the 2<sup>nd</sup> brood cycle the brood termination rates in both test item groups were comparable with controls.</p> <p>The brood and compensation indices in both treatment groups were comparable with controls over the whole study period with exception of the compensation index of cells initially containing young larvae during the 1<sup>st</sup> brood cycle on 21 BFD in the T2 group. However, at that observation time this index was lower in all test groups comparing to other brood assessment days and it seems that in case of T2 group the more pronounced reduction was to low CI in a single replicate.</p> <p>No information on the presence of the queens is given in the study report, but presence of eggs over the entire study period in all control and treatment groups (with exception of the last brood assessment on 16<sup>th</sup> of October, when no eggs were observed in some of replicates of all test groups due to the time of the season) indicates that queens were present in the hives.</p> <p>Overall, the study is considered acceptable and its results indicate that application of GF3308 at rate corresponding to 65 g a.s./ha has no effect on bees or bee colony. Application at 130 g a.s./ha may have adverse effects on colony strength and brood development.</p> <p>The Applicant is kindly reminded that for future assessments respective tables presenting results and graphs extracted from the report should be presented in the study summary in order to facilitate independent validation by the concerned Member States.</p>
Reference:	KCP 10.3.1.5/1



Report:	Kleinhenz, M.; 2017; GF-3308 (XDE-777): Brood Development of the Honey Bee ( <i>Apis mellifera</i> L.) in a Semi-Field Tunnel Study in <i>Phacelia tanacetifolia</i> in Germany 2016; Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH, Niefern-Öschelbronn, Germany; Lab Study No. S16-02036; DAS Study No. 160515 ; 30 March 2017; Unpublished
Guideline(s):	OECD Guidance Document No. 75 (2007); OEPP/EPPO Guideline No. 170(4) (2010); EC Guidance Document 7029/VI/95 rev. 5 (1997)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): GF-3308 ~~XDE-777~~  
Purity: 4.92% g XDE-777 (nominal)  
49 g XDE-777/L (4.8%) (analysed)  
Description (physical state): Emulsifiable concentrate (EC)  
Lot/batch no.: E3240-85-1 [TSN311166]

### Test System

Organism (*Species*): Honey bee (*Apis mellifera*)  
Study type: Effects on honeybee brood development  
Study design: Tunnel test under semi-field conditions  
Test concentrations: Treatment T1: 65 g XDE-777/ha  
Treatment T2: 130 g XDE-777/ha  
Dosing method: Spray application on *Phacelia tanacetifolia* plants. Direct exposure of adults to the crop, indirect exposure by consumption of nectar and pollen collected from the treated crop.  
Environmental conditions: Field phase in the tunnels (2DBA to 7DAA): Daily rainfall from 0 to 9 mm, relative humidity (RH) from 29.0% to 100%, temperatures from 11.6°C to 35.1°C.  
Further observation at the monitoring site (8DAA to 86DAA): Daily rainfall from 0 to 23 mm, RH from 29.6% to 100%, temperatures from 1.4°C to 35.5°C.  
Reference substance: Insegar

### Methodology

The aim of the study was to determine potential effects of GF-3308 (active substance: XDE-777, 49 g a.i./L formulation) on the honeybee and honeybee brood over two brood cycles and to determine the magnitude of residues of XDE-777 in honey stomachs and pollen from forager bees following a spray application, to evaluate the potential exposure to honeybees. *Phacelia* plants were also collected and analysed but these data were not plausible in all cases and are not reported or evaluated.

The effect of the test item was examined on commercial honeybee colonies (*Apis mellifera carnica* L.; Hymenoptera, Apidae) under semi-field conditions.

The trial consisted of four treatment groups: two groups (T1 and T2) consisting of GF-3308 treatment, each replicated five times (T1a – T1e, T2a – T2e) plus one additional replicate for sampling (T1s, T2s), one group (C) consisting of five replicates of a water-treated control (Ca – Ce) plus one watertreated replicate for sampling (Cs) and one group consisting of an Insegar (active substance: fenoxycarb) treated reference group. Each replicate comprised one tunnel (approximately 100 m<sup>2</sup>) containing the target crop *Phacelia tanacetifolia*.

The test item, XDE-777 (formulation GF-3308), was applied at 65 g a.i./ha in T1 and at 130 g a.i./ha in T2. All applications were made with a calibrated, portable boom sprayer to deliver 100 L/ha spray volume (target) to the crop. All applications were applied within the limit of  $\pm 10\%$  deviation to the target spray volume. The application of the treatments was made to the crop during flowering and daily bee-flight.

The colonies were kept inside the tunnels and exposed to the crop from 3DBA to 7DAA and were then brought to a monitoring site for further observation until up to 86DAA.

Assessments of mortality and behaviour were carried out daily from 3DBA to 37DAA, and assessments of foraging activity on the crop were carried out daily from 3DBA to 7DAA.

The condition of the colonies and the development stage of the bee brood were assessed twice before application (8DBA, 1DBA; DBA = Days before application), once during exposure (5DAA; DAA = Days after application) and 12 times after exposure during further monitoring (9DAA, 15DAA, 20DAA, 26DAA, 32DAA, 37DAA, 44DAA, 51DAA, 58DAA, 65DAA, 77DAA and 86DAA). In total, 15 assessments were carried out.

The development of brood in individually marked cells (target:  $\geq 200$  cells containing eggs,  $\geq 200$  cells containing young larvae and  $\geq 200$  cells containing old larvae) was photographed and evaluated in detail over two complete brood cycles (5 assessment dates per brood cycle). The first brood cycle started shortly before the applications were performed (1DBA = BFD0; BFD = brood area fixing day) and lasted until BFD+21. The second brood cycle started shortly before the completion of the first brood cycle (15DAA = BFD+16 of the first brood cycle = BFD0 of the 2<sup>nd</sup> brood cycle) and lasted until BFD+22.

Samples of forager bees were collected for residue analysis of XDE-777 in pollen and nectar once before the applications at 1DBA (DBA = days before application) and three times during exposure to the treated crop: once shortly after the application on the same day (0DAA) and at 2DAA and 6DAA (DAA = days after application). Whole *Phacelia* plants were also collected and analysed but these data were not plausible in all cases and are not reported or evaluated.

Additionally, pupae from combs were collected once towards the end of the first brood cycle (16DAA=BFD+17) to assess their weight and check them for abnormal development.

After start of the first treatment against *Varroa* mites in autumn, mites fallen on the hive floor were counted six times over a period of 21 days.

## RESULTS AND DISCUSSION

### Mortality of adult worker bees:

Before application (2DBA to 0DBA), the mean daily worker bee mortality was 52.9 dead bees/colony/day in the control, 35.8 in T1, 43.4 in T2 and 34.5 in R.

On the day of the application, there were 89.8 dead bees/colony in the control, 92.2 in test item treatment T1, 107.0 in test item treatment T2 and 91.2 in the reference item group R.

Mean daily worker bee mortality during the exposure period (0DAA to 7DAA) was 99.2 dead worker bees/colony/day in the control, 82.4 in T1, 94.6 in T2 and 70.9 in the reference item group R. There were no statistically significant differences of the test item treatments T1 and T2 or the reference item treatment R compared to the control on any day during this period.

During further monitoring (8DAA to 37DAA), mean daily worker bee mortality was 44.0 dead bees per day in the control, 51.9 in T1, 41.0 in T2 and 43.4 in the reference item group R. Data in test item treatment T1 were not statistically different from the control on any day during this period.

In R, adult worker bee mortality was significantly higher than the control on 8DAA but this probably resulted from transport to the monitoring site during the night before. Mortality in R on 8DAA was still on a rather low level and the slight difference to the control is not considered as biologically relevant or treatment related.

Increased mortality that was observed in all treatments on 16DAA and 17DAA resulted from opening the hives, marking of additional combs and comb photography during the colony assessments on 15DAA (=BFD0/start of the 2<sup>nd</sup> brood cycle) and from collection of pupae from combs on 16DAA. After this period of clearly increased mortality in all treatments, mortality was on a normal level though significantly different from the control in T2 and R on 18DAA. These single observations in these treatments are considered an aftermath of the recent disturbances described above and they are not considered as treatment related.

Increased mortality that was observed in all treatments from 19DAA to 21DAA resulted from feeding sucrose solution to the colonies and the presence of scavenging bees at the hives.

**Overall, there was no effect of the test item treatment in T1 or T2 or of the reference item treatment R on adult worker bee mortality throughout the whole observation period (2DBA to 37DAA).** With regard to the reference item, this is not unusual since the reference item Insegar (a.s.: fenoxycarb) acts mainly on honeybee brood, and no effect on adult worker bee mortality is expected.

Summary of mean daily mortality of adult worker bees is presented in table below.

**Table 65: Mean daily mortality of adult worker bees per treatment group**

Date	Timing	Mortality (mean number of dead adult worker bees/day)			
		C (tap water)	T1 (GF-3308 at 65 g XDE-777/ha)	T2 (GF-3308 at 130 g XDE-777/ha)	R (Insegar)
18 Jul 2016	2DBA	29.0 ± 36.4	9.6 ± 3.4	12.6 ± 3.8	16.4 ± 13.9
19 Jul 2016	1DBA	77.4 ± 69.9	54.8 ± 43.1	46.6 ± 36.5	46.2 ± 44.2
20 Jul 2016	0DBA	52.4 ± 30.4	43.0 ± 21.0	71.0 ± 53.7	40.8 ± 26.5
Mean pre-exposure period (2DBA to 0DBA)		52.9 ± 19.8	35.8 ± 9.3	43.4 ± 21.4	34.5 ± 21.8
20 Jul 2016	Sum 0DAA	89.8 ± 28.8	92.2 ± 33.2	107.0 ± 45.9	91.2 ± 75.1
21 Jul 2016	1DAA	18.6 ± 11.7	15.2 ± 9.1	18.6 ± 11.8	15.2 ± 5.6
22 Jul 2016	2DAA	105.6 ± 45.9	58.8 ± 16.6	96.8 ± 46.8	57.6 ± 27.1
23 Jul 2016	3DAA	106.4 ± 43.6	74.0 ± 11.6	92.4 ± 41.1	67.8 ± 39.1
24 Jul 2016	4DAA	141.0 ± 62.9	100.8 ± 50.8	124.0 ± 54.6	96.2 ± 42.5
25 Jul 2016	5DAA	99.0 ± 32.2	96.2 ± 42.9	79.2 ± 25.9	66.8 ± 43.6
26 Jul 2016	6DAA	127.4 ± 61.3	120.0 ± 72.7	119.8 ± 43.5	95.6 ± 53.7
27 Jul 2016	7DAA	106.0 ± 52.9	102.2 ± 43.3	119.2 ± 39.0	76.8 ± 39.2
Mean exposure period (0DAA to 7DAA)		99.2 ± 35.1	82.4 ± 27.3	94.6 ± 33.3	70.9 ± 38.7
Mean post-exposure period (8DAA to 37DAA)		44.0 ± 5.9	51.9 ± 14.9	41.0 ± 11.1	43.4 ± 8.6

DBA/DAA = Days before/after application

#### Mortality of worker pupae and larvae:

Mortality of worker pupae in the test item treatments T1 and T2 was on a low level and not significantly higher than the control on any day during the observation period (2DBA to 37DAA).

Before exposure (2DBA to 0DBA), there were 0.2 dead worker pupae/day in the control, 0.3 in T1, 1.0 in T2 and 0.7 in the reference item group R. During exposure (0DAA to 7DAA) there were 0.5 dead worker pupae/day in the control, 1.3 in test item treatment T1, 1.2 in test item treatment T2 and 0.6 in R. During further monitoring after exposure (8DAA to 37DAA) there were 1.1 dead worker pupae/day in the control, 0.5 in test item treatment T1, 1.1 in test item treatment T2 and 28.1 in the reference item group R.

In the reference item treatment R, mortality of worker pupae was significantly higher than the control on each day during the period from 8DAA to 28DAA and on 31DAA, 32DAA and 35DAA. Moreover, malformed pupae were frequently observed during the post-application period. Thus, there was a clear impact of the reference item on worker pupae mortality, confirming exposure of the honeybees and their brood to the treated crop and its products and confirming suitability of the study design.

**Overall, there was no effect of the test item treatments T1 and T2 on the mortality of worker pupae and larvae whereas there was a clear impact in the reference item treatment R.**

Summary of mean daily mortality of worker pupae is presented in table below.

**Table 66: Mean daily mortality of worker pupae per treatment group**

Date	Timing	Mortality (mean number of dead worker pupae/day)			
		C (tap water)	T1 (GF-3308 at 65 g XDE-777/ha)	T2 (GF-3308 at 130 g XDE-777/ha)	R (Insegar)
18 Jul 2016	2DBA	0.0 ± 0.0	0.2 ± 0.4	0.6 ± 0.5	0.8 ± 1.3
19 Jul 2016	1DBA	0.4 ± 0.9	0.2 ± 0.4	1.2 ± 1.6	0.8 ± 1.8
20 Jul 2016	0DBA	0.2 ± 0.4	0.4 ± 0.9	1.4 ± 2.2	0.4 ± 0.5
Mean pre-exposure period (2DBA to 0DBA)		0.2 ± 0.3	0.3 ± 0.4	1.0 ± 1.4	0.7 ± 1.2
20 Jul 2016	Sum 0DAA	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5	0.6 ± 0.9
21 Jul 2016	1DAA	0.4 ± 0.5	0.8 ± 1.8	0.8 ± 1.3	0.0 ± 0.0
22 Jul 2016	2DAA	0.2 ± 0.4	1.6 ± 2.1	2.4 ± 3.2	0.6 ± 0.5
23 Jul 2016	3DAA	0.2 ± 0.4	1.0 ± 1.4	0.4 ± 0.5	0.2 ± 0.4
24 Jul 2016	4DAA	0.4 ± 0.5	2.6 ± 5.3	1.0 ± 1.7	0.8 ± 1.3
25 Jul 2016	5DAA	1.0 ± 1.4	2.2 ± 3.5	2.6 ± 2.1	0.0 ± 0.0
26 Jul 2016	6DAA	0.8 ± 1.3	1.8 ± 3.5	0.4 ± 0.5	1.0 ± 1.7
27 Jul 2016	7DAA	0.6 ± 0.9	0.4 ± 0.5	1.4 ± 1.5	1.8 ± 2.5
Mean exposure period (0DAA to 7DAA)		0.5 ± 0.4	1.3 ± 2.1	1.2 ± 1.3	0.6 ± 0.7
Mean post-exposure period (8DAA to 37DAA)		1.1 ± 1.0	0.5 ± 0.4	1.1 ± 1.2	28.1* ± 7.4

DBA/DAA = Days before/after application

\* Significantly different from the control (Student's t-test (method: pooled), one-sided,  $p \leq 0.05$ )

#### Mortality of male bees and male pupae:

Dead male bees (drones) and dead male pupae were found very infrequently in the bee traps and on the hive floors during the whole study. On most days there were no dead male bees or pupae found in the majority of hives. In those cases where dead male bees or pupae were found, their number was usually only 1-2 in single replicates except T2e where slightly higher male mortality was observed from the beginning.

Before exposure (2DBA to 0DBA), the daily mean of dead male bees and pupae was 0.3 in the control, 0.1 in T1, 1.0 in T2 and 0.3 in R. During exposure (0DAA to 7DAA), the daily mean of dead male bees and pupae was 0.1 in the control, 0.1 in test item treatment T1, 1.2 in T2 and 0.0 in R. At the monitoring site (8DAA to 37DAA), there were on average 0.1 dead male bees and pupae per day in the control, 0.1 in T1, 0.1 in T2 and 0.0 in R.

The higher mean values in T2 during the pre-exposure and exposure period resulted mainly from replicate T2e whereas no unusual observations were made in the other four replicates. The higher values in T2 are clearly not due to the test item treatment since this difference was already present during the pre-application period (2DBA to 0DBA).

None of the observed differences in the test item treatments T1 and T2 or the reference item treatment R were statistically significantly different from the control on any day of the observation period (2DBA to 37DAA).

**Overall, there was no effect of the test item treatment on mortality of male bees and male pupae.**

#### Foraging Activity of the honeybees:

Flight activity in the test item treatments T1 and T2 was not significantly different from the control on any observation day from 2DBA to 7DAA except on 0DAA.

In the test item treatment T1, foraging activity on 0DAA was slightly reduced during the first 2 hours after application (until 2HAA; significantly different from the control). Although the assessment 4 hours after application (4HAA; 19.5 forager bees/m<sup>2</sup>) was also significantly different from the control (24.0 forager bees/m<sup>2</sup>), it was on the same level as before application (18.4 forager bees/m<sup>2</sup> on 0DBA) in T1, and the slight difference to the control is not considered as biologically relevant.

In test item treatment T2, foraging activity on 0DAA was slightly reduced during the first hour after application (significantly different from the control until 1HAA). Although the values from 2HAA until 6HAA were also significantly different from the control, this is not considered an effect of the test item: At 2HAA and 4HAA, foraging activity was on the same level (12.2 and 12.7 forager bees/m<sup>2</sup>) as shortly before application in T2 (12.9 forager bees/m<sup>2</sup> on 0DBA). Low flight activity in T2 at 6HAA (8.1 forager bees/m<sup>2</sup>) clearly resulted from the late start of applications in T2 and the late timing of these assessments (19:29 to 20:36) when the end of the daylight period and of daily honeybee foraging were close.

On the following days, there were no statistically significant differences of foraging activity in T1, T2 or R compared to the control on any day. The mean foraging activity over the whole post-application exposure period in the tunnels (0DAA to 7DAA) was 17.1 forager bees/m<sup>2</sup> in C, 15.8 in T1, 14.5 in T2 and 14.8 in R. These slight differences to the control were statistically not significant.

**Overall, test item treatments T1 and T2 had an effect on honeybee foraging activity on the day of application (0DAA).**

Summary of bee foraging activity is presented in table below.

**Table 67: Mean daily foraging activity per treatment group**



Date	Timing	Foraging activity (mean number forager bees/m <sup>2</sup> /10-15 sec)			
		C (tap water)	T1 (GF-3308 at 65 g XDE-777/ha)	T2 (GF-3308 at 130 g XDE-777/ha)	R (Insegar)
18 Jul 2016	2DBA	12.5 ± 5.4	10.3 ± 2.8	11.3 ± 6.5	7.4 ± 5.0
19 Jul 2016	1DBA	14.8 ± 2.3	13.8 ± 2.7	12.5 ± 2.4	13.7 ± 3.5
20 Jul 2016	0DBA	15.8 ± 2.8	18.4 ± 6.6	12.9 <sup>1)</sup> ± 0.4	17.5 ± 5.1
Mean pre-exposure period (2DBA to 0DBA)		14.4 ± 1.9	14.0 ± 2.9	12.6 ± 3.1	13.1 ± 3.8
20 Jul 2016	Mean 0DAA	24.5 ± 3.2	13.2* ± 2.1	8.7* ± 1.0	18.7* ± 2.7
21 Jul 2016	Mean 1DAA	11.3 ± 2.4	11.9 ± 3.0	10.8 ± 2.1	9.0 ± 2.8
22 Jul 2016	2DAA	18.3 ± 6.6	17.2 ± 4.0	15.0 ± 2.1	16.7 ± 6.1
23 Jul 2016	3DAA <sup>2)</sup>	4.9 ± 3.0	6.0 ± 1.2	5.1 ± 2.3	2.6 ± 2.6
24 Jul 2016	4DAA	22.9 ± 8.3	22.3 ± 6.0	23.3 ± 6.1	21.7 ± 5.3
25 Jul 2016	5DAA	22.7 ± 6.4	22.2 ± 1.8	21.0 ± 3.7	20.6 ± 5.1
26 Jul 2016	6DAA	17.7 ± 7.5	18.6 ± 5.8	15.2 ± 3.0	15.8 ± 3.2
27 Jul 2016	7DAA	14.1 ± 5.2	15.1 ± 1.8	17.1 ± 4.8	12.7 ± 1.4
Mean exposure period (0DAA to 7DAA)		17.1 ± 4.3	15.8 ± 2.3	14.5 ± 1.2	14.8 ± 3.0

DBA/DAA = Days before/after application

<sup>1)</sup> Results of a 2<sup>nd</sup> assessment on this day, shortly before start of the application

<sup>2)</sup> Low flight activity in all treatments due to poor weather conditions on 3DAA

\* Significantly different from the control (Dunnett's t-test or Student's t-test (method: pooled), one-sided, p≤0.05)

#### Behaviour of the honeybees:

In all treatment groups (C, T1, T2, R), no unusual behaviours were observed during the preapplication period (2DBA to 0DBA).

During the post-application period in the tunnels (0DAA to 7DAA), no unusual behaviour was observed in the control.

In T1, 70 bees hanging on the edge of flowers and 11 cramping bees were observed on the day of application (0DAA). Additionally, few observations of bees with locomotion problems (3), inactive bees (7), trembling bees (4) and bees cleaning themselves intensively (3) were made during the exposure period (mainly on 0DAA).

In T2, there were 38 bees hanging on the edge of flowers on 0DAA, and few observations of cramping bees (8), bees with locomotion problems (5) or bees cleaning themselves intensively (2) during the exposure period (0DAA to 7DAA).

At the monitoring site (8DAA to 37DAA), very few cases of bees with locomotion problems (6 cases) or cramping bees (3 cases) were observed in the control. In T1, there were 29 observations of cramping bees, 26 observations of bees with locomotion problems and 7 trembling bees during this period. In T2, there were 60 observations of bees with locomotion problems, 34 cramping bees and 26 trembling bees.

In the reference item group R, in total 8 observations of cramping bees, bees with locomotion problems or bees hanging on the edge of flowers were recorded during the exposure period (0DAA to 7DAA). At the monitoring site (8DAA to 37DAA) there were 31 observations of bees with locomotion problems, 30 cramping bees and 6 trembling bees.

**Overall, test item treatments T1 and T2 had an effect on the behaviour of worker bees.**

Condition of the colonies (colony size):

At start of the study (8DBA) the mean colony size (number of honeybees per colony) was on the same level in all treatment groups with 7397 bees/colony in C and in T1, 7254 bees/colony in T2 and 7202 bees/colony in R (not significant).

At the first assessment after installation of the bee colonies in the tunnels (1DBA, one day before the applications), mean colony sizes were still on almost the same level in C, T1 and T2 and a slight decrease was observed in R. Mean colony sizes slightly grew in all treatment groups during the exposure period in the tunnels (5DAA). After relocation of the colonies out of the tunnels and installation at the monitoring site, the mean colony sizes were 8788 bees/colony in C, 9334 in T1, 7930 in T2 and 7410 in R on 9DAA (not significant).

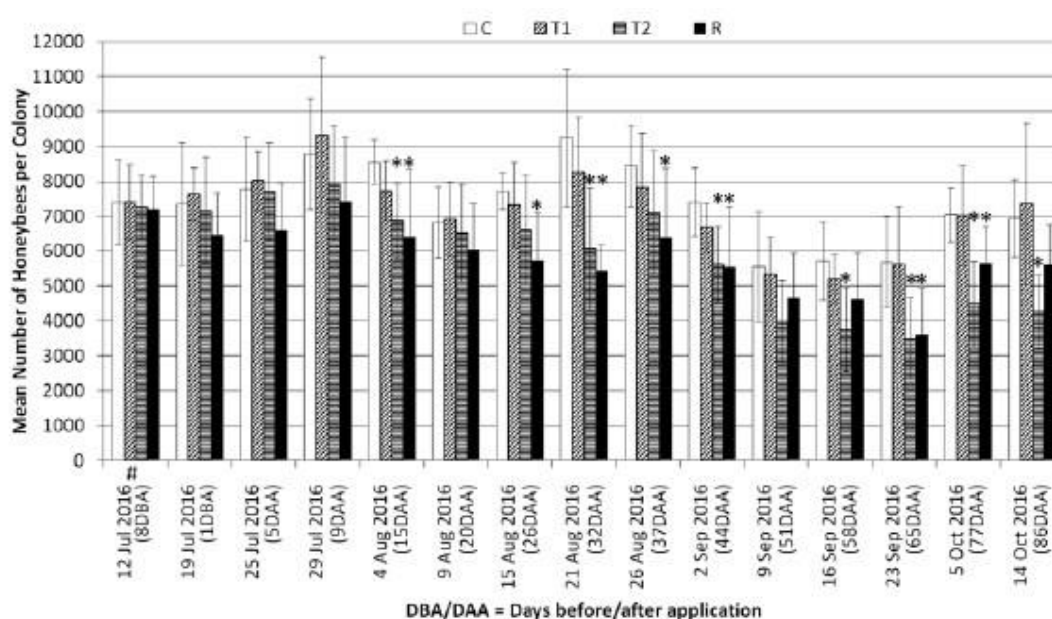
In the test item treatment T1, the mean number of honeybees per colony followed the changes in the control throughout the observation period. At the final assessment (86DAA), the mean colony sizes were 6929 bees/colony in C and 7358 bees/colony in T1. There were no statistically significant differences between T1 and C on any day from 8DBA to 86DAA.

Except for a slight intermittent increase on 37DAA, the mean colony size in test item treatment T2 slowly decreased from 7930 bees/colony on 9DAA to 3497 bees/colony on 65DAA. At the final assessment (86DAA), the mean colony size was 4264 bees/colony in T2 compared to 6929 bees/colony in C. During observation at the monitoring site, the mean number of honeybees per colony was lower in T2 than control colony sizes. These differences were statistically significant on 15DAA, 32DAA, 44DAA and at the four assessments from 58DAA to 86DAA.

Mean colony sizes in R decreased from 7410 bees/colony (9DAA) to 3601 bees/colony on 65DAA, with a slight intermittent increase on 37DAA. Mean colony size in R was smaller than in the control on all assessment dates (statistically significant on 15DAA, from 26DAA to 44DAA, 65DAA and 77DAA). At the final assessment the mean colony size in R was 5603 bees/colony.

**Overall, the test item treatment T2 had an effect on the colony size during the post-exposure monitoring period. There was no effect of the test item treatment T1 throughout the entire study period.**

The colony size is presented on the below figure.



**Figure 1: Mean colony size (number of bees per hive) per treatment group**



Condition of the colonies (amount of brood):

Brood of all stages (eggs, larvae, pupae) was present in all colonies at all assessment dates from 8DBA to 65DAA (end of September 2016) except the intermittent lack of larvae in hive T2b and Rc (and low number of larvae in the other hives of these treatments) on 44DAA, and the lack of eggs in Rd on 65DAA. Additionally, the number of larvae (15DAA and 37DAA) and pupae (15DAA) was significantly reduced in T2. Pupae in T2 were also significantly reduced on 51DAA and 65DAA but these findings were preceded by start of the first anti-*Varroa* treatment on 37DAA and should not be evaluated.

During the last two assessments in October 2016 (77DAA, 86DAA) certain brood stages were missing or on a low level in several hives of all treatment groups including the control. This was due to the seasonal end of brood rearing and is not considered as treatment related.

The total number of brood cells of all stages per colony was 22560 in C, 22640 in T1, 20160 in T2 and 19800 in R at start of the study (8DBA) and increased to 23400 in C, 24840 in T1, 21800 in T2 and 21880 in R until the first assessment inside the tunnels (1DBA, before application). None of these slight differences to the control were statistically significant.

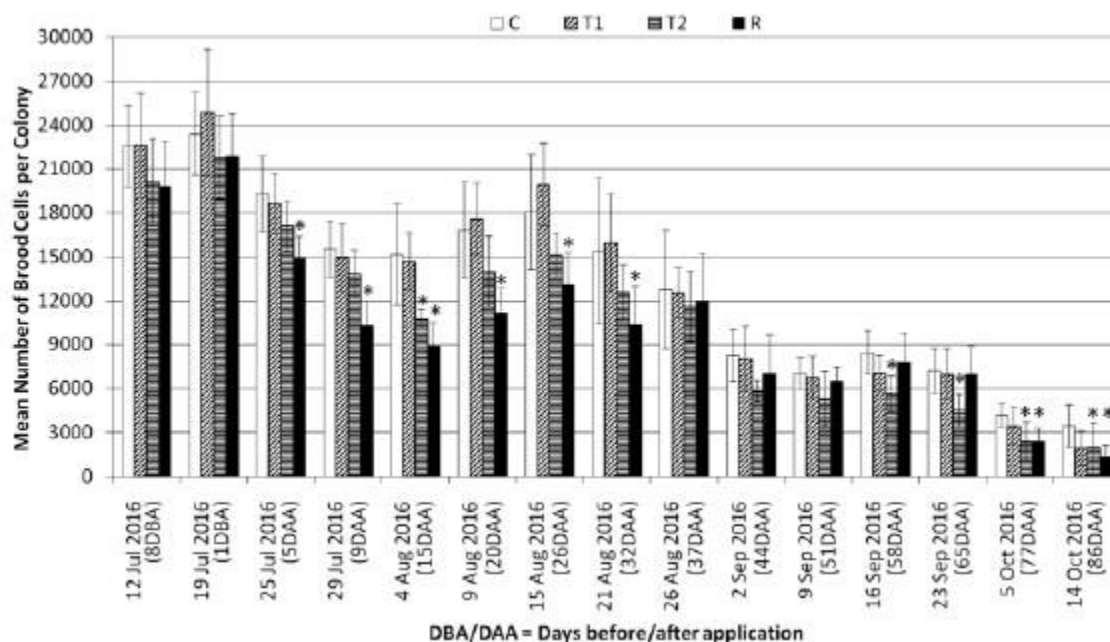
During the post-application exposure period and the following two assessments after relocation of the hives from the tunnels to the monitoring site, the total amount of brood decreased in all treatments, namely to 15160 in C, 14680 in T1, 10760 in T2 and 8880 in R on 15DAA. Statistically significant differences to the control were observed in T2 on 15DAA and in R on 5DAA, 9DAA and 15DAA. The general decrease of the amount of brood in all treatments is probably due to the confinement conditions inside the tunnels and seasonal scarcity of natural food sources at the monitoring site, although differences between the groups may be due to the treatment. After moderate feeding of 2.5 kg sucrose solution to each hive on 18DAA, the amount of brood increased during the following two assessments and reached its post-exposure maximum of 18080 brood cells per colony in C, 19920 brood cells in T1, 15080 in T2 and 13080 in R on 26DAA. Data in R were significantly different from the control on 20DAA and 26DAA.

The following slow decrease of the number of brood cells in all treatments including the control on 32DAA and 37DAA is considered due to the season (mid to end of August, i.e., the end of honeybee brood season coming close) and not due to the treatment. During the period between the 1<sup>st</sup> and 2<sup>nd</sup> treatments against *Varroa* mites (37DAA to 65DAA), the mean number of brood cells was on a low though rather stable level within each treatment group (significantly different from the control in T2 on 58DAA and 65DAA). Considering the anti-*Varroa* treatment using formic acid and its potential effects on the brood, these findings should not be interpreted regarding possible treatment effects.

At the last two assessments in October (77DAA and 86DAA), the amount of brood was low in all treatments. Differences in T2 and R compared to the control were statistically significant on these days but this is not considered as biologically relevant or treatment related: The lack of eggs in two control hives and low number of eggs and larvae in the other control hives clearly shows that the main brood rearing season had come to an end until 86DAA (14 October 2016).

**Overall, there was no effect of the test item treatment T1 and a slight effect of T2 on the total amount of brood or certain brood stages during the post-exposure period.**

Amount of brood is presented on the below figure.



**Figure 2: Mean number of brood cells (all brood stages) per treatment group**

Condition of the colonies (amount of nectar):

The mean number of nectar cells per colony was similar in all treatment groups at start of the study (8DBA): 8240 nectar cells/colony in C, 11400 in T1, 10040 in T2 and 9120 in R. Since in some individual hives the total amount of nectar cells was in the lower range and equaled only approximately one full honeycomb (=6400 cells) or less (e.g., hives Ca, Rd, T1d), moderate feeding of all hives with 2.5 kg sucrose solution was done on 5DBA.

Since some individual hives had very low levels of nectar after installation at the monitoring site and were at risk of starving (e.g., hives Ca, Cb and Rd on 15DAA), moderate feeding of all hives was done on 18DAA (2.5 kg sucrose solution per hive). Before feeding, the colonies in T1 and T2 had a better nutritional status than those of the control and R (T1: 10080 nectar cells per colony; T2: 8840 cells; C: 7520 cells; R: 7520 cells on 15DAA), i.e. no negative effect of the test item treatment could be observed within 15 days after the application and start of exposure.

In preparation of the first treatment against *Varroa* mites and to prevent the risk of starving in some hives (generally low amount of nectar in 4 hives of the R group and no nectar stores in hive Ca on 32DAA) all hives were fed with 3 kg sucrose solution each on 34DAA. Before feeding, the mean number of nectar cells in T1 (10960 cells per colony) was similar to the control (10480 cells) whereas the number of nectar cells in T2 (7160 cells per colony) and R (4320 cells) were lower than the control on 32DAA (not significant; in R this statistical result is clearly due to lack of nectar in one control replicate which creates a high standard deviation).

Intensive feeding of all colonies was done on 44DAA and 51DAA to prepare the colonies for overwintering according to good beekeeping practice. The number of nectar cells in T2 was significantly lower than the control on 51DAA, 77DAA and 86DAA but was still on a very high level.

Since previous intensive feeding of all hives does not allow for evaluation of the colonies' ability to collect food, these findings are not considered as biologically relevant or test item related. Moreover, the smaller colony size in T2 may have made these colonies more vulnerable to scavenging bees during this period.

**Overall, no negative effect of the test item treatments T1 and T2 on the mean number of nectar cells per colony was observed.**

#### Condition of the colonies (amount of pollen):

The mean amount of pollen per hive was similar in all treatments before installation of the colonies in the tunnels: There were 5440 pollen cells per hive in C, 6680 cells in T1, 4880 in T2 and 6120 in R on 8DBA/5DBA (adjustment of pollen stores in the individual hives was carried out on 5DBA to compensate for initial differences between the treatments and hives).

At all assessments during the study period, pollen was available in all hives except a temporary lack of pollen in Ca on 32DAA and in Re on 20DAA.

In the test item treatments T1 and T2 there were no significant differences of the pollen supply compared to the control except a single record of reduced amounts of pollen in T1 (51DAA) and two records in T2 (44DAA and 51DAA) which was probably due to seasonal scarcity of natural pollen sources in September and is not considered as treatment related.

In the reference item treatment R, the number of pollen cells was significantly reduced on 32DAA, 37DAA, 44DAA, 51DAA and 77DAA.

**Overall, there was no negative effect of the test item treatments T1 or T2 on the pollen storage of the honeybee colonies.**

#### Photographic Evaluation of Brood Development in Individual Cells

##### First brood cycle:

During the 1<sup>st</sup> brood cycle (1DBA to 20DAA), the development of the termination rate, brood index and compensation index of cells containing eggs, young larvae or old larvae on BFD0 was very similar in T1, T2 and the control except replicate Cd over the whole development cycle of this brood. In Cd an unexpected high termination rate (67.63%, compared to the range from 11.71% to 23.02% in the other control replicates), low brood indices (1.62 in Cd on BFD+21, compared to the range from 3.85 to 4.41 in the other control replicates) and low compensation indices (2.13 on BFD+21, compared to the range from 4.26 to 4.67 in the other control replicates) of eggs were observed. Since data in Cd weakened statistical evaluation of eggs and these data were identified as outliers at all assessment dates (Grubbs' test, one-sided,  $p \leq 0.05$ ), exclusion of this replicate from evaluation of egg development during the 1<sup>st</sup> brood cycle is justified. Grubbs' test was also applied to the brood indices, compensation indices and termination rates of young larvae and old larvae in the control during the first brood cycle but Cd was not an outlier in these categories and was not excluded from evaluation. Since no other unusual observations were made in Cd and this colony performed well during the 2<sup>nd</sup> brood cycle and further observation at the monitoring location until autumn 2016, the unexpected data of eggs during the 1<sup>st</sup> brood cycle probably resulted from poor adaptation of this colony to the temporary confinement in the tunnels.

At the completion of this brood cycle (BFD+21) the mean brood index for eggs was 4.15 in the control (4 replicates), 4.35 in T1, 3.93 in T2 and 2.32 in R. The mean compensation indices were 4.44 in the control (4 replicates), 4.57 in T1, 4.26 in T2 and 2.90 in R. The mean termination rates were 16.99% in C (4 replicates), 12.99% in T1, 21.41% in T2 and 53.56% in R. None of the differences of T1 or T2 to the control were statistically significant on any assessment day during the first brood cycle. Data for eggs in R and the control were statistically significant on all assessment days of the 1<sup>st</sup> brood cycle (BFD+6, BFD+10, BFD+16, BFD+21; Student's t-test (method: pooled) or Satterthwaite t-test, onesided,  $p \leq 0.05$ ).

At BFD+21, the brood index of young larvae was 3.36 in C, 3.82 in T1, 3.24 in T2 and 3.66 in R and the compensation index was 2.58 in C, 2.05 in T1, 1.77 in T2 and 1.95 in R. Data of young larvae were not significantly different from the control on any assessment day except for the compensation index of

T2 and R on the last day (BFD+21; Dunnett's t-test or Student's t-test (method: pooled), onesided,  $p \leq 0.05$ ). However, the decrease of the compensation index in T2 from 3.32 on BFD+16 to 1.77 on BFD+21 or in the control from 3.78 to 2.58 is not considered as biologically relevant. This development is within the expected range for young larvae and mainly indicates successful emergence of the adults during the intermittent period. The lower value in T2 compared to the control on BFD+21 may result from a smaller number of cells that had been used again for rearing new brood in the meantime, or because new eggs had been laid in these cells only recently, both resulting in a lower value for the compensation index.

The termination rates for young larvae were 32.90% in the control, 23.63% in T1, 35.28% in T2 and 26.92% in R. None of the differences to the control were statistically significant in any treatment group on any day of the 1<sup>st</sup> brood cycle.

The development of cells containing old larvae on BFD0 is expected to be complete until BFD+16( $\pm 1$ ) of the brood cycle. On BFD+16, the brood index was 4.63 in C, 4.82 in T1, 4.38 in T2 and 3.31 in R and the compensation index was 4.76 in C, 4.84 in T1, 4.46 in T2 and 3.61 in R. The termination rates of old larvae were 7.32% in C, 3.67% in T1, 12.53% in T2 and 33.72% in R. There were no statistically significant differences of T1 or T2 to the control on any assessment day. Data in R (brood index, compensation index, brood termination rate) were significantly different from the control on BFD+10 and BFD+16.

**Overall, there was no effect of the test item treatments T1 or T2 on the development of eggs, young larvae and old larvae in individual cells during the 1<sup>st</sup> brood cycle. A clear effect was observed in the reference item treatment R, confirming suitability of the test design and exposure of the bee colonies to the treated crop.**

#### Second brood cycle:

There was no effect of the test item treatments T1 and T2 or of the reference item treatment R on the brood indices, compensation indices or termination rates of eggs, young larvae or old larvae on any assessment day of the 2<sup>nd</sup> brood cycle (BFD0 to BFD+22 = 15DAA to 37DAA). None of the differences to the control were statistically significant on any day during this period.

The brood index of eggs was 3.68 in the control C, 3.69 in T1, 3.59 in T2, 3.57 in R and the compensation index was 4.09 in C, 4.07 in T1, 4.01 in T2 and 4.21 in R at the end of the 2<sup>nd</sup> brood cycle (BFD+22). The termination rates of eggs were 26.50% in C, 26.18% in T1, 28.13% in T2 and 28.60% in R.

The brood index of young larvae was 3.93 in C, 4.70 in T1, 3.89 in T2, 4.12 in R and the compensation index was 2.17 in C, 1.63 in T1, 1.79 in T2 and 2.46 in R on BFD+22. The termination rates of young larvae were 21.37% in C, 6.09% in T1, 22.17% in T2 and 17.60% in R on BFD+22.

The development of old larvae is expected to be complete until BFD+16( $\pm 1$ ) of the brood cycle. On BFD+17 of the 2<sup>nd</sup> cycle, the brood index was 4.68 in C, 4.76 in T1, 4.64 in T2, 4.61 in R and the compensation index was 4.81 in C, 4.82 in T1, 4.72 in T2 and 4.79 in R. The termination rates of old larvae were 6.29% in C, 4.71% in T1, 7.27% in T2 and 7.71% in R on BFD+17.

**Overall, there was no effect of the test item treatments T1 and T2 or the reference item treatment R on the development of individual brood cells during the 2<sup>nd</sup> brood cycle.**

#### Determination of weight and assessment of morphological abnormalities of pupae:

The weight of pupae collected from combs on 16DAA ranged from 0.1161 to 0.1490 g (mean: 0.1372 g) in the control, from 0.1171 to 0.1516 g (mean: 0.1353 g) in T1, from 0.1225 to 0.1541 g (mean: 0.1358 g) in T2 and from 0.0806 to 0.1563 g (mean: 0.1342 g) in the reference item group R. None of these mean pupal weights were statistically significantly different from the control.

No malformations were observed in the control or in the test item treatments T1 and T2. In the reference item treatment R, 5 out of 90 pupae collected from 3 hives showed malformations.

In two replicates of the reference item group (Rb and Re) no pupae could be collected since only freshly capped brood cells (containing larvae before pupation) were present, indicating the intermittent removal of young brood due to the reference item treatment.

Summary of the obtained results is provided in the table below.

**Table 68: Effects of GF-3308 (XDE-777) on honey bee brood under semi-field conditions – tunnel test**

Treatment	Untreated control	GF-3308 (XDE-777)		Toxic standard
Rate <sup>1</sup>	-	65 g a.s./ha	130 g a.s./ha	1200 g/ha
Brood termination rate (1 <sup>st</sup> brood cycle)				
Eggs: <sup>#</sup>	16.99	12.99	21.41	53.56* 26.92
Young larvae:	32.90	23.63	35.28	33.72*
Old larvae:	7.32	3.67	12.53	
Brood index (1 <sup>st</sup> brood cycle)				
Eggs:	4.15	4.35	3.93	2.32*
Young larvae:	3.36	3.82	3.24	3.66
Old larvae:	4.63	4.82	4.38	3.31*
Compensation index (1 <sup>st</sup> brood cycle)				
Eggs:	4.44	4.57	4.26	2.90*
Young larvae:	2.58	2.05	1.77*	1.95*
Old larvae:	4.76	4.84	4.46	3.61*
Brood termination rate (2 <sup>nd</sup> brood cycle) Eggs:				
Young larvae:	26.50	26.18	28.13	28.60
Old larvae:	21.37	6.09	22.17	17.60
	6.29	4.71	7.27	7.71
Brood index (2 <sup>nd</sup> brood cycle)				
Eggs:	3.68 3.93	3.69	3.59 3.89	3.57
Young larvae:	4.68	4.70	4.64	4.12
Old larvae:		4.76		4.61
Compensation index (2 <sup>nd</sup> brood cycle)				
Eggs:	4.09	4.07	4.01	4.21
Young larvae:	2.17	1.63	1.79	2.46
Old larvae:	4.81	4.82	4.72	4.79
Dead worker bees <sup>2</sup>				
Exposure:	99.2	82.4	94.6	70.9
Monitoring:	44.0	51.9	41.0	43.4
Dead pupae <sup>2</sup>				
Exposure:	0.5	1.3	1.2	0.6
Monitoring:	1.1	0.5	1.1	28.1*

<sup>1</sup> Delivered in 100 L/ha of water

<sup>2</sup> Over the post-application period (exposure period in the tunnels 8 days (0DAA to 7DAA), further monitoring 30 days (8DAA to 37DAA); mean value per hive per day (5 replicates))

# one replicate (Cd) excluded from evaluation of eggs during the 1<sup>st</sup> brood cycle (outlier) \*

statistically significant (Student's t-test, method: pooled, one-sided, p≤0.05)

Counting of Varroa mites after anti-Varroa treatment:

During the period over 21 days after start of the anti-*Varroa* treatment (37DAA to 58DAA), the mean values of fallen mites per colony were 600.2 in the control, 504.4 in T1, 821.0 in T2 and 562.8 in R. None of these values were statistically significantly different from the control.

### **Residue Analysis**

#### **Untreated and control samples:**

There were no residues of XDE-777 at or above the limit of quantification (LOQ) levels (0.01 mg XDE-777/kg) in any of the untreated samples taken from the control at any sampling date or in samples from the treatment groups T1 and T2 that were collected before the application was carried out (1DBA).

#### **Pollen from forager bees:**

In the treatment group T1, residue levels in pollen from forager bees were 13.2 to 19.9 mg XDE777/kg on 0DAA and clearly declined to 0.176 to 0.191 mg XDE-777/kg on 2DAA and 0.0152 to 0.0223 mg XDE-777/kg on 6DAA.

In the treatment group T2, residue levels in pollen from forager bees were 0.248 to 0.334 mg XDE777/kg on 2DAA and declined to 0.0684 mg XDE-777/kg on 6DAA. No pollen sample could be obtained in this group on 0DAA.

#### **Nectar from forager bees:**

Residue levels in nectar from forager bees were 0.0403 mg XDE-777/kg in T1 and 0.0572 in T2 on 0DAA. On the following sampling days (2DAA and 6DAA) residues in nectar declined and were below the limit of detection (< LOD of 0.003 mg/kg) in T1 and T2.

### **CONCLUSION**

One application of GF-3308 at rates of 65 g XDE-777 a.i./ha (treatment T1) or 130 g XDE-777 a.i./ha (treatment T2), applied at full flowering and during daily honeybee flight activity, had effects as follows:

1. There was no effect on the mortality of adult worker bees, worker bee pupae or male adult bees and male pupae.
2. Foraging activity in T1 and T2 decreased on the day of application (0DAA).
3. Test item treatments T1 and T2 had an effect on the behaviour of honeybees, mainly on the day of the application during bee-flight, and to a lesser extent during the following observation period.
4. There was no effect of the test item treatment T1 on the colony size and total number of brood cells throughout the entire study period. Test item treatment T2 had an effect on the colony size and a slight effect on the total number of brood cells or certain brood stages (larvae) during the postexposure monitoring period.
5. There was no effect of the test item treatments T1 or T2 on the storage of nectar and pollen.
6. There was no effect of the test item treatments T1 or T2 on the brood index, compensation index or termination rate of eggs, young larvae or old larvae during the 1<sup>st</sup> (1DBA to 20DAA) or 2<sup>nd</sup> (15DAA to 37DAA) brood cycle.
7. There was no effect of the test item treatments T1 or T2 on the weight or malformations of pupae sampled from combs towards the end of the 1<sup>st</sup> brood cycle (16DAA).
8. There was no effect on the number of *Varroa* mites fallen on the hive floor after anti-*Varroa* treatment in autumn.

#### ***Residue analysis:***

During the exposure period (0DAA, 2DAA and 6DAA), residue levels of XDE-777 in pollen and nectar from forager bees were as follows:

1. In the treatment group T1, residue levels in pollen from forager bees were 13.2 to 19.9 mg XDE777/kg on 0DAA and clearly declined to 0.176 to 0.191 mg XDE-777/kg on 2DAA and 0.0152 to 0.0223 mg XDE-777/kg on 6DAA.

2. In the treatment group T2, residue levels in pollen from forager bees were 0.248 to 0.334 mg XDE777/kg on 2DAA and declined to 0.0684 mg XDE-777/kg on 6DAA. No pollen sample could be obtained in this group on 0DAA.
3. Residue levels in nectar from forager bees were 0.0403 mg XDE-777/kg in T1 and 0.0572 in T2 on 0DAA. On the following sampling days (2DAA and 6DAA) residues in nectar declined and were below the limit of detection (< LOD of 0.003 mg/kg) in T1 and T2.

#### A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

#### A 2.3.2 KCP 10.3.2.1 Effects on non-target arthropods other than bees

#### A 2.3.2.1 KCP 10.3.2.1 Tier 1 NTA studies A 2.3.2.1.1 Study 1 - GF-3308: Effects on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory (Tier I) - Dose Response Test

Comments of zRMS:	<p>The study was performed in line with the respective guidelines with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- mortality in the control should not exceed 13 % (actually was 2.5 %),</li> <li>- corrected mortality in the toxic reference treatment should be &gt; 50 % (actually was 100 %),</li> <li>- wasps in the control should produce <math>\geq 5</math> mummies per female (mean value; actually was 24.1),</li> <li>- in the control there should be no more than 2 parasitoids producing zero values (actually one parasitoid produced zero values).</li> </ul> <p>Overall the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR<sub>50</sub> = 314 mL product/ha ER<sub>50</sub> &gt; 200 mL product/ha</p>
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Reference:	KCP 10.3.2.1/1
Report:	Moll, M.; 2016; GF-3308: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> in the Laboratory (Tier I) - Dose Response Test -; ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 111271001; DAS Study No. 160185; 11 May 2016; Unpublished
Guideline(s):	Mead-Briggs <i>et al.</i> 2000 and Mead-Briggs <i>et al.</i> 2010
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

ISO Common name: Not applicable

Test item (chemical/other name): GF-3308

Purity: XDE-777: 4.8 % (49 g/L)

Description (physical state): Brown

Lot/batch no.: E3240-85-1 (TSN311166)  
CAS no.: Not applicable

### Test System

Organism (*Species*): Parasitic wasp (*Aphidius rhopalosiphi*), adults not older than 48 hours

Study type: Tier 1 laboratory study, glass plates for mortality and barley plants for fecundity

Study design: Assessments of mortality measured 48 hrs after treatment and parasitisation 14 - 15 days after treatment. 4 replicates, each consisting of 10 wasps (7 females and 3 males) in one arena per test concentration for mortality phase.

Test concentrations: 0 (control), 12.8, 32.0, 80.0, 200 and 500 mL product/ha

Environmental conditions: Temperature: 19 - 21 °C Relative humidity: 76 - 80 % (acclimatisation, exposure period) 68 - 70 % (post-exposure period, within the test units) Photoperiod: 16 h light: 8 hr dark 650 - 2180 lux (acclimatisation, exposure, parasitisation period) 7520 - 14970 lux (post-parasitisation period) Feeding: A 10 %-fructose solution (acclimatisation and exposure)

Reference substance: 0.3 mL Perfekthion/ha (nominal: 400 g dimethoate/L)

### Methodology

The study comprised 7 treatment groups (5 dose rates of the test item, control and reference item) with 4 replicates each containing 10 parasitoids. The parasitoids were exposed to fresh, dried residues on treated glass plates. Survival of the parasitoids was assessed after approximately 2, 24 and 48 hours. At 48 hours, for treatment groups where there was less than 50.0 % corrected mortality, female wasps were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult parasitoids were removed after 24 hours and the aphid-infested plants left for a further 11 - 12 days before the numbers of aphid mummies that had developed were assessed.

### RESULTS AND DISCUSSION

At 12.8, 32.0, 80.0 and 200 mL product/ha there was no test item related mortality compared to the control. At 500 mL product/ha, there was statistically significant mortality compared to the control. Reproduction was tested at 12.8, 32.0, 80.0 and 200 mL product/ha. There was no statistically significant adverse effect on the reproduction (parasitisation efficiency) of surviving females up to and including 200 mL product/ha compared to the control.

**Table 69: Effects of GF-3308 on the survival of *Aphidius rhopalosiphi***

Test concentrations (mL GF-3308/ha)	% Mortality	Abbott corrected % mortality
Control	2.5	-
12.8	0.0	-2.6
32.0	0.0	-2.6
80.0	15.0	12.8
200	0.0	-2.6



500	87.5	87.2 *
Toxic Reference	100.0	100.0 *

\* Statistically different from the control

(Negative values indicate better survivorship compared to control)

**Table 70: Effects of GF-3308 on the parasitism rate of *Aphidius rhopalosiphi***

Test concentrations (mL GF-3308/ha)	Mean no. of mummies per female	% Difference compared to control
Control	24.1	-
12.8	15.0	37.8
32.0	28.1	-16.3
80.0	16.5	31.4
200	25.8	-6.8

(Negative values indicate better performance compared to control)

## CONCLUSION

Under worst case laboratory conditions the 48-hour LR<sub>50</sub> of GF-3308 on *Aphidius rhopalosiphi* is 314 mL product/ha in 200 L water/ha.

Reproduction (mummies per female) was tested at 12.8, 32.0, 80.0 and 200 mL product/ha. There was no adverse effect on the reproduction (parasitisation efficiency) of surviving females up to and including 200 mL product/ha compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Parasitic Wasp	<i>Aphidius rhopalosiphi</i>	GF-3308	14 days	LR <sub>50</sub>	314	mL/ha

### A 2.3.2.1.2 Study 2 - GF-3308: Effects on the Predatory Mite *Typhlodromus pyri* in the Laboratory (Tier I)- Dose Response Test

Comments of zRMS:	<p>The study was performed in line with the respective guideline with minor deviations.</p> <p>It was noted that the study was performed on 20 individuals per treatment group in 3 replicates which is lower than the guideline recommended 20 individuals in each of 5 replicates. Even though a lower number of individuals was tested than required, this deviation is considered to have no impact on the outcome of the study</p> <p>It was also noted that although the corrected mortality at the test item concentration of 400 mL product/ha was 56% (guideline recommended cutoff is ≤ 50%), the reproduction assessment was performed. This deviation had no impact on the outcome of the study.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- in the control the arithmetic mean mortality should not exceed 20 % (observed 16.7 %),</li> <li>- in the control the cumulative mean number of eggs per female should be ≥ 4 (observed 4 eggs/female),</li> <li>- in the toxic reference treatment the cumulative mean corrected mortality should be between 50 and 100 % (observed 74.0 %).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>LR<sub>50</sub> = 306 mL product/ha ER<sub>50</sub> &gt; 400 mL product/ha</p>
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Reference:	KCP 10.3.2.1/2
Report:	Moll, M.; 2016; GF-3308: Effects on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory (Tier 1) - Dose Response Test -; ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 111271063; DAS Study No. 160188; 11 May 2016; Unpublished
Guideline(s):	Blümel <i>et al.</i> , 2000
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

ISO Common name:	Not applicable
Test item (chemical/other name):	GF-3308
Purity:	XDE-777: 4.8 % (49 g/L)
Description (physical state):	Brown
Lot/batch no.:	E3240-85-1 (TSN311166)
CAS no.:	Not applicable

### Test System

Organism ( <i>Species</i> ):	Predatory mite ( <i>Typhlodromus pyri</i> )
Study type:	Tier 1 laboratory study, glass plates for mortality and fecundity
Study design:	Assessments of mortality measured 7 days after treatment and egg production 14 days after treatment. 3 replicates, each consisting of 20 mites in one arena per test concentration.
Test concentrations:	0 (control), 64.0, 160, 400, 1000 and 2500 mL product/ha
Environmental conditions:	Temperature: 24 - 26 °C Relative humidity: 74 - 76 % Photoperiod: 16 h light : 8 h dark light intensity: 240 - 470 lux Feeding: A mixture of pine ( <i>Pinus nigra</i> ) and birch ( <i>Betula</i> sp.) pollen (3:1) ad libitum on the day of the test start and on each assessment day except for the last one ( <i>i.e.</i> at least every four days).
Reference substance:	8 mL Perfekthion/ha (nominal: 400 g dimethoate/L)

### Methodology

The study comprised 7 treatment groups (5 dose rates of the test item, control and reference item) with 3 replicates each containing 20 mites. The mites were exposed to fresh, dried residues on treated glass plates. Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where there was equal or less than 50 % corrected mortality were sexed and the number of eggs per female was recorded on 3 assessment days within one week.

## RESULTS AND DISCUSSION

There was no treatment-related mortality at a rate of 64.0 mL product/ha. At 160, 400, 1000 and 2500 mL product/ha mortality was statistically significantly higher compared to the control. Reproduction of *T. pyri* was assessed in the control and at 64.0, 160 and 400 mL product/ha. There was no statistically significant adverse effect on the reproduction (eggs produced per female) of the mites up to and including 400 mL product/ha compared to the control.

**Table 71: Effects of GF-3308 on the survival of *Typhlodromus pyri***

Test concentrations (mL GF-3308/ha)	% Mortality	Abbott corrected % mortality
Control	16.7	-
64.0	25.0	10.0
160	31.7	18.0 *
400	63.3	56.0 *
1000	93.3	92.0 *
2500	100.0	100.0 *
Toxic Reference	78.3	74.0 *

\* Statistically different from the control

**Table 72: Effects of GF-3308 on the fecundity of *Typhlodromus pyri***

Test concentrations (mL GF-3308/ha)	Mean no. of mummies per female	% Difference compared to control
Control	4.0	-
64.0	4.1	-2.3
160	4.4	-8.9
400	4.2	-4.6

(Negative values indicate better performance compared to control)

## CONCLUSION

Under worst case laboratory conditions the 7-day LR<sub>50</sub> of GF-3308 on *Typhlodromus pyri* is 306 mL product/ha (95 % CL: 187 - 491 mL product/ha) in 200 L water/ha.

Reproduction of *T. pyri* was assessed in the control and at 64.0, 160 and 400 mL product/ha. There was no adverse effect on the reproduction (eggs produced per female) of the mites up to and including 400 mL product/ha compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Predatory mite	<i>Typhlodromus pyri</i>	GF-3308	14 days	LR <sub>50</sub>	306	mL/ha

### A 2.3.2.1.3 Study 3 - GF-3308: A laboratory test to evaluate the effects of fresh residues on the green lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae)

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- mortality in the control group was <math>\leq 20</math> % (observed 10 %),</li> <li>- mortality in the reference item group was <math>\geq 50</math> % (observed 100 %),</li> <li>- fecundity in the control (mean number of eggs per female per day) was <math>\geq 15</math> (observed 30.2),</li> <li>- fertility in the control (mean hatching rate) was <math>\geq 70</math> % (observed 89.8 %).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 3400 mL product/ha ER<sub>50</sub> &gt; 3400 mL product/ha</p>
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Reference:	KCP 10.3.2.1/3
Report:	Vaughan, R.; 2016; Summary of GF-3308: A laboratory test to evaluate the effects of fresh residues on the green lacewing, <i>Chrysoperla carnea</i> (Neuroptera, Chrysopidae); Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, UK; Lab Study No. DOW-16-3; DAS Study No. 160216 ; 08 July 2016; Unpublished
Guideline(s):	Vogt et al. (2000). Laboratory method to test effects of plant protection products on larvae of <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae)
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name):	GF-3308
Purity:	49 g/L (4.8% w/w) XDE-777 (analysed)
Description (physical state):	Clear liquid (Emulsifiable concentrate formulation)
Lot/batch no.:	TSN311166 (E3240-85-1)

### Test System

Organism ( <i>Species</i> ):	Lacewing ( <i>Chrysoperla carnea</i> )
Study type:	Tier I laboratory study, glass plates for mortality and boxes for fecundity.
Study design: (No. of replicates, assessments made etc.)	Assessment of the survival of larvae and pupae, the number of eggs laid per female (fecundity) and the larval hatching rate (fertility). 40 replicates, consisting of 1 lacewing in each arena per test concentration for mortality phase.
Test concentrations:	0 (Control), 3400, 2000, 1176.5, 692.0 and 407.1 mL product/ha
Environmental conditions:	Temperature: 22.9-25.6°C Relative humidity: 61-80% Photoperiod: 16 h (2500-4400 lux) Feeding: lacewing larvae fed every 1-3 days with untreated UVkilled eggs of the Angoumois grain moth, <i>Sitotroga cerealella</i> .
Reference substance:	Dimethoate, nominally 400 g/L, applied at 80 mL product/ha.

## Methodology

All treatments were applied at a volume rate of 200 L spray solution/ha to 7.5 cm x 7.5 cm glass plates. Larvae of *C. carnea* (2-3 days old, n = 40 per treatment) were individually confined on the freshly-dried, treated surface of these plates. The larvae were fed every 1-3 days with eggs of the moth, *Sitotroga cerealella*, and the pre-imaginal mortality of the lacewings was assessed. To determine if there had been any sub-lethal effects on the reproductive capacity of the test insects, the egg-laying activity of the matured adult lacewings was monitored over a 1-week period for the control treatment and the three highest test item treatments that had < 60% corrected mortality. For these reproduction assessments, both the numbers of eggs laid over two 24-h sampling periods and the viability of these eggs were recorded.

## RESULTS AND DISCUSSION

Pre-imaginal mortality in the control treatment was 10.0% and that in the toxic reference treatment was 100% (100% corrected mortality). For GF-3308, corrected pre-imaginal mortality was 41.7%, 33.3%, 38.9%, 33.3% and 30.6% for the 3400, 2000, 1176.5, 692 and 407.1 mL product/ha treatment rates, respectively. The reproductive performance in the control and the highest three test-item treatments exceeded the thresholds of  $\geq 15$  eggs/female/day and  $\geq 70\%$  hatching rate, currently viewed as being indicative of no harmful treatment effects. All validity criteria imposed for the study were therefore met.

**Table 73: Effects of GF-3308 on the survival of *Chrysoperla carnea***

Test concentrations (mL product/ha)	% Mortality	Abbott corrected % mortality
Control	10.0	-
3400	47.5 *	41.7
2000	40.0 *	33.3
1176.5	45.0 *	38.9
692	40.0 *	33.3
407.1	37.5 *	30.6
Toxic Reference	100 *	100

\* Statistically different from the control ( $\alpha = 0.05$ ).

**Table 74: Effects of GF-3308 on the fecundity and fertility of *Chrysoperla carnea***

Test concentrations (mL product/ha)	Mean no. of eggs per female per day (fecundity)	Mean % larval hatching rate (fertility)
Control	30.2	89.8
3400	28.4	89.9
2000	29.4	87.5
1176.5	34.7	83.9
692	~	~
407.1	~	~
Toxic Reference	~	~

~ not assessed.

## CONCLUSION

The effects of fresh, dry residues of GF-3308 on the green lacewing *Chrysoperla carnea* were evaluated under laboratory test conditions. At application rates up to and including 3400 mL product/ha, GF-3308 had no unacceptable effects on either the survival of larvae or the subsequent reproductive capacity of the adult lacewings.

Common name	Species	Test item	Endpoint	Value	Toxicity value	Units of test item
Lacewing	<i>Chrysoperla carnea</i>	GF-3308	Pre-imaginal mortality	LR <sub>50</sub>	> 3400	mL product/ha
Lacewing	<i>Chrysoperla carnea</i>	GF-3308	Pre-imaginal mortality	NOER	3400	mL product/ha

Lacewing	<i>Chrysoperla carnea</i>	GF-3308	Pre-imaginal mortality	LOER	> 3400	mL product/ha
Lacewing	<i>Chrysoperla carnea</i>	GF-3308	Fecundity	NOER	3400	mL product/ha
Lacewing	<i>Chrysoperla carnea</i>	GF-3308	Fecundity	LOER	> 3400	mL product/ha

**A 2.3.2.2 KCP 10.3.2.2 Higher-tier NTA studies (Tier II) A 2.3.2.2.1 Study 1 - GF-3308: Effects on the Parasitoid *Aphidius rhopalosiphi*, Extended Laboratory Study (Tier II) - Dose Response Test**

Comments of zRMS:	<p>The study was performed in line with the respective guideline with a minor deviation.</p> <p>It was noted that the study was performed on 30 individuals per test group (5 female wasps in 6 replicates) which is lower than the guideline recommended 40 individuals per test group (preferably 10 wasps in a minimum of 4 replicates). Even though a lower number of individuals were tested than required, this deviation is considered to have no impact on the outcome of the study.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- mortality in the control was <math>\leq 10\%</math> (observed 0%),</li> <li>- the corrected mortality in the reference item was <math>\geq 50\%</math> (observed 100%),</li> <li>- mean reproduction per female in the control was <math>\geq 5</math> mummies per female (observed 24.2),</li> <li>- number of surviving wasps in the control producing zero values for reproduction was <math>\leq 2</math> (observed 0).</li> </ul>
	<p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> = 1636 mL product/ha ER<sub>50</sub> &gt; 1176 mL product/ha</p>

Reference:	KCP 10.3.2.2/1
Report:	Moll, M.; 2016; GF-3308: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> , Extended Laboratory Study (Tier II) - Dose Response Test; ibacon GmbH, 64380 Rossdorf, Germany; Lab Study No. 111271002; DAS Study No. 160186 ; 29 August 2016; Unpublished
Guideline(s):	Mead-Briggs et al. 2000 and Mead-Briggs et al. 2010
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test item(s)

Test item (Common name): GF-3308  
Purity: XDE-777: 4.8% (49 g/L)  
Description (physical state): Brown liquid  
Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism ( <i>Species</i> ):	Parasitic wasp ( <i>Aphidius rhopalosiphi</i> ), adults less than 48 hours old
Study type:	Tier 2 extended laboratory study, barley plants for mortality and fecundity
Study design:	Assessments of mortality measured 48 hrs after treatment and parasitisation 14 - 15 days after treatment. 6 replicates, each consisting of 5 wasps in one arena per test concentration for mortality phase.
Test concentrations:	Control, 407, 692, 1176, 2000 and 3400 mL product/ha. All treatments were applied in 400 L water/ha.
Environmental conditions:	Temperature: 19 - 22 °C Relative humidity: 79 - 88 % (acclimatisation, exposure) 78 - 79 % (post-exposure period, within the test units) Photoperiod: 16 h light : 8 h dark; light intensity: 430 - 760 lux (acclimatisation, exposure) 1700 - 2100 lux (parasitisation period) 9690 - 13450 lux (post-parasitisation period) Feeding: A 10 %-fructose solution (acclimatisation and exposure)
Reference substance:	10.0 mL Perfekthion/ha (nominal: 400 g dimethoate/L)

## Methodology

This study comprised 7 treatment groups (5 dose rates of the test item, control, reference item) with 6 replicates each containing 5 female parasitoids. The parasitoids were exposed to dried residues on treated plant surfaces (barley plants). Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups where there was less than 50 % corrected mortality, female wasps were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult parasitoids were removed after 24 hours and the aphid-infested plants left for a further 11 - 12 days before the numbers of aphid mummies that had developed were assessed.

## RESULTS AND DISCUSSION

At 407 and 692 mL product/ha there was no test item related mortality compared to the control. At 1176, 2000 and 3400 mL product/ha, there was statistically significant mortality compared to the control. No repellent effect of the test item was observed compared to the control. Reproduction was tested at 407, 692 and 1176 mL product/ha. At 407 and 692 mL product/ha there was no statistically significant effect on reproduction (parasitisation efficiency) compared to the control. At 1176 mL product/ha reproduction was statistically significantly lower compared to the control, but the effect on reproduction was below the trigger value of 50 % (44.1 %). Therefore it can be summarised that there was no effect on reproduction up to and including 1176 mL product/ha.

**Table 75: Effects of GF-3308 on the survival of *Aphidius rhopalosiphi***

Test concentrations (mL product/ha)	% Mortality	Abbott corrected % mortality
Control	0.0	-
407	6.7	6.7
692	3.3	3.3
1176	36.7	36.7 *
2000	66.7	66.7 *

3400	80.0	80.0 *
Toxic Reference	100.0	100.0 *

\* Statistically different from the control

**Table 76: Effects of GF-3308 on the parasitism rate of *Aphidius rhopalosiphi***

Test concentrations (units)	Mean no. of mummies per female	% Difference compared to control
Control	24.2	-
407	19.6	19.3
692	18.7	22.8
1176	13.5	44.1 *

\* Statistically different from the control

## CONCLUSION

Under extended laboratory conditions the LR<sub>50</sub> of GF-3308 is 1636 mL product/ha in 400 L water/ha. No repellent effect of the test item was observed compared to the control.

The reproductive capacity of *A. rhopalosiphi* was tested at 407, 692 and 1176 mL product/ha. There was no adverse effect on the reproduction (parasitisation efficiency) of surviving females up to and including 1176 mL product/ha compared to the control.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Parasitic wasp	<i>Aphidius rhopalosiphi</i>	GF-3308	14 day	LR <sub>50</sub>	1636	mL/ha

### A 2.3.2.2.2 Study 2 - GF-3308: Effects on the Predatory Mite *Typhlodromus pyri*, Extended Laboratory Study (Tier II) – Dose Response Test

Comments of zRMS:	<p>The study was performed in line with the respective guideline with a minor deviation.</p> <p>It was noted that the study was performed with 10 protonymphs in 6 replicates per treatment group instead of 20 individuals in 5 replicates per treatment. Even though a lower number of individuals were tested than required, this deviation is considered to have no impact on the outcome of the study.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- the arithmetic mean mortality rate (dead and escaped mites) in the control was ≤ 20% on day 7 after treatment application (observed 13.3%),</li> <li>- the cumulative mean number of eggs per female (reproduction) in the control (from day 7 to day 14) was ≥ 4 eggs/female (observed 5.2 eggs/female),</li> <li>- the cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the toxic reference item was between 50 and 100% (observed 100%).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 3400 mL product/ha ER<sub>50</sub> &gt; 3400 mL product/ha</p>
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Reference:	KCP 10.3.2.2/2
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Report:	Moll, M.; 2016; GF-3308: Effects on the Predatory Mite <i>Typhlodromus pyri</i> , Extended Laboratory Study (Tier II) - Dose Response Test; ibacon GmbH, 64380 Rossdorf, Germany; Lab Study No. 111271062; DAS Study No. 160189 ; 29 August 2016; Unpublished
Guideline(s):	Blümel et al., 2000 and Oomen 1988
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: XDE-777: 4.8% (49 g/L)  
Description (physical state): Brown liquid  
Lot/batch no.: E3240-85-1 (TSN311166)

Control, 87.0, 218, 544, 1360 and 3400 mL product/ha.  
All treatments were applied in 200 L water/ha.

### Test System

Organism (*Species*): Predatory mite *Typhlodromus pyri*, protonymphs less than 24 hours old

Study type: Tier 2 laboratory study, leaf discs for mortality and fecundity

Study design: Assessments of mortality measured 7 days after treatment and egg production 14 days after treatment. 6 replicates, each consisting of 10 mites in one arena per test concentration.

Test concentrations:

Environmental conditions: Temperature: 24 - 26 °C  
Relative humidity: 74 - 77 %  
Photoperiod: 16 h light : 8 h dark Light intensity: 280 - 580 lux

Feeding: A mixture of pine (*Pinus sp.*) and birch (*Betula sp.*) pollen (3:1) *ad libitum* on the day of the test start and on each assessment day except for the last one *resp.* at least every four days.

Reference substance:

40.0 mL Perfekthion/ha (nominal: 400 g dimethoate/L)

## Methodology

This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 6 replicates each containing 10 mites. The mites were exposed to dried residues on treated leaf surfaces (bean leaves). Survival of the mites was assessed after 2 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where the corrected mortality was < 50 % were sexed and the number of eggs per females was recorded on 3 assessment days within one week.

## RESULTS AND DISCUSSION

At all dose rates, up to and including 3400 mL product/ha there was no test item related mortality compared to the control. Reproduction was tested at all dose rates. At 87.0, 218, 544 and 3400 mL product/ha the reproduction (eggs produced per female) was not statistically significantly affected compared to the control. At 1360 mL product/ha reproduction was statistically significantly affected compared to the control, but the reduction in reproduction was below the trigger value of 50 % (44.2 %). Therefore it can be summarised that there was no effect on reproduction up to and including 3400 mL product/ha.

**Table 77: Effects of GF-3308 on the survival of *Typhlodromus pyri***

Test concentrations (mL product/ha)	% Mortality	Abbott corrected % mortality
Control	13.3	-
87.0	15.0	1.9
218	16.7	3.8
544	6.7	-7.7
1360	8.3	-5.8
3400	20.0	7.7
Toxic Reference	100.0	100.0 *

(Negative values indicate better survivorship compared to control) \*

Statistically different from the control

**Table 78: Effects of GF-3308 on the fecundity of *Typhlodromus pyri***

Test concentrations (mL product/ha)	Mean no. of mummies per female	% Difference compared to control
Control	5.2	-
87.0	5.8	-12.3
218	4.3	17.9
544	7.3	-40.3
1360	2.9	44.2 *
3400	5.8	-12.0

(Negative values indicate better performance compared to control)

\* Statistically different from the control

## CONCLUSION

Under extended laboratory conditions the LR<sub>50</sub> of GF-3308 is estimated to be greater than 3400 mL product/ha in 200 L water/ha.

The reproductive capacity of *T. pyri* was tested at all dose rates. There was no adverse effect on reproduction (eggs produced per female) up to and including 3400 mL product/ha.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
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Predatory mite	<i>Typhlodromus pyri</i>	GF-3308	14 day	LR <sub>50</sub>	> 3400	mL/ha
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### A 2.3.2.2.3 Study 3 - GF-3308: Effects on mortality and reproduction to *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) under extended laboratory conditions

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no deviations to the guideline but with the following deviation to the study plan.</p> <p>According to the study plan collecting eggs and assessing egg fertility should have been carried out on day 3 after treatment. However, due to organisational reasons no mortality assessment took place on that day. This deviation is considered to have no impact on the outcome of the study as all the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- pre-imaginal mortality in the control was <math>\leq 30\%</math> (observed 22.5 %),</li> <li>- number of viable eggs per female per day in the control was <math>&gt; 2</math> (observed 12.4),</li> <li>- pre-imaginal mortality in the reference treatment was <math>&gt; 40\%</math> (observed 100%).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> &gt; 2000 mL product/ha ER<sub>50</sub> = 939 mL product/ha</p>
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Reference:	KCP 10.3.2.2/3
Report:	Schmidt, T.; 2016; GF-3308: Effects on mortality and reproduction to <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae) under extended Laboratory Conditions; Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland; Lab Study No. 20160012; DAS Study No. 160162 ; 09 December 2016; Unpublished
Guideline(s):	Schmuck R., Candolfi M. P., Kleiner R., Mead-Briggs M., Moll M., Kemmeter F., Jans D., Waltersdorfer A. and Wilhelmy H.: A laboratory test system for assessing effects of plant protection products on the plant dwelling insect <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae). IOBC/WPRS 2000, pages 45-56.
Deviations:	None to the guideline, minor to the study plan (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: XDE-777 (49 g/L, 4.8% wt%)  
Description (physical state): Brown liquid  
Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism (*Species*): Ladybird beetle (*Coccinella septempunctata*), three to five days old  
Study type: Tier II laboratory study, bean leaves for mortality and filter paper in reproduction cages for reproduction

Study design: (No. of replicates, assessments made etc.):	Assessment of the survival of larvae and pupae, the number of eggs laid per female (fecundity) and the larval hatching rate (fertility) 40 replicates per treatment consisting of one <i>C. septempunctata</i> larva in each arena per test item rate for mortality phase
Test rates:	0 (Control), 125, 250, 500, 1000 and 2000 mL product/ha
Plant substrate:	Whole leaves from potted French bean plants ( <i>Phaseolus vulgaris</i> )
Environmental conditions:	Temperature: mean 25.0° C (range 21.7* – 26.6° C) Relative humidity: mean 66.8% (range 43.6* – 78.9%) *Short-term deviations from the set range of 23-27° C (two times for ≤ 1 hour outside the recommended range) and 60-90% relative humidity (for ≤ 1 hour at 13 occasions) are due to handling of the test units and do not impact the outcome of the study. Photoperiod: 16 h/8 h light/dark Lighting: 1382 to 2654.5 Lux for the mortality phase; 2149 to 2655 Lux for the reproduction phase Feeding: aphids of the species <i>Acyrtosiphon pisum</i>
Reference substance: Roxion (60 mL product/ha) (corresponding to 24.6 g dimethoate/ha, based on a content of the a.i. of 39.07 % w/w and a density of 1.05 g/mL)	

## Methodology

The test consisted of two major phases: an exposure phase (mortality assessment: DAT 0 to DAT 14) and a reproduction phase (fecundity assessment: DAT 29 to DAT 48; fertility assessment: DAT 32 to DAT 48) which were separated by 11 to 29 days of a pre-reproduction phase. After application of the test rates of 125, 250, 500, 1000 and 2000 mL product/ha and after the spray deposits on the bean leaves had dried, the test units were assembled and the larvae were individually transferred onto the surface of the treated substrate. Total pre-imaginal mortality during the exposure phase was assessed daily. On day 14 after application at least 90% of the viable pupae had hatched in the control treatment. All beetles were sexed and pooled within their respective treatment groups and placed into the reproduction test units. At DAT 22 the control beetles started to lay eggs and another 7 days later the assessment of the reproductive performance was initiated.

## RESULTS AND DISCUSSION

Larvae in the control and all test item treatment groups reached the pupation after 8

exception (1 larva at 2000 mL/ha was alive until day 13 and was found dead at day 14)

-days with one with the most frequent duration of 6 days. The mean corrected mortality in the test item treatments ranged between 3.2 and 45.2%. The reproduction performance of females in the test item treatments ranged from 4.3 to 11.3 fertile eggs per female per day.

**Table 79: Effects of GF-3308 on survival of *Coccinella septempunctata* L.**

Test concentrations (mL product/ha)	% Mortality	Abbott corrected % mortality
Control	22.5	-
125	25	3.2
250	25	3.2
500	30	9.7
1000	37.5	19.4
2000	57.5 *	45.2 *
Toxic Reference	100	100

(Negative values indicate better survivorship compared to control)

\* Statistically different from the control

**Table 80: Effects of GF-3308 on fecundity and fertility of *Coccinella septempunctata* L.**

Test concentrations (mL product/ha)	Mean no. of eggs per female per day (fecundity)	Mean % larval hatching rate (fertility)	Mean no. of fertile eggs per female per day (fertility)
Control	14.9	83.2 <del>84</del>	12.4
125	11.9	87.4	10.4
250	14.3	79	11.3
500	12.6	74.6 <del>75</del>	9.4
1000	8.5	51.8 <del>52</del> a	4.4 a
2000	8.5	50.6 <del>51</del> a	4.3 a

a: No statistical analysis possible due to low number of replicates

**CONCLUSION**

After 14 days of exposure of *Coccinella septempunctata* larvae to dried residues of GF-3308 on bean leaves, the LR<sub>50</sub> was determined to be > 2000 mL product/ha. At application rates up to and including 500 mL product/ha, GF-3308 had no adverse effect on the subsequent reproductive capacity of the adult ladybird beetles.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3308	14 days	NOER	1000	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3308	14 days	LR <sub>50</sub>	> 2000	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3308	48 days	NOER	500	mL product/ha
Lady bird beetle	<i>Coccinella septempunctata</i>	GF-3308	48 days	ER <sub>50</sub>	939	mL product/ha

**A 2.3.2.2.4 Study 4 - GF-3308: Toxicity to the Parasitoid Rove Beetle *Aleochara bilineata* (Coleoptera: Staphylinidae) under Extended Laboratory Conditions**

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- average number of beetles emerging from the fly pupae was &gt; 400 (observed 947),</li> <li>- reduction of the reproductive capacity in the reference item treatment relative to the control was ≥ 50 % (observed 97.6 %).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER<sub>50</sub> &gt; 4000 ml product/ha</p>
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Reference:	KCP 10.3.2.2/4
Report:	Schmidt, T.; 2016; GF-3308: Toxicity to the Parasitoid Rove Beetle <i>Aleochara bilineata</i> (Coleoptera: Staphylinidae) under Extended Laboratory; Innovative Environmental Services (IES) Ltd, Witterswil, Switzerland; Lab Study No. 20160013; DAS Study No. 160161 ; 09 December 2016; Unpublished
Guideline(s):	Grimm C. et al. (2000): A test for evaluating the chronic effects of plant protection products on the rove beetle <i>Aleochara bilineata</i> Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions. IOBC/WPRS, Gent.
Deviations:	None

GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: XDE-777 (49 g/L, 4.8 wt%)  
Description (physical state): Brown liquid  
Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism (*Species*): Rove beetle *Aleochara bilineata*  
Study type: Tier 2 extended laboratory study  
Study design: Assessments of mortality of beetles exposed for 28 days to dried test item residues freshly sprayed onto standard soil (Lufa 2.1); emergence of beetles up to 37 days after exposure to residues (test units with the introduced fly pupae were left to dry for one week after the exposure phase (28 days) and the removal of the adults).  
(No. of replicates, assessments made etc.): 4 replicates with 10 pairs of beetles per replicate (10 female and 10 male beetles).

Test Rates: 0 (Control), 250, 500, 1000, 2000 and 4000 mL product/ha

Environmental conditions: Temperature: 16.9 to 25.1 °C\*, with a mean of 19.8 °C  
Relative humidity: 47.1 to 86.9%\*, with a mean humidity of 71.8%.  
\*The variations of temperature and relative humidity observed were either only occasional (< 2 hours) due to the opening of the climate chambers in order to handle the test units or of technical reason, but are not considered to have an influence on biological results, as documented by the acceptable performance of the beetles in the control.  
Photoperiod: 16 h/8 h light/dark  
Lighting: 595 to 1139 Lux with a mean of 832 Lux  
Feeding: defrosted mealworm larvae of the species *Tenebrio molitor*  
The mean water content of the test substrate at test initiation was 11.4 % which corresponds approximately to 35.0 % of MWHC.

Reference substance: Roxion (8000 mL product/ha) (nominal: 400 g dimethoate/L)

### Methodology

The test consisted of two major phases: an exposure phase (mortality assessment: 28 days) and a hatching phase (hatching assessment: 37 days) which were separated by 7 days of a pre-hatching phase. After the spray deposits on the substrate had dried, the pre-selected beetles were transferred into each test unit of each treatment. After 28 days of exposure, surviving beetles were removed from the test units by sieving the soil. Afterwards, the soil of each replicate was transferred back to the test units and

was left to dry for one week. After 35 days, parasitized fly pupae were regained from the substrate by sieving and transferring them to the hatching test units. On the same day and before soil disposal, the substrate was checked carefully in order to find more adults in it either alive or dead. The hatching of the beetles was monitored every one to three days until less than two beetles hatched per replicate in the control treatment per day (37 days).

## RESULTS AND DISCUSSION

The values of mean hatching rate in the test item treatments ranged from 943 to 1027 beetles per replicate resulting in percentages on reproduction of between 99.5 and 108.5% relative to the control.

There was no statistically significant difference in reproduction between the control and all test item treatments (Step-down Jonckheere-Terpstra Test Procedure, one-sided smaller,  $\alpha = 0.05$ ). As reproduction in the test item treatments was similar or higher than in the control, the corresponding  $ER_{50}$  was determined (directly from the raw data) to be > 4000 mL GF-3308/ha.

**Table 81: Effects of GF-3308 on the mortality and reproduction of *Aleochara bilineata***

Test rates (mL/ha)	Mean mortality (%)	Mean hatching rate (# of beetles/treatment)	% reduction relative to Control
Control	20	947	-
250	14	991 n.s.	-4.7
500	14	1027 n.s.	-8.5
1000	19	1001 n.s.	-5.7
2000	16	1001 n.s.	-5.7
4000	18	943 n.s.	0.5
Toxic Reference (Roxion)	99	22.5	97.6

(Negative values indicate better hatching rates compared to control)

n.s.: Not statistically significant different compared to the control (Step-down Jonckheere-Terpstra Test Procedure, one-sided smaller,  $\alpha = 0.05$ )

## CONCLUSION

After 28 days of exposure of adults of *Aleochara bilineata* to dried residues of GF-3308 on natural soil, the  $ER_{50}$  for reproduction was determined to be higher than the highest rate tested (> 4000 mL GF-3308/ha; obtained directly from the raw data). At application rates up to and including 4000 mL product/ha, GF-3308 had no adverse effect on the reproductive capacity of the adult rove beetles.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rove beetle	<i>Aleochara bilineata</i>	GF-3308	28 day	$ER_{50}$	> 4000	mL/ha
Rove beetle	<i>Aleochara bilineata</i>	GF-3308	28 day	NOER	4000	mL/ha

### A 2.3.2.2.5 Study 5 - GF-3308: Toxicity to the Parasitoid Rove Beetle *Aleochara bilineata* (Coleoptera: Staphylinidae) under Extended Laboratory Conditions

Comments of zRMS:	<p>The study was performed in line with the respective guideline with minor deviations.</p> <p>It was noted that on one occasion there were 2 consecutive hourly temperature readings that exceeded the intended temperature range of 18-22.0°C, reaching a maximum of 22.1°C. Also, the beetles were to be fed every 1 to 3 days depending on the food consumption, and on one occasion during the bioassay, due to an oversight, the beetles were fed on the fourth day. These deviations are considered to have no impact on the outcome of the study since all the validity criteria were met.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER<sub>50</sub> &gt; 4000 mL product/ha</p>
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Reference:	KCP 10.3.2.2/5
Report:	Tew, G; 2020; GF-3308: A Rate-Response Extended Laboratory Study of the Effects of Freshly Treated Substrate on the Rove Beetle, <i>Aleochara bilineata</i> (Coleoptera, Staphylinidae) ; Mambo Tox, A Division of Cawood Scientific Ltd., Southampton, UK; Lab Study No. COR-20-34; DAS Study No. 200611 ; 10 November 2020; Amendment 1
	19 November 2020; Unpublished
Guideline(s):	Grimm C. et al. (2000). A test for evaluating the chronic effects of plant protection products on the rove beetle <i>Aleochara bilineata</i> Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory test conditions.
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name):	GF-3308
Purity:	fenpicoxamid 5.1 % w/w (51 g/L)
Description (physical state):	Clear liquid
Lot/batch no.:	ENBK-169309-002 (TSN313638)

### Test System

Organism ( <i>Species</i> ):	Rove beetle <i>Aleochara bilineata</i>
Study type:	Tier 2 extended laboratory study
Study design:	Assessments of mortality of beetles exposed for 28 days; emergence of beetles up to 72 days after exposure to residues.
No of replicates:	Each treatment group consisted of 4 replicates with 10 pairs of beetles per replicate (10 female and 10 male beetles).
Test concentrations:	0 (control), 4000, 2000, 1000, 500 and 100 mL GF3308/ha.
Soil type:	LUFA 2.1
Application method of test item to soil:	Sprayer
Environmental conditions:	Temperature: 18.0-22.1 °C Relative humidity: 63 - 86%



Lighting: 800-1000 lux, 16 h photoperiod

Feeding: Raw minced beef

Reference substance:

BAS 152 65 I (Perfekthion), containing nominally 400g/L dimethoate, applied at 3800 mL/ha.

## Methodology

GF-3308 was evaluated in a extended laboratory bioassay at five application rates, equivalent to 4000, 2000, 1000, 500 and 100 mL test item/ha. Also included in the test were a water-treated control and a toxic reference treatment of BAS 152 65 I (nominally 400 g/L dimethoate), applied at a rate of 3800 mL product per 400 L water/ha.

All treatments were applied using a laboratory track-sprayer calibrated to deliver the equivalent of 400 L spray solution/ha. The individual treatments were each applied to four replicate arenas containing a natural sandy soil (LUFA 2.1). Immediately following treatment, twenty adult *A. bilineata* of equal sex ratio were introduced into each replicate arena. The beetles were fed with raw minced beef approximately 1 hour after treatment and at least every fourth day, thereafter. Assessments of the condition of the original beetles were made at 1, 7 and 28 days after treatment (DAT). The parasitic success of the larval offspring of the original beetles was assessed by the provision of 500 onion fly pupae (*Delia antiqua*) in each replicate box on three, weekly occasions, i.e. at 7, 14 and 21 DAT. The original adult beetles were removed from the arenas at 28 DAT. After a 7-day soil drying period the number of new adults (F1 progeny) that subsequently developed from the parasitised fly pupae was recorded over a further 37 -day period, until 72 DAT.

## RESULTS AND DISCUSSION

At 28 DAT, there was 18.8% mortality in the control treatment, compared with 27.5%, 25.0%, 18.8%, 27.5%, and 32.5% mortality in the 4000, 2000, 1000, 500 and 100 mL product/ha treatment rates of GF-3308, respectively. Therefore, the LR<sub>50</sub> value was > 4000 mL GF-3308/ha. None of the test item treatment rates differed significantly from the control (Multiple sequentially-rejective Fisher Test after Bonferroni-Holm, one-sided, > control,  $\alpha = 0.05$ ). Therefore, the NOER value for beetle survival was 4000 mL GF-3308, the highest rate tested.

The mean number of progeny produced per replicate was 932.8 in the control treatment, compared to values of 960.5, 948.3, 921.3, 946.3 and 898.5 in the 4000, 2000, 1000, 500 and 100 mL product/ha treatment rates of GF-3308, respectively. Therefore, the ER<sub>50</sub> value was > 4000 mL GF-3308/ha. None of the test item treatment rates differed significantly from the control (Dunnett's multiple t-test procedure, one-sided, > control,  $\alpha = 0.05$ ). Therefore, the NOER value for beetle reproduction was 4000 mL GF-3308, the highest rate tested.

All of the study validity criteria were met: a) The mean number of beetles emerging from parasitised fly pupae in the control treatment should be > 400 per replicate (actual value was 932.8); b) The mean number of beetles emerging in the toxic reference treatment should be reduced by > 50%, relative to the control (actual value was 100%).

**Table 82: Effects of test item on the mortality and reproduction of *Aleochara bilineata***

Test item rates (mL/ha) GF-3308	% Mortality	Abbott corrected % mortality	Mean hatching rate (number of beetles/treatment)	% of Control
Control	18.8	--	932.8	-
100	32.5	16.9	898.5	3.7
500	27.5	10.8	946.3	-1.4
1000	18.8	0.0	921.3	1.2
2000	25.0	7.7	948.3	-1.7
4000	27.5	10.8	960.5	-3.0
Toxic Reference	100 *	100	0.0*	100.0

(Negative values indicate better hatching rates compared to control)

\* Statistically different from the control

## CONCLUSION

In an extended laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to a natural soil substrate freshly treated with GF-3308, the ER<sub>50</sub> value was >4000 mL GF-3308/ha. The NOER value for reproduction was 4000 mL GF-3308/ha, the highest rate tested.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Rove beetle	<i>Aleochara bilineata</i>	GF-3308	72 day	ER <sub>50</sub>	>4000	mL/ha
Rove beetle	<i>Aleochara bilineata</i>	GF-3308	72 day	NOEC	4000	mL/ha

### A 2.3.2.3 KCP 10.3.2.3 Aged-residues NTA studies A 2.3.2.3.1 Study 1 – GF-3308: Effects on the Parasitoid *Aphidius rhopalosiphi* Extended Laboratory Study (Tier II) – Aged Residue Test

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- mortality in the control was ≤ 10% (observed 0% in 1<sup>st</sup> and 3<sup>rd</sup> bioassay; 2.5% in 2<sup>nd</sup> bioassay),</li> <li>- the corrected mortality in the reference item was ≥ 50% (observed 100%),</li> <li>- mean reproduction per female in the control was ≥ 5 mummies per female (observed 34.9 in 2<sup>nd</sup> bioassay; 35.8 in 3<sup>rd</sup> bioassay),</li> <li>- number of surviving wasps in the control producing zero values for reproduction was ≤ 2 (observed 0 in 2<sup>nd</sup> and 3<sup>rd</sup> bioassays).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>ER<sub>50</sub> &gt; 2.0 L product/ha</p>
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Reference:	KCP 10.3.2.3/1
Report:	Moll, M.; 2016; GF-3308: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> , Extended Laboratory Study (Tier II) - Aged Residue Test; ibacon GmbH, 64380 Rossdorf, Germany; Lab Study No. 111271003; DAS Study No. 160187 ; 27 October 2016; Unpublished
Guideline(s):	Mead-Briggs <i>et al.</i> 2000 and Mead-Briggs <i>et al.</i> 2010
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308

Purity: XDE-777: 4.8% (49 g/L)

Description (physical state): Brown liquid

Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism ( <i>Species</i> ):	Parasitic wasp ( <i>Aphidius rhopalosiphi</i> )
Study type:	Aged residue extended laboratory study, French bean leaves for mortality and barley plants for fecundity.
Study design:	Assessments of mortality measured 48 hrs after treatment and parasitisation 13 days after treatment. 4 replicates, each consisting of 10 wasps in one arena per test concentration for mortality phase.
Test concentrations:	0 (Control), 2.00 L product/ha
Environmental conditions:	Temperature: 18 - 22 °C Relative humidity: 66 % - 85 % (acclimatisation, exposure) 68 % - 74 % (post-parasitisation period; within the test units) Photoperiod: 16 h light : 8 h dark light intensity: 710 - 990 lux (acclimatisation, exposure) 1150 - 2250 lux (parasitisation period) 5480 - 14190 lux (post-parasitisation period) Feeding: A 10 %-fructose solution (acclimatisation and exposure)
Reference substance:	50.0 mL Perfekthion/ha (nominal: 400 g dimethoate/L)

### Methodology

Three bioassays were performed in this aged residue study. The 1<sup>st</sup> bioassay was started on the day of the 2<sup>nd</sup> application; the 2<sup>nd</sup> bioassay was started 13 days and the 3<sup>rd</sup> bioassay was started 27 days after the 2<sup>nd</sup> application, respectively. The study encompassed 3 treatment groups (1 test item dose rate, control, reference item) in the 1<sup>st</sup> bioassay and 2 treatment groups (1 test item dose rate, control) in the 2<sup>nd</sup> bioassay and 3<sup>rd</sup> bioassay with 4 replicates each containing 7 female and 3 male parasitoids. The parasitoids were exposed to freshly dried and aged residues on leaves from field treated bean plants. Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups with < 50 % corrected mortality survived females were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult parasitoids were removed after 24 hours and the aphid-infested plants left for further 10 days before the numbers of aphid mummies that had developed were assessed.

### RESULTS AND DISCUSSION

In the 1<sup>st</sup> bioassay mortality of *Aphidius rhopalosiphi* was statistically significantly affected by GF3308 when exposed to freshly dried residues on the day of the 2<sup>nd</sup> application. The corrected mortality was above the trigger value of 50 % (90.0 %). In the 2<sup>nd</sup> bioassay mortality of *Aphidius rhopalosiphi* was statistically significantly affected by GF-3308 when exposed to aged residues 13 days after the 2<sup>nd</sup> application, but the corrected mortality was below the trigger value of 50 % (46.2 %). In the 3<sup>rd</sup> bioassay mortality of *Aphidius rhopalosiphi* was not statistically significantly affected by GF-3308 when exposed to aged residues 27 days after the 2<sup>nd</sup> application and the corrected mortality was below the trigger value of 50 % (7.5 %).

No repellent effect of the test item was observed in all three bioassays. The settling rate in the test item treatment group was not statistically significantly lower compared to the control in all bioassays. Reproduction was assessed in the 2<sup>nd</sup> and 3<sup>rd</sup> bioassay and the reproductive capacity of *Aphidius rhopalosiphi* was statistically significantly affected in both bioassay. The effect on reproduction was below the trigger value of 50 % in both bioassays (33.8 and 29.9 %). Therefore reproduction is considered unaffected in both bioassays.

**Table 83: Effects of GF-3308 on the survival of *Aphidius rhopalosiphi***

Bioassay initiated	Test concentrations (L product/ha)	% Mortality	Abbott corrected % mortality
0 DAT	Control	0.0	-
	2.00	90.0	90.0 *
	Toxic Reference	100.0	100.0 *
13 DAT	Control	2.5	-
	2.00	47.5	46.2 *
27 DAT	Control	0.0	-
	2.00	7.5	7.5

\* Statistically different from the control

DAT = day(s) after treatment (2<sup>nd</sup> application)**Table 84: Effects of GF-3308 on the parasitism rate of *Aphidius rhopalosiphi***

Bioassay initiated	Test concentrations (L product/ha)	Mean no. of mummies per female	% Difference compared to control
13 DAT	Control	34.9	-
	2.00	23.1	33.8 *
27 DAT	Control	35.8	-
	2.00	25.1	29.9 *

\* Statistically different from the control DAT

= day(s) after treatment (2<sup>nd</sup> application)

## CONCLUSION

Mortality of adult *Aphidius rhopalosiphi* was statistically significantly affected compared to the control by exposure to freshly dried residues (day of 2<sup>nd</sup> application) of GF-3308 applied at 2.00 L product/ha. The corrected mortality was above the trigger value of 50 % (90.0 %).

Thirteen days after the 2<sup>nd</sup> application mortality was statistically significantly affected compared to the control, but the corrected mortality was below the trigger value of 50 % (46.2 %).

Twenty-seven days after the 2<sup>nd</sup> application the aged residues caused no statistically significant effect on survival compared to the control and the corrected mortality was below the trigger value of 50 % (7.5 %).

Reproduction was assessed in the 2<sup>nd</sup> and 3<sup>rd</sup> bioassay and the reproductive capacity of *Aphidius rhopalosiphi* was statistically significantly affected in both bioassay. The effect on reproduction, however was below the trigger value of 50 % in both bioassays (33.8 and 29.9 %). Therefore reproduction is considered to be unaffected in both bioassays.

Overall, the effects of GF-3308 applied twice with an application rate of 2.00 L product/ha on survival and reproduction were less than the ESCORT 2 trigger of 50 % after 13 and 27 days of aging.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
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Parasitic wasp	<i>Aphidius rhopalosiphii</i>	GF-3308	0, 13, and 27 DALT*	NA	Mortality at 2 L/ha x 2 applications with a 15 day interval: 90.0 % at 0 DALT 47.5 % at 13 DALT 7.5 % at 27 DALT  Red. Of reproduction at 2 L/ha: 33.8 % at 13 DALT 29.9 % at 27 DALT	L/ha
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\*days after last application

#### A 2.3.2.3.2 Study 2 – GF-3308: Aged-residue extended laboratory tests to determine effects on the ladybird beetle, *Coccinella septempunctata* (Coleoptera, Coccinellidae)

Comments of zRMS:	<p>The study was performed in line with the respective guideline with no deviations.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- pre-imaginal mortality in the control was <math>\leq 30\%</math> (observed 0 and 25 %),</li> <li>- number of viable eggs per female per day in the control was <math>&gt; 2</math> (observed 20.9 and 11.8),</li> <li>- pre-imaginal mortality in the reference treatment was <math>&gt; 40\%</math> (observed 97.5%).</li> </ul> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>LR<sub>50</sub> <math>&gt; 2.0</math> L product/ha  ER<sub>50</sub> <math>&gt; 2.0</math> L product/ha</p>
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Reference:	KCP 10.3.2.3/2
Report:	Vaughan R.; 2017; GF-3308: Aged-residue extended laboratory tests to determine effects on the ladybird beetle, <i>Coccinella septempunctata</i> (Coleoptera, Coccinellidae); Mambo-Tox Ltd., 2 Venture Road, University Science Park, Southampton SO16 7NP, UK; Lab Study No. DOW-17-7; DAS Study No. 170779; 12 January 2018; Unpublished
Guideline(s):	Schmuck et al. (2000). A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect <i>Coccinella septempunctata</i> L. (Coleoptera: Coccinellidae).
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308

Purity: 51 g/L (5.1% w/v) fenpicoxamid

Description (physical state): Pale brown liquid (emulsifiable concentrate formulation)

Lot/batch no.: ENBK-169309-002 (TSN313638)

### Test System

Organism (*Species*): Seven-spotted ladybird (*Coccinella septempunctata* L.)

Study type: Tier 2 extended laboratory study, exposure to both fresh dry residues and field-aged residues on leaves of the French bean plant (*Phaseolus vulgaris* L.).

Study design: (No. of replicates, assessments made etc.) Assessment of the survival of larvae and pupae, and, where < 50% corrected mortality in the test-item treatment, the number of eggs laid per female (fecundity) and the larval hatching rate (fertility). 40 replicates, consisting of 1 ladybird larva in each arena per test concentration for mortality phase.

Test item concentrations: 0 (control), 2.0 L product/ha.  
Treatments applied to plants twice (times T1 and T2), with a 14-day interval in-between.

Environmental conditions: Temperature: 23.6-25.5°C  
Relative humidity: 50-81%  
Photoperiod: 16 h (2800-4400 lux)  
Feeding: insects provided daily with untreated pea aphids (*Acyrtosiphon pisum* (Harris)).

Toxic reference substance: BAS 152 11 I (Perfekthion), nominally 400 g a.s./L dimethoate, applied at 200 mL product/ha. This treatment was applied to plants once (at time T2 only).

### Methodology

Treatments were applied at a volume rate of 400 L spray solution/ha to potted French bean plants on two occasions (T1 and T2), 14 days apart. Both in-between applications and following the second one (at T2), the treated plants were maintained outdoors, but protected from any rainfall, until foliage was collected for bioassays. These were initiated at 0 and 14 days after the second treatment application (DAT). The toxic reference treatment was included in the 0 DAT bioassay only, but the water control was included in all bioassays.

For each bioassay, the ladybird larvae (3-4 days old, n = 40 per treatment) were individually confined on excised leaves. The larvae were fed daily with untreated pea aphids and the pre-imaginal mortality of the ladybirds was assessed. To determine if there had been any sub-lethal effects on the reproductive capacity of the test insects, the egg-laying activity of the matured adult ladybirds was monitored over a 2-week period. For these reproduction assessments, both the numbers of eggs laid over a two-week sampling period and the viability of these eggs were recorded daily.

### RESULTS AND DISCUSSION

Pre-imaginal mortality for the control treatment was 25% and 0.0% in the bioassays initiated 0 and 14 DAT, respectively, and it was 97.5% (96.7% corrected) for the toxic reference treatment in the bioassay at 0 DAT. Therefore, all validity criteria imposed for the study were met. For the GF-3308 treatment, corrected pre-imaginal mortality was -16.7% and 5.0% for the 0 and 14 DAT bioassays, respectively.

**Table 85: Effects of GF-3308 on the survival of *Coccinella septempunctata*.**

Bioassay initiated (days after 2 <sup>nd</sup> treatment application)	Test concentrations (L product/ha, applied on two occasions)	% Mortality	Abbott-corrected % mortality
0	Control	25.0	-
	2.0	12.5	-16.7
	Toxic Reference	97.5 *	96.7
14	Control	0.0	-

	2.0	5.0	5.0
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\* Statistically different from the control ( $\alpha = 0.05$ ).

**Table 86: Effects of GF-3308 on the reproductive capacity of *Coccinella septempunctata*.**

Bioassay initiated (days after 2 <sup>nd</sup> treatment application)	Test concentrations (L product/ha, applied on two occasions)	Mean number eggs/female/day	Mean percentage egg viability	Mean viable eggs/female/day	Effects on reproduction[%]
0	Control	30.0	69.7	20.9	-
	2.0	29.0	62.6	18.2	13.2
14	Control	16.7	70.6	11.8	-
	2.0	15.9	73.3	11.7	1.0

## CONCLUSION

The effects of both freshly-dried and field-aged residues of GF-3308 on the ladybird beetle, *Coccinella septempunctata*, were evaluated under extended laboratory test conditions. Following two applications of GF-3308 to French bean plants at a rate of 2.0 L product/ha, with a 14-day interval between applications, no significant adverse effects on the survival or reproductive capacity of the ladybirds were observed in bioassays initiated 0 and 14 days after the second treatment application.

Common name	Species	Test item	Endpoint	Value	Toxicity value	Units of test item
Ladybird	<i>Coccinella septempunctata</i>	GF-3308	Preimaginal mortality	Time to pre-imaginal mortality being < 50%	0 days following 2 x 2.0	L product/ha

**A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna****A 2.4.1 KCP 10.4.1 Earthworms A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects A 2.4.1.1.1 Study 1 - GF-3308: Effects on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil with 10% Peat**

Comments of zRMS:	<p>The study was performed in line with OECD 222 with no deviations.</p> <p>The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to lack of the dose-response.</p> <p>All the validity criteria for the control group were met:</p> <ul style="list-style-type: none"> <li>- each replicate (containing 10 adults) should have produced <math>\geq</math> 30 juveniles by the end of the test (observed 180 to 265),</li> <li>- the coefficient of variation of reproduction should be <math>\leq</math> 30% (observed 12.3%),</li> <li>- adult mortality over the initial 4 weeks of the test should be <math>\leq</math> 10% (observed 0%).</li> </ul> <p>It is noted that at test concentrations of 51.4 and 167 mg product/kg dws clear promotion of the reproductive performance was observed. It is, however, considered to be incidental and not treatment related since promotion was not observed at higher and lower test concentrations.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>NOEC = 972 mg product/kg soil d.w.</p>
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Reference:	KCP 10.4.1.1/1
Report:	Ganßmann, M.; 2016; GF-3308: Effects on Reproduction and Growth of Earthworms <i>Eisenia fetida</i> in Artificial Soil with 10% peat; ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 111271022; DAS Study No. 160193 ; 15 September 2016; Unpublished
Guideline(s):	OECD 222, 2004 and ISO 11268-2, 2012
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

**MATERIALS AND METHODS****Test Item(s)**

Test item (Common name):	GF-3308
Purity:	XDE-777: 4.8% (49 g/L) (analysed)
Description (physical state):	Brown liquid
Lot/batch no.:	E3240-85-1, (TSN311166)



## Test System

Organism ( <i>Species</i> ):	Earthworm ( <i>Eisenia fetida</i> ), adult worms (with clitellum and weight range 301 to 567 mg), approximately 10 months old
Study type:	56 day earthworm reproduction study
Study design:	Assessment of the survival, behaviour and weight change of worms after 28 days exposure. Assessment of the number of offspring 56 days after treatment. 4 replicates, consisting of 10 worms in each vessel per test concentration. 8 replicates, consisting of 10 worms in each vessel for the control.
Test concentrations:	Control, 15.9, 28.6, 51.4, 92.6, 167, 300, 540 and 972 mg GF-3308/kg soil <sup>4</sup>
Soil parameters:	Artificial soil according to OECD 222. pH at initiation: 5.7 pH at termination: 5.9 Water content at initiation: 30.7% to 31.2% (52.0% to 52.9% of the maximum water holding capacity) Water content at termination: 29.5% to 35.5% (49.9% to 60.1% of the maximum water holding capacity)
Environmental conditions:	Temperature: within the range of 18°C to 22°C Relative humidity: - Light intensity: within the range of 400 lux to 800 lux Photoperiod: 16 h light : 8 h dark Feeding: Finely ground cattle manure was used as food and was added each week for the first 4 weeks of the experiment.

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<sup>4</sup> All concentrations are indicated per kg soil dry weight.

Reference substance:

Carbendazim 600 g/L SC (600 g/L nominal). The effects of the reference item were investigated in a separate study.

## Methodology

56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each.

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).

## RESULTS AND DISCUSSION

GF-3308 did not cause any statistically significant effects on mortality or body weight of the earthworms, up to and including the concentration of 972 mg test item/kg soil (Williams t-test,  $\alpha = 0.05$ ). No statistically significant effects on reproduction were observed up to and including the concentration of 972 mg test item/kg soil compared to the control group (Williams t-test,  $\alpha = 0.05$ , see Table 1). No behavioural abnormalities were observed in any of the treatment groups and the feeding activity in all the treated groups was comparable to the control.

**Table 87: Effects of GF-3308 on earthworm survival and biomass and reproduction**

Test concentrations (mg/kg)	% Mortality after 28 days	% Bodyweight change after 28 days	Mean no. of juveniles at day 56	% Change in number of juveniles compared to control
Control	0	47.8	212	-
15.9	0	48.0	240	113.3
28.6	0	45.8	195	92.1
51.4	0	45.4	280	132.2
92.6	0	43.9	207	97.8
167	0	43.1	254	119.8
300	0	45.0	209	98.8
540	0	49.3	207	97.6
972	0	48.6	210	99.4

\* Statistically different from the control

## CONCLUSION

In a 56 day earthworm reproduction and growth study with GF-3308, the  $LC_{50}$   $EC_{10}$  as well as the  $EC_{20}$  for reproduction were estimated to be greater than 972 mg test item/kg soil dry weight. The noobserved-effect-concentration (NOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined to be 972 mg test item/kg soil dry weight.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Earthworm	<i>Eisenia fetida</i>	GF-3308	56 day	NOEC	>972	mg/kg
Earthworm	<i>Eisenia fetida</i>	GF-3308	56 day	$EC_{10}$ reproduction	>972	mg/kg
Earthworm	<i>Eisenia fetida</i>	GF-3308	56 day	$EC_{20}$ reproduction	>972	mg/kg

**A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies A 2.4.2 KCP 10.4.2**  
**Effects on non-target soil meso- and macrofauna (other than earthworms)**  
**A 2.4.2.1 Study 1 – GF-3308: Effects on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat**

Comments of zRMS:	<p>The study was performed in line with OECD 232 with no deviations.</p> <p>The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). Reliability of the EC<sub>10</sub> value was evaluated in line with the recommendations of EFSA Supporting publication 2019:EN-1673:</p> <ul style="list-style-type: none"> <li>- NW (normalised width) of 0.37 was calculated, which results in rating “good” in line with Table E9 in EFSA Supporting publication 2019:EN-1673, - the dose-response curve is steep with steepness of 0.73 (i.e. &gt; 0.66).</li> <li>- median EC<sub>10</sub> (79.1 mg/kg soil d.w.) is not lower than EC<sub>20,low</sub> (73.7 mg/kg soil d.w.) but is lower than EC<sub>50,low</sub> (99.4 mg/kg soil d.w.) which indicates a medium rating for the certainty of the protection level.</li> </ul> <p>Taking the above results into account and in line with Table E10 in EFSA Supporting publication 2019:EN-1673, the calculated EC<sub>10</sub> is considered to be sufficiently reliable.</p> <p>All the validity criteria in the untreated controls were met:</p> <ul style="list-style-type: none"> <li>- mean adult mortality should not exceed 20% at the end of the test (observed 4%),</li> <li>- the mean number of juveniles per vessel should be at least 100 at the end of the test (observed 432 to 685),</li> <li>- the coefficient of variation calculated for the number of juveniles should be less</li> </ul>
	<p>than 30% at the end of the test (observed 13.4%).</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>NOEC (mortality) = 92.6 mg product/kg soil d.w. NOEC (reproduction) = 51.4 mg product/kg soil d.w. EC<sub>10</sub> = 79.1 mg product/kg soil d.w. EC<sub>20</sub> = 88.3 mg product/kg soil d.w. EC<sub>50</sub> = 108.8 mg product/kg soil d.w.</p>

Reference:	KCP 10.2.4.2/1
Report:	Ganßmann, M.; 2016; GF-3308: Effects on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat; ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 111271016; DAS Study No. 160191 ; 15 September 2016; Unpublished
Guideline(s):	OECD 232, 2009 and ISO 11267, 2014
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308

Purity: XDE-777: 4.92% (nominal), 4.8% (49 g/L) (analysed)

Description (physical state): Brown liquid

Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism (*Species*): Collembola (*Folsomia candida*), 10-12 days old

Study type: 28 day reproduction study

Study design: Assessment of survival and reproduction.  
4 replicates, consisting of 10 organisms in each vessel per test concentration, 8 replicates for the control

Test concentrations: Control, 4.90, 8.82, 15.9, 28.6, 51.4, 92.6, 167 and 300 mg GF-3308/kg soil dry weight

Soil parameters: Soil type: Artificial soil according to OECD 232 pH at initiation: 5.8  
pH at termination: 5.8 to 5.9  
Water content at initiation: 20.3% to 20.8% (53.3% to 54.8% of the maximum water holding capacity) Water content at termination: 18.9% to 19.7% (49.7% to 51.7% of the maximum water holding capacity)  
WHCmax: 38%

Environmental conditions: Temperature: within the range of 18°C to 22°C  
Lighting: 16 h light : 8 h dark (within the range of 400 to 800 lux)  
Feeding: Feeding: With *ca.* 2 mg dry yeast for each test vessel at the beginning of the test and on day 14.

Reference substance: Boric acid (conducted and reported as a separate study)

### Methodology

28 days exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the Collembola were introduced on top of the soil; 8 concentrations; 4 replicates/concentration (8 for the control) with 10 Collembola each. Feeding of Collembola with *ca.* 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioral effects and reproduction after 28 days.

## RESULTS AND DISCUSSION

A statistically significantly increased mortality was observed in the test item treated groups of 167 and 300 mg test item/kg soil compared to the control.

Reproduction of the collembolans exposed to GF-3308 was not statistically significantly different compared to the control up to and including the test concentration of 51.4 mg test item/kg soil. At all concentrations tested above 51.4 mg test item/kg soil the reproduction was statistically significantly reduced.

No behavioural abnormalities were observed in any of the treatment groups.

**Table 88: Effects of GF-3308 on *Folsomia candida* survival and reproduction**

Test concentrations (mg/kg)	Mean mortality of adults (%)	Mean no. of juveniles	% Change in no. of juveniles compared to control
Control	4	575	-
4.90	8	457	79
8.82	5	561	98
15.9	3	583	101
28.6	3	472	82
51.4	8	509	89
92.6	15	429*	75*
167	98*	20*	4*

300	100*	0*	0*
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\* Statistically different from the control

## CONCLUSION

GF-3308 caused no statistical significant effects on mortality of *Folsomia candida* up to and including the concentration of 92.6 mg test item/kg soil. On reproduction no effects were observed up to and including the concentration of 51.4 mg test item/kg soil.

Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be 92.6 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for mortality was determined to be 167 mg test item/kg soil. The NOEC for reproduction was determined to be 51.4 mg test item/kg soil. The LOEC for reproduction was determined to be 92.6 mg test item/kg soil. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> were determined to be 79.1 (95% CI: 58.3 to 87.9), 88.3 (95% CI: 73.7 to 96.5) and 108.8 (95% CI: 99.4 to 134.3) mg test item /kg soil, respectively.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Collembola	<i>Folsomia candida</i>	GF-3308	28 day	EC <sub>10</sub> reproduction	79.1	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3308	28 day	EC <sub>20</sub> reproduction	88.3	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3308	28 day	EC <sub>50</sub> reproduction	108.8	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3308	28 day	NOEC reproduction	51.4	mg/kg
Collembola	<i>Folsomia candida</i>	GF-3308	28 day	NOEC mortality	92.6	mg/kg

### A 2.4.2.2 Study 2 – GF-3308: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat

Comments of zRMS:	<p>The study was performed fully in line with OECD 226 with no deviations.</p> <p>The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). However, the ECx values could not be calculated due to lack of the dose-response.</p> <p>All the validity criteria in the untreated controls were met:</p> <ul style="list-style-type: none"> <li>- mean adult female mortality should not exceed 20% at the end of the test (observed 6%),</li> <li>- the mean number of juveniles per replicate (with 10 adult females introduced) should be at least 50 at the end of the test (observed 113 to 240),</li> <li>- the coefficient of variation calculated for the number of juveniles per replicate should not be higher than 30% at the end of the test (observed 21.7%).</li> </ul> <p>It is noted that at test concentration of 28.6 mg product/kg dws clear promotion of the reproductive performance was observed. It is, however, considered to be incidental and not treatment related since promotion was not observed at higher and lower test concentrations.</p> <p>Overall, the study is considered acceptable with the following endpoints relevant for the risk assessment:</p> <p>NOEC = 300 mg/kg soil d.w.</p>
Reference:	KCP 10.4.1.2/2

Report:	Ganßmann, M.; 2016; GF-3308: Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat; ibacon GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany; Lab Study No. 111271089; DAS Study No. 160192 ; 26 October 2016; Unpublished
Guideline(s):	OECD 226, 2008
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: XDE-777: 4.92% (nominal), 4.8% (49 g/L) (analysed)  
Description (physical state): Liquid Brown  
Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Organism (*Species*): Predatory soil mite (*Hypoaspis aculeifer*)  
Study type: Reproduction study  
Study design: Assessment of survival and reproduction.  
4 replicates, consisting of 10 organisms (females) in each vessel per test concentration, 8 replicates for the control  
Test concentrations: Control, 4.90, 8.82, 15.9, 28.6, 51.4, 92.6, 167 and 300 mg GF-3308/kg soil dry weight  
Soil parameters: Soil type: Artificial soil according to OECD 226.  
pH at initiation: 5.8 pH  
at termination: 5.7  
Water content at initiation: 20.3% to 20.8% (53.3% to 54.8% of the maximum water holding capacity) Water content at termination: 18.2% to 19.7% (47.9% to 51.8% of the maximum water holding capacity) WHCmax: 38%  
Environmental conditions: Temperature: within the range of 18°C to 22°C  
Lighting: 16 h light : 8 h dark (within the range of 400 to 800 lux)  
Feeding: Cheese mites (*Tyrophagus putrescentiae* cultured by ibacon), after the introduction of the test organisms and on day 2, 4, 7, 9 and 11.  
Reference substance: Perfekthion (a.s. dimethoate, 400 g/L, nominal) (conducted and reported as a separate study)

### Methodology

14-day exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the predatory mites were introduced on top of the soil; 8 concentrations; 4 replicates/concentration and 8 replicates for the control, with 10 female predatory mites each. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae*) *ad libitum* at test start and on days 2, 4, 7, 9 and 11. Assessment of adult mortality and reproduction after 14 d (counted after extraction on day 16 after application).

## RESULTS AND DISCUSSION

After 14 days of exposure, GF-3308 caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the concentration of 300 mg test item/kg soil. No behavioural effects were observed in any treatment group.

**Table 89: Effects of GF-3308 on *Hypoaspis aculeifer* survival and reproduction**

Test concentrations (mg/kg)	Mean mortality of adults (%)	Mean no. of juveniles	% change in no. of juveniles compared to control
Control	6	184	-
4.90	8	199	108
8.82	3	195	106
15.9	0	208	113
28.6	0	233	127
51.4	0	189	103
92.6	5	198	108
167	3	214	117
300	3	206	112

\* Statistically different from the control

## CONCLUSION

GF-3308 caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the concentration of 300 mg test item/kg soil. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 300 mg test item/kg soil. The overall Lowest Observed Effect Concentration (LOEC) as well as the EC<sub>10</sub>, EC<sub>20</sub>, EC<sub>50</sub> and the LC<sub>50</sub> were estimated to be greater than 300 mg test item/kg soil.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Predatory soil mite	<i>Hypoaspis aculeifer</i>	GF-3308	14 day	NOEC reproduction	300	mg/kg
Predatory soil mite	<i>Hypoaspis aculeifer</i>	GF-3308	14 day	EC <sub>10</sub> reproduction	>300	mg/kg

**A 2.4.2.3**  
**testing A 2.4.2.4**

**KCP 10.4.2.1**  
**KCP 10.4.2.2**

**Species level**  
**Higher tier**  
**testing**

**A 2.5 KCP 10.5 Effects on soil nitrogen transformation****A 2.5.1 Study 1 – GF-3308: Effects on the Activity of the Soil Microflora in the Laboratory**

Comments of zRMS:	<p>The study was performed fully in line with OECD 216 with no deviations.</p> <p>Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason the part of the study pertaining to carbon mineralisation was not validated by the zRMS and was struck through.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- the variation between replicate control samples was <math>\leq 15</math> % (observed max. 10.85 %).</li> </ul> <p>Overall, the study is considered acceptable.</p> <p>It may be concluded that the effects of the test item on soil nitrogen formation rates were <math>&lt; 25</math> % at the end of the study period (28 days) up to 13.5 mg product/kg soil d.w.</p>
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Reference:	KCP 10.5/1
Report:	Hammesfahr, U.; 2016; GF-3308: Effects on the Activity of the Soil Microflora in the Laboratory; ibacon GmbH, Rossdorf, Germany; Lab Study No. 111271080; DAS Study No. 160194 ; 05 July 2016; Unpublished
Guideline(s):	OECD Guideline 216 – Soil Microorganisms – Nitrogen Transformation Test
Deviations:	None
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

**MATERIALS AND METHODS****Test Item(s)**

Test item (Common name): GF-3308  
Purity: Analytical: XDE-777: 4.8wt% (49 g/L)  
Description (physical state): Emulsifiable concentrate (EC)  
Lot/batch no.: E3240-85-1 (TSN311166)

**Test System**

Organism (*Species*): Soil micro-organisms  
Study type: Laboratory study with OECD guideline natural soil, assessed for: Nitrate formation  
Microbial respiration  
Study duration: 28 days



Parameters measured:	<p>Nitrogen transformation: analysis of nitrate, nitrite and ammonium in extracted soil samples, via Continuous Flow Analyser (AA3, XY-2 / XY-3Sampler); limits of quantification:</p> <p>NO<sub>3</sub>-N: 0.096 mg/kg soil dry weight</p> <p>NO<sub>2</sub>-N: 0.077 mg/kg soil dry weight</p> <p>NH<sub>4</sub>-N: 0.125 mg/kg soil dry weight soil</p> <p>water content 46% to 48%</p> <p>pH 6.6 to 6.8</p> <p><del>Microbial respiration: soil respiration rates after addition of glucose</del></p> <p><del>soil water content 45% to 48%</del></p> <p><del>pH 6.6 to 6.8</del></p>
Observation intervals:	0, 7, 14 and 28 days
Test concentrations:	2.69 and 13.5 mg GF-3308/kg soil dry weight
Toxic reference:	<p>Sodium Chloride</p> <p>16 g/kg soil dry weight</p> <p>The inhibition of <del>soil respiration</del> and nitrogen transformation by sodium chloride at a concentration of 16 g/kg soil dry weight was determined at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly.</p>
Method of test item application:	Incorporation into the soil
Environmental conditions:	<p>Conducted in the dark.</p> <p>Temperature: 20 ± 2°C (SD) pH:</p> <p>6.6 to 6.8</p>
Soil properties	<p>Soil source: The soil batch used in this study was according to the Guidelines and was taken from fallow grassland:</p> <p>District authority: Darmstadt-Dieburg</p> <p>Municipality: 64380 Rossdorf, Germany</p> <p>Geographical position:</p> <p>longitude 8° 44' 38.70" E</p> <p>latitude 49° 51' 59.59" N</p> <p>Moisture content of soil at start: 46 - 48% of MWHC</p> <p>Moisture content of soil at end: 45 - 46% of MWHC</p> <p>Clay (%): 8.6</p> <p>Silt (%): 30.2</p> <p>Sand (%): 61.2</p> <p>Organic Carbon (%): 0.95</p> <p>Textural classification: Loamy Sand</p>

## Methodology

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH<sub>4</sub><sup>+</sup>-, NO<sub>2</sub><sup>-</sup>- and NO<sub>3</sub><sup>-</sup>-nitrogen formed from the nitrification process were determined by means of a Continuous Flow Analyser (AA3, XY-2 / XY-3Sampler).

~~Determination of soil respiration in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A BSB Sensomat System® was used to determine the CO<sub>2</sub> production over a period of up to 24 hours at different sampling intervals.~~

## RESULTS AND DISCUSSION

The soil nitrate formation rates were below the 25% trigger value given by the OECD 216 guideline. In the last interval between days 14 and 28, the deviations from control were 1.79% and 2.62% for the low and high test rate of GF-3308.

The soil respiration rates were within the trigger value of  $\pm 25\%$  set by OECD guideline 217 at day 28; on day 28 the values differed by 2.38% and 2.11% from the control for the low and high test rate of GF-3308, respectively.

**Table 90: Effects of GF-3308 on the nitrate formation rate**

Interval sampling days	Control	2.69 mg GF-3308 /kg soil dry weight				13.5 mg GF-3308 /kg soil dry weight		
	[mg/kg/day <sup>1</sup> ]	[mg/kg/day <sup>1</sup> ]	[% <sup>2</sup> ]	[sig <sup>3</sup> ]		[mg CO <sub>2</sub> /kg <sup>1</sup> ]	[% <sup>2</sup> ]	[sig <sup>3</sup> ]
0-7	-1.120	-1.136	1.43	n.s.		-1.099	-1.88	n.s.
7-14	1.564	1.675	7.10	n.s.		1.663	6.33	n.s.
14-28	1.450	1.476	1.79	n.s.		1.488	2.62	n.s.

1 mean mg NO<sub>3</sub>-N/kg soil dry weight per day

2 deviation from control

3 statistical significance

**Table 91: Effects of GF-3308 on the respiration rate**

Sampling days	Control	2.69 mg GF-3308 /kg soil dry weight				13.5 mg GF-3308 /kg soil dry weight		
	[mg CO <sub>2</sub> /kg/h <sup>1</sup> ]	[mg CO <sub>2</sub> /kg/h <sup>1</sup> ]	[% <sup>2</sup> ]	[sig <sup>3</sup> ]		[mg CO <sub>2</sub> /kg/h <sup>1</sup> ]	[% <sup>2</sup> ]	[sig <sup>3</sup> ]
0	12.179	12.385	1.69	n.s.		12.787	4.99	n.s.
7	11.181	10.772	-3.66	n.s.		10.042	-10.19	n.s.
14	10.538	10.306	-2.20	n.s.		10.609	0.67	n.s.
28	9.324	9.546	2.38	n.s.		9.521	2.11	n.s.

1 mean mg CO<sub>2</sub>/kg soil dry weight and hour

2 deviation from control

3 statistical significance

## CONCLUSION

Based on the results of this study, it is concluded that GF-3308 had no significant impact on soil microorganisms (carbon and nitrogen transformation) when applied at test item concentrations up to 13.5 mg/kg soil dry weight.

It can be concluded that GF-3308 will not have any long term influence on soil microorganisms.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
Soil micro organisms	N/A	GF-3308	28 day – nitrogen transformation	NOEC	13.5	mg/kg soil
Soil micro organisms	N/A	GF-3308	28 day – carbon respiration	NOEC	13.5	mg/kg soil

### A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

#### A 2.6.1 KCP 10.6.1 Summary of screening data A 2.6.2 KCP 10.6.2

##### Testing on non-target plants A 2.6.2.1 Study 1 - GF-3308 (XDE-777 50 g as/L, EC):

#### A Vegetative Vigour Test with ten Non Target Plant Species, GLP Terrestrial Non Target Plants (based on OECD Guideline 227) – Europe 2016

Comments of zRMS:	<p>The study was performed in line with OECD 227 with minor deviations in the test conditions.</p> <p>It was noted that the temperature range of 12°C to 32°C was not kept during the test. The temperature was higher than 32°C for: sugar beet (5 times for 1-4 hours, 35 °C in maximum), oat and onion (1 time for 2 hours, 33 °C in maximum), tomato (11 times for 1-5 hours, 35 °C in maximum), cucumber (8 times for 1-3 hours, 35 °C in maximum), soybean (9 times for 2-7 hours, 39 °C in maximum), ryegrass, oilseed rape, carrot, sunflower (9 times for 1-4 hours, 37 °C in maximum). For that reason ventilation flaps were fully opened and shadow system was activated. Since the untreated control plants were not damaged and remained healthy throughout the test, the temperature deviations are considered to have had no negative influence on plant development of all treatments and plant species and no impact on the outcome of the study.</p> <p>It was also noted that the number of plants per pot (14 cm diameter) was 4-6 for all plant species while the guideline recommends 1-2 seeds for bigger plants and 5-10 for smaller plants (15 cm diameter pot). However, since all the control plants in the study survived and no phytotoxic effects were observed, the overcrowding potential is not considered to have had an impact on the outcome of the study.</p> <p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- the seedling emergence was <math>\geq 70\%</math> (observed 78 to 100 %),</li> <li>- the control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibited only normal variation in growth and morphology for that particular species,</li> <li>- the mean survival of emerged control seedlings was <math>\geq 90\%</math> (observed 100 %),</li> <li>- the environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.</li> </ul> <p>The analytical measurements confirmed that the substance concentration was maintained within 80-120% of nominal.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER<sub>50,shoot fresh weight</sub> &gt; 4.0 L product/ha (corresponding to 200.0 g a.s./ha)</p> <p>The phytotoxic effects were qualitatively estimated using phytotoxicity rating system, so it would be difficult to calculate actual phytotoxicity endpoints from the study. Nevertheless, in case score 10 would be considered to represent 0% visual injury and each point would be considered to represent 10% effects, with the lowest score of 7 observed on sugar beet at 4.0 L/ha the maximum effect would be representative for 30% effect. Since this is &lt;50%, the ER<sub>50</sub> for phytotoxicity is estimated to be &gt;4.0 L product/ha.</p>
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Reference:	KCP 10.6.2/1
Report:	Strömel, C., Friedemann, A.; 2016; GF-3308 (DE-777 50 g a.s./L, EC): A Vegetative Vigour Test with ten Non Target Plant Species, GLP Terrestrial Non Target Plants (based on OECD Guideline 227) – Europe 2016; agro-check Dr. Teresiak & Erdmann GbR, Dorfstr. 15, 16833 Lentzke, Germany; Lab Study No. AC/DOW/16/02; DAS Study No. 160372 ; 16 December 2016; Unpublished
Guideline(s):	OECD Guideline for the Testing of Chemicals; Test No. 227 Terrestrial Plant Test: Vegetative Vigour Test, 19 July 2006

Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## Deviations:

## Deviation No. 1

## 1. Description

The Temperature range of 12°C to 32°C should be kept in trial period.

The temperature was higher than 32°C for :

Sugar beet (5 times for 1-4 hours, 35 °C in maximum), oat and onion (1 time for 2 hours, 33 °C in maximum), tomato (11 times for 1-5 hours, 35 °C in maximum), cucumber (8 times for 1-3 hours, 35 °C in maximum), soybean (9 times for 2-7 hours, 39 °C in maximum), ryegrass, oilseed rape, carrot, sunflower (9 times for 1-4 hours, 37 °C in maximum).

2. Measures taken: Ventilation flaps were fully opened and shadow system was activated.

3. Impact on the study: None. Untreated control plants were not damaged and healthy throughout the trial period. Therefore it can be assumed that the temperatures >32 °C had no negative influence on plant development of all treatments and plant species.

OECD 227 is vague with regard to the number of plants per pot and pot size as it relates to overcrowding potential. The number of plants per pot and pots per treatment are listed below. The density did not impact the study outcome as the ER50 was > 200 g XDE-777/ha, the highest rate tested, and validity criteria for controls were met. Pictures are provided in the full report.

Species	Plants per pot	Pots per treatment
Oat	5	5
Ryegrass	6	5
Onion	5	5
OSR	6	5
Soybean	5	5
Carrot	5	5
cucumber	4	6
Sugar beet	6	5
Sunflower	6	5
tomato	6	5

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308

Purity: XDE-777 4.8%

Description (physical state): Clear amber liquid

Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Monocotyledonous species: Oat, ryegrass, onion  
 Dicotyledonous species: Oilseed rape, soybean, carrot, cucumber, sugar beet, sunflower, tomato  
 Study type: Greenhouse study assessing Vegetative Vigour  
 Parameters measured: Number of living and dead plants: 7, 14 and 21 days after application  
 Foliar fresh weight: 21 days after application

Phytotoxicity rating system, if used:

Rating	Description of main categories	Detailed description
10	No effects	Excellent, good colour, no defects, no crop reduction or injury
9	Slight effects	Very slight marginal phytotoxic effects, no stunting
8		Slight, but identifiable phytotoxic effects, slight stunting
7		Crop injury more pronounced, but not lasting, slight stunting
6	Moderate effects	Moderate injury, crop usually recovers, stunting
5		Crop injury more lasting, recovery doubtful, stunting
4		Lasting crop injury, no recovery, stunting
3	Severe effects	Heavy crop injury and stunting
2		Crop nearly destroyed – a few surviving plants, stand loss
1		Only occasional live crop plants left, almost dead
0	Complete effects	Complete crop destruction, all dead

Growth conditions: Temperature (range): daily mean average 18.3 - 27.4 °C  
 Photoperiod: 16/8  
 Light intensity (range): adding artificial light for 16 hours in maximum if outdoor light intensity was lower than 10 klux  
 Relative humidity: 47.4 – 72.4 %  
 Water regime and schedules: as needed  
 Water source/type: rain water  
 Pest control method /fertilisation, if used: Pest control none, Fertiliser was added to each pot as required with a NPK-fertiliser (Hakaphos blau) as a 0.3 % solution. Kfertiliser (Gabi Plus K) was added as needed. All pots of one plant species and treatment rate obtained the same level of fertiliser during the trial  
 Growth medium: Soil type: Light loamy sand  
 Details of nutrient medium, if used: Hakaphos blau, Gabi Plus K pH: 7.4  
 Test concentrations: Nominal: 0.25, 0.50, 1.00, 2.00 and 4.00 L test item /ha corresponding to 12.5, 25.0, 50.0, 100.0 and 200.0 g a.s./ha  
 Mean calculated concentrations: 0.25 to 4.00 L test item /ha  
 Analytical verification: Mean recovery of XDE-777 in the application solution accounted for 100 % of the target concentration and thus reflected the theoretical value. In the untreated specimen (control) no residues of XDE-777 were found.  
 Test material application: Method: track sprayer

Application interval: 1 timing  
 Reference chemical (if used): none/ plain tap water  
 Seeds: Source: breeders  
 Method of seeding: by hand  
 Prior seed treatment/sterilisation: For sugar beet cultivation the soil was pre-treated by steaming ( $> 70^{\circ}\text{C}$ ) for more than 12 hours to reduce soil-borne diseases.  
 Number of seeds per replicate: 4-6  
 Growth stage at application: two to four leave stage (BBCH 12-14)  
 Number of control replicates: 5-6  
 Number of test concentration replicates: 5-6

## Methodology

Greenhouse trial, dose response design; GF-3308 was applied at BBCH 12 – 14; plants were cultivated for 21 days under greenhouse conditions. Assessments for plant injury (phytotoxicity) and plant survival were done 7, 14 and 21 days after treatment (DAT) for all species. Above-ground shoot fresh weight was determined at study termination 21 DAT.

## RESULTS AND METHODS

The trial was conducted under stable and controlled environmental conditions. Temperature conditions ensured a good growth of plants. Additional light ensured a light supply determined to represent a  $\geq 16$  hour day length (additional light supply for 16 hours in maximum if outdoor illumination was less than 10 klux). All maintenance was done according to good horticultural practice and was adapted to each pot. Plant growth and biomass production of all crops showed no problems in the untreated control. Summarising these aspects there are no unusual test conditions affecting the study. Conclusively the study is suited to determine effect rates for the tested plant species.

All control plants remained healthy throughout the entire trial period. No control mortality was observed. Thus any adverse influences on the study results can be excluded and the study can be considered as valid.

GF-3308 did not influence plant survival of all tested plant species up to the highest tested rate of 200.0 g DE-777/ha (4.00 L GF-3308/ha).

No phytotoxic effect occurred for oat, ryegrass and onion. Very slight damages were found for oilseed rape, soybean, carrot, cucumber and tomato (chlorosis, necrosis). Slight deformations were observed for oilseed rape, soybean and cucumber. Most affected species were sugar beet and sunflower with additional necrosis, stunting and deformation at rates  $\geq 2.00$  L GF-3308/ha.

No negative impact of GF-3308 on fresh biomass production was found for all tested species except sugar beet and sunflower with biomass reductions of 21 % after application of 4.00 L GF-3308/ha (200.0 g DE-777/ha) at BBCH 12-14. For sugar beet a significant reduction (6%) in biomass was recorded at the 2.00 L GF-3308/ha (100.0 g DE-777/ha) rate at BBCH 12-14 growth stage.

**Table 92: Observations of % survival, visual injury and shoot fresh weight (g): Monocotyledonous species**

	Oat			Ryegrass			Onion		
Application rate [g DE-777/ha]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]

0.0	100	10.0	98.350	100	10.0	26.467	100	10.0	105.887
12.5	100	10.0	78.756	100	10.0	25.354	100	10.0	101.323
25.0	100	10.0	101.173	100	10.0	25.354	100	10.0	97.518
50.0	100	10.0	87.475	100	10.0	28.169	100	10.0	90.119
100.0	100	10.0	95.110	100	10.0	23.346	100	10.0	98.396
200.0	100	10.0	90.515	100	10.0	25.245	100	10.0	96.996

**Table 93: Observations of % survival, visual injury and shoot fresh weight (g): Dicotyledonous species**

	Oilseed rape			Soybean			Carrot		
Application rate [g XDE777/ha]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]
0.0	100	10.0	471.745	100	10.0	161.762	100	10.0	69.396
12.5	100	9.0	505.833	100	10.0	162.478	100	9.4	70.380
25.0	100	9.0	480.996	100	9.0	158.442	100	9.2	59.893
50.0	100	9.2	467.344	100	9.0	162.682	100	9.0	72.500
100.0	100	9.0	495.391	100	9.0	158.672	100	9.0	72.150
200.0	100	9.0	462.840	100	9.0	150.456	100	9.0	73.481

	Cucumber			Sugar beet			Sunflower		
Application rate [g XDE777/ha]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]
0.0	100	10.0	860.445	100	10.0	334.710	100	10.0	723.288
12.5	100	10.0	1042.625	100	10.0	333.540	100	9.0	708.208
25.0	100	10.0	947.954	100	9.6	326.955	100	9.0	716.790
50.0	100	9.0	996.173	100	9.0	326.156	100	9.0	731.995
100.0	100	9.0	1026.415	100	8.0	313.558**	100	8.4	689.853
200.0	100	9.0	897.830	100	7.0	262.717**	100	7.0	569.303 *

	Tomato		
Application rate [g XDE-777/ha]	Survival [%]	Visual injury	Shoot fresh weight [g/replicate]
0.0	100	10.0	292.364
12.5	100	10.0	305.707
25.0	100	9.2	318.086
50.0	100	9.4	297.910
100.0	100	9.0	296.408
200.0	100	9.0	296.835

\*significantly different to the untreated control (Dunnett's t-test,  $\alpha=0.05$ )\*\*significantly different to the untreated control (Welch-t test with Bonferroni adjustment,  $\alpha=0.05$ )**Table 94: Reported ER<sub>50</sub> values for shoot fresh weight g a.s./ha**

Species	Shoot fresh weight ER <sub>50</sub>
Oat	> 200.0
Ryegrass	> 200.0
Onion	> 200.0
Oilseed rape	> 200.0
Soybean	> 200.0
Carrot	> 200.0
Cucumber	> 200.0
Sugar beet	> 200.0
Sunflower	> 200.0
Tomato	> 200.0

## CONCLUSION

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that GF-3308 applied post emergence with rates up to 4.00 L/ha (200.0 g DE-777/ha) did not cause adverse effects to plant mortality of all tested species.

No negative impact of GF-3308 on fresh biomass production was found for all species except sugar beet and sunflower. The most sensitive plant species were found to be sugar beet and sunflower with biomass reductions of 21 % each after application of 4.00 L GF-3308/ha (200.0 g DE-777/ha) at BBCH 12-14. No ER50 for fresh biomass could be calculated for all tested species and it is considered to be greater than 200.0 g DE-777/ha, the highest rate tested.

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
All tested monocots	All tested monocots	GF-3308	N/A	Shoot weight ER <sub>50</sub>	>200.0	g XDE-777/ha
Sunflower	<i>Helianthus annuus</i> L. (dicot)	GF-3308	N/A	Shoot weight ER <sub>50</sub>	>200.0	g XDE-777/ha

### A 2.6.2.2 Study 2 - GF-3308 (XDE-777 50 g as/L, EC): A Seedling Emergence and Seedling Growth Test with ten Non Target Plant Species, GLP Terrestrial Non Target Plants (based on OECD Guideline 208) – Europe 2016

Comments of zRMS:	<p>The study was performed in line with OECD 208 with minor deviations in the test conditions.</p> <p>It was noted that the temperature range of 12°C to 32°C was not kept during the test. The temperature was higher than 32°C for: ryegrass, sugar beet (5 times for 1-5 hours, 34 °C in maximum), onion, carrot (7 times for 1-6 hours, 35 °C in maximum), sunflower, oat (1 time for 1 hour, 33 °C in maximum), cucumber (10 times for 1-4 hours, 39 °C in maximum), soybean, tomato (4 times for 1-4 hours, 39 °C in maximum). For that reason ventilation flaps were fully opened and shadow system was activated. Since the untreated control plants were not damaged and remained healthy throughout the test, the temperature deviations are considered to have had no negative influence on plant development of all treatments and plant species and no impact on the outcome of the study.</p> <p>It was also noted that the number of seeds per pot (14 cm diameter) was 4-10 for all plant species while the guideline recommends 1-2 seeds for bigger plants and 5-10 for smaller plants (15 cm diameter pot). However, since all the control plants in the study survived and no phytotoxic effects were observed, the overcrowding potential is not considered to have had an impact on the outcome of the study.</p> <p>It was further noted that one pot of one replicate was excluded from the biomass measurements because the pot/shoot was damaged. That exclusion from analysis did not have any negative impact on the outcome of the study.</p>
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	<p>All the validity criteria were met:</p> <ul style="list-style-type: none"> <li>- the seedling emergence was <math>\geq 70\%</math> (observed 78 to 100 %),</li> <li>- the control seedlings of each species did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibited only normal variation in growth and morphology for that particular species,</li> <li>- the mean survival of emerged control seedlings was <math>\geq 90\%</math> (observed 100 %),</li> <li>- the environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.</li> </ul> <p>The analytical measurements confirmed that the substance concentration was maintained within 80-120% of nominal.</p> <p>Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment:</p> <p>ER<sub>50,shoot fresh weight</sub> &gt; 4.0 L product/ha (corresponding to 200.0 g a.s./ha)</p> <p>No phytotoxic effects were observed on any of the tested species, hence the ER<sub>50</sub> for phytotoxicity is estimated to be &gt;4.0 L product/ha.</p>
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Reference:	KCP 10.6.2/2
Report:	Strömel, C., Friedemann, A.; 2016/17; GF-3308 (DE-777 50 g a.s/L, EC): A Seedling Emergence and Seedling Growth Test with ten Non Target Plant Species, GLP Terrestrial Non Target Plants (based on OECD Guideline 208) – Europe 2016; agrocheck, Dr. Teresiak & Erdmann GbR, Dorfstr. 15, 16833 Lentzke, Germany; Lab Study No. AC/DOW/16/01; DAS Study No. 160373 ; 12 January 2017; Unpublished
Guideline(s):	OECD Guideline for the Testing of Chemicals; Test No. 208 Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, July 19, 2006
Deviations:	Minor (see the commenting box above)
GLP:	Yes
Acceptability:	Acceptable
Duplication (if vertebrate study)	NA

## Deviations:

## Deviation No. 1

Description: The Temperature range of 12°C to 32°C should be kept in trial period. The temperature was higher than 32°C for:

ryegrass, sugar beet (5 times for 1-5 hours, 34 °C in maximum), onion, carrot (7 times for 1-6 hours, 35 °C in maximum), sunflower, oat (1 time for 1 hour, 33 °C in maximum), cucumber (10 times for 1-4 hours, 39 °C in maximum), soybean, tomato (4 times for 1-4 hours, 39 °C in maximum).

Measures taken: Ventilation flaps were fully opened and shadow system was activated.

Impact on the study: None. Untreated control plants were not damaged and healthy throughout the trial period. Therefore it can be assumed that the temperatures >32 °C had no negative influence on plant development of all treatments and plant species.

OECD 208 is vague with regard to the number of seeds planted per pot and pot size as it relates to overcrowding potential. The number

of seeds per pot and pots per treatment are listed below. The density did not impact the study outcome as the ER<sub>50</sub> was > 200 g XDE777/ha, the highest rate tested, and validity criteria for controls were met. Pictures are provided in the full report.

Species	Seeds per pot	Pots per treatment
Oat	5	5
Ryegrass	10	5
Onion	10	5
OSR	5	5
Soybean	5	5
Carrot	8	5
cucumber	4	6
Sugar beet	5	5
Sunflower	5	5
tomato	5	5

## MATERIALS AND METHODS

### Test Item(s)

Test item (Common name): GF-3308  
Purity: XDE-777 4.8%  
Description (physical state): Clear amber liquid  
Lot/batch no.: E3240-85-1 (TSN311166)

### Test System

Monocotyledonous species: Oat, ryegrass, onion  
Dicotyledonous species: Oilseed rape, soybean, carrot, cucumber, sugar beet, sunflower, tomato  
Study type: Greenhouse study assessing Seedling Emergence and Seedling Growth  
Parameters measured: Emergence, number of living and dead plants: 7, 14 and 21 days after application (14, 21 and 28 days after application for onion and carrot)  
Shoot fresh weight: 21 days after application (28 days after application for onion and carrot) Phytotoxicity rating system, if used:

Rating	Description of main categories	Detailed description
10	No effects	Excellent, good colour, no defects, no crop reduction or injury
9	Slight effects	Very slight marginal phytotoxic effects, no stunting
8		Slight, but identifiable phytotoxic effects, slight stunting
7		Crop injury more pronounced, but not lasting, slight stunting
6	Moderate effects	Moderate injury, crop usually recovers, stunting
5		Crop injury more lasting, recovery doubtful, stunting
4		Lasting crop injury, no recovery, stunting
3	Severe effects	Heavy crop injury and stunting
2		Crop nearly destroyed – a few surviving plants, stand loss
1		Only occasional live crop plants left, almost dead
0	Complete effects	Complete crop destruction, all dead

Growth conditions:

Temperature (range): daily mean average 21.6 °C -

	27.1 °C
	Photoperiod: 16/8
	Light intensity (range): adding artificial light for 16 hours in maximum if outdoor light intensity was lower than 10 klux
	Relative humidity: 46.4 % to 65.6 %
	Water regime and schedules: as needed
	Water source/type: rain water
	Pest control method /fertilisation, if used: Pest control none, Fertiliser was added to each pot as required with a NPK-fertiliser (Hakaphos blau) as a 0.2 % or 0.3 % solution. K-fertiliser (Gabi Plus K) was added as needed. All pots of one plant species and treatment rate obtained the same level of fertiliser during the trial
Growth medium:	Soil type: Light loamy sand
	Details of nutrient medium, if used: Hakaphos blau, Gabi Plus K pH: 7.4
Test concentrations:	Nominal: 0.25, 0.50, 1.00, 2.00 and 4.00 L test item /ha corresponding to 12.5, 25.0, 50.0, 100.0 and 200.0 g a.s./ha
	Mean calculated concentrations: 0.25 to 4.00 L test item /ha
Analytical verification:	Mean recovery of XDE-777 in the application solution accounted for 98 % or 100 % of the target concentration and thus reflected the theoretical value. In the untreated specimen (control) no residues of XDE-777 were found.
Test material application:	Method: track sprayer
	Application interval: 1 timing
	Reference chemical (if used): none/ plain tap water
Seeds:	Source: breeders
	Method of seeding: by hand
	Prior seed treatment/sterilisation: For sugar beet cultivation the soil was pre-treated by steaming (> 70 °C) for more than 12 hours to reduce soil-borne diseases.
	Number of seeds per replicate pot: 4-10
	Growth stage at application: applied pre emergence shortly after seeding (BBCH 00)
Number of control replicates:	5
Number of test concentration replicates:	5

## Methodology

Greenhouse trial, dose response design; applied pre emergence shortly after seeding; plants were cultivated for 21 days (28 days for onion and carrot) under greenhouse conditions. Assessments for plant injury (phytotoxicity) and plant stand (emergence and mortality) were done 7, 14 and 21 days after treatment (DAT) (carrot and onion 14, 21 and 28 days). Above-ground shoot fresh weight was determined at study termination 21 DAT (28 DAT for carrot and onion).

## RESULTS AND DISCUSSION

The trial was conducted under stable and controlled environmental conditions. Temperature conditions ensured a good growth of plants. Additional light ensured a light supply determined to represent a  $\geq 16$  hour day length (additional light supply for 16 hours in maximum if outdoor illumination was less than 10 klux). All maintenance was done according to good horticultural practice and was adapted to each

pot. Plant growth and biomass production of all plant species showed no problems in the untreated control. Summarising these aspects there are no unusual test conditions affecting the study. Conclusively the study is suited to determine effect rates for the tested plant species.

All emerged control plants remained healthy throughout the entire trial period. No control mortality was observed. The rate of emergence in the controls was  $\geq 70\%$  for all tested plant species. Thus any adverse influences on the study results can be excluded and the study can be considered as valid.

The seedling emergence, plant survival and biomass production (fresh weight) of all tested species were not influenced by the test item up to the highest tested rate of 4.00 L GF-3308/ha (200.0 g XDE777/ha).

**Table 95: Observations of % emergence, % survival, visual injury and shoot fresh weight (g): Monocotyledonous species**

	Oat				Ryegrass				Onion			
Application rate [g XDE- 777/ha]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]
0.0	96	100	10.0	12.341	92	100	10.0	5.321	90	100	10.0	11.560
12.5	96	100	10.0	14.421	90	100	10.0	5.364	90	100	10.0	12.227
25.0	96	100	10.0	13.455	96	100	10.0	5.904	90	100	10.0	11.741
50.0	100	100	10.0	13.894	92	100	10.0	5.769	90	100	10.0	12.451
100.0	96	100	10.0	13.289	86	100	10.0	5.503	90	100	10.0	12.278
200.0	100	100	10.0	13.104	92	100	10.0	4.810	94	100	10.0	12.641

**Table 96: Observations of % emergence, % survival, visual injury and shoot fresh weight (g): Dicotyledonous species**

	Oilseed rape				Soybean				Carrot			
Application rate [g XDE- 777/ha]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]
0.0	84	100	10.0	42.923	88	100	10.0	29.578	78	100	10.0	14.412
12.5	88	100	10.0	44.732	84	100	10.0	25.764	78	100	10.0	15.010
25.0	100	100	10.0	49.335	88	100	10.0	30.538	88	100	10.0	15.512
50.0	88	100	10.0	43.413	84	100	10.0	28.990	90	100	10.0	14.904
100.0	96	100	10.0	48.763	84	100	10.0	24.719	80	100	10.0	13.044
200.0	96	100	10.0	44.806	84	100	10.0	30.472	80	100	10.0	15.071

	Cucumber				Sugar beet				Sunflower			
Application rate [g XDE- 777/ha]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g /replicate]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]	Emergence [%]	Survival [%]	Visual injury	Shoot fresh weight [g/ replicate]
0.0	100	100	10.0	52.229	100	100	10.0	28.855	100	100	10.0	67.680
12.5	100	100	10.0	52.788	96	100	10.0	30.268	96	100	10.0	66.584
25.0	96	100	10.0	50.075	100	100	10.0	35.315	100	100	10.0	68.576
50.0	96	100	10.0	49.996	96	100	10.0	32.458	96	100	10.0	65.849
100.0	100	100	10.0	50.777	96	100	10.0	31.532	96	100	10.0	68.696
200.0	100	100	10.0	52.235	100	100	10.0	33.793	96	100	10.0	68.199

	<b>Tomato</b>
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<b>Application rate [g XDE-777/ha]</b>	<b>Emergence [%]</b>	<b>Survival [%]</b>	<b>Visual injury</b>	<b>Shoot fresh weight [g/ replicate]</b>
0.0	92	100	10.0	32.775
12.5	92	100	10.0	32.254
25.0	96	100	10.0	33.091
50.0	84	100	10.0	31.481
100.0	92	100	10.0	29.293
200.0	88	100	10.0	31.196

**Table 97: Reported ER<sub>50</sub> values based on shoot fresh weight g a.s./ha**

Species	Shoot fresh weight ER <sub>50</sub>
Oat	> 200.0
Ryegrass	> 200.0
Onion	> 200.0
Oilseed rape	> 200.0
Soybean	> 200.0
Carrot	> 200.0
Cucumber	> 200.0
Sugar beet	> 200.0
Sunflower	> 200.0

## CONCLUSION

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that the fungicide GF-3308 did not cause adverse effects to the seedling emergence, plant survival and biomass of all tested plant species up to the highest tested rate of 4.00 L GF-3308/ha (200.0 g XDE-777/ha).

Common name	Species	Test item	Time-scale	Endpoint	Toxicity value	Units of test item
All tested monocots	All tested monocots	GF-3308	N/A	Shoot weight ER <sub>50</sub>	>200.0	g XDE-777/ha
All tested dicots	All tested monocots	GF-3308	N/A	Shoot weight ER <sub>50</sub>	>200.0	g XDE-777/ha

### A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No new data submitted.

### A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No new data submitted.

### A 2.8 KCP 10.8 Monitoring data

Studies of this type are not required and no data are submitted.